The effect of remifentanil on cardiovascular dynamics during anaesthesia

Thesis submitted to the University of Leicester for the degree of Doctor of Medicine by

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Dr Jonathan Thompson

Alterations in cardiovascular parameters are common during general anaesthesia, owing to the combined effects of anaesthetic drugs, surgical stimuli, changes in intravascular volume and baroreflex activity. In healthy patients, these changes may be well tolerated with minimal morbidity. However, in patients with limited cardiovascular function, hypotension, hypertension and tachycardia may lead to cardiac arrhythmias, myocardial ischaemia and infarction. Certain phases of anaesthesia and surgery are associated with particular increased risk for cardiovascular instability, especially induction of anaesthesia, instrumentation of the airway, skin incision, emergence and awakening from anaesthesia. Therefore specific measures may be required to attenuate cardiovascular responses to noxious stimuli. Remifentanil is a new opioid introduced into clinical practice in 1996, whose pharmacokinetic and pharmacodynamic properties suggested it might be useful to attenuate adverse autonomic and cardiovascular responses. This thesis describes studies of these possible effects of remifentanil.

Remifentanil attenuated the cardiovascular and plasma catecholamine responses to laryngoscopy and tracheal intubation in healthy young adults. However, bradycardia and hypotension were observed in some individuals, and further studies confirmed that lower doses than originally recommended were effective. Remifentanil was also effective in elderly patients, and hypertensive patients, but observations of myocardial ischaemia confirmed that these groups are at risk. There was significant inter-individual variation in cardiovascular responses, confirming that remifentanil and other drugs used during anaesthesia should be titrated to effect. Remifentanil administered as a bolus attenuated the cardiovascular responses to emergence from anaesthesia and tracheal extubation, without compromising clinical recovery. Future studies should also address its place as an analgesic/sedative outside the operating room, for example in Intensive Care.

This thesis details the effects of remifentanil on responses to noxious stimuli during anaesthesia and adds to our collective understanding of cardiovascular responses during anaesthesia in different groups of patients.
Acknowledgements

This thesis is based on studies performed in the University of Leicester Department of Anaesthesia, Critical Care and Pain Management at the Leicester Royal Infirmary between April 1997 and September 2000. I am grateful to the many members of the University and NHS Departments of Anaesthesia who have helped along the way. In particular Drs Andrew Hall, Nisha Kumar, Apsara Leslie, Andrew Fox, Ashraf Habib and Andrew Gregg have all assisted greatly with the recruitment of patients and data collection. Jim Strupish, Chief Technician in the University Department of Anaesthesia has worked tirelessly to perform the assays of plasma catecholamine concentrations, and his contribution is greatly appreciated.

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<th>Full Form</th>
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<tbody>
<tr>
<td>5'AMP</td>
<td>5' adenosine monophosphate</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>ACh</td>
<td>acetyl choline</td>
</tr>
<tr>
<td>AChE</td>
<td>acetylcholinesterase</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ASA</td>
<td>American Society of Anesthesiologists</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AV</td>
<td>atrioventricular</td>
</tr>
<tr>
<td>AVN</td>
<td>atrioventricular node</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>calcium ions</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CO_{2}</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-O-methyl transferase</td>
</tr>
<tr>
<td>CSHT</td>
<td>context-sensitive half-time</td>
</tr>
<tr>
<td>CTOP</td>
<td>D-Phe-Sys-Tyr-D-Trp-Orn-Thr-Pen-Thr amide</td>
</tr>
<tr>
<td>CVLM</td>
<td>caudal ventrolateral medulla</td>
</tr>
<tr>
<td>DA_{1,2}</td>
<td>dopamine subtypes 1&amp;2</td>
</tr>
<tr>
<td>DAMGO</td>
<td>[d-Ala^{2-}, Me-Phe^{4}Gly-ol]-enkephalin</td>
</tr>
<tr>
<td>DAP</td>
<td>diastolic arterial pressure</td>
</tr>
<tr>
<td>DHB</td>
<td>dihydroxybenzylamine</td>
</tr>
<tr>
<td>DOP</td>
<td>delta opioid peptide</td>
</tr>
<tr>
<td>DPDPE</td>
<td>[D-Pen^{2,3}]-enkephalin</td>
</tr>
<tr>
<td>EC_{50}</td>
<td>median effective concentration of drug</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiograph</td>
</tr>
<tr>
<td>ED_{50}</td>
<td>median effective dose of drug</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma amino butyric acid</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>G_{i}, G_{io}, G_{q}</td>
<td>guanine nucleotide binding protein (i/o, inhibitory and q, stimulatory)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Term</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>GTP</td>
<td>guanosine triphosphate</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>IML</td>
<td>intermediolateral cell column</td>
</tr>
<tr>
<td>IP₃</td>
<td>inositol triphosphate,</td>
</tr>
<tr>
<td>K⁺</td>
<td>potassium ions</td>
</tr>
<tr>
<td>kₐ₀</td>
<td>constant for rate of equilibration between plasma and effect site of a drug</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>KOP</td>
<td>kappa opioid peptide</td>
</tr>
<tr>
<td>kPa</td>
<td>kilopascal</td>
</tr>
<tr>
<td>Li⁺</td>
<td>lithium ions</td>
</tr>
<tr>
<td>M₁-M₅</td>
<td>muscarinic receptors subtypes 1-5</td>
</tr>
<tr>
<td>MAC</td>
<td>minimum alveolar concentration</td>
</tr>
<tr>
<td>MANOVA</td>
<td>multivariate analysis of variance for repeated measures</td>
</tr>
<tr>
<td>MAO</td>
<td>monamine oxidase</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>mcg</td>
<td>microgram</td>
</tr>
<tr>
<td>mcl</td>
<td>microlitre</td>
</tr>
<tr>
<td>mcM</td>
<td>micromolar</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>magnesium ions</td>
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<tr>
<td>min</td>
<td>minute</td>
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<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm Hg</td>
<td>millimetres of mercury</td>
</tr>
<tr>
<td>MOP</td>
<td>mu opioid peptide</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>nM</td>
<td>nanomolar</td>
</tr>
<tr>
<td>NOP</td>
<td>nociceptin opioid peptide</td>
</tr>
<tr>
<td>NTS</td>
<td>nucleus tractus solitarius</td>
</tr>
<tr>
<td>O₂</td>
<td>oxygen</td>
</tr>
<tr>
<td>PAG</td>
<td>periaqueductal grey matter</td>
</tr>
<tr>
<td>pH</td>
<td>log concentration of hydrogen ions in solution</td>
</tr>
<tr>
<td>PIP₂</td>
<td>phosphatidylinositol biphosphate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>pKa</td>
<td>negative log of the dissociation constant; pH at which a drug is 50% ionised</td>
</tr>
<tr>
<td>POMC</td>
<td>pro-opiomelanocortin</td>
</tr>
<tr>
<td>RVLM</td>
<td>rostral ventrolateral medulla</td>
</tr>
<tr>
<td>RVMM</td>
<td>rostral ventromedial medulla</td>
</tr>
<tr>
<td>s</td>
<td>seconds</td>
</tr>
<tr>
<td>SA</td>
<td>sinoatrial</td>
</tr>
<tr>
<td>SAN</td>
<td>sinoatrial node</td>
</tr>
<tr>
<td>SAP</td>
<td>systolic arterial pressure</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SPN</td>
<td>sympathetic preganglionic neurone</td>
</tr>
<tr>
<td>SpO₂</td>
<td>peripheral oxygen saturation</td>
</tr>
<tr>
<td>t₁/₂ ( k_{e0} )</td>
<td>half-time for ( k_{e0} )</td>
</tr>
<tr>
<td>TRH</td>
<td>thyrotropin</td>
</tr>
<tr>
<td>( V_{De} )</td>
<td>central compartment volume of distribution</td>
</tr>
<tr>
<td>( V_{Des} )</td>
<td>volume of distribution at steady state</td>
</tr>
<tr>
<td>VLM</td>
<td>ventrolateral medulla</td>
</tr>
<tr>
<td>VSM</td>
<td>vascular smooth muscle</td>
</tr>
</tbody>
</table>
CHAPTER 1: INTRODUCTION

1.1 Stress responses during anaesthesia

1.2 Scope of this thesis
1.1 Anaesthesia and stress responses

The history of modern anaesthesia is relatively short, and began with the introduction into clinical practice in the 1840s of ether, nitrous oxide and chloroform. Before this time, surgeons and practitioners had attempted to alleviate the pain of surgery by various methods, including the administration of alcohol, herbal remedies and plant extracts or the topical administration of ice or pressure. Morphine was commercially available by the early 1820s in Europe, and was widely used as an analgesic but its use for surgery was limited because of the risks of severe respiratory depression and death. Pain was therefore considered an inevitable consequence of surgery and the scope of surgery was limited because of the lack of appropriate anaesthetic drugs or techniques (Toski et al. 2001). With the advent of modern anaesthesia 160 years ago the range of feasible surgery increased dramatically to encompass progressively more invasive and traumatic procedures. However, it became apparent that surgical trauma or stress is associated with physiological responses that can themselves be harmful, and that attenuation of these responses is not only desirable but is a fundamental part of good anaesthetic practice.

The quest for safer methods of anaesthesia, which allow increasingly invasive surgery in progressively sicker patients, has been a prominent feature of anaesthesia in the 20th century. This has led to an increased understanding of the physiological responses to surgery, and has fuelled the search for techniques or drugs that will effectively attenuate these responses. Although ‘anaesthesia’ is strictly defined as ‘absence of sensation’, it may be defined as a process of modification of the physiological responses to noxious environmental stimuli, in particular the responses to surgery. Rees and Gray classified surgical anaesthesia as comprising three basic components: narcosis, analgesia, and relaxation (Rees & Gray 1950), although Gray later redefined this triad as consisting of narcosis, reflex suppression, and relaxation. In this context, reflex suppression refers to inhibition of the reflex physiological autonomic and cardiovascular responses to surgical stimuli.

These autonomic and cardiovascular responses are very similar to those involved in the mammalian ‘fight or flight’ reaction to pain, fear or rage, and include an increase in heart
rate, arterial pressure, cardiac output, plasma catecholamine concentrations, myocardial contractility and oxygen consumption, and diversion of blood flow to the brain, heart and skeletal musculature, away from other vital organs (Guyton & Hall 2000). The responses involve interaction with many anatomical sites, but are co-ordinated by the autonomic and cardiovascular control centres in the spinal cord, brain stem and hypothalamus, which are in turn influenced by higher cortical centres and by visceral reflexes. Surgery, trauma or stressful stimuli also initiates a series of neuro-endocrine and metabolic changes in response to afferent neuronal input and tissue damage, mediated partly by the secretion of hormones from the hypothalamus, pituitary and adrenal glands (Table 1). The hormonal changes have secondary effects on other target organs and include increased secretion of adrenocorticotrophic hormone, growth hormone, arginine vasopressin, cortisol, aldosterone and decreased secretion of insulin (Derbyshire & Smith 1984, Desborough 2000). The overall effect of these endocrine and metabolic responses is to increase catabolism (to mobilise energy substrates) and activate mechanisms to retain salt and water (to maintain fluid volume and cardiovascular homeostasis). However, it has been suggested that these autonomic and endocrine stress responses are not only superfluous but may be harmful for some patients undergoing surgery and therefore many attempts have been made to modify them (Desborough 2000).

Suppression of reflex autonomic responses during and after surgery was part of the triad of anaesthesia defined over 50 years ago, but it has been suggested more recently that attenuation of these autonomic responses may reduce morbidity and mortality (Kehlet 1991). Anaesthetic drugs may interact with the cardiovascular system at a number of potential sites, including the central cardiovascular control centres, via effects on autonomic nervous activity or via direct effects on the heart and peripheral vasculature. A number of techniques have been used to attenuate perioperative stress responses, including the use of regional anaesthesia, opioids, anti-inflammatory, adrenergic or cytokine blocking drugs. The evidence for improved outcome after a single therapeutic manoeuvre is conflicting, but most data are derived from studies in which postoperative outcome was not a primary endpoint, and are therefore limited (Liu et al. 1995, Chumbley & Hall 1997, Kehlet 1997, Sheeran & Hall
Recent studies have shown that reduction of the perioperative stress responses by alpha and beta-adrenergic blockade or intense analgesia improves cardiovascular outcome in high risk patients (Mangano et al. 1992, Mangano et al. 1996, Martin et al. 1997, Wallace et al. 1998). It is likely that the wider benefits of perioperative stress reduction are greatest in certain patient subgroups in particular those at risk of myocardial ischaemia and cardiovascular morbidity, but the attenuation of reflex autonomic responses during anaesthesia and surgery remains a basic goal of anaesthesia.

### Table 1  Systemic responses to surgery

<table>
<thead>
<tr>
<th>Sympathetic nervous system activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine stress response:</td>
</tr>
<tr>
<td>Anterior and posterior pituitary hormones</td>
</tr>
<tr>
<td>Pancreatic hormones</td>
</tr>
<tr>
<td>Thyroid hormones</td>
</tr>
<tr>
<td>Immunological and haematological changes:</td>
</tr>
<tr>
<td>Cytokine production</td>
</tr>
<tr>
<td>Acute phase reaction</td>
</tr>
<tr>
<td>Neutrophil leucocytosis</td>
</tr>
<tr>
<td>Lymphocyte proliferation</td>
</tr>
</tbody>
</table>

A number of biological cascade systems are also activated in response to major physiological insults such as trauma, infection or surgery. They interact with the autonomic and endocrine responses at a number of levels and include increased production of cytokines, complement, arachidonic acid metabolites, nitric oxide, and oxygen free radicals. These compounds mediate both the inflammatory and immune responses to trauma and some of the changes in organ function (Sheeran & Hall 1997, Herskowitz & Mangano 1996). The systemic responses include neutrophilia, fever, adrenocorticotropic hormone release, increased hepatic production of 'acute phase' proteins and altered concentrations of serum transport proteins and ions. Together they are known as the acute phase response (Table 2).
Table 2  Features of the acute phase response

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophilia</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td>Increased hepatic production of 'acute phase' proteins:</td>
<td></td>
</tr>
<tr>
<td>C reactive protein, fibrinogen and alpha₂ macroglobulin</td>
<td></td>
</tr>
<tr>
<td>Changes in serum concentrations of transport proteins:</td>
<td></td>
</tr>
<tr>
<td>Increased caeruloplasmin</td>
<td></td>
</tr>
<tr>
<td>Decreased transferring, albumin, alpha₂ macroglobulin</td>
<td></td>
</tr>
<tr>
<td>Changes in serum ion concentrations:</td>
<td></td>
</tr>
<tr>
<td>Increased copper</td>
<td></td>
</tr>
<tr>
<td>Decreased iron and zinc</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Desborough (2000).

Whilst these inflammatory responses are advantageous in a biological context, their benefit in patients undergoing surgery is less certain, and in many circumstances may be detrimental, as may the autonomic and endocrine responses to trauma or surgery. For example, exaggerated or prolonged stress responses in surgical or Intensive Care patients can lead to the subsequent development of a more widespread systemic inflammatory response syndrome or to multi-organ failure (Rangelfrausto et al. 1995, Nystrom 1998, Nastkolb et al. 1998). There has been increasing interest in the modification of the inflammatory responses to surgery by anaesthesia, opioids or sympatholytic drugs although most studies have demonstrated little effect and the effect of this approach on outcome is not established (Eriksson-Mjoberg et al. 1997, Brix-Christensen et al. 1998, Helmy et al. 1999). However, since the acute phase response is directly related to the magnitude of tissue trauma, it is unlikely to be affected by anaesthesia (Desborough 2000). The work detailed in this thesis therefore concentrates on attenuation of the sympatho-adrenal response to noxious stimuli, as these responses are amenable to therapeutic manipulation (Derbyshire & Smith 1984).

The technique of laryngoscopy and tracheal intubation became increasingly popular approximately 50 years ago following the introduction of neuromuscular blocking drugs, which permitted tracheal intubation at a lighter plane of anaesthesia than had previously been
possible (Bourne 1947). The mammalian larynx and trachea are protected by a number of physiological reflexes designed to prevent the passage of noxious substances into the lungs (Wyke & Kirschner 1976). These reflexes are extremely sensitive, responding to minute gaseous or liquid particles, and primarily consist of coughing, and glottic closure, but are also associated with increased sympathetic nervous activity and cardiovascular stimulation. The mechanisms underlying these reflex responses are co-ordinated by the brain stem nuclei involved in cardiovascular and autonomic nervous control, including a number of sites possessing opioid receptors. It was soon recognised that laryngoscopy and tracheal intubation was a potent stimulus to these physiological responses, even under general anaesthesia, and it could cause significant morbidity (King et al. 1951). Therefore many methods have since been used to attenuate the responses to laryngoscopy and tracheal intubation (Kovac 1996, Ng 1997), although all of the methods or techniques have disadvantages. Endogenous opioid systems are involved in cardiovascular and autonomic function, and opioid drugs have been successfully used to attenuate these responses to laryngoscopy and tracheal intubation.

The cardiovascular effects of opioids are mostly mediated by mu opioid receptors. Most opioid drugs used in clinical practice are predominantly mu receptor agonists, and they therefore have effects on the cardiovascular system and on reflex autonomic and cardiovascular responses. In clinical practice, opioids may produce bradycardia, hypotension, decreased myocardial contractility and vasodilatation by a combination of effects on the central and autonomic nervous systems, and both direct and indirect effects on the heart and vascular smooth muscle. Endogenous opioid peptides and their receptors are distributed widely throughout the nervous system, including a number of locations within the central cardiovascular control pathways (particularly the nucleus tractus solitarius, locus caeruleus, dorsal motor nucleus of the vagus, and the arcuate nucleus of the hypothalamus), autonomic ganglia, the heart, and adrenal medulla (Van Giersbergen et al. 1992, Little et al. 1998). The physiological role of endogenous opioids in cardiovascular homeostasis is complex, but they are involved in circadian cardiovascular regulation, and the modulation of reflex cardiovascular responses. They have a number of effects in vitro, primarily inhibiting autonomic nervous system activity. Different opioid peptide subtypes (endorphins,
enkephalins, dynorphins, nociceptin and endomorphins) have different effects, via direct
effects on the heart and vascular smooth muscle, and indirectly via interaction with the
autonomic nervous system.

1.2 Scope of this thesis

Remifentanil is a potentially useful drug for use as an infusion during anaesthesia. The
overall aim of this thesis is to examine the effect of remifentanil on cardiovascular responses
at induction of anaesthesia and to evaluate the role of remifentanil in the attenuation of
responses to noxious stimuli.

The physiological mechanisms underlying the autonomic and cardiovascular responses to
stressful or noxious stimuli, such as those occurring during surgery, are detailed. The effects
of endogenous opioid peptides on cardiovascular and autonomic function are described. The
precise role of these endogenous opioid peptides in cardiovascular homeostasis is uncertain
and the available data are reviewed. The effects of anaesthesia and of opioid drugs on the
cardiovascular system are examined.

Laryngoscopy and tracheal intubation produce similar physiological responses to other
noxious stimuli. The anatomical and physiological basis for this is explored, and the
pathophysiological consequences of laryngoscopy and tracheal intubation are described.
Laryngoscopy and tracheal intubation can be used as a model for the investigation of
cardiovascular responses to stressful stimuli. The data relating to previous studies of
laryngoscopy and tracheal intubation and the effects of different drugs or therapeutic
manoeuvres in modifying these responses are reviewed.

The mechanism of action of opioids is described, and previous work relating to the in vitro
and in vivo effects of opioid drugs is evaluated. Remifentanil is a new opioid which was
introduced into clinical practice in 1996 and is metabolised by plasma and tissue esterase
enzymes. This metabolic pathway results in a rapid offset of action, independent of the
duration of infusion, or organ function. It pharmacokinetic properties also result in a rapid
onset of clinical effect; these features allow it to be used in a different manner to previously available opioids, and are examined in this thesis. The available clinical data on remifentanil are reviewed.

The results of clinical studies of the effects of remifentanil on cardiovascular dynamics at induction of anaesthesia, laryngoscopy and tracheal intubation, emergence from anaesthesia and tracheal extubation are reported. The effect of remifentanil on plasma catecholamine concentrations is also described. In view of the high incidence of bradycardia, the effect of different doses of remifentanil, and of pre-treatment with glycopyrrolate is examined. Having determined a suitable infusion regimen, the effect of remifentanil in higher risk patients is evaluated.
CHAPTER 2. CARDIOVASCULAR HOMEOSTASIS

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2.7 Conclusions
Regulation of blood flow, heart rate, contractility and cardiac output is controlled by nervous and local mechanisms. Local mechanisms include the production of metabolites that influence local vascular tone and blood flow within tissues or organs. Nervous control affects more global functions including the redistribution of blood to different areas of the body, and co-ordination of the mechanisms involved in the control of cardiac output, heart rate and contractility (Guyton & Hall 2000). The nervous elements of cardiovascular control mechanisms are controlled by the autonomic nervous system, in particular the sympathetic nervous system, described below.

2.1 Physiology of the Autonomic Nervous System

The autonomic nervous system is controlled by centres in the spinal cord, brain stem and hypothalamus, which are in turn influenced by higher centres in the cerebral and particularly the limbic cortex. In addition, the autonomic nervous system may be influenced by visceral reflexes. Sensory signals entering the autonomic ganglia, spinal cord, hypothalamus or brain stem can elicit appropriate reflex responses directly to the visceral organs. The efferent autonomic signals are transmitted through the body to two major sub-divisions, the sympathetic nervous system and the parasympathetic nervous system.
Figure 2.1 Principal efferent pathways of the Autonomic nervous system.
Pre- and postganglionic fibres are shown by solid and dashed lines respectively.
2.2 The Sympathetic Nervous System

The sympathetic nervous system consists of nerves which originate in the spinal cord between the first thoracic and second lumbar segments (T1 to L2). Most preganglionic neurone cell bodies lie in the intermediolateral cell column (IML) of the spinal cord and adjacent lateral funiculus; fibres pass into an anterior root of the cord into the corresponding spinal nerve (Stoelting 1999). On leaving the spinal canal, the sympathetic preganglionic fibres pass into the paravertebral sympathetic chain, via the prevertebral (coeliac, mesenteric or hypogastric) ganglia. The preganglionic fibres synapse with unmyelinated neurones (C fibres) either at the level of the ganglia they enter, or travel upwards or downwards for some distance and synapse, or they terminate outside the sympathetic chain in one of the abdominal prevertebral ganglia before travelling to their effector organ with the relevant arteries (Figures 2.1 and 2.2) (Ebert & Stowe 1996, Guyton & Hall 2000). Some sympathetic preganglionic neurones (SPNs) from T10 and T11 travel directly in splanchnic nerves to the adrenal medulla, before synapsing with chromaffin cells, causing catecholamine release into the circulation.

Postganglionic sympathetic fibres travel from paravertebral ganglia in sympathetic nerves, (to supply the internal viscera including the heart) and spinal nerves (that enervate the peripheral vasculature and sweat glands). Sympathetic nerves principally contain vasoconstrictor fibres, which are distributed throughout the circulation, particularly the kidneys, the spleen, the gut and the skin. In skeletal muscle, coronary and cerebral vessels, sympathetic vasodilator fibres may predominate. Therefore sympathetic stimulation causes predominantly vasoconstriction but also a redistribution of blood flow to skeletal muscle; constriction of venous capacitance vessels can decrease their volume and thereby increase venous return. Stimulation of sympathetic fibres to the heart (see below) causes increased heart rate, increased contractility and increased cardiac output. SPNs directly supplying the adrenal medulla are usually co-stimulated with cardiac and vasomotor fibres, causing the release of epinephrine and norepinephrine into the circulation. The distribution of sympathetic nervous fibres to an organ or region may differ from the sensory or motor supply, according to its embryonic origin. For example, sympathetic fibres to the heart arise
from T1 to T5, (but predominantly from T1 to T4); the neck is supplied by fibres from T2, the chest by fibres from T3 to T6, and the abdomen by fibres from T7 to T11 (Stoelting 1999, Guyton & Hall 2000).

Figure 2.2 Principal efferent sympathetic nervous pathways
Pre- and postganglionic fibres are shown by solid and dashed lines respectively. Postganglionic vasomotor fibres travel with the corresponding spinal nerves.
2.2.1 Sympathetic neurotransmitters

It is well established that the SPNs contain acetyl choline (ACh) as their neurotransmitter; these and other neurones containing ACh are termed cholinergic. Postganglionic sympathetic neurones secrete norepinephrine and are termed adrenergic (except postganglionic sympathetic nerve fibres to sweat glands, pilo-erector muscles and some blood vessels which are cholinergic) (Guyton & Hall 2000). However, a number of other neuropeptides such as enkephalin, neurotensin, substance P, somatostatin, nitric oxide and dopamine may be present in SPNs (Dampney 1994, Ebert & Stowe 1996). In addition, SPNs are influenced by other synaptic inputs containing gamma amino butyric acid (GABA) and glycine (both inhibitory), and glutamate (facilitatory). Different peptides are present in different SPNs at different segmental levels within the spinal cord, (although more than one peptide may exist within the same neurone), so that different target organs may be influenced by different transmitters. SPNs are influenced by a number of synaptic inputs, which modulate their activity. Evidence from electrophysiological, immunochemical and pharmacological studies suggest that GABA and glycine inhibit, and glutamate augments SPN activity. Several other neurotransmitters and peptides, including serotonin, enkephalin, substance P, neuropeptide Y and catecholamines are present in axons synapsing with SPNs although their precise functions are unclear (Grundemar & Hakanson 1993, Dampney 1994).

ACh is the transmitter at all preganglionic synapses, acting via nicotinic receptors, as in the parasympathetic ganglia. At the postganglionic sympathetic endings, transmission is mediated by norepinephrine, which is present in the presynaptic terminals as well as the adrenal medulla. Activation of preganglionic nicotinic fibres to the adrenal medulla causes the release of epinephrine. Epinephrine is released primarily as a circulating hormone and is only found in insignificant amounts in the nerve endings. Endogenous catecholamines (epinephrine, norepinephrine and dopamine) are synthesized from the essential amino acids tyrosine and phenylalanine (Stoelting 1999).
The action of norepinephrine released from sympathetic nerve endings is terminated by re-uptake into the nerve terminal, diffusion into the circulation or enzymatic destruction. Most norepinephrine released from sympathetic nerves is taken back into the presynaptic nerve ending for storage and subsequent re-use. Reuptake is by active transport back into the nerve terminal cytoplasm and then into cytoplasmic vesicles. This mechanism of presynaptic reuptake, termed uptake₁, is dependent on adenosine triphosphate (ATP) and magnesium (Mg²⁺) ions, is enhanced by lithium (Li⁺) ions and may be blocked by cocaine and tricyclic antidepressants (Stoelting 1999). Endogenous catecholamines entering the circulation, by diffusion from their site of action at sympathetic nerve endings or by release from the adrenal gland, are rapidly metabolised by the enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) in the liver, kidneys, gut and many other tissues. The metabolites are conjugated before being excreted in the urine as 3-methoxy-4-hydroxymandelic acid, metanephrine (from epinephrine) and normetanephrine (from norepinephrine). Norepinephrine taken up into the nerve terminal may also be deaminated by cytoplasmic MAO. Another mechanism for the postsynaptic cellular reuptake of catecholamines, termed uptake₂, is present predominantly at the membrane of smooth muscle cells. Uptake₂ may be responsible for the termination of action of catecholamines released from the adrenal medulla (Stoelting 1999).

2.2.2 Adrenergic receptor pharmacology

The actions of catecholamines are mediated by specific postsynaptic cell surface receptors. Classification of these receptors into two groups (α and β adrenergic receptors) was first suggested by Ahlquist based upon the effects of epinephrine at peripheral sympathetic sites, α receptors being responsible for vasoconstriction and β receptors mediating effects on the heart, bronchial and intestinal smooth muscle (Ahlquist 1948). However, it is now apparent that this anatomical subdivision of the receptor subtypes is an oversimplification: there are several subtypes of α and β receptors in addition to receptors specific for dopamine (DA₁ and DA₂ subtypes). Two subtypes of α and β receptors have been well characterised to date on functional, anatomical and pharmacological grounds (α₁ and α₂, β₁ and β₂) (Stoelting
A third subtype of β receptor, β3, has been well documented in humans, and at least three further subtypes of both α1 and α2 receptor and five subtypes of DA receptor have been identified, although their precise functions are unclear (Calvey & Williams 1997). Differentiation of receptor subtypes is now based more directly on the effects of various catecholamine agonist compounds (including endogenous catecholamines). Epinephrine and norepinephrine are equipotent at β1 receptors, but β2 receptors are much more sensitive to epinephrine. Norepinephrine and epinephrine are agonists at both α1 and α2 receptors, although α1 receptors are more sensitive to norepinephrine and mediate most of its physiological actions.

Until recently, it was thought that β1 receptors predominated in the heart, mediating increases in force and rate of contraction and that β2 receptors existed in bronchial, uterine and vascular smooth muscle, mediating relaxation. In fact, most organs and tissues contain both β1 and β2 receptors, which may even serve the same function. For example, up to 25% of cardiac β receptors in the normal individual are of the β2 subtype, and this proportion may be increased in patients with cardiac failure (Calvey & Williams 1997). It is now apparent that β1 receptors in tissues are situated on the postsynaptic membrane of adrenergic neurones and respond to released norepinephrine. β2 receptors are presynaptic and when stimulated principally by circulating catecholamines, they modulate autonomic activity by promoting neuronal norepinephrine release. β3 receptors are present on adipocytes and several other tissues including the heart, intestines and lungs (Varghese et al. 2000). α1 receptors are present on the postsynaptic membrane, whereas α2 receptors are predominantly presynaptic, responding to circulating epinephrine but also mediating feedback inhibition of sympathetic neuronal activity. Postsynaptic α2 receptors, present on platelets and in the CNS, mediate platelet aggregation and membrane hyperpolarisation respectively (Table 2.1).

Dopamine receptors (DA1) are present postsynaptically in vascular smooth muscle of the renal, splanchnic, coronary and cerebral circulations, where they mediate vasodilatation. They are also situated on renal tubules, where they inhibit sodium reabsorption, causing natriuresis and diuresis. DA2 receptors are widespread in the CNS, (where dopamine is an
important neurotransmitter), occur on the presynaptic membrane of sympathetic nerves and in the adrenal gland. Stimulation of presynaptic DA₂ receptors inhibits dopamine release by negative feedback (Calvey & Williams 1997).

Postganglionic sympathetic fibres supplying sweat glands and arterioles in some areas of skin and skeletal muscle are cholinergic. Vascular smooth muscle also contains non-innervated cholinergic receptors which can mediate vasodilatation in response to circulating agonists, although cholinergic effects on vascular smooth muscle are usually minimal.
<table>
<thead>
<tr>
<th>Organ</th>
<th>Receptor subtype</th>
<th>Effect</th>
<th>Receptor subtype</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>β₁ also β₂, β₃, also DA₁</td>
<td>↑ Heart rate, ↑ Force of contraction, ↑ Conduction velocity, ↑ Automaticity (β₂), ↑ Excitability</td>
<td>M</td>
<td>↓ Heart rate, ↓ Force of contraction, Slight ↓ conduction velocity</td>
</tr>
<tr>
<td>Arteries</td>
<td>β₁</td>
<td>Coronary vasodilatation</td>
<td>M**</td>
<td>Vasodilatation in skin, skeletal muscle, pulmonary and coronary circulations</td>
</tr>
<tr>
<td></td>
<td>β₂</td>
<td>Vasoconstriction (skeletal muscle)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α₁, α₂</td>
<td>Vasoconstriction (coronary, pulmonary, renal and splanchnic circulations, skin and skeletal muscle)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DA₁</td>
<td>Coronary, splanchnic and renal vasodilatation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veins</td>
<td>α₁, also α₂</td>
<td>Vasoconstriction ++</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₁</td>
<td>Vasodilatation +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>β₂</td>
<td>Bronchodilatation + (Inhibition of secretions)</td>
<td>M₃</td>
<td>Bronchoconstriction ++ Stimulation of secretions +</td>
</tr>
<tr>
<td>GI tract</td>
<td>α₁,α₂, β₁,β₂</td>
<td>Decreased motility</td>
<td>M</td>
<td>Increased motility +++ Relaxation of sphincters Stimulation of secretions</td>
</tr>
<tr>
<td></td>
<td>α₁,α₂,β₁,β₂</td>
<td>Contraction of sphincters Inhibition of secretions Increased insulin release</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>β₁</td>
<td>Renin secretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂, α</td>
<td>Glycogenolysis Gluconeogenesis</td>
<td>M</td>
<td>Glycogen synthesis</td>
</tr>
<tr>
<td>Kidney</td>
<td>β₁</td>
<td>Renin secretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β₂, α</td>
<td>Glycogenolysis Gluconeogenesis</td>
<td></td>
<td></td>
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<td>Bladder</td>
<td>β₁</td>
<td>Detrusor relaxation</td>
<td>M</td>
<td>Detrusor contraction Sphincter relaxation</td>
</tr>
<tr>
<td>Uterus</td>
<td>α₁</td>
<td>Myometrial contraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α₂</td>
<td>Myometrial relaxation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipocytes</td>
<td>β₁</td>
<td>Lipolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>α₁</td>
<td>Mydriasis (radial muscle contraction) Ciliary muscle relaxation (far vision)</td>
<td>M</td>
<td>Miosis, ciliary muscle contraction (for near vision)</td>
</tr>
<tr>
<td>Platelets</td>
<td>α₂</td>
<td>Promote platelet aggregation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweat</td>
<td>M*</td>
<td>Sweating</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.1. Sympathetic and parasympathetic effects on peripheral organs, and receptor subtypes mediating these functions.**

* Sympathetic cholinergic fibres supply sweat glands and arterioles in some sites.

** Muscarinic receptors are present on vascular smooth muscle, but they are independent of parasympathetic innervation and have little or no physiological role in the control of vasomotor tone.
2.2.3 Presynaptic receptors

Autonomic nerve terminals (whether adrenergic, dopaminergic, or cholinergic) possess other receptors on the presynaptic membrane, in addition to the $\alpha_2$, $\beta_2$, and DA$_2$ receptors outlined above, which modulate neuronal transmission. These respond to a variety of endogenous substances that may facilitate or inhibit activity of the nerve, and include endogenous opioid peptides (Barron 2000). The function of presynaptic $\beta_2$ receptors may include maintaining basal levels of norepinephrine release when sympathetic activity is low and augmenting neuronal norepinephrine transmission in response to high circulating catecholamine concentrations. A number of compounds may be stored in vesicles within the presynaptic sympathetic nerve terminal. These include enkephalins, substance P, vasoactive intestinal peptide, somatostatin, nitric oxide, dopamine and neuropeptide Y. The function of these substances is not yet defined but they may modulate the synthesis or release of particular neurotransmitters. Alternatively, they may be released alone or in conjunction with the neurotransmitter to act directly on the post-synaptic receptor (Calvey & Williams 1997). The role of opioid peptides in autonomic nervous system activity and cardiovascular regulation is discussed in Chapter 3.

2.2.4 Structure of adrenergic receptors and intracellular messengers

Both $\alpha$ and $\beta$ adrenergic receptors are proteins with a similar basic structure, comprising seven hydrophobic transmembrane domains, with three intracellular loops and a $\text{COOH}$ terminal. Differences in amino acid sequences of the intracellular loops differentiate $\alpha$ and $\beta$ receptors. Both are linked to guanine nucleotide binding proteins (G proteins) in the cell membrane, which mediate the generation of second messengers that activate intracellular events. These second messenger systems include enzymes (adenylate cyclase, phospholipases) and ion channels (for calcium and potassium). In addition to functional differences, $\alpha$ and $\beta$ receptors differ in the intracellular mechanisms by which they act. Stimulation of $\beta_1$ and $\beta_2$ receptors activates $G_\alpha$ proteins which activate adenylate cyclase and cause the generation of intracellular cyclic adenosine monophosphate (cAMP). cAMP activates intracellular enzyme pathways to produce the associated alteration in cell function, (e.g. increased force of cardiac muscle contraction, liver
glycogenolysis, bronchial smooth-muscle relaxation) (Figure 2.3). In cardiac myocytes, the intracellular pathway involves the activation of protein kinases to phosphorylate intracellular proteins, increased activation of protein kinases to phosphorylate intracellular proteins, and increased intracellular Ca\textsuperscript{2+} concentrations by augmentation of the L-type calcium current. Increased intracellular calcium contractions in cardiac myocytes enhances excitation-contraction coupling and causes increased force of muscle contraction.

Intracellular cAMP concentrations are modulated by the enzyme phosphodiesterase, which breaks down cAMP to inactive 5′AMP. The balance between production and degradation of cAMP is an important regulatory system for cell function. α\textsubscript{2} and cholinergic M\textsubscript{2} receptors interact with G\textsubscript{i} proteins to inhibit adenylate cyclase and Ca\textsuperscript{2+} channels, but activate both K\textsuperscript{+} channels, phospholipase C and phospholipase A\textsubscript{2}. Somatostatin affects G\textsubscript{i} proteins in the same way (Guyton & Hall 2000). Muscarinic M\textsubscript{2} receptors also cause the G\textsubscript{i} protein to stimulate the production of nitric oxide via the enzyme nitric oxide synthase\textsubscript{3}. Nitric oxide stimulates soluble guanylyl cyclase to increase the production of cyclic GMP, which has opposite effects to cAMP. In the case of cardiac inotropy, parasympathetic (muscarinic) and sympathetic (β\textsubscript{1} and β\textsubscript{2} adrenergic) stimulation therefore have opposite effects, which are effected through separate pathways. β\textsubscript{3} adrenergic stimulation has negative inotropic effects which are accompanied by increases in nitric oxide and production of cyclic GMP. The mechanism may also involve G\textsubscript{i} proteins in a similar manner to cholinergic M\textsubscript{2} receptors. The physiological role of β\textsubscript{3} receptors may be in the negative feedback of β\textsubscript{1}- and β\textsubscript{2}- stimulated positive inotropic effects by a nitric oxide-dependent mechanism (Varghese et al. 2000). β\textsubscript{3} receptors are also upregulated in cardiac failure. G\textsubscript{i0} proteins also mediate the intracellular effects of opioid peptides. This is detailed further in Chapter 5.1.

In contrast, α\textsubscript{1} receptor stimulation does not affect cAMP levels within the cell directly, but causes coupling with another G protein (G\textsubscript{q}), to activate membrane-bound phospholipase C. This in turn hydrolyses phosphatidylinositol biphosphate (PIP\textsubscript{2}) to inositol triphosphate (IP\textsubscript{3}), which produces changes in intracellular Ca\textsuperscript{2+} concentration (Figure 2.3). This leads, for example, to vascular smooth muscle contraction (Calvey & Williams 1997).
Figure 2.3  Intracellular mechanisms of action of adrenergic receptors

Activation of $\beta$ receptors stimulates adenylate cyclase to catalyse the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) via a membrane bound $G_S$ protein. Activation of $\alpha_2$ receptors inhibits adenylate cyclase via an inhibitory ($G_i$) protein. $\alpha_2$ stimulation also activates $K^+$ channels and inhibits $Ca^{2+}$ channels. $\alpha_1$ adrenergic stimulation interacts with a $G_q$ protein to activate phospholipase $C$ and thereby promote the production of inositol triphosphate ($IP_3$). $G_q$ protein activation also stimulates the activation of protein kinase $C$ and phospholipase $A_2$. 
2.2.5 Cardiovascular effects of sympathetic stimulation

The central cardiovascular neurones influence cardiac activity via the sympathetic nerves to the heart. Preganglionic fibres originate in the intermediolateral cell column of the upper five or six thoracic spinal segments and terminate in the corresponding ganglia of the sympathetic trunk and cervical ganglia. Postganglionic fibres reach the heart via the superior, middle and inferior cardiac branches of the cervical portion of the sympathetic trunk and through varying numbers of thoracic cardiac nerves. The vagus nerve supplying the heart contains sympathetic fibres from the inferior cervical ganglion as well as parasympathetic fibres. Sympathetic fibres terminate in the sinoatrial and atrioventricular nodes, on cardiac muscle fibres in the atria and ventricles and in coronary vessels. When stimulated they increase heart rate, contractility and cause coronary vasodilatation, mediated predominantly by $\beta_1$ receptors, although $\beta_2$, $\alpha$ and possibly DA receptors are also involved. In contrast to parasympathetic effects on the heart, sympathetic fibres control myocardial contractility, although other intrinsic properties of the myocardium are involved which are responsible for the Starling, Anrep and Bowditch effects (Lake 2001). Sympathetic nerve impulses are usually transmitted at the same time to the adrenal medulla causing the secretion of epinephrine and norepinephrine into the circulation. These hormones cause predominantly vasoconstriction, although epinephrine may cause vasodilatation in certain areas of the body including skeletal and coronary muscle. The diameter of blood vessels (and thus the resistance to blood flow) depends on the state of contraction of vascular smooth muscle, which is mediated by $\alpha$ adrenergic neurones. Therefore, sympathetic activity at $\alpha$ receptors controls the peripheral resistance to blood flow (by effects on the arterial side of the circulation), and the circulating volume (venous constriction increases blood flow returning to the heart). The redistribution of blood between skeletal muscle and other tissues is determined by activation of $\beta$ receptors and is mediated physiologically by circulating epinephrine: $\beta$ stimulation produces vasodilatation in skeletal muscle, the brain and the coronary circulation. Dopaminergic ($DA_1$) and $\beta_2$ receptors in the splanchnic and renal circulations also mediate regional vasodilatation. The overall effect of catecholamines on cardiac output and arterial pressure depends on the interaction between effects on cardiac rate, contractility and peripheral vascular resistance.
2.3 The Parasympathetic Nervous System

Parasympathetic neurones arise from cell bodies of the motor nuclei of the cranial nerves, III, VII, IX and X in the brain stem and from the sacral segments of the spinal cord (the 'craniosacral outflow'). The preganglionic fibres run almost to the organ innervated and synapse in ganglia within the organ, giving rise to postganglionic fibres, which supply the relevant tissues (Figure 2.4). The ganglion cells may be well organised, (e.g. in the myenteric plexus of the intestine), or diffuse (e.g. in the bladder or blood vessels) (Guyton & Hall 2000).

Figure 2.4 The Parasympathetic nervous system
2.3.1 Parasympathetic neurotransmitters

The chemical neurotransmitter at both pre- and postganglionic synapses is acetyl choline (ACh), though transmission at postganglionic synapses may be modulated by other substances including GABA, serotonin and opioid peptides. ACh is synthesised in the cytoplasm of cholinergic nerve terminals by the combination of choline and acetate (in the form of acetyl CoA, which is synthesised in the mitochondria as a product of normal cellular metabolism). ACh is stored in specific agranular vesicles and released from the presynaptic terminal in response to neuronal depolarisation to act at specific receptor sites on the postsynaptic membrane. It is rapidly metabolised by the enzyme acetylcholinesterase (AChE) to produce acetate and choline. Choline is then taken up into the presynaptic nerve ending for the regeneration of ACh. AChE is synthesised locally at cholinergic synapses but is also present in erythrocytes and parts of the CNS. Butyryl cholinesterase (also known as plasma cholinesterase or pseudocholinesterase) is synthesised in the liver and is found in the plasma, skin, GI tract and parts of the CNS but not at cholinergic synapses or the neuromuscular junction. It can metabolise ACh, as well as certain neuromuscular blockers (e.g. suxamethonium and mivacurium), and its physiological role probably involves the breakdown of other choline esters which may be present in the intestine (Calvey & Williams 1997).

2.3.2 Parasympathetic receptor pharmacology

Parasympathetic receptors have been classified according to the actions of the alkaloids muscarine and nicotine. The actions of ACh at the postganglionic membrane site are mimicked by muscarine and are termed muscarinic, whereas preganglionic transmission is termed nicotinic. ACh is also the neurotransmitter at the neuromuscular junction, via nicotinic receptor sites. Five subtypes of muscarinic receptors (M₁-M₅) have been characterised by molecular cloning techniques and specific antagonists developed for M₁-M₃ receptors (Lambert & Appadu 1995). All five receptors subtypes exist in the CNS, but there are differences in their peripheral distribution and function. M₁ receptors are found in the stomach where they mediate acid secretion, whereas M₂ receptors predominate in the myocardium, where they modulate heart rate and impulse conduction. Prejunctional M₂ receptors may also be involved in the regulation of synaptic norepinephrine and ACh release. M₃ receptors are present in classical postsynaptic sites in glandular tissue (of the
gastrointestinal and respiratory tracts) and probably in bronchial smooth muscle. M₄ receptors have been isolated in cardiac and lung tissue in animal models, and may have inhibitory effects, but the distribution and functions of M₃ receptors are not yet defined (Table 2.2).

Like adrenergic receptors, muscarinic receptors are coupled to membrane-bound G proteins and thus comprise seven transmembrane domains, of which the third intracellular domain interacts with the G protein. The subtypes differ in the second messenger system with which they interact. M₁, M₃ and M₅ receptors couple to phospholipases C via a G₉ protein to generate IP₃. M₂ and M₄ receptors inhibit adenylate cyclase via interaction with a Gᵢ protein and decrease the formation of cAMP, activate K⁺ channels and inhibit Ca²⁺ channels.

Table 2.2 Properties of muscarinic (m₁-m₅) receptors

<table>
<thead>
<tr>
<th></th>
<th>m₁</th>
<th>m₂</th>
<th>m₃</th>
<th>m₄</th>
<th>m₅</th>
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<tr>
<td>Second messenger</td>
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<td>cAMP</td>
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</tr>
<tr>
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<td>CNS</td>
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</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>CNS</td>
<td>Gland</td>
<td>Heart</td>
<td>?</td>
</tr>
<tr>
<td>Important effects</td>
<td>Gastric acid</td>
<td>Bradycardia</td>
<td>Secretion</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Clinically selective agent</td>
<td>Pirenzepine</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Adapted from Lambert & Appadu (1995).

IP₃ = stimulates inositol triphosphate production

cAMP = inhibits adenylate cyclase to decrease cAMP formation
2.3.3 Cardiovascular functions of the parasympathetic nervous system

The parasympathetic nervous system plays only a minor role in efferent circulatory regulation overall and is involved principally in the regulation of heart rate via the vagus nerve. Cell bodies of preganglionic neurones involved in cardiovascular function are mainly in the nucleus ambiguus and dorsal motor nucleus of the vagus; fibres enter cardiac branches of the vagus and synapse with the postganglionic neurones in the cardiac plexuses and scattered cell clusters in the walls of the atria. Postganglionic fibres principally supply the sinoatrial (SA) and atrioventricular (AV) nodes. There are also fibres supplying atrial and ventricular muscle and coronary arteries, which cause decreased contractility and vasoconstriction. However, the main effect of parasympathetic stimulation is to decrease SA node discharge, decrease the passage of impulses through the AV node and decrease ventricular automaticity; these effects combine to decrease heart rate. There is usually little effect on contractility, although intense vagal stimulation may decrease atrial and ventricular contractility, at least in part by decreasing sympathetic activity at these sites (Lake 2001).

Vagal postganglionic fibres receive afferent inputs from peripheral baroreceptors via the nucleus tractus solitarius and are inhibited during inspiration by respiratory neurones originating in the medulla. They can be stimulated by peripheral chemoreceptors, cardiac receptors, and trigeminal receptors activated during the 'diving' reflex (Dampney 1994). Inhibition of cardiac vagal neurones during inspiration may be mediated by ACh, since it is blocked by atropine but other neurotransmitters may be involved in neurotransmission or modulation, including GABA, serotonin and opioid peptides (Dampney 1994). Muscarinic receptors mediating vasodilatation are present on vascular smooth muscle but are independent of parasympathetic nerves. They have minimal effects on the physiological control of vascular tone but may be involved in the mechanism of vasovagal attacks (Calvey & Williams 1997).
Overall, cardiovascular homeostasis is co-ordinated by 5 specific regions in the medulla, lower third of the brain stem and the hypothalamus. The ventrolateral medulla (VLM) is often referred to as the vasomotor centre because it is the primary site regulating sympathetic outflow. However, more recently it has been recognised that cardiovascular function is controlled by a number of regions or groups of cells with specific interconnections, which may be responsible for specific aspects of cardiovascular activity, although these act in a co-ordinated manner (Dampney 1994). These regions are the rostral ventrolateral medulla (RVLM), rostral ventromedial medulla (RVMM), caudal raphe nuclei, A5 noradrenergic cell group and the paraventricular nucleus of the hypothalamus (Figure 2.5). They control the activity of SPNs, which supply the adrenal medulla and all major sympathetic ganglia. In addition, neurones in the caudal ventrolateral medulla (CVLM), area postrema, cerebellar nuclei, locus coeruleus and the midbrain periaqueductal grey matter are involved in cardiovascular control, by mechanisms not directly served by SPNs (Dampney 1994). The activity of these brain stem neurones is influenced by centres in the lateral and posterior hypothalamus, anteroventral 3rd ventricle, amygdala and cerebral cortex. Some of these pathways are described in more detail below. Under normal conditions, there is continuous stimulation of sympathetic vasoconstrictor nerves mediated by RVLM neurones, causing constant slow firing of these fibres. This continual firing is often termed sympathetic vasomotor 'tone' (Stoelting 1999) but it is now apparent that this notion of a uniform response throughout the body is inaccurate and that differential effects occur in different parts of the cardiovascular system in response to different stimuli (Ebert & Stowe 1996). For example, the RVLM contains excitatory neurones and the CVLM contains inhibitory neurones. The effect on a particular organ of sympathetic stimulation also depends on the balance of adrenergic receptors present (Table 2.1)
Figure 2.5 Main centres involved in the central control of cardiovascular function

DMNV = dorsal motor nucleus of the vagus, LRN = lateral reticular nuclei, RN = (caudal) raphe nuclei. Other abbreviations are defined in the text.
2.4.1 Cardiovascular afferent pathways

Control of cardiovascular function within the brainstem is influenced by signals from a number of peripheral receptors. These include sensory afferent fibres from nonencapsulated visceral nerve endings in the aortic arch and carotid sinus at the carotid bifurcation (arterial baroreceptors) whose cell bodies are in the inferior ganglia of the vagus (X<sup>th</sup>) and glossopharyngeal (IX<sup>th</sup>) nerves, respectively. Other afferent signals from atrial and ventricular walls (cardiac baroreceptors) and arterial chemoreceptors in the carotid and aortic bodies travel via the vagus and glossopharyngeal nerves to the nucleus tractus solitarius (see below). These vagal cardiopulmonary baroreceptors and chemoreceptors were viewed as the predominating influences on cardiovascular function but inputs from numerous somatic and visceral receptors (including peripheral nociceptors, chemoreceptors, mechanical and stretch receptors) are also involved. Signals from these receptors enter the spinal cord via the sympathetic chain and synapse in the dorsal horn. They may cause reflex changes in local or segmental sympathetic activity but impulses are also transmitted centrally to the medulla via spinoreticular pathways (Dampney 1994). Descending influences from higher centres in the CNS (e.g. the cerebral cortex) also synapse in dorsomedial medulla and influence cardiovascular function. In addition, circulating neuropeptides or neuromodulators may affect sympathetic activity by effects on VLM neurone activity, SPN and postganglionic sympathetic neuronal transmission, or by direct effects on vascular tone or renal function (Ebert & Stowe 1996).

2.4.1.1 Nucleus tractus solitarius

The nucleus tractus solitarius (NTS), in the dorsomedial region of the medulla, controls the activity of the brain stem cardiovascular efferent neurones. As described above, it receives many cardiovascular afferents, mainly via the vagus and glossopharyngeal nerves, as well as second order neurones which receiving inputs from visceral and somatic receptors. The NTS projects to various regions in the spinal cord, lower brain stem and forebrain including the nucleus ambiguus, RVLM, CVLM, midbrain periaqueductal grey matter, the paraventricular nucleus, area postrema, lateral hypothalamus and the intermediolateral (IML) cell column of the spinal cord (Van Giersbergen et al. 1992). Different types of afferent fibres within the NTS have specific
terminal sites, with some crossover. However, a number of other neurotransmitters are synthesised locally and contained within the NTS terminal fibres. These include catecholamines (norepinephrine, epinephrine, dopamine), other amines (histamine, serotonin, ACh), amino acids (glutamate, GABA), neuropeptides (substance P, neuropeptide Y, somatostatin, atrial natriuretic peptide, cholecystokinin) and opioid peptides (β-endorphin, enkephalin and dynorphin), although their physiological role is uncertain (Calaresu & McKitrick 1991, Van Giersbergen et al. 1992, Dampney 1994).

2.4.2 Cardiovascular efferent pathways

2.4.2.1 Rostral ventrolateral medulla (RVLM)

The RVLM plays a crucial role in the tonic and acute phasic regulation of blood pressure. RVLM cells project to SPNs at all levels of the spinal cord and receive inputs from baroreceptors, chemoreceptors, cardiopulmonary receptors, somatic receptors as well as from several regions of the brain, in particular the nucleus tractus solitarius, periaqueductal grey matter, lateral and paraventricular hypothalamic nuclei. Excitation of RVLM cells produces increases in arterial pressure, heart rate and adrenal catecholamine release but no non-cardiovascular effects. Inhibition of RVLM cells produces a marked decrease in blood pressure and activity in sympathetic nerves supplying the kidney, splanchnic bed and skeletal muscle. Some RVLM neurones exhibit spontaneous regular activity and the RVLM is responsible for sympathetic vasomotor tone, under the influence of synaptic inputs mediated by glutamate (excitatory) and GABA (inhibitory) receptors (Dampney et al. 2000). Other neurotransmitters regulating the RVLM cardiovascular neurones include ACh, opioids, catecholamines, angiotensin, vasopressin, serotonin and nitric oxide (Hirooka et al. 1997, Potts et al. 1999), although the precise function of each has not been fully elucidated. ACh, via muscarinic M₂ receptors, and angiotensin are involved in the maintenance of vasomotor tone, either directly or via inhibition of GABA-ergic neurones (Kubo 1998). In addition to involvement in vasomotor tone, GABA receptors mediate inhibition of RVLM activity in response to stimulation of arterial baroreceptors or cardiopulmonary receptors (the baroreceptor reflex) (Dampney 1994). RVLM cardiovascular neurones are also chemosensitive and respond to hypoxaemia and hypercapnia (Shapoval 1992, Horiuchi et al. 1999, Dampney et al. 2000). RVLM neurones are also involved in anti-nociceptive reflexes by a
mechanism which may involve direct projections to the dorsal horn of the spinal cord (Siddall \textit{et al.} 1994). Opiate receptors are also involved in the activity of the RVLM. Injection of enkephalin analogues produces a decrease in blood pressure and heart rate which is reversed by naloxone. The C1 group of catecholamine cells in the RVLM and is sometimes termed the vasoconstrictor area. C1 neurones directly excite cardiovascular SPNs, releasing glutamate as an excitatory transmitter (Dampney 1994).

2.4.2.2 RVMM and caudal raphe nuclei

The rostral ventromedial medulla and caudal raphe nuclei contain neurones with similar projections to those in the RVLM, ie SPNs innervating the major sympathetic ganglia and the adrenal medulla, although their chemical properties differ. They contain a number of potential neurotransmitters particularly 5-HT, as well as substance P, thyrotropin (TRH) and enkephalin, in various combinations. The function of RVMM neurones is not known but at least some of the caudal raphe neurones are sympato-inhibitory (Dampney 1994).

2.4.2.3 Other brain stem nuclei

In addition to sympathetic premotor nuclei, cardiovascular function is modified by several other cell groups which may or may not project directly to SPNs. These include the caudal ventrolateral medulla (CVLM), nucleus ambiguus, area postrema, cerebellar nuclei, the locus caeruleus and higher centres.

\textit{Caudal Ventrolateral Medulla}

The caudal ventrolateral medulla (CVLM) receives projections from the NTS. CVLM activity results in a widespread decrease in peripheral resistance accompanied by inhibition of sympathetic vasomotor activity and a decrease in cardiac contractility. There is evidence for tonic inhibition of the RVLM as well as a possible role in baroreceptor-mediated inhibition of sympathetic activity. The mechanisms whereby CVLM neurones inhibit sympathetic activity are not clear, but may be GABA-mediated (Dampney 1994).
**Nucleus Ambiguus**

The nucleus ambiguus contains preganglionic cardiac vagal neurones which receive afferents from arterial baroreceptors as well as projections from the NTS. Excitation of these neurones may be mediated by glutamate, and produces bradycardia.

**Area postrema**

The area postrema lies on the dorsal surface of the medulla outside the blood brain barrier and is therefore readily accessible to circulating peptides and hormones; its role in vomiting is well known. The area postrema also projects to the nucleus tractus solitarius and the RVLM and it may have a role in cardiovascular regulation. Stimulation by circulating angiotensin and vasopressin both cause a rise in arterial pressure (Dampney 1994).

**Periaqueductal grey matter**

The periaqueductal grey matter (PAG) in the midbrain has a critical role in mediating defence reactions and anti-nociception. It also controls the cardiovascular responses to noxious stimuli. Stimulation of different sites within the PAG causes different patterns of cardiovascular responses, with vasodilatation and increased blood flow in some vascular beds and vasoconstriction in others. This is probably achieved via highly specific connections to RVLM SPNs (Snowball et al. 1997).

**Higher centres**

Neurones in the cerebral cortex, hypothalamus, amygdala and other parts of the limbic system connect with brain stem nuclei including the NTS and the RVLM and are involved in the integration of autonomic and behavioural responses, although the exact function of different regions is not known.
Reflex cardiovascular responses are primarily co-ordinated in the NTS of the medulla but are influenced by a number of inputs from suprabulbar and spinal afferents. For example, the defence reaction to fear or rage is co-ordinated in the hypothalamus, and may be inhibited by influences from the cerebral cortex. Baroreceptors in the carotid body and aortic arch co-ordinate reflex alterations in sympathetic and parasympathetic cardiovascular activity in response to changes in arterial pressure. Somatosensory reflexes (for example the sympathetic response to pain or noxious stimuli, or the bradycardic response to visceral traction) are initiated by spinal afferents. Chemoreceptors are also involved in the responses to hypoxaemia or hypercapnia. This variety of inputs means that the sympathetic nervous system is capable of responding to different stimuli in a differentiated manner; the physiological response produced depends on the quality and intensity of the afferent stimulus (Herd 1991, Damney 1994, Ebert & Stowe 1996). The previous view that sympathetic stimulation produces an undifferentiated response to pain, haemorrhage or mental stress has been superceded. For example, the circulatory adjustments to isotonic or isometric exercise, haemorrhage or mental stress are quite distinct (Martner & Biber 1982). They also depend on other factors such as age: baroreceptor function declines with age so that haemodynamic responses to a given stimulus are decreased (Ferrari 1992). The extent to which different organs or vascular beds respond to different stimuli is determined by inputs operating at several levels within the central nervous system but all are regulated by sympathetic efferents.

The sympathetic and parasympathetic nervous systems are constantly active in regulating cardiovascular function. This tonic activity, previously termed sympathetic 'tone', allows rapid two-way regulation of physiological effect. For example, a decrease in sympathetic tone will rapidly cause decreased vasoconstriction i.e. vasodilatation. This would occur more slowly if the blood vessels were not partly vasoconstricted already. Increased heart rate in response to haemorrhage can be achieved by increased sympathetic or decreased vagal tone and the mechanism depends on the initial balance between the two (Martner & Biber 1982). In addition, the vascular endothelium itself influences local vascular tone. It has stretch receptors which respond to changes in shear stress or pressure, and chemoreceptors for peptides, kinins, arachidonic acid metabolites and nucleotides. It also responds to substances produced by vascular smooth muscle e.g. adenosine and potassium.
ions and produces several substances which alter vascular tone, including nitric oxide, prostacyclin, adenosine and endothelin. Some of these substances cause relaxation of vascular smooth muscle and vasodilatation (e.g. nitric oxide, prostacyclin) whereas others cause vasoconstriction (e.g. endothelin). The vascular endothelium can therefore affect local blood flow and blood pressure, although the precise physiological roles of different mediators is unclear (Stowe & Ebert 1996).

The cardiovascular response to laryngoscopy and tracheal intubation comprises increased heart rate, contractility and myocardial oxygen consumption, increased arterial pressure, increased adrenal release of catecholamines and renal and splanchnic vasoconstriction, with a variable degree of vasodilatation in skeletal muscle (see Chapter 4). It is similar to the pressor response to noxious or painful somatic or visceral stimuli and to the defence reaction provoked by pain, fear or rage. It is initiated within a few seconds of the stimulus and is mediated by the medulla, although it may be modulated by higher influences from the hypothalamus and cerebral cortex. The somatic response to more distal noxious stimuli also involves spinal reflexes (Martner & Biber 1982). The mechanism mediating the physiological response to laryngoscopy and tracheal intubation is discussed in Chapter 4.
2.6 Opioid peptides and cardiovascular regulation

Opioid peptides and their receptors are distributed widely throughout the central, peripheral and autonomic nervous systems. They are found at a number of locations within the central cardiovascular control pathways (particularly the nucleus tractus solitarius, locus caeruleus, dorsal motor nucleus of the vagus, and the arcuate nucleus of the hypothalamus), autonomic ganglia, the heart, and adrenal medulla, where beta endorphin is co-released with epinephrine (Van Giersbergen et al. 1992, Little et al. 1998). The role of opioids in cardiovascular homeostasis is complex and it is possible that opioids act at a number of central and peripheral sites, having direct effects on the heart and vascular smooth muscle, and indirect effects by interaction with the autonomic nervous system. However, the physiological significance of the role of opioid peptides in cardiovascular homeostasis has not been fully elucidated.

A number of different endogenous opioid peptides have been characterised, but they have been traditionally classified as enkephalins, endorphins, and dynorphins. Although there are differences in molecular structure, they all contain the initial amino acid sequence Tyr-Gly-Gly-Phe, which is found in leu-enkephalin or met-enkephalin. More recently, the endogenous peptides endomorphin$_1$ and endomorphin$_2$, which are selective mu opioid receptor agonists, have been discovered. In addition, another endogenous ligand, nociceptin, has been characterised, which is selective for an endogenous opioid-like receptor, the NOP receptor (formerly termed the nociceptin/orphanin FQ receptor) and has distinct pharmacological effects (Alexander & Peters 2000, Calo et al. 2000). The different endogenous opioids interact with different opioid receptors, with differing affinity. The classification and mechanism of action of opioid peptides is discussed further in Chapter 5, but in this thesis, the traditional terminology will be used. Enkephalins are most active at delta (DOP) receptors, endorphins act mainly at mu (MOP) receptors, and dynorphins act mainly at kappa (KOP) receptors (Calvey & Williams 1997). Endomorphins act only at mu (MOP) receptors and nociceptin acts only at NOP receptors.
Within the central nervous system, neurones containing enkephalin are found in the pre-optic, paraventricular and lateral hypothalamic nuclei, the central nuclei of the amygdala and the hippocampus, the periaqueductal grey matter, the pons and medulla oblongata (eg RVLM, caudal raphe nuclei and RVMM) (Feuerstein & Siren 1987). These areas serve the SPNs which supply the adrenal medulla and sympathetic ganglia (see above). In particular, the C₁ group of catecholamine cells in the RVLM (whose cell bodies arise in the NTS and are responsible for excitation of SPNs in the spinal cord) contains opioid receptors; enkephalin released from RVLM neurones inhibits SPN activity, causing a decrease in heart rate and arterial pressure (Dampney 1994). Cardiac vagal neurones in the nucleus ambiguus also contain high concentrations of opioid receptors (Laubie et al. 1979). There is variation of distribution of receptors, with mu receptors predominating in the NTS, the locus caeruleus and the dorsal motor nucleus of the vagus, (as well as areas associated with nociception such as the dorsal horn of the spinal cord, the periaqueductal grey and raphe nuclei). Delta receptors are mostly found in the limbic system, whilst kappa receptors are also associated with areas subserving cardiovascular control (eg supraoptic and paraventricular nuclei, caudal nucleus tractus solitarius, locus caeruleus and RVMM). Neurones containing enkephalin are also found in autonomic ganglia and neurones, including the stellate ganglia and vagus (Barron 2000).

Cardiac vagal nerve endings also contain enkephalin and opioid-specific binding sites are found in the myocardium of the atria and ventricles (Barron 2000). Recent studies have shown that myocardial cells can synthesise enkephalins (Younes et al. 2000) and dynorphins (Ventura et al. 1998). There is some evidence to suggest that opioid peptides act to modulate the effects of catecholamines and may modulate myocardial calcium homeostasis (Ventura et al. 1998). For instance enkephalins may decrease the tachycardic response to catecholamines by inhibiting the accumulation of atrial intracellular calcium (Feuerstein & Siren 1987). Kappa and delta opioid receptors are coupled via a G<sub>ι/o</sub> protein which inhibits adenylyl cyclase and attenuates the increase in cAMP in response to norepinephrine (Pepe et al. 1997). Opioid peptides are co-released with norepinephrine in cardiac sympathetic nerves and they attenuate the increase in intracellular calcium which occurs in response to norepinephrine. They may also modulate norepinephrine release from sympathetic nerves (Xiao et al. 1993, 1997). However, the cardiac effects of
parasympathetic stimulation appear to independent of opioid receptors, as ACh release is unaffected (Hung et al. 2000). Other work using selective ligands has suggested that cardiac atrial opioid receptors are of the delta- and kappa- but not mu- subtypes, and opioid peptides selective for mu, delta and kappa opioid receptors may have different physiological effects at other sites (Paakkari & Feuerstein 1995). The effects of stimulation of different opioid receptor subtypes are discussed in sections 2.6.1–2.6.4.

The physiological significance of these pathways is still uncertain, and when studied in animal models the administration of different opioid peptides has produced differing and sometimes opposite effects, depending on the dose, species, receptor subtype, route of administration and concurrent anaesthesia (Feuerstein & Siren 1987, Siren & Feuerstein 1992, Van Giersbergen et al. 1992, Paakari et al. 1992). However, there are data from numerous in vitro and in vivo studies suggesting that endogenous opioids have many potential effects on the cardiovascular system. These effects are mediated at least partly by a decrease in sympathetic nervous activity and opioids may act to modulate the sympathetic nervous response to stress (Lowenstein et al. 1972, Hsu et al. 1979, Flacke et al. 1983, Flacke et al. 1985, Feuerstein & Siren 1987, Paakkari & Feuerstein 1987, Xiao et al. 1997, Barron 2000). Exogenous opioids also have direct effects on vascular smooth muscle. For example, morphine produces naloxone-sensitive, calcium-dependent contraction of aortic VSM (Lee & Berkowitz 1976) but relaxation of vascular smooth muscle in other sites (Altura et al. 1978, Lowenstein et al. 1972). Some of the actions of opioids on vascular smooth muscle are mediated by opioid receptors but others are indirect (eg histamine-mediated) (Feuerstein & Siren 1987). The actions of exogenous opioids on cardiovascular function are discussed in detail in Chapter 3.

2.6.1 Mu opioid (MOP) receptor effects

It is thought that the mu receptor is the most important opioid receptor subtype involved in cardiovascular control (Paakari & Feuerstein 1995). The systemic administration of morphine and other opioids can cause hypotension and bradycardia, but direct CNS injection of opioids into the lateral ventricles causes a well-characterised pressor
response, comprising tachycardia and hypertension. This response is though to be mediated by mu opioid receptors. It is biphasic, and bradycardia occurs at higher doses (Siren & Feuerstein 1992, Barron 2000). The cardiovascular effects of proenkephalin peptides (enkephalins and dynorphins) in the rat are mediated by peripheral mu opioid receptors and act on vagal afferents to alter CNS activity (Douglas & Kitchen 1992). Others have confirmed that mu receptors are the predominant subtype regulating autonomic outflow and the changes in cardiovascular activity induced by opioid peptides (Kiritsyroy et al. 1989). Paakkari and colleagues hypothesised that there may be differences in opioid action at different mu receptor subtypes, high affinity mu_1 receptors mediating tachycardia and lower affinity mu_2 receptors mediating bradycardia in response to centrally injected selective mu agonists (Paakkari et al. 1992). However, this has not been confirmed, and there is in fact no molecular biological evidence for existence of different mu receptor subtypes (Harrison 2000). Nevertheless, as they have been described, they will be included in this thesis. Several workers have suggested that the pressor response to central nervous system activation of mu opioid receptors is mediated by activation of the sympathetic nervous system and adrenal medulla, based on observations that CNS mu opioid receptor activation is associated with increased plasma catecholamine concentrations, increased postganglionic sympathetic nervous activity and is blocked by adrenergic blocking drugs (Siren & Feuerstein 1992, Paakkari & Feuerstein 1995). Conversely, others have found that the selective mu agonist DAMGO obtunds the response to afferent baroreceptor stimulation and produces bradycardia, hypotension and decreased sympathetic nerve activity. There is also evidence for involvement of the parasym pathetic nervous system in the central actions of opioids. At higher doses, DAMGO causes bradycardia, which is abolished by atropine. Stimulation of endorphinergic neurones in the CNS causes bradycardia and cardiovascular depression by a mechanism mediated by the vagus (Paakkari & Feuerstein 1995).

Studies of the effects of selective opioid agonists in isolated heart preparations have also produced conflicting results. However, mu agonists have been shown to decrease heart rate, stroke volume, contractility and cardiac output in vitro, similar to the in vivo effects of opioids used in clinical practice (see Chapter 3).
2.6.2 **Kappa opioid (KOP) receptor effects**

Both CNS and peripheral administration of selective kappa opioid peptide agonists produces bradycardia and hypotension in anaesthetised animals, although differing effects have been found in conscious animals and different species. Kappa opioid receptors are found in the heart, vasculature and adrenal medulla, and kappa agonists inhibit presynaptic norepinephrine release. It has therefore been suggested that their cardiovascular depressant effects are mediated at peripheral receptors (Siren & Feuerstein 1992). However, they are also widely distributed in the hypothalamic nuclei and parts of the limbic system involved in cardiovascular regulation. Kappa agonists inhibit myocardial norepinephrine release and cardiac myocyte contraction in response to neural stimulation. In addition, they stimulate hypothalamic vasopressin release (Barron 2000).

2.6.3 **Delta opioid (DOP) receptor effects**

Delta opioid receptors are found predominantly in the limbic system and the substantia gelatinosa of the spinal cord. They are present also in the heart where stimulation causes increased myocardial contractility in vitro. There is little information regarding the cardiovascular effects of delta opioid stimulation, but they are generally much less than those of mu receptor stimulation (Paakkari & Feuerstein 1995). However, Osadchi and Pokrovskii found that met-enkephalin, an endogenous delta and mu agonist, produced bradycardia in anaesthetised cats, which was prevented by atropine or the selective delta antagonist naltrindole but not by naloxonazine (a selective mu antagonist) (Osadchi & Pokrovskii 1998). They suggested that as most cardiac opioid receptors are of the delta subtype, delta receptors are responsible for initial cardiac rhythm whereas both mu and delta activation are involved in tonic inhibitory vagal control of heart rate, perhaps by presynaptic augmentation of ACh release. This suggestion has not yet been confirmed. Peripheral activation of delta receptors is responsible for the phenomenon of ischaemic preconditioning in the myocardium (Schultz *et al.* 1997a) and the possible cardio-protective effects of opioid drugs may be mediated by action at delta receptors (Schultz *et al.* 1997b).
2.6.4 **NOP receptor effects**

Nociceptin causes transient hypotension, bradycardia and decreased peripheral resistance when administered intravenously to anaesthetised rats (Champion et al. 1997), by a mechanism different from that of delta and kappa opioid receptor agonists (Madeddu et al. 1999, Salis et al. 2000). These effects are abolished by bilateral cervical vagotomy and guanethidine pre-treatment, suggesting that they are mediated by the autonomic nervous system (Guilani et al. 1997). Similar cardiovascular effects occurred in conscious rodents and are not affected by naloxone but were attenuated by administration of the synthetic nociceptin antagonist [F/G]NC(1-13)NH$_2$ (Madeddu et al. 1999). [F/G]NC(1-13)NH$_2$ had no effect on basal blood pressure or heart rate or the hypotension and bradycardia produced by bradykinin, and specific mu, delta and kappa opioid receptor antagonists (endomorphin$_1$, leu-enkephalin or U504885 respectively). In contrast, nociceptin produced an increase in arterial pressure and heart rate after intravenous administration, suggesting that there may be differences in its cardiovascular effects between species (Arndt et al. 1999). Nociceptin also produces vasodilatation by a direct endothelium-independent mechanism that is independent of opioid or muscarinic receptors and nitric oxide. The mechanism involves increased production of cAMP and subsequent activation of ATP-sensitive and calcium-sensitive $K^+$ channels (Czapla et al. 1997, Champion et al. 1998a, Armstead 1999). Nociceptin also modulates noradrenergic neurotransmission by acting on prejunctional receptors located on nerve terminals innervating the rat tail artery by interaction with prejunctional $\alpha_2$ adrenergic receptors (Bucher 1998). However, although nociceptin has several effects on cardiovascular function in experimental situations, its physiological role in cardiovascular regulation and in pathological states is not known (Salis et al. 2000).
2.6.5 Summary

The role of opioids in cardiovascular homeostasis is complex and they act at a number of central and peripheral sites. Opioid systems are involved in the circadian aspects of cardiovascular control (Porttaluppi et al. 1996), the modulation of reflex cardiovascular responses (e.g., baroreflexes, the responses to injury and haemorrhage), the pathophysiology of systemic hypertension and ischaemic preconditioning. It is thought that opioids exert their cardiovascular actions by inhibition of sympathetic activity and by increased parasympathetic activity. Opioid receptor activation results in primarily inhibitory effects on autonomic nervous activity, presynaptic transmitter release and postsynaptic transmitter function. For example, opioids inhibit the release of catecholamines in response to β adrenergic stimulation. Endogenous opioid peptides also alter cardiac responsiveness to both vagal and sympathetic stimulation. In addition, there is interplay between opioid receptors and autonomic activity; cardiac opioid receptor numbers and affinity are increased by adrenergic stimulation and opioid receptor blockade increases the cardiac response to β adrenergic stimulation (Barron 2000). Furthermore, opioid peptides have direct effects on the heart and vascular smooth muscle (Paakkari & Feuerstein 1995). The acute and chronic effects of opioids may be different (Barron 2000), and there are differences in effect between opioid receptor subtypes. However, mu receptors are probably the most important subtype with regard to cardiovascular function, and most opioid drugs used clinically are also predominantly mu receptor agonists. The mechanism of action of opioid drugs and opioid receptor pharmacology is discussed in Chapter 5.

The physiological significance of the role of opioid peptides in cardiovascular homeostasis is not fully elucidated. The opioid antagonist naloxone has no effect per se on heart rate and blood pressure except in models of haemorrhage or traumatic shock and it has been suggested that opioids may have a limited role in cardiovascular homeostasis except in situations of physiological stress. However, it is certain that opioid peptides are intimately involved with autonomic nervous and cardiovascular function at many levels, and even if the physiological role of opioids is limited to autonomic and cardiovascular stress responses, it is clear that exogenous opioids may affect both these responses and cardiovascular function per se. The cardiovascular effects of opioid drugs administered exogenously are discussed further in Chapter 3.
2.7 Conclusions

Central control of the cardiovascular system and its responses is co-ordinated by a number of interconnected regions of the brain stem, which receive afferent inputs from the periphery via connections with autonomic nerves, spinal neurones and higher centres. Cardiovascular function is predominantly controlled via the sympathetic nervous system, regulated by parasympathetic and circulating influences. The activity of sympathetic preganglionic and vagal neurones is controlled by a number of synaptic inputs, although descending inputs from the RVLM predominate. Although most of these nerves contain a dominant neurotransmitter (which may be excitatory or inhibitory), a number of neuropeptides and monoamines, including endogenous opioids, co-exist and are thought to have a modulatory role. The traditional concept that global activation of efferent sympathetic activity occurs in response to different stimuli has been superseded. It has been demonstrated that different sympathetic nerves supplying different regions or vascular beds may be differentially activated, with differential patterns of response, depending on the nature of the afferent stimulus and the autonomic receptors within that region.

All of these influences on the cardiovascular system may be affected by opioids and other drugs used during anaesthesia. Opioid receptors are distributed widely in the central nervous system, the heart and vasculature, as well as autonomic pathways, where they are co-transmitters at pre- and post-synaptic adrenergic neurones. They are involved in circadian cardiovascular regulation and in the modulation of reflex cardiovascular responses. The cardiovascular effects of opioids are mostly mediated by mu receptors. Most opioid drugs used in clinical practice are predominantly mu receptor agonists, and it is therefore logical that opioid drugs have effects on the cardiovascular system and on reflex cardiovascular responses. This is discussed further in Chapter 3.
CHAPTER 3. EFFECTS OF ANAESTHESIA ON THE CARDIOVASCULAR SYSTEM

3.1. Potential interactions between anaesthesia and the cardiovascular system

3.2. Cardiovascular effects of volatile anaesthetic agents
   3.2.1. Volatile anaesthetic agents
   3.2.2. Nitrous oxide

3.3. Cardiovascular effects of intravenous anaesthetic drugs

3.4. Effects of opioid drugs on cardiovascular function
   3.4.1. The effects of opioids on cardiovascular function in vitro
      3.4.1.1. Effects of opioids on heart rate
      3.4.1.2. Effects of opioids on myocardial contractility
      3.4.1.3. Effects of opioids on vascular smooth muscle tone
   3.4.2. Clinical studies of the cardiovascular effects of opioids

3.5. Effects of other factors on cardiovascular function during anaesthesia and surgery

3.6. Summary
EFFECTS OF ANAESTHESIA ON THE CARDIOVASCULAR SYSTEM

All the studies detailed in this thesis were performed in patients undergoing general anaesthesia. In order to predict the actions of opioids, the actions of general anaesthesia must be appreciated. General anaesthesia affects cardiovascular function in a number of ways. It causes general depression of the central nervous system (CNS), including the vital centres involved in cardiovascular control. However, cardiovascular function is determined by many different physiological factors. These physiological factors act at several different levels within the central nervous system and peripherally at the heart and peripheral vasculature. They include effects on preload, afterload, cardiac contractility and vascular tone. Anaesthesia potentially affects all of these factors, and in addition, the different drugs used during anaesthesia have different effects at these different sites.

3.1 Potential interactions between anaesthesia and the cardiovascular system

Drugs used during anaesthesia may have effects on the higher CNS centres involved in cardiovascular control, on cardiovascular control centres in the medulla or any of the receptor systems, afferent or efferent pathways or neuroendocrine connections involved in cardiovascular reflexes. These include baroreceptor reflexes, somatosensory reflexes, chemoreceptor reflexes or the defence reactions to rage or fear. They can also affect the effector organs (heart and peripheral vasculature) directly or indirectly via interaction with vasoactive substances (Reiz et al. 1994, Martner & Biber 1982).

The precise effects of anaesthesia in an individual depends on the prevailing cardiovascular function and activity of cardiovascular reflexes, circulating volume, interactions with other drugs, and the presence or absence of other stimuli, for example pain (Chiu & White 2001). In order to consider the actions of anaesthesia on cardiovascular function, the possible effects will be considered in isolation, although in practice, all effects will be co-existing and interacting at any given time.

General anaesthesia causes global depression of CNS functions, including those involved with regulation of vital body functions. However, it is thought that supramedullary centres (including those involved with the regulation of baroreceptor function), are generally more susceptible to the effects of anaesthesia than centres in the medulla.
Martner & Biber 1982). Consequently, the minimum alveolar concentration (MAC) of anaesthetic required to blunt autonomic responses to a noxious stimulus is approximately 50% higher than the MAC to prevent movement, and is approximately three times higher than the MAC to produce unconsciousness (Roizen et al. 1981). Although volatile, intravenous and local anaesthetic drugs attenuate baroreceptor and somatosensory reflexes in a dose dependent manner, they may do so at several potential sites. The relative importance of the different suggested sites (afferent or efferent limbs, the medulla or higher centres) is not established, but they probably differ between different drugs, and depend on prevailing conditions. However, these reflexes are co-ordinated by the sympathetic nervous system and sympathetic tone is decreased or modified under general anaesthesia. General, regional, local anaesthetics and opioids all attenuate the reflex sympathetic responses to noxious stimuli but by different mechanisms. For example, opioids decrease the transmission of afferent impulses in CNS pain pathways, so decreasing sympathetic responses to pain. However, they may have little effect in the absence of pain stimulus and have little effect on baroreflexes. Local or regional anaesthesia both decrease afferent and efferent sympathetic activity, but in contrast, volatile anaesthetic drugs have additional direct effects on the heart and peripheral vasculature.

3.2 Cardiovascular effects of inhalational anaesthetic agents

3.2.1 Volatile anaesthetic agents

Volatile anaesthetic agents affect cardiovascular function by actions on autonomic cardiovascular efferent pathways, the heart and the vasculature, although different agents have differing effects at these sites. This section will concentrate on the cardiovascular effects of the volatile anaesthetic isoflurane as this agent was used exclusively in the studies outlined in Chapters 7-12 of this thesis. All volatile anaesthetic agents depress arterial pressure in a dose-related manner, via several different mechanisms. Efferent sympathetic nervous activity is decreased, and consequently the baroreflex control of heart rate and sympathetic outflow to the heart and blood vessels is depressed (Skovstedt & Saphavichaikul 1977, Seagard et al. 1983). Isoflurane has more effect than halothane on the reflex control of heart rate (Kotrly et al. 1984) and is also thought to augment
baroreceptor afferent activity (Seagard et al. 1984). This has the effect of lowering sympathetic tone and decreasing arterial pressure. In addition, isoflurane and other volatile agents affect transmission at multiple sites in the baroreflex arc, including at autonomic ganglia and so the chronotropic responses to direct sympathetic and parasympathetic nervous stimulation are decreased (Seagard et al. 1983). In vitro, all volatile anaesthetic agents slow sinoatrial node (SAN) discharge and prolong conduction in the His-Purkinje system and ventricles, but these effects may be masked in vivo (Atlee & Bosjnak 1990). Halothane decreases myocardial contractility, heart rate and cardiac output, whereas isoflurane causes a decrease in arterial pressure, which is frequently accompanied by a reflex tachycardia. Myocardial contractility is decreased to a lesser extent with isoflurane, although all volatile agents have the potential to cause negative inotropic effects by effects on intracellular calcium (Ebert & Schmid 2001). However, cardiac output is maintained with most agents except halothane. Isoflurane causes vasodilatation and a decrease in arterial pressure by a direct action on vascular smooth muscle (Ostman et al. 1985), although different vascular beds are affected to different extents (Reiz et al. 1994). The mechanism of vascular smooth muscle relaxation may be related to increased cAMP formation (Reiz et al. 1994): isoflurane has no effects at α or β adrenergic receptors (Ostman et al. 1986). Vascular relaxation causes a decrease in systemic vascular resistance; heart rate increases but central venous pressure and cardiac output are maintained (Ebert & Schmid 2001). Isoflurane causes marked dose-dependent coronary vasodilatation by an action at the vascular endothelium of coronary resistance vessels (Blaise et al. 1987, Sill et al. 1987). The precise mechanism is uncertain but it may be mediated by nitric oxide release (Blaise et al. 1987). Coronary vasodilatation may predispose to the development of myocardial ischaemia in susceptible individuals by the phenomenon of coronary steal (Reiz et al. 1994, Ebert & Schmid 2001).

Unusually, desflurane (and to a lesser extent isoflurane) may cause a sympathetically-mediated increase in heart rate during rapid increases in inspired concentrations. Epinephrine concentrations are increased in parallel and the effect is attenuated by opioids. Both these volatile agents are pungent and this response may be mediated by activation of airway receptors (Weiskopf et al. 1994). Volatile anaesthetic agents also depress chemoreceptor reflexes, including the ventilatory and cardiovascular responses to hypoxia (Martner & Biber 1984).
The cardiovascular effects of isoflurane therefore comprise dose-dependent decreases in arterial pressure mediated by direct vasodilatation and decreases in sympathetic tone. Coronary vasodilatation and reflex tachycardia also occur, and myocardial contractility is relatively maintained at clinically used doses.

### 3.2.2 Nitrous oxide

Nitrous oxide produces dose-dependent myocardial depression in vitro (Motomura et al. 1984). However, in vivo it causes sympathetic stimulation with an increase in sympathetic nervous activity, vascular resistance and plasma catecholamine concentrations (Ebert & Kampine 1984). It causes activation of sympathetic nervous activity to skeletal muscle blood vessels but decreases baroreflex tachycardia in response to sodium nitroprusside (Ebert 1990). Its effects in vivo are sometimes difficult to separate from the effects of other co-administered drugs, but it can cause depression of cardiovascular function when administered with opioids (Bovill et al. 1984, Stanley et al. 1977). Decreases in myocardial contractility may be more marked in patients with coronary artery disease and impaired left ventricular function (Eisele et al. 1976), and may be apparent at low (10%) inspired concentrations (McDermott & Stanley 1974).
3.3 Cardiovascular effects of intravenous anaesthetic drugs

All intravenous anaesthetic drugs produce cardiovascular depression by a combination of direct effects on the heart and peripheral vasculature, and depression of central and peripheral nervous system activity and responses, including baroreceptor reflexes. The degree of cardiovascular depression depends on the individual drug, dose and speed of injection. It also depends on the patient's age, pre-existing cardiovascular function, intravascular volume, resting sympathetic tone and the co-administration of other cardiovascular drugs (Chiu & White 2001). In general, all intravenous anaesthetic drugs potentially cause hypotension by decreasing sympathetic nervous activity, direct myocardial depression, decreased peripheral vascular resistance and dilatation of venous capacitance vessels, although benzodiazepines and etomidate have less cardiovascular effects than other agents (Ebert et al. 1990, Scheffer et al. 1993, Reiz et al. 1994). The intravenous anaesthetic drug used in all the studies outlined in this thesis was propofol, and the cardiovascular effects of propofol will be considered in more detail.

Propofol causes dose-dependent cardiovascular depression by several mechanisms. These include decreased central sympathetic tone, attenuation of baroreflex activity and direct effects on the heart and peripheral vasculature. It causes bradycardia, and has a direct negative inotropic action on the myocardium by decreasing intracellular calcium availability. This is thought to be secondary to inhibition of calcium influx across the myocyte sarcoplasmic reticulum, although this direct negative inotropic effect may be modest (Brussel et al. 1989, Riou et al. 1992). Studies in animal models have suggested that bradycardia is caused by dose-related depression of the SAN and His-Purkinje conducting systems (Colson et al. 1988, Pires et al. 1996), with a possible effect on the AV node mediated by M2 cholinergic receptors (Alphin et al. 1995), although the evidence is conflicting (Ikeno et al. 1999) and has not been confirmed in humans (Romano et al. 1994). It is probable that inhibition of central sympathetic nervous activity, (Ebert et al. 1992, Ebert & Muzi 1994, Xu 2000) and effects on baroreflex mechanisms also contribute to bradycardia (Kamijo et al. 1992, Scheffer et al. 1993). Attenuation of baroreflex mechanisms results in a smaller increase in heart rate and less increase in peripheral vascular resistance for a given decrease in arterial pressure (Cullen et al. 1987, Sellgren et al. 1994).
Vasodilatation is an important factor in the cardiovascular depression caused by propofol. In addition to causing arterial and venous dilatation by inhibition of sympathetic nervous activity, propofol may also have direct effects on the vascular smooth muscle (Bentley et al. 1989, Park et al. 1992), via a direct effect on intracellular calcium mobilization or increased nitric oxide production in vascular smooth muscle (Calvey & Williams 1997, Chiu & White 2001). Systemic vascular resistance is decreased and venodilatation results in decreased venous return (Muzi et al. 1992). Propofol has greater cardio-depressant effects in comparison with other intravenous anaesthetic drugs (Brussel et al. 1989, Mulier et al. 1991, Ebert et al. 1992), and attenuates the cardiovascular response to laryngoscopy and tracheal intubation more effectively than thiopental (Lindgren et al. 1992, Chan & Chung 1996). In common with other intravenous anaesthetic drugs, the cardiovascular depressant effects of propofol are more marked in the elderly (Olmos et al. 2000).

3.4 The effects of opioid drugs on cardiovascular function

As detailed in Chapter 2, endogenous opioid peptides act at many of the sites involved in cardiovascular control, including sites in the central nervous system, the afferent and efferent limbs of the autonomic nervous system, and directly on the heart and vasculature. Opioid peptides are involved in the modulation of reflex cardiovascular responses to injury, haemorrhage and the circadian aspects of cardiovascular control (Portaluppi et al. 1996). In addition they may be involved in the pathophysiology of systemic hypertension. The mechanism of action of opioid drugs is described in Chapter 5.

It has traditionally been held that exogenous opioids exert their cardiovascular actions by inhibition of sympathetic nervous activity and by increased parasympathetic activity, and that the site of action is within the CNS (Coda 2001). However, the evidence for this is largely indirect and in some cases is conflicting. Some data suggest that opioids have specific peripheral actions on the heart and vasculature. There are also differences in opioid actions between species, and the effects of anaesthesia or other co-administered drugs may have confounded the results of some studies. Furthermore, although most opioids used in clinical practice are mu opioid receptor agonists, there are differences between them with respect to their cardiovascular effects. This is partly because of
differences in affinity and intrinsic efficacy at different opioid receptors, but also because of differences in auxiliary effects (Bowdle 1998). For example meperidine is a phenylpiperidine derivative, like fentanyl (and its analogues alfentanil, sufentanil and remifentanil). However, meperidine has other effects including anticholinergic activity and histamine release (Flacke et al. 1987). Consequently, meperidine causes tachycardia, vasodilatation and hypotension, and it also has more marked negative inotropic effects compared with other opioids (Calvey & Williams 1997). In contrast, the other phenylpiperidine-derived opioids (such as fentanyl and its derivatives), may cause bradycardia and hypotension, but are often used in high doses in clinical practice with few adverse cardiovascular effects (see below). Morphine causes arteriolar and venous dilatation, with a decrease in peripheral vascular resistance, largely because of dose-dependent histamine release (Feuerstein & Siren 1987), although it also has a direct effect on vascular smooth muscle (Altura et al. 1978).

The data regarding the cardiovascular effects of opioids will therefore be considered in more detail, with respect to in vitro studies, those performed in animal models and human studies.

3.4.1 The effects of opioids on cardiovascular function in vitro

A number of studies have examined the effects of opioids on cardiovascular function in vitro and in animal models, and have attempted to separate the effects on heart rate, myocardial contractility, and vascular smooth muscle tone. These data are considered separately below.

3.4.1.1 Effects of opioids on heart rate

Early studies suggested that morphine has little or no effect on heart rate (Lowenstein et al. 1969). However, when injected directly into the sinus node in anaesthetised dogs, morphine produced dose-dependent bradycardia, which was maximal after 2 minutes and had resolved after 20 minutes. This was unaffected by atropine, ACh, vagotomy, beta blockade or norepinephrine, suggesting that the bradycardia produced by morphine was independent of vagal and sympathetic nervous function. In this study, intravenous
morphine 0.2 mg kg$^{-1}$ also produced bradycardia (Urthaler et al. 1973). However, in a later study the authors found that in awake dogs, administration of morphine in the sinus node produced a transient tachycardia lasting for 7-10 minutes, followed by a more prolonged bradycardia which had a nadir at 25-30 minutes after injection (Urthaler et al. 1975). The changes in heart rate were unaffected by beta blockade, and when atropine was administered no further initial tachycardia was produced by subsequent sinus node morphine injection. Intravenous morphine in these awake animals produced only bradycardia, which was apparent after approximately 10 minutes. The initial tachycardia after sinus node injection of morphine was therefore attributed to a local vagolytic action, whereas the subsequent prolonged bradycardia was attributed to a centrally mediated increase in vagal tone. The authors attributed the differences between these two studies to the confounding effects of anaesthesia and surgery on autonomic function (Urthaler et al. 1975). Reitan and colleagues found that fentanyl produced dose-dependent bradycardia and hypotension in anaesthetized dogs, which were markedly attenuated by prior cervical vagotomy (Reitan et al. 1978). Bradycardia associated with fentanyl is reversed by atropine (Eisele et al. 1975) and naloxone (Freye 1974). Others have supported the view that bradycardia associated with opioids is more marked in anaesthetised subjects (Tammisto et al. 1970, Bovill et al. 1984). It is also related to the dose (Freye 1974, Reitan et al. 1978, Crawford et al. 1987), and the speed of injection (Bovill et al. 1984). Earlier studies suggested that the bradycardia produced by fentanyl was largely caused by central vagal stimulation but was also related to attenuation of sympathetic activity (Reitan et al. 1978). It has been suggested that different mu opioid receptor subtypes may mediate the tachycardia and bradycardia produced by opioids, tachycardia being mediated by $\mu_1$ and delta agonists, and bradycardia being mediated by $\mu_2$ and kappa agonists (Paakkari et al. 1992, Paakkari & Feuerstein 1995), although the evidence for the existence of $\mu_1$ and $\mu_2$ receptors is uncertain. The current understanding of opioid receptors and their subtypes is discussed further in Chapters 2 and 5.

Other data suggest that morphine directly depresses conduction in the SA and AV nodes (Bovill et al. 1984). A study in rat cardiac ventricular microsomes suggested that the bradycardia produced by fentanyl occurs by a mechanism which is independent of $\beta$ adrenergic receptors and does not involve inhibition of $\beta$ adrenergic receptor-G$\text{G}_{\text{i,0}}$ protein coupling (Locker et al. 1999). Morphine also has a protective effect against ventricular fibrillation, which is abolished by atropine or vagotomy (De Silva et al. 1978). Others
have suggested that the anti-arrhythmic effect of morphine may be partially caused by a
direct effect on sodium channels (Hung et al. 1998). However, other studies have
suggested that opioids (with the exception of meperidine) cause only mild bradycardia or
have no effect on heart rate (Bovill et al. 1984).

3.4.1.2 Effects of opioids on myocardial contractility

Early studies suggested that exogenous opioids have little or no effect on myocardial
contractility in vitro at clinically relevant concentrations, although dose-dependent
depression of contractility occurred at higher concentrations (Goldberg & Padget 1969,
examined the effects of morphine, piritramide, fentanyl, pentazocine and meperidine in an
isolated cat papillary muscle preparation and found that morphine and piritramide had
some positive inotropic effects at low concentrations (Strauer 1972, 1974). In higher
concentrations, all opioids studied produced dose-dependent depression of myocardial
contractility, as demonstrated by decreases in the force, velocity and extent of muscle
shortening and relaxation. In the case of morphine, fentanyl and piritramide, this negative
inotropic effect is relatively minor and in comparative terms approximates to their relative
analgesic potency. However, the negative inotropic effects of pentazocine were 50 fold
greater and the effects of meperidine were 100-200 fold greater than the other opioids
studied. In an isolated rat heart preparation, fentanyl produced negative inotropic effects
which were additive with those of diazepam (Reves et al. 1984). However, the EC50 for
deression of myocardial contractility by fentanyl was 400 times greater than the plasma
concentrations after large intravenous doses of fentanyl in humans. Furthermore, the
clinical relevance of these in vitro data is uncertain, as compensatory baroreceptor and
other physiological circulatory reflex effects occur in vivo.

Fentanyl in large doses (20 mcg kg⁻¹) caused a decrease in heart rate but little effects on
contractility or arterial pressure in anaesthetized dogs. These effects were attenuated by
atropine (Eisele et al. 1975). In another study in anaesthetised dogs, fentanyl 50 mcg kg⁻¹
causde decreases in heart rate, contractility, arterial pressure and plasma catecholamine
concentrations. These cardiovascular changes were reversed by naloxone, but only
slightly attenuated by atropine 20 mcg kg⁻¹, suggesting that they were largely independent
of vagal function. Administration of the centrally-acting α2 adrenergic agonist clonidine,
which decreases central vasomotor activity, augmented the cardiodepressant effect of fentanyl, but these effects were reversed by tolazoline, a non-selective peripherally-acting $\alpha_2$ and $\alpha_1$ adrenergic antagonist. These workers therefore concluded that the cardiovascular effects of fentanyl were caused by a reduction of central sympathetic activity (Flacke et al. 1983).

Alfentanil has also been shown to have negative chronotropic effects in a rabbit isolated heart preparation (Zhang et al. 1990). The effects of alfentanil were different between with left and right atria, and between atria and ventricles. Force of atrial contraction was increased but there was no effect on papillary muscle contraction. However, the threshold alfentanil concentrations required to produce positive inotropic effects were over 50 times higher than those achieved after the highest therapeutic concentrations of alfentanil used in clinical practice, and this positive inotropic effect has not been confirmed. When used in high doses in clinical practice, alfentanil (like fentanyl) is usually associated with bradycardia and decreased arterial pressure (Bovill et al. 1984, Coda 2001).

3.4.1.3 Effects of opioids on vascular smooth muscle tone

Morphine causes venous (Ward et al. 1972) and arterial dilatation by centrally-mediated neural effects and a direct local effect on vascular smooth muscle (Lowenstein et al. 1972). The venous effects are more marked than the arterial effects (Ward et al. 1972). The direct arterial effects are rapid and dose-dependent, and can be observed in denervated muscle preparations. They are mostly attenuated by promethazine but not naloxone and are unaffected by prior administration of atropine, suggesting that they are independent of parasympathetic nervous function and may be mediated by histamine release (Hsu et al. 1979, Feuerstein & Siren 1987). However, vasodilatation in response to intravenous morphine is more marked when sympathetic tone is high, and is absent in the presence of low vascular resistance or alpha-adrenergic blocking drugs. This suggests that the neural mechanism is central inhibition of sympathetic nervous activity (Lowenstein et al. 1972, Hsu et al. 1979).

Fentanyl and its derivatives are considered to have less cardiovascular effects in comparison to other opioids (Liu et al. 1976, Bovill et al. 1984). However, when large
bolus doses of fentanyl were administered to anaesthetised dogs who had also received subarachnoid anaesthesia to eliminate sympathetic tone, no changes in arterial pressure, heart rate, cardiac output or systemic vascular resistance were observed, suggesting that previous observations of cardiovascular depression were caused by a reduction in central sympathetic outflow (Flacke et al. 1985). Fentanyl does not cause histamine release (Rosow et al. 1984).

3.4.2 Clinical studies of the cardiovascular effects of opioids

In general, opioids have little effect on arterial pressure and heart rate in normovolaemic supine patients. They are used in clinical anaesthesia because of their perceived lack of adverse cardiovascular effects in comparison with volatile anaesthetic agents. When given to healthy awake patients, fentanyl in doses of up to 70 mcg kg\(^{-1}\) (which can induce unconsciousness) produces no significant changes in heart rate, arterial pressure, cardiac output or stroke volume (Graves et al. 1975, Sebel et al. 1981b, Bailey et al. 1985). However, the precise cardiovascular effects of opioids in clinical practice are related to dose and speed of injection and bradycardia or decreases in arterial pressure are frequently observed (Bovill et al. 1984). Bradycardia, hypotension and decreases in cardiac output are more common after fentanyl when it is co-administered with diazepam, nitrous oxide or other drugs used during anaesthesia (Tammisto et al. 1970, Liu et al. 1976, Eisele et al. 1975, Reves et al. 1984, Motomura et al. 1984, Bailey et al. 1985). Cardiovascular effects are also dose-related; during balanced anaesthesia with nitrous oxide and enflurane, fentanyl had no effects on cardiovascular function at doses of 50-100 mcg but produced decreases in stroke volume, cardiac output and mean arterial pressure with no change in heart rate or peripheral resistance at a dose of 200 mcg (Bennett & Stanley 1979). These effects depend on underlying cardiac function and cardiovascular depression is more likely in those with impaired cardiac function (Eilele et al. 1976). In higher doses, opioids attenuate or abolish the neuroendocrine and cardiovascular stress responses during surgery (Stanley et al. 1979, 1980, Sebel et al. 1981a, Bovill et al. 1984, Giesecke et al. 1988). High dose intravenous opioid infusions have been used in an attempt to produce unconsciousness before surgery (Bailey et al. 1985). However, although deep sedation and characteristic EEG changes occur (Sebel et al. 1981b), this
technique does not predictably produce anaesthesia (Bailey et al. 1985). Fentanyl has no effects on the coronary circulation or myocardial metabolism (Blaise et al. 1990), and coronary autoregulation is preserved during high-dose fentanyl infusions (Reiz et al. 1994). Fentanyl also attenuates the increase in heart rate, cardiac index and oxygen delivery produced by the β adrenergic agonist dobutamine, by a mechanism independent of β adrenergic receptors ( Locker et al. 1999). Bradycardia and hypotension may be prevented or treated in clinical practice by the use of atropine or intravenous fluids (Coda 2001).

The cardiovascular effects of other opioids are similar to those of fentanyl but more marked when equi-analgesic doses are compared (Strauer 1972, Liu et al. 1976). Morphine may produce vasodilatation and histamine release, whereas meperidine causes tachycardia, and has anticholinergic effects (section 3.1.3). However, all the fentanyl-derived opioids have similar effects on the cardiovascular system in vivo. These are dose-dependent bradycardia, and a mild decrease in myocardial contractility. Conversely, cardiovascular depression may occur when these and other opioids are co-administered with other vasoactive drugs or drugs which depress CNS function. In contrast to morphine, the fentanyl-derived opioids do not cause histamine release. Although morphine may produce venodilatation and arteriolar dilatation by a combination of histamine release and direct effects on vascular smooth muscle, the direct effects of fentanyl-derived opioids on vascular smooth muscle is not known.

There is ample evidence for the involvement of endogenous opioid peptides in cardiovascular homeostasis. This is discussed in detail in Chapter 2, although the physiological relevance of these data is uncertain. It is probable that the cardiovascular effects of opioid drugs are mediated by several mechanisms. These include the attenuation of sympathetic afferent and efferent activity, direct central or peripheral vagal stimulation, direct and indirect effects on the myocardium and vascular smooth muscle. The cardiovascular effects of opioids are dose-dependent, and vary between opioids because of differences in receptor affinity and intrinsic efficacy, and differences in auxiliary effects. The effects in an individual also depend on several factors including pre-existing cardiovascular function, sympathetic tone and the effects of other co-administered drugs.
3.5 The effects of other factors on cardiovascular function during anaesthesia and surgery

In addition to direct pharmacological effects, cardiovascular depression can be produced by a number of other factors during anaesthesia and surgery. Pertinent to the studies outlined in Chapters 7-12 are the effects of positive pressure ventilation, which by increasing mean intrathoracic pressure, causes a decrease in venous return. This can decrease cardiac output and arterial pressure, depending on the intrathoracic pressure generated, and the duration of the inspiratory and expiratory phases. The cardiovascular effects of positive pressure ventilation are also determined by other factors including cardiovascular status, sympathetic tone and circulating intravascular volume (Lin & Hanning 1994). In healthy individuals, decreased venous return is compensated by baroreceptor-mediated increases in sympathetic nervous activity, producing tachycardia, vasoconstriction and restoration of cardiac output and arterial pressure (Guyton & Hall 2000). However, this compensation may be incomplete, particularly in the elderly or those with cardiovascular disease. In clinical practice, intravenous fluid loading is frequently used to minimise the cardiovascular depressant effects of intravenous anaesthetic drugs and positive pressure ventilation at induction of anaesthesia in at risk patients. This technique was employed in a standardised manner in Studies 5 and 6, where elderly and hypertensive patients were studied.
3.6 Summary

Cardiovascular function is determined by many different physiological factors acting within the CNS and the peripheral effector organs (heart and peripheral vasculature). Drugs used during anaesthesia may have effects on the higher CNS centres involved in cardiovascular control, on cardiovascular control centres in the medulla or any of the receptor systems, afferent or efferent pathways or neuroendocrine connections involved in cardiovascular reflexes. The precise effects depend on individual cardiovascular function and activity of cardiovascular reflexes, circulating volume, interactions with other drugs, and the presence of noxious stimuli. However, general anaesthesia is commonly induced using a combination of several drugs and manoeuvres, all of which can depress cardiovascular function. Induction of anaesthesia is therefore a period of risk for acute cardiovascular instability.

Opioid receptors are distributed widely in the central nervous system, the heart and vasculature, as well as autonomic pathways. They are involved in circadian cardiovascular regulation, and the modulation of reflex cardiovascular responses. It is therefore logical that opioid drugs have cardiovascular effects, although they are usually administered to produce analgesia. The cardiovascular effects of opioids vary and are dose-dependent. However, they include attenuation of sympathetic nervous activity, vagal stimulation and effects on the myocardium and VSM. Although the physiological responses to noxious stimuli have advantages in evolutionary terms, they may be detrimental in patients undergoing surgery, particularly in those with cardiovascular disease. Attenuation of potentially harmful autonomic responses is a basic part of good anaesthetic practice and opioids are widely used for this purpose in clinical anaesthesia.

Induction of anaesthesia also causes respiratory depression and loss of protective airway reflexes, and is immediately followed by manoeuvres to protect and maintain the integrity of the patient’s airway. One of these manoeuvres is laryngoscopy and tracheal intubation, a noxious stimulus that produces potent autonomic and cardiovascular effects. The combination of acute cardiovascular depression caused by induction of anaesthesia and intense autonomic stimulation caused by laryngoscopy and tracheal intubation may be harmful to susceptible individuals. The pathophysiological effects of laryngoscopy and tracheal intubation are discussed in Chapter 4.
CHAPTER 4. LARYNGOSCOPY AND TRACHEAL INTUBATION

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   4.1.1. The pharynx
   4.1.2. The larynx
   4.1.3. Nervous supply of the larynx
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4.2. Physiological role of laryngeal reflexes

4.3. Laryngoscopy and tracheal intubation
   4.3.1. Historical aspects
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4.4. Emergence from anaesthesia and tracheal extubation

4.5. Mechanisms underlying the cardiovascular response to laryngoscopy and tracheal intubation

4.6. Mechanisms underlying the cardiovascular responses to emergence from anaesthesia and tracheal extubation

4.7. Summary
LARYNGOSCOPY AND TRACHEAL INTUBATION

Laryngoscopy and tracheal intubation is commonly performed during anaesthesia for surgical procedures, resuscitation and the care of the critically ill patient. Tracheal intubation literally refers to the passage of a tube into the trachea, and laryngoscopy is the process by which an instrument (the laryngoscope) is placed into the larynx to elevate the tongue and laryngopharyngeal structures in order to pass the tracheal tube through the vocal cords under direct vision. Despite the introduction of newer devices to assist airway management in the unconscious patient, tracheal intubation under direct vision is accepted as the most reliable method of securing and protecting the airway, and it is performed routinely throughout the world. However, laryngoscopy and tracheal intubation has a number of pathophysiological consequences which are harmful for some patients. This chapter will describe the pertinent anatomy of the upper airway and the physiological effects associated with laryngoscopy and tracheal intubation.

4.1 Anatomy of the pharynx and larynx

4.1.1 The pharynx

The pharynx is 12-15 cm long in adults and extends from the base of the skull to the level of the cricoid cartilage, where it becomes continuous with the oesophagus. The pharyngeal muscles include the superior, middle and inferior constrictors, which act in a co-ordinated fashion during swallowing. The pharynx communicates anteriorly with the nasal cavity (nasopharynx), the oral cavity (oropharynx) and the larynx (hypopharynx or laryngopharynx) (Ellis & Feldman 1997) (Figure 4.1).

The oropharynx is bounded superiorly by the soft palate, anteriorly by the posterior third of the tongue, inferiorly by the epiglottis, and posteriorly by the bodies of the 2nd and 3rd cervical vertebrae. On the lateral wall of the oropharynx is the tonsillar fossa with its anterior and posterior folds. The hypopharynx lies at the level of the 4th to 6th cervical vertebrae, between the superior border of the epiglottis and the inferior border of the cricoid cartilage. The pyriform fossae or sinuses form the lowest border of the hypopharynx and lie lateral to the larynx. The sensory innervation of the mucous membranes of the oropharynx is from the glossopharyngeal nerve, via pharyngeal nerves.
(sensory to the pharyngeal wall and oropharyngeal isthmus), tonsillar nerves (sensory to
the soft palate, tonsils and pharyngeal arches) and sensory branches to the posterior third
of the tongue and roof of the pharynx. The anterior two thirds of the tongue is supplied by
the mandibular division of the trigeminal nerve. The hypopharynx close to the entrance
of the larynx is supplied by the vagus nerve, via the internal laryngeal branch of the
superior laryngeal nerve. The motor supply of the pharynx is from the pharyngeal plexus,
which is primarily formed from branches of the vagus but has contributions from the
glossopharyngeal nerve and sympathetic ganglia (Graney & Flint 1999).

4.1.2 The larynx

The larynx extends from the root of the tongue to the trachea. In the adult male it lies
opposite the 3rd to 6th cervical vertebrae and opens into the laryngopharynx above and
the trachea below (Figure 4.1). It comprises a framework of cartilages (thyroid, cricoid,
arytenoids and epiglottis) connected by ligaments and membranes, and to which the
extrinsic and intrinsic muscles of the larynx are attached. The epiglottis lies anteriorly in
the larynx, and is attached at its inferior aspect to the thyroid cartilage via the
thyroepiglottic ligament. Posteriorly, it is attached to the hyoid bone. The anterior surface
of the epiglottis is free and descends to the vallecula, into which the laryngoscope blade is
usually inserted during laryngoscopy (see below).

The laryngeal mucous membrane is continuous above with that of the mouth and
pharynx, and below with that of the trachea. It is loosely attached to the anterior surface
of the epiglottis and to the adjacent tissues in the valleculae. It covers the aryepiglottic
folds, which limit the inlet of the larynx. On the anterior surface and the upper half of the
posterior surface of the epiglottis, the upper part of the aryepiglottic folds and the vocal
folds, the epithelium of the mucous membrane is of the stratified squamous type. The
remainder of the laryngeal mucous membrane is covered by ciliated columnar respiratory
epithelium (Wyke & Kirschner 1976).
Figure 4.1 Sagittal section of the head and neck showing the anatomy of the upper airway

4.1.2 Nervous supply of the larynx

The motor fibres supplying the muscles of the larynx are derived from the nucleus ambiguus in the medulla. Axonal processes from the nucleus ambiguus contribute to both the vagus (Xth) and accessory (XIth) cranial nerves, but accessory nerve fibres join the vagus shortly after it emerges from the jugular foramen, so all the motor innervation of the larynx travels together in the vagus nerve. Two branches of the vagus nerve leave the main nerve as it travels down the neck in the carotid sheath: the superior laryngeal and recurrent laryngeal nerves. The superior laryngeal nerve divides into the internal and external laryngeal nerves. However, the motor fibres in the superior laryngeal nerve leave and are carried in the external laryngeal nerve, so the internal laryngeal nerve is entirely
sensory. The external laryngeal nerve supplies the cricothyroid muscle and inferior constrictor muscles of the pharynx. All the intrinsic muscles of the larynx except the cricothyroid are supplied by the recurrent laryngeal nerve. The laryngeal nerves also contain sympathetic and parasympathetic fibres originating in the superior and middle cervical ganglia, and the dorsal nucleus of the vagus respectively. These autonomic fibres regulate the calibre of laryngeal blood vessels and the secretion of mucus from laryngeal glands (Graney & Flint 1999).

The sensory supply of the larynx is carried in both the internal and recurrent laryngeal nerves, and hence the vagus nerve. The internal branch of the superior laryngeal nerve supplies the larynx above the vocal cords, and the recurrent laryngeal nerve supplies the larynx below the vocal cords (i.e. the false vocal cords and subglottic mucosa) and the upper part of the oesophagus. All these sensory fibres ascend in the vagus nerve to their cell bodies in the inferior ganglion of the vagus. From the ganglia, the central processes of these neurones ascend superiorly in the vagus to enter the medulla where they synapse in the nucleus tractus solitarius. The vagus is primarily a parasympathetic nerve but also contains some cervical sympathetic fibres and motor fibres to laryngeal muscles. The commonest type of nerve ending found in the larynx is free nerve endings in the mucosa and submucosa, with myelinated or non-myelinated fibres most densely distributed in the posterior supraglottic region, and inferior surfaces of the vocal cords (Graney & Flint 1999).

4.1.4 Receptor systems in the larynx

Epiglottic and other laryngeal nerve endings are extremely sensitive to a range of stimuli, particularly to light touch and other mechanical stimuli (Sato & Koyano 1987). The mucosa of the larynx above the glottis contains glomerular corpuscular nerve endings which are thought to function as low-threshold mechanoreceptors. There are also free nerve endings which form a plexus of unmyelinated fibres responsible for pain sensation, and chemoreceptors. Stimulation of the corpuscular mechanoreceptors in the supraglottic region causes rapid transmission of impulses via large diameter nerve fibres to the nucleus tractus solitarius via the superior laryngeal nerve, causing reflex laryngeal occlusion. More intense stimulation causes activation of smaller diameter fibres via the same route, leading to autonomic responses consistent with the sensation of pain (Wyke...
& Kirschner 1976). Most of these impulses can be abolished by topical anaesthesia of the laryngeal mucosa. The subglottic mucosa contains similar mechanoreceptors and pain receptors which are transmitted via the recurrent laryngeal nerve.

In addition, the fibrous capsules of the cartilaginous joints in the larynx contain low threshold corpuscular mechanoreceptors and pain-sensitive free nerve endings. These receptors are highly sensitive and relay afferents to the nucleus ambiguus to cause reflex activity of the laryngeal muscles. The intrinsic laryngeal muscles also contain nerve endings which function as stretch-sensitive mechanoreceptors, and miniature tendon organs at their tendinous attachments to the laryngeal muscles. These are also highly sensitive and their activity is unaffected by topical anaesthesia of the larynx, although it is depressed by general anaesthesia (Wyke & Kirschner 1976).

4.2 Physiological role of laryngeal reflexes

The nuclei of the vagus nerve are situated close together in the medulla oblongata. The nucleus ambiguus provides efferent fibres to the muscles of the larynx, pharynx and the upper oesophagus. The dorsal efferent nucleus supplies branches to the vagus and glossopharyngeal nerves, and the nucleus tractus solitarius receives afferents from viscera supplied by the vagus, in addition to afferents relaying cardiovascular control (Haxhiu et al. 1993). Other nearby structures include the medullary coughing and respiratory centres, which are connected directly with the vagal nuclei and also via connections through the brainstem reticular formation. The functions of the larynx include coughing, swallowing and phonation; all these are co-ordinated by the vagus nerve. Afferent impulses travel to the nucleus tractus solitarius, connecting to the nucleus ambiguus both directly and via the reticular formation. Efferents via the vagus, phrenic, glossopharyngeal and thoracic nerves travel to the relevant effector muscles, but the reflex is co-ordinated within the medulla. The swallowing reflex is triggered by stimulation of the mechanoreceptors at the level of the anterior fauces and is coordinated by efferents travelling in the glossopharyngeal nerve. Coughing, swallowing and phonation can also be controlled from the cerebral cortex, with corticobulbar fibres travelling via the internal capsule to the nucleus ambiguus (Graney & Flint 1999). The laryngeal nerves also contain afferent receptors originating in pulmonary mechanoreceptors and aortic baroreceptors. These
ascend along the wall of the trachea, where they enter the recurrent laryngeal nerve at the level of the upper tracheal rings, and are easily stimulated by tracheal distention which occurs during tracheal intubation (Wyke & Kirschner 1976). The physiological role of these pulmonary mechanoreceptors (and also chemoreceptors and J receptors) is in airway protection, and controlling the continuous phasic activity of the laryngeal muscles during respiration. The activity of the intrinsic muscles of the larynx is also modulated by afferents originating from mechanoreceptors in the tongue.

Stimulation of the receptors described above leads to a number of reflex responses principally concerned with protection of the airway including coughing and glottic closure. However, bronchodilatation, bronchoconstriction and laryngeal spasm may also occur. In addition, cardiovascular efferents may be activated with an increase in autonomic activity. In a series of experiments in cats, Tomori and Widdecombe showed that mechanical stimulation of the nose and epipharynx caused reflex bronchodilatation, whereas stimulation of the laryngopharynx and tracheobronchial tree caused reflex bronchoconstriction. Stimulation at all sites caused a large increase in heart rate and blood pressure, especially in response to pharyngeal stimulation associated with increased efferent cervical sympathetic nervous activity (Tomori & Widdecombe 1969).
4.3 **Laryngoscopy and tracheal intubation**

4.3.1 *Historical aspects*

Tracheal insufflation was first described in animals by Andreas Vesalius of Padua in 1555. John Snow performed intubation through a tracheotomy in anaesthetised animals in 1858, and this method of tracheal intubation was first described in humans by Friedrich Trendelenberg in 1871 (Trendelenberg 1871). In 1878, William MacEwen used a tracheal tube passed blindly through the mouth to relieve airway obstruction in laryngeal diphtheria, and later used it to administer chloroform during anaesthesia (MacEwen 1880). Nonetheless, intubation via a tracheostomy was the accepted method up to the turn of the 20th century, as it was supposed that a laryngeal tube would not be tolerated at the levels of inhalational anaesthesia (and without neuromuscular blocking drugs) conventionally used at that time. The first cuffed tracheal tube was described by Eisenmenger in 1893 (Eisenmenger 1893) and a cuffed orotracheal tube, similar to the ones in use today, was first described in 1910 (Dorrance 1910). Tracheal intubation was used during anaesthesia for a number of years but was often performed using a blind nasal technique as pioneered by Magill and Rowbotham (Rowbotham & Magill 1921, Magill 1930). The laryngoscopes available were designed for use by endoscopists or laryngologists. These were cumbersome, offered poor views of the larynx, and gave little protection against dental injury (Toski *et al.* 2001). However, tracheal intubation increased in popularity with the advent of neuromuscular blocking drugs in the 1940s (Bourne 1947) and the availability of newer laryngoscopes (Miller 1941, Macintosh 1943). Management of the airway during anaesthesia by tracheal intubation became commonplace after the polio epidemic in Copenhagen in 1952, when the benefits of manual positive pressure ventilation were appreciated (Lassen 1953). Many variations of tracheal tube and other airway aids are now available (Latto & Vaughan 1997). However, tracheal intubation under direct vision with a cuffed tracheal tube remains the most reliable way of securing the airway of an unconscious patient, and it is widely practised by anaesthetists and emergency medical practitioners throughout the world.

Although a number of methods and pieces of equipment may be employed to aid tracheal intubation, the commonest method today is orotracheal intubation under direct vision,
using a laryngoscope with a curved blade. The blade is inserted into the right side of the mouth, advanced over the right side of the tongue, (moving the tongue towards the left) and on towards the tongue base. The base of the tongue is elevated, and the blade advanced further towards the epiglottis. The tip of the blade is then advanced into the vallecula, and the laryngoscope is pushed anteriorly, perpendicular to the axis of the laryngoscope blade, so that the epiglottis is lifted and the vocal cords and trachea visualised (Figures 4.2 and 4.3). The tracheal tube is then placed under direct vision and the tracheal tube cuff inflated at a level below the vocal cords (Murrin 1997). This manoeuvre has some significant physiological consequences, which are detailed below.

Figure 4.2  Position of the curved blade laryngoscope during laryngoscopy

The dashed line represents the line of view of the larynx when the laryngoscope is lifted in the direction of the arrow.
4.3.2 Pathophysiological consequences of laryngoscopy and tracheal intubation

Laryngoscopy and tracheal intubation are associated with increases in arterial pressure and heart rate which usually peak at 1-2 minutes and last for 5-10 minutes after intubation (Kovac 1996, Ng 1997), although tachycardia may persist for longer (Singh et al. 1995). This pressor response is now widely appreciated by anaesthetists but tracheal intubation had been performed for several decades during anaesthesia, usually with the patient breathing spontaneously (Magill 1930), before the potential physiological consequences were realised. It is likely that the depth of ether or cyclopropane anaesthesia required to tolerate tracheal intubation using this technique would also have attenuated any cardiovascular responses (Bedford 1988). However, with the advent of neuromuscular blocking drugs, laryngoscopy and tracheal intubation was possible at lighter level of general anaesthesia and the first reports of associated cardiovascular disturbances appeared (Burstein et al. 1950, Noble & Derrick 1950, King et al. 1951). The availability of direct intra-arterial pressure measurement allowed the discovery that heart rate and arterial pressure typically increased soon after the start of laryngoscopy (i.e. before tracheal intubation) and that the changes were less apparent at deeper levels of anaesthesia (King et al. 1951). These findings were confirmed by several groups (Ng 1997), and the characteristic pattern is of an increase in heart rate of 15-30 beats min\(^{-1}\) and an increase in arterial pressure of 25-50 mm Hg from baseline values before induction of anaesthesia (Forbes & Dally 1970, Stoelting 1979, Brossy et al. 1994, Korpinen et al.
1995). In published studies of this response, some investigators have made comparisons with baseline values before anaesthesia but have not reported cardiovascular values immediately before intubation. Since arterial pressure usually decreases after induction of anaesthesia, the absolute changes at laryngoscopy and tracheal intubation may be greater than reported (Dahlgren & Messeter 1981, Martin et al. 1982).


### 4.3.3 Myocardial ischaemia associated with laryngoscopy and tracheal intubation

The primary determinants of myocardial oxygen consumption are heart rate, contractility, degree of ventricular filling (preload), and resistance to ventricular ejection (afterload) (Warltier et al. 2000). Tachycardia causes both increased oxygen demand and can reduce oxygen supply by decreasing the time available for coronary perfusion during diastole. Hypertension and systemic vasoconstriction, secondary to increased sympathetic nervous system activity and increased plasma catecholamine concentrations, cause increases in afterload, intraventricular wall tension and therefore increase myocardial oxygen demand (Guyton & Hall 2000). Other factors, including the existence of coronary collaterals, blood haematocrit and rheology, and autonomic nervous influences determine the balance between individual myocardial oxygen supply and demand. In healthy individuals increased myocardial oxygen demand causes the release of vasodilator metabolites, including adenosine, which mediate compensatory coronary vasodilatation. Myocardial oxygen supply is thereby increased and this local autoregulation in response to oxygen demand is the major determinant of myocardial blood flow (Guyton & Hall 2000) within the physiological range of coronary perfusion pressures (approximately 50-120 mm Hg) (Lake 2001). However, if myocardial oxygen demand is already increased (because of left ventricular hypertrophy causing increased ventricular wall tension), or supply is impaired (by coronary artery disease), myocardial ischaemia occurs.
Myocardial ischaemia may also be caused by increased coronary vascular resistance (decreasing myocardial oxygen supply) as well as by increased oxygen demand. Silent myocardial ischaemia occurs commonly in patients with coronary artery disease during daily life (Deanfield et al. 1983, Knight et al. 1989) as well as during the perioperative period (Knight et al. 1988). Many episodes of perioperative myocardial ischaemia in patients with coronary artery disease are unrelated to haemodynamic disturbances (Slogoff & Keats 1985, Kleinman et al. 1986, Thomson 1989, Knight et al. 1988). Moffitt and colleagues found that in patients with coronary artery disease, laryngoscopy and tracheal intubation increased myocardial oxygen consumption by up to 45%, associated with a decrease in coronary sinus oxygen content. Systemic vascular resistance and coronary sinus blood flow also increased, and myocardial lactate concentrations remained constant, with no evidence of myocardial ischaemia (Moffitt et al. 1985). Giles and colleagues found that mean left ventricular ejection fraction decreased by 16% at intubation in both patients with coronary artery disease, with smaller changes in healthy patients. The changes persisted for at least 10 minutes after intubation in over 25% of these patients. These authors suggested that either increased afterload (causing increased myocardial work in those unable to increase coronary blood flow) or increased coronary vascular resistance were responsible for myocardial ischaemia and consequent dysfunction (Giles et al. 1982). Kleinman and colleagues found that myocardial hypoperfusion and ischaemia occurred after tracheal intubation even when heart rate, arterial and pulmonary capillary wedge pressures were unchanged (Kleinman et al. 1986), implying that myocardial ischaemia was related to decreased oxygen supply, consistent with coronary vasoconstriction rather than increased demand. Myocardial ischaemia is associated with increased plasma norepinephrine concentrations (Backlund et al. 1999) and systemic vasoconstriction, both of which can occur at intubation. Lowenstein and Reiz found that coronary blood flow decreased very rapidly in patients who developed electrocardiographic or metabolic evidence of myocardial ischaemia at laryngoscopy and tracheal intubation. In contrast, coronary blood flow was unchanged in patients who did not develop ischaemia. There were no differences in heart rate in the two sets of patients and the authors suggested that in patients with coronary artery disease ischaemia at laryngoscopy and tracheal intubation was caused by vasospasm rather than increased myocardial oxygen consumption (Lowenstein & Reiz 1987). This suggestion was supported by Hagglmark and colleagues, who found that myocardial ischaemia at induction of anaesthesia and during surgery was common, and often undetected by the
ECG (Haggmark et al. 1989). However, despite the fact that some episodes of myocardial ischaemia may be unrelated to haemodynamic disturbances in patients with coronary artery disease, several groups have demonstrated that the tachycardia and hypertension associated with laryngoscopy and tracheal intubation does cause myocardial ischaemia in susceptible individuals (Roy et al. 1979, Slogoff & Keats 1985, Edwards et al. 1994). Other cardiorespiratory effects of laryngoscopy and tracheal intubation include hypoxaemia, hypercapnia, increased central venous pressure and bronchospasm, which may exacerbate myocardial ischaemia (Ng 1997).

The pathophysiological consequences of laryngoscopy and tracheal intubation include hypertension, tachycardia, increased plasma catecholamine concentrations, increased intracranial and intra-ocular pressures. Laryngoscopy and tracheal intubation can cause myocardial ischaemia in susceptible individuals, and may be associated with significant morbidity. Similar cardiovascular changes also occur at emergence from anaesthesia and tracheal extubation, although the magnitude of these changes may be lower (Miller et al. 1995, Edwards et al. 1994). It is considered desirable for most patients and mandatory for those at risk to attenuate excessive cardiovascular responses during anaesthesia and surgery (Kovac 1996). This is discussed in detail below.

4.3.4 Morbidity associated with laryngoscopy and tracheal intubation

Early reports suggested that laryngoscopy and tracheal intubation is associated with a risk of cardiac arrhythmias (Burstein et al. 1950, Noble & Derrick 1950, King et al. 1951). Sudden death and acute coronary insufficiency with pulmonary oedema have also been reported (Fox et al. 1977). Several groups have shown that laryngoscopy and tracheal intubation can be associated with myocardial ischaemia (Roy et al. 1979, Edwards et al. 1994), particularly if tachycardia occurs (Slogoff & Keats 1985, Stone et al. 1988a, 1988b). Myocardial ischaemia may also occur after laryngoscopy and tracheal intubation when haemodynamic values are normal (Kleinman et al. 1986, Slogoff & Keats 1986). In this case it is likely to be caused by decreases in myocardial oxygen supply, caused by coronary or systemic vasoconstriction (Kleinman et al. 1986) or redistribution of coronary blood flow in patients with coronary artery disease (Khambatta et al. 1988). In addition to myocardial ischaemia, acute left ventricular dysfunction and intracerebral
haemorrhage have been associated with laryngoscopy and tracheal intubation (Fox et al. 1977).

It is now well established that perioperative tachycardia can lead to myocardial ischaemia (Coriat et al. 1982, London et al. 1988, Mangano 1990). Furthermore, perioperative myocardial ischaemia is associated with myocardial necrosis and infarction (Slogoff & Keats 1985, Mangano et al. 1990, Fleisher et al. 1995, Rapp et al. 1999), and increased long-term cardiac morbidity (Mangano et al. 1992b). Despite the fact that some episodes of myocardial ischaemia may be unrelated to haemodynamic disturbances in patients with coronary artery disease, attempts to minimise undue episodes of ischaemia should be made. Perioperative myocardial ischaemia may be prevented by changes in anaesthetic management. In particular, perioperative administration of beta blocking drugs has been shown to decrease the incidence of myocardial ischaemia (Stone et al. 1988b, Wallca et al. 1998) and improve long-term outcome after major surgery (Mangano et al. 1996, Poldermans et al. 1999). Other strategies effective in decreasing ischaemia include attenuating sympathetic and adrenal stress responses to surgery by the use of alpha adrenergic agonists (Martin et al. 1997) or intensive analgesia with opioids (Mangano et al. 1992a). It has also been suggested that volatile anaesthetic agents and opioid agonists may have specific cardioprotective effects against ischaemia (Warltier et al. 2000).

4.3.5 Groups at risk

Whilst these responses may not be significant for healthy patients, several groups of patients are at increased risk of morbidity and mortality associated with cardiovascular responses to induction of anaesthesia, laryngoscopy and tracheal intubation (Kovac 1996). These include patients with exaggerated cardiovascular responses, for example those with arterial hypertension (Prys-Roberts et al. 1971a, 1971b, Forbes & Dally 1970, Bedford & Feinstein 1980, Low et al. 1986, Stone et al. 1988b), pre-eclampsia (Lavies et al. 1989, Allen et al. 1991) or the elderly (Bullington et al. 1989). The other category of patients are those in whom a ‘normal’ response puts them at increased risk of morbidity because of co-existing cardiovascular disease (for example patients with a recent myocardial infarction, diminished cardiovascular reserve or coronary insufficiency), or
those with raised intracranial pressure or cerebrovascular pathology (Kovac 1996, Ng 1997).

4.4 Mechanisms underlying the cardiovascular response to laryngoscopy and tracheal intubation

There is detailed knowledge of the nervous supply of the upper airway, including the afferent and efferent limbs of the relevant sensory and autonomic pathways, and the anatomical and physiological aspects of the cardiovascular control centres in the medulla. However, the precise physiological mechanisms involved in the cardiovascular response to laryngoscopy and tracheal intubation are less well defined.

Early reports suggested that bradycardia associated with laryngoscopy and tracheal intubation is caused by an increase in vagal tone (Reid & Brace 1940). This is supported by the widespread observation that bradycardia frequently accompanies laryngoscopy in infants and children and is occasionally seen in adults. The common explanation for this is that epiglottis in infants and children is relatively larger and is positioned more anteriorly, so laryngoscopy in children is often performed by inserting the laryngoscope behind the epiglottis rather than in the vallecula as in adults. The sensory innervation of the posterior surface of the epiglottis is from the internal laryngeal branch of the superior laryngeal nerve, and the larynx and airways distal to the vocal cords is supplied by the recurrent laryngeal nerve. Both these are branches of the vagus nerve. The motor supply to the larynx is from the vagus nerve via its recurrent laryngeal and exterior laryngeal branches. The reflex responses of the larynx and pharynx to stimulation (which include glottic closure or laryngospasm, gagging, swallowing, or vomiting) are thus transmitted by vagal afferents and efferents. Noxious stimuli arising from elsewhere within the distribution of the vagus nerve (e.g. within the gastrointestinal or genitourinary system) result in reflex bradycardia by a direct parasympathetic action at the sino-atrial and atrioventricular nodes (Guyton & Hall 2000). Stimulation of the posterior surface of the epiglottis by the laryngoscope also causes reflex bradycardia via a similar mechanism (Hatch 1994).
However, the usual response to laryngoscopy and tracheal intubation in adults is of tachycardia and increased arterial pressure. King and colleagues noted that laryngoscopy alone caused an increase in heart rate and arterial pressure, which was augmented when intubation occurred, and suggested that either decreased vagal activity or increased sympathetic nervous activity was responsible (King et al. 1951). Studies in animals have indicated that the increases in heart rate and arterial pressure occurring at tracheal intubation and extubation are accompanied by increased sympathetic efferent activity (Seagard et al. 1984). This has been confirmed in human volunteers. Ebert and colleagues found that muscle postganglionic sympathetic nervous activity (recorded directly from the peroneal nerve) and plasma norepinephrine concentrations decreased after induction of anaesthesia with thiopental. Laryngoscopy and tracheal intubation produced a significant increase in sympathetic nervous activity (Ebert et al. 1990). Akbar and coworkers showed that peroneal nerve sympathetic nerve activity increased by 600% at intubation (Akbar et al. 1996). In both these studies, laryngoscopy and tracheal intubation was accompanied by tachycardia and hypertension suggesting a generalised increase in sympathetic nervous activity. Studies using the skin vasomotor response as an index of sympathetic nervous activity have also shown that laryngoscopy and tracheal intubation is associated with a marked increase in efferent sympathetic activity (Shimoda 1997, 1998).

Furthermore, laryngoscopy and tracheal intubation in humans is accompanied by increased plasma norepinephrine concentrations, which parallel the cardiovascular response (Russell et al. 1981, Derbyshire et al. 1983, Shribman et al. 1987, Achola et al. 1988, Scheinin et al. 1989). It is known that plasma catecholamine concentrations reflect sympathetic nervous system activity during stress (Derbyshire & Smith 1984) and when high doses of opioids or deep anaesthesia are used to attenuate the pressor response to laryngoscopy and endotracheal intubation, the increase in catecholamine concentrations is also suppressed (Stanley et al. 1980, Hoar et al. 1981, Crawford et al. 1987, Scheinin et al. 1989, Miller et al. 1993, Chraemmer-Jorgensen et al. 1992).

Involvement of the sympathetic nervous system in the response to laryngoscopy and tracheal intubation is explained by the anatomy of the upper airway. During direct laryngoscopy, the laryngoscope blade comes into contact with the tongue, hypopharynx, epiglottis and vallecula. It will therefore stimulate the low threshold mechanoreceptors
and free nerve endings present therein. Afferent impulses stimulated by light touch or pressure are transmitted primarily in branches of the vagus nerves, but also in the trigeminal nerve (from the tongue) and glossopharyngeal nerves (from the pharynx). These impulses may be attenuated by topical anaesthesia of the larynx (Wyke & Kirchner 1976). When the laryngoscope is used to lift the epiglottis forward in order to view the laryngeal inlet, mechanoreceptors and free nerve endings present in cartilaginous joints, tendinous attachments and within the laryngeal muscles are stimulated. These impulses are not affected by topical anaesthesia. Afferent impulses from the larynx and trachea are transmitted via the superior laryngeal nerve to the inferior ganglion of the vagus and thence to the nucleus tractus solitarius (NTS) and the nucleus ambiguus (Haxhiu et al. 1993). The NTS and nucleus ambiguus are intimately involved with sympathetic nervous regulation and cardiovascular control mechanisms (see Chapter 2). Efferent impulses from the nucleus ambiguus and NTS are transmitted in the laryngeal nerves, which also contain sympathetic fibres from the superior and middle cervical ganglia. The superior and middle cervical ganglia also supply the sympathetic fibres to the heart (Lake 2001). Epipharyngeal stimulation in cats causes increased cervical sympathetic activity (Tomori & Widdecombe 1969). Gentle stimulation of supraglottic mechanoreceptors in animals also produces reflex glottic closure. This is abolished by topical anaesthesia, whereas more intense stimulation is unaffected and causes additional autonomic responses consistent with pain (Wyke & Kirchner 1976). In addition, tracheal intubation stimulates mechanoreceptors in the vocal cords and tracheal wall. Afferents are transmitted via the recurrent laryngeal nerves, also to the nucleus tractus solitarius, and the efferent pathways are identical to those stimulated by laryngeal and pharyngeal stimulation.

These considerations are supported by clinical data. Local anaesthesia is only partially effective in attenuating the pressor response to laryngoscopy and tracheal intubation (Kautto & Heinonen 1982). The magnitude of the response depends on the laryngoscopic technique, in particular the duration and forces applied during laryngoscopy (Bucx et al. 1992a, 1992b, Hassan et al. 1991, Stoelting 1977). Hassan and colleagues found that the cardiovascular and catecholamine responses to laryngoscopy depended both on the forces applied and the duration of laryngoscopy. Subsequent tracheal intubation was associated with a greater increase in heart rate, arterial pressure and plasma catecholamine concentrations than laryngoscopy alone. Sympathetic nervous activity, as assessed by the skin vasomotor responses, was greater with laryngoscopy and tracheal intubation.
compared to laryngoscopy alone (Shimoda et al. 1997). Conversely, Shribman found that the increase in arterial pressure and plasma catecholamine concentrations after laryngoscopy was similar whether or not tracheal intubation was performed (Shribman et al. 1987).

It is therefore likely that the cardiovascular response to laryngoscopy and tracheal intubation is mediated by both sympathetic and parasympathetic nervous systems. Afferent impulses travel predominantly in the vagus nerve to the medulla where they synapse with vagal and sympathetic nuclei. Reflex vagal efferent activity may occasionally be manifest as reflex bradycardia but this is usually overridden by the sympathetic efferent response of tachycardia, hypertension and other signs of increased sympathetic nervous system activity.

4.5 Emergence from anaesthesia and tracheal extubation

Tracheal extubation is usually performed during a light stage of anaesthesia and is also associated with a significant pressor response (Prys-Roberts 1971a, Fuhrman et al. 1992), which may persist into the postoperative period (Dyson et al. 1990). Plasma catecholamines are increased (Lowrie et al. 1992) and myocardial ischaemia may occur (Edwards et al. 1994). The magnitude of the response may be less in comparison with the response to laryngoscopy and tracheal intubation: increases in heart rate of 12-20 min⁻¹ and arterial pressure of 15-40 mm Hg are typical (Bidwai et al. 1979, Lowrie et al. 1992, O'Dwyer et al. 1993). However, different considerations apply, particularly after certain types of surgery, for example vascular or reconstructive surgery, when cardiovascular instability is particular undesirable as rapid increases in heart rate and arterial pressure may compromise the integrity of vascular anastomoses. After carotid endarterectomy or neurosurgical procedures, cerebral pressure autoregulation may be impaired and a significant pressor response can cause potentially disastrous increases in cerebral blood flow and intracranial pressure. In addition, methods to attenuate cardiovascular responses to laryngoscopy and tracheal intubation which rely on sedation or deepening of anaesthesia are inappropriate because they may simply delay rather than attenuate an inevitable consequence of emergence from anaesthesia, or encourage extubation before the restoration of protective airway reflexes.
The cardiovascular response to emergence from anaesthesia and tracheal extubation has been much less studied than the response to laryngoscopy and tracheal intubation. Possible reasons for this include the view that tracheal extubation is less of a clinically significant problem than laryngoscopy and tracheal intubation, difficulties in standardising the depth of anaesthesia at extubation during clinical studies, and the lack of suitable pharmacological agents to attenuate these responses without producing adverse (principally sedative) effects. Previous methods used to attenuate the pressor response to emergence from anaesthesia and tracheal extubation have included beta blockers (Dyson et al. 1990, O’Dwyer et al. 1990), alfentanil (Fuhrman et al. 1992), fentanyl (Nishina et al. 1995a), local anaesthetics (Bidwai et al. 1979) and calcium channel blockers (Nishina et al. 1995b, Mikawa et al. 1996a, 1996b). Others have used combinations of these (Mikawa 1997, Nishina 1997) or have avoided the issue by performing tracheal extubation under deep anaesthesia (Miller et al. 1995). However, these methods are not universally effective, and cause hypotension, delayed recovery from anaesthesia, and postoperative respiratory depression. The ideal agent or technique to attenuate the cardiovascular responses at emergence from anaesthesia and tracheal extubation would be effective, without compromising postoperative airway maintenance, respiratory function or conscious level (Miller et al. 1995).

4.6 Mechanisms underlying the cardiovascular responses to emergence from anaesthesia and tracheal extubation

The mechanisms underlying the cardiovascular responses to emergence from anaesthesia and tracheal extubation are likely to be similar to those that mediate the responses to laryngoscopy and tracheal intubation, (detailed in Chapter 4.4), although their aetiology has not been examined specifically. However, the anatomical origin and basis for the responses are identical, with the exception that laryngoscopy is often not performed at tracheal extubation. Conversely, the oropharynx is aspirated of saliva and secretions before extubation, although the forces applied are less than during laryngoscopy and the magnitude of the noxious stimulus during oropharyngeal aspiration is therefore likely to be less. Cuff deflation and physical removal of the tracheal tube from the trachea stimulates touch, pressure and mechanoreceptors in the tracheal wall, vocal cords and larynx. As discussed in Chapter 4.4, afferent impulses are transmitted from these
receptors via the recurrent and superior laryngeal branches of the vagus nerve to the inferior ganglion of the vagus and thence to the nucleus tractus solitarius and the nucleus ambiguus in the brain stem. As the tube is withdrawn further, afferent impulses from the tongue and pharynx are transmitted via the trigeminal and glossopharyngeal nerves. Efferent impulses from these medullary nuclei are transmitted in the laryngeal nerves, which also transmit sympathetic impulses from the superior and middle cervical ganglia to the heart (Lake 2001). The cardiovascular response to emergence from anaesthesia and tracheal extubation is therefore also mediated by the sympathetic and parasympathetic nervous systems. Although the evidence for this involvement of the sympathetic nervous system is based largely on anatomical deductions, airway stimulation causes increased cervical sympathetic nerve activity in cats (Tomori & Widdecombe 1969). Furthermore, tracheal extubation in humans causes a significant increase in heart rate arterial pressure and plasma catecholamine concentrations (Bidwai et al. 1979, Lowrie et al. 1992, O'Dwyer et al. 1993).

In the absence of laryngoscopy, the afferent stimulus may be less than that during laryngoscopy and tracheal intubation, and in comparative terms the cardiovascular response is usually less. This is reflected in the number of studies performed in this area. The residual effects of surgery and of anaesthetic drugs may also confound available data. Nevertheless, tracheal extubation may be associated with significant morbidity (Edwards et al. 1994), and in contrast to laryngoscopy and tracheal intubation, appropriate techniques must have a rapid offset, to avoid residual central nervous, cardiovascular or respiratory effects. The ideal agent or technique to attenuate the cardiovascular responses at emergence from anaesthesia and tracheal extubation would be effective, without compromising postoperative airway sensation, reflexes or airway maintenance, respiratory function or conscious level. None of the previously published methods had satisfied all these criteria. The pharmacological characteristics of remifentanil suggested that it would be ideal for this purpose, which was the rationale behind Study 2.
4.7 Summary

In healthy mammals, the larynx and pharynx perform a number of complicated physiological reflexes which occur phasically during respiration, and co-ordinate functions such as speech, swallowing and airway protection. The receptor systems serving these nervous activities are extremely sensitive. Mechanoreceptors respond to pressure exerted by very small amounts of water or mucus and chemoreceptors respond to minute quantities of inhaled noxious irritants. The afferent limbs of these reflexes are carried predominantly in the vagus nerve and its branches to the brain stem where efferent reflexes are coordinated. The brain stem nuclei are in close association with nuclei involved with cardiovascular regulation and autonomic function, and the reflex responses to noxious stimuli in the upper airway include increased autonomic activity and stimulation of cardiovascular function.

In a biological context, laryngoscopy and tracheal intubation are extremely potent stimuli, causing reflex cardiovascular changes, which are manifest even during general anaesthesia. These changes differ amongst different groups of patients but may be harmful in some, and methods to attenuate them are commonly employed. However, the pressor response to laryngoscopy and tracheal intubation is reproducible between individuals and therefore can be used as a model for interventions aimed at the attenuation of noxious stimuli. Remifentanil was a newly introduced opioid drug with unique pharmacokinetic properties and a pharmacodynamic profile that made it theoretically suitable for this purpose. These properties are discussed in Chapter 5.
CHAPTER 5. THE PHARMACOLOGY OF REMIFENTANIL

5.1 Mechanism of action of opioids
   5.1.1 Opioid receptor pharmacology

5.2 Endogenous opioid ligands
   5.2.1 Endorphins, enkephalins and dynorphins
   5.2.2 Endomorphin
   5.2.3 Nociceptin

5.3 Summary

5.4 Pharmacokinetic considerations
   5.4.1 Factors affecting onset of effect of intravenous drugs
   5.4.2 Factors affecting offset of effect of intravenous drugs

5.5 Pharmacology of remifentanil
   5.5.1 Physicochemical characteristics of remifentanil
   5.5.2 Metabolism of remifentanil
      5.5.2.1 Effect of abnormal cholinesterase activity
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5.7 Summary and aims of this thesis
THE PHARMACOLOGY OF REMIFENTANIL

5.1 Mechanism Of Action Of Opioids

Like most other opioid drugs used in clinical practice, remifentanil is a mu opioid receptor agonist. The mechanism of action of opioid drugs is by binding to opioid receptors, which are served physiologically by a number of endogenous opioid peptides. An understanding of the pharmacology of opioid drugs requires an appreciation of the pharmacology of opioid receptors and their endogenous ligands. This chapter describes the endogenous peptides, their physiological mechanism of action, and the pharmacology of remifentanil.

5.1.1 Opioid receptor pharmacology

Endogenous and exogenous opioids produce their effects by interaction with a number of cell surface opioid receptors. Different classification systems have been used to characterise opioid receptors, but they are traditionally divided into mu, delta and kappa receptors based primarily on the actions of exogenous ligands (Pasternak 1993). The recent discovery of an opioid-like receptor and its endogenous ligand nociceptin, both of which are widely distributed in the central nervous system, has led to its inclusion in the most recent classification (Table 5.1) (Alexander & Peters 2000). In line with this classification, the conventional nomenclature (mu, delta, kappa and NOP receptors) will be used in this thesis.
### Table 5.1 Classification of opioid and opioid-like receptors

<table>
<thead>
<tr>
<th></th>
<th>Delta opioid peptide</th>
<th>Kappa opioid peptide</th>
<th>Mu opioid peptide</th>
<th>N/OFQ receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred abbreviation</td>
<td>DOP</td>
<td>KOP</td>
<td>MOP</td>
<td>NOP</td>
</tr>
<tr>
<td>Other names</td>
<td>δ, OP₁</td>
<td>κ, OP₂</td>
<td>µ, OP₃</td>
<td>ORL₁</td>
</tr>
<tr>
<td>Endogenous ligands</td>
<td>enkephalins,</td>
<td>dynorphins A&amp;B,</td>
<td>β-endorphin,</td>
<td>nociceptin</td>
</tr>
<tr>
<td></td>
<td>β-endorphin,</td>
<td>α-neoendorphin</td>
<td>enkephalins,</td>
<td>endomorphins₁ &amp; ₂</td>
</tr>
<tr>
<td>Potency of endogenous ligands</td>
<td>β-end = leu = dynA &gt;&gt; β-end &gt; met = leu &gt; dynA leu &gt; met</td>
<td>β-end &gt; dynA &gt; met nociceptin &gt;&gt; nociceptin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective agonists</td>
<td>DPDPE</td>
<td>enadoline</td>
<td>DAMGO,</td>
<td>NC(1-13)NH₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>endomorphins₁ &amp; ₂</td>
<td>morphine</td>
</tr>
<tr>
<td>Selective antagonists</td>
<td>Naltrindole</td>
<td>Nor-binaltorphine</td>
<td>CTOP</td>
<td>J113397</td>
</tr>
<tr>
<td>Coupling</td>
<td>Gᵢₒ</td>
<td>Gᵢₒ</td>
<td>Gᵢₒ</td>
<td>Gᵢₒ</td>
</tr>
</tbody>
</table>

Adapted from Alexander & Peters (2000).

**KEY:** β-end=β-endorphin, leu=leu-enkephalin, met=met-enkephalin, dynA=dynorphin A, DPDPE=cyclic[DPen²DPen⁵]enkephalin, DAMGO=D-Ala-NMePhe-Glyol, NC=nociceptin, CTOP=DPhe-Cys-Thr-DTrp-Orn-Thr-Pen-Thr-NH₂, J113397=1[(3R4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one

The human N/OFQ receptor exhibits a high degree of structural homology with the conventional opioid receptors but displays a distinct pharmacology responding to very few of the agents active at the classical opioid receptors and is therefore considered ‘opioid-related’ rather than opioid. The existence of mu, delta and kappa receptor subtypes has been proposed. The physiological actions of endogenous opioid ligands are discussed in section 5.2 and the effects of opioid receptor stimulation summarised in Table 5.2.
It is generally agreed that mu (MOP) receptors mediate the major effects of opioids used in clinical practice e.g. analgesia and respiratory depression. Kappa (KOP) receptors mediate analgesia, sedation and dysphoria. The actions of delta (DOP) and NOP receptors are outlined below. In addition, there may be subtypes of each receptor, with two mu subtypes, two delta subtypes, and three kappa subtypes (Pasternak 1993). It has also been suggested that additional opioid receptors exist, including morphine-6-glucuronide receptors and orphan receptors (Bowdle 1998). However, the evidence for these receptor subtypes and for additional opioid receptors has been questioned as only one subtype of mu, kappa, and delta receptors have been cloned from any one species and their existence is not generally supported by molecular biological studies (Harrison 2000). The actions of different opioid receptors are summarised in Table 5.2. In addition, mu, kappa, and delta receptors are widely distributed within the parts of the central nervous system involved in cardiovascular control mechanisms, the autonomic nervous system, and the heart. Although their physiological role is uncertain, they are ideally situated to be involved in cardiovascular regulation, and opioid peptides are known to have a number of effects on the cardiovascular and autonomic nervous systems (Siren & Feuerstein 1992, Paakkari & Feuerstein 1995, Barron 2000). The role of opioid peptides in cardiovascular homeostasis is discussed in Chapter 2.

The anatomical distribution of opioid receptors and subtypes varies between species. All opioid receptors are typical 7 domain transmembrane receptors coupled to a G protein and activation of the opioid receptor usually inhibits neuronal activity (Dhawan et al. 1996). Cell surface receptors have no direct contact with their effector molecules and G proteins relay information from the cell surface receptor to the final effector molecules via interaction with intracellular second messenger systems. Activation of the receptor causes G\textsubscript{\text{io}} protein activation, which results in exchange of GTP for GDP and the regulation of second messengers. There are several types of G\textsubscript{\text{io}} protein, and opioid receptor activation can activate more than one type of G\textsubscript{\text{io}} protein (Law 1995). G\textsubscript{\text{io}} proteins regulate the activity of adenylyl cyclase (which is responsible for the generation of cyclic AMP from ATP), phospholipase C, inositol triphosphate, protein kinases and ion channels (Huang 1995).
Table 5.2 Known and suggested opioid receptor actions

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>mu (MOP)</th>
<th>delta (DOP)</th>
<th>kappa (KOP)</th>
<th>NOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesia</td>
<td>peripheral, spinal &amp; supraspinal analgesia</td>
<td>peripheral, spinal &amp; ?supraspinal analgesia</td>
<td>spinal analgesia</td>
<td>supraspinal hyperalgesia, spinal analgesia</td>
</tr>
<tr>
<td>Endocrine</td>
<td>prolactin release, ↓ vasopressin release</td>
<td>growth hormone release</td>
<td>vasopressin release</td>
<td>various</td>
</tr>
<tr>
<td>Other</td>
<td>cardiovascular depression, respiratory depression, ↓ GI secretions &amp; motility, sedation, catalepsy, urinary retention, miosis, euphoria, dependence, muscle rigidity</td>
<td>?cardiovascular depression, ?respiratory depression, miosis, nausea and vomiting, urinary retention</td>
<td>?cardiovascular depression, dysphoria, sedation, diuresis, miosis</td>
<td>cardiovascular depression, anxiolysis, antinatriuresis, diuresis, hyperphagia</td>
</tr>
</tbody>
</table>
These second messengers are responsible for the cellular effects of opioid receptor activation (Standifer & Pasternak 1997) which vary according to the receptor subtype and its anatomical location. Opioid receptors, via G_{i/o} proteins, couple negatively to adenylyl cyclase to cause an inhibition of cAMP formation (Childers 1991) but different opioid receptors may produce different intracellular effects. For example, activation of presynaptic opioid receptors produces a decrease in cyclic AMP formation, closure of voltage operated Ca^{2+} channels and activation of outward K^+ currents. This causes hyperpolarisation of the cell membrane and decreases synaptic neurotransmitter release (Figure 5.1). Mu opioid receptors are thought to affect potassium currents predominantly, whereas kappa opioid receptors affect voltage operated calcium channels. Delta opioid receptors have actions on both K^+ and Ca^{2+} conductance (McFadzean 1988). The differences in actions may be related to differential effects on the subunits of G_{i/o} proteins (Standifer & Pasternak 1997, Huang 1995), although this is not certain. Opioid receptors are also located post-synaptically and may 'dampen down' neurotransmission by having similar effects on postsynaptic neurons.

A new type of opioid-like receptor has recently been cloned. It is sensitive to an endogenous peptide ligand, termed orphanin FQ or nociceptin (Bunzow et al. 1994, Reinscheid et al. 1995), and is now termed the NOP receptor (Table 5.2). This receptor is also a 7 transmembrane receptor with 60% homology with mu, delta or kappa receptors and is distributed throughout the brain and the spinal cord (Calo et al. 2000). NOP receptors have the greatest structural similarities with kappa receptors, and nociceptin is similar to Dynorphin A in structure, although it has very little affinity for mu, delta or kappa opioid receptors (Butour et al. 1997). However, like traditional opioid receptors, NOP receptors are also coupled to G_{i/o} proteins and have similar intracellular transduction mechanisms, causing inhibition of adenylyl cyclase, reduction of cAMP formation, activation of ATP sensitive and calcium-sensitive K^+ channels and inhibition of voltage gated L-type Ca^{2+} channels, causing an increase in outward K^+ current and an inhibition of Ca^{2+} current to decrease neuronal transmission (Meunier 1997, Hawes et al. 2000, Calo et al. 2000). NOP receptors have negligible affinity for naloxone or opioid drugs, but nociceptin is thought to serve functional roles related to those of the endogenous opioids. NOP receptors are now considered to be a non-opioid branch of the family of opioid receptors (Alexander & Peters 2000).
Pre-synaptic opioid receptors are thought to decrease neurotransmission via a reduction in cAMP levels, closure of voltage-operated calcium channels and activation of inwardly rectifying potassium currents. These coordinated changes lead to a reduction in the release of excitatory neurotransmitters and analgesia. Post-synaptic opioid receptors may have similar effects.
5.2 Endogenous opioid ligands

5.2.1 Endorphins, enkephalins and dynorphins

Following the discovery of opioid receptors in brain tissues, the first endogenous opioid peptides (leu- and met-enkephalin) were characterised in 1975 (Hughes et al. 1975) and a number of opioid peptides have since been defined (Calo et al. 2000). These are mostly derived from three prohormones; pro-enkephalin, pro-dynorphin and pro-opiomelanocortin (POMC), which give rise to the peptides enkephalins, dynorphins and endorphins respectively. Each precursor is coded by a separate gene, and the opioid peptides derived from them differ in their anatomical distribution, receptor affinity, and physiological effects. The precursor for the recently discovered endomorphins is not known. However, enkephalins, dynorphins and endorphins all share the same amino acid sequence (Tyr-Gly-Gly-Phe) found in leu- and met-enkephalin (Figure 5.2).

Figure 5.2

Amino acid sequences of representative endogenous opioid peptides with common sequences highlighted

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met/Leu-enkephalin</td>
<td>Tyr-Gly-Gly-Phe-Met/Leu</td>
</tr>
<tr>
<td>β endorphin</td>
<td>Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Lys-Gly-Gly</td>
</tr>
<tr>
<td>Endomorphin 1/2</td>
<td>Tyr-Pro-Trp-Phe/Tyr-Pro-Phe-Phe</td>
</tr>
</tbody>
</table>
With the exception of nociceptin and endomorphins, endogenous opioid peptides are relatively unselective in their action at individual opioid receptors and their subtypes and all endogenous ligands have some activity at mu, delta and kappa opioid receptors. However, dynorphins bind preferentially to kappa opioid receptors, enkephalins have higher affinity for delta opioid receptors, whilst endorphins bind with approximately equal affinity to mu and delta sites, (although is slightly preferential for mu opioid receptors). Endomorphins are highly selective for mu opioid receptors (Table 5.3).

### Table 5.3

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Precursor</th>
<th>Receptor selectivity</th>
<th>Other active opioid peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynorphin</td>
<td>Pro-dynorphin</td>
<td>kappa</td>
<td>Dynorphin B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dynorphin A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>α and β neo-endorphin</td>
</tr>
<tr>
<td>Enkephalin</td>
<td>Pro-enkephalin</td>
<td>delta</td>
<td></td>
</tr>
<tr>
<td>β-endorphin</td>
<td>Pro-opioimelanocortin</td>
<td>mu and delta</td>
<td></td>
</tr>
<tr>
<td>Endomorphin</td>
<td>unknown</td>
<td>mu</td>
<td></td>
</tr>
<tr>
<td>Nociceptin</td>
<td>Pre-pro-nociceptin</td>
<td>NOP</td>
<td></td>
</tr>
</tbody>
</table>

Beta-endorphin is synthesised in the pituitary, but it is also found in other parts of the brain and the gastrointestinal tract. It is secreted from the pituitary in response to pain, injury or stress. The enkephalins are distributed more widely in the CNS (including several sites in the brain and substantia gelatinosa of the spinal cord), throughout the gastrointestinal tract, and in autonomic ganglia and the adrenal medulla. The enkephalins often co-exist with other hormones, for example, with epinephrine in the adrenal medulla, with norepinephrine in sympathetic nerve terminals, and with acetyl choline in preganglionic sympathetic nerves. They may be co-secreted with these other hormones and modify their action.
5.2.2 *Endomorphin*

Endomorphin<sub>1</sub> and endomorphin<sub>2</sub> are endogenous peptides which are potent, selective μ opioid receptor agonists. Endomorphin<sub>1</sub> has a 4000 and 15000 fold preference for the μ opioid receptor over delta and kappa receptors respectively, and endomorphin<sub>2</sub> has a 13000 fold and 15000 selectivity over delta and kappa receptors respectively (Zadina *et al.* 1997). They were originally isolated from bovine brain, and have subsequently been found to occur in high concentrations in many areas of the human brain which have high concentrations of μ opioid receptors. These include medulla, thalamus, striatum, frontal cortex as well as in the periaqueductal grey matter and the spinal cord (Hackler *et al.* 1997, Martin-Schild *et al.* 1997, Zadina *et al.*1999) and it has been suggested that the endomorphins could be endogenous ligands at the μ opioid receptor. Their mechanism of action is similar to other μ opioid receptor agonists i.e. by activation of G<sub>ia</sub> proteins and by inhibition of inward calcium currents. However, to date no precursor for either of these two peptides has been isolated. Endomorphin<sub>2</sub> is found primarily in afferent nociceptive fibres in the dorsal horn of the spinal cord, and in the lower brain stem, whereas endomorphin<sub>1</sub> is distributed more widely and densely in the brain. Endomorphins produce potent and prolonged analgesia, and they may have diverse functions including the modulation of responses to pain and stress (Zadina *et al.* 1999).

In common with other μ receptor agonists, endomorphins produce analgesia and dose-dependent, naloxone-reversible decreases in heart rate and arterial pressure in animal models (Champion *et al.* 1998a, Czapla *et al.* 2000). The decrease in arterial pressure is ten-fold less than equivalent doses of nociceptin (Champion *et al.* 1998b), and appears to be caused by direct vascular smooth muscle relaxation (Hugghins *et al.* 2000). The mechanism is endothelium-dependent, and is attenuated by the nitric oxide synthase inhibitor N-omega-nitro-L-arginine methyl ester, suggesting that it is mediated by nitric oxide (Hugghins *et al.* 2000, Champion & Kadowitz 1998), similar to the effects of acetylcholine and the selective mu opioid agonist DAMGO (Champion *et al.*1998c). In contrast, nociceptin produces vasodilatation by a different mechanism which is independent of nitric oxide and is unaffected by naloxone (Champion *et al.* 1998b, Champion & Kadowitz 1999, Madeddu *et al.* 1999). These effects of both endomorphins and nociceptin are independent of prostaglandins and ATP-sensitive potassium channels (Champion *et al.* 1999).
5.2.3 Nociceptin

The physiological role of nociceptin and the NOP receptor system is not yet fully elucidated but they have effects on mood, gut motility, feeding behaviour, immune function and the modulation of CNS neurotransmitter release (Meunier 1997, Calo et al. 2000, Meunier 2000). The cardiovascular effects of nociceptin are detailed in section 2.6.4. It is thought that most of the effects of nociceptin are produced by inhibition of presynaptic neurotransmitter release or by decreasing postsynaptic neuronal excitability, and they are mostly unaffected by the opioid receptor antagonist naloxone (Meunier 1997). Nociceptin has a role in pain mechanisms and in some studies has been shown to decrease pain thresholds i.e. have an antinociceptive effect. Several other studies have shown nociceptin to counteract the analgesic effects of endogenous opioids, exogenous opioids or selective opioid receptor agonists (Calo et al. 2000). The mechanism by which this occurs is unclear but it may be because of differential localisation of NOP and classical opioid receptors within the CNS.
5.3 Summary

Most opioid drugs used in anaesthesia are selective for the mu receptor, which is widely distributed in the central nervous system, the spinal cord and the periphery. All opioid receptors are responsible for analgesia at the spinal or supraspinal level, but mu receptors are predominantly responsible for the cardiovascular effects. The cardiovascular actions of opioid peptides are discussed further in Chapter 2.

Different opioids used in clinical practice produce different clinical effects (because of differences in receptor affinity and intrinsic efficacy, and in auxiliary effects) but the effects of remifentanil on cardiovascular function would be expected to be similar to those of other fentanyl-derived mu opioid agonists. However, remifentanil has pharmacokinetic properties which are unique among opioid drugs and which determine its clinical activity and applications. These properties and the available data regarding the cardiovascular effects of remifentanil are reviewed in the following sections.
5.4 Pharmacokinetic considerations

In order to understand the pharmacology of remifentanil, some understanding of the factors affecting onset and offset of effect of intravenous anaesthetic and opioid drugs is required.

5.4.1 Factors affecting onset of effect of intravenous drugs

For a drug whose effect is in the CNS, such as opioids or intravenous anaesthetic drugs, the onset of clinical effect depends on the time taken to achieve adequate CNS concentrations. The factors that determine the attainment of effective concentrations within the brain include the characteristics of the drug itself, the dose administered, and factors depending on the individual including blood flow to the brain and the integrity or otherwise of the blood-brain barrier (Calvey & Williams 1997). Important drug characteristics are drug pKa (and therefore the degree of ionisation at body pH), its lipid solubility and the volume of distribution of the central compartment. A drug that has a small central volume of distribution will achieve high central concentrations relatively quickly and therefore have a rapid onset. Highly lipid soluble drugs penetrate the blood-brain barrier more easily and have a rapid onset of effect. A drug which is largely unionised at body pH can also penetrate lipid membranes more rapidly and so have a faster onset of effect compared with one which is more ionised. The time to onset can be defined according to the constant describing drug equilibration between the central plasma compartment and its (hypothetical) effect site (Sheiner et al. 1979). If a drug is administered as an infusion or a rapid bolus, whilst its plasma concentration and some measure of clinical effect are measured simultaneously (e.g. effect on minute ventilation or EEG), the rapid increase and decrease in plasma concentrations is followed by the clinical effect. Although the relationship between the two is not parallel (as there is hysteresis), a constant can be calculated which represents the rate of equilibration between plasma and effect site (Glass 1998). This constant is the $k_{eo}$ (Hull et al. 1978, Sheiner et al. 1979). The $t_{1/2}$ $k_{eo}$ $(0.693/ k_{eo})$ is the time for half this equilibration to occur. Complete equilibration will occur in 4 to 5 multiples of $t_{1/2}$ $k_{eo}$. The time to peak effect after a bolus is also a function of $t_{1/2}$ $k_{eo}$ (as well as the drug's disposition) and drugs with a short $t_{1/2}$ $k_{eo}$ have a rapid onset (Glass 1998).
5.4.2 Factors affecting offset of effect of intravenous drugs

The offset of effect of general anaesthetic drugs and the rapidly-acting intravenous opioids (e.g. fentanyl, alfentanil, remifentanil and sufentanil) after a bolus is initially caused by redistribution from the central compartment and their site of action (Calvey & Williams 1997). Their clinical effect has disappeared long before they have been metabolised or eliminated from the body. After repeated bolus administration or a prolonged intravenous infusion, these other factors (metabolism and elimination) become more important, but are not the sole determinants of offset of effect. Previous assumptions regarding the offset of effect have tended to assume that offset of effect is simply related to the elimination half-life of the drug, which is dependent on the terminal clearance. However, these assumptions ignore the effects of redistribution of drug between its effect site compartment and between different peripheral compartments. Clinical examples of this include all the intravenous anaesthetic agents, and most opioids including fentanyl and alfentanil. All of these drugs have a prolonged effect when given by intravenous infusion, which far exceeds their elimination half-life (Shafer & Varvel 1991). This is because drugs distribute throughout different pharmacokinetic compartments within the body, as determined by their lipid solubility and other factors such as cardiac output and tissue blood flow. This is a dynamic process and begins to occur as soon as the intravenous bolus or infusion is administered. The plasma and effect site drug concentrations at any given moment depend on the administered dose and the distribution of the drug throughout the body, as well as the processes of drug metabolism and elimination. Distribution between compartments depends not only on the relevant intercompartmental clearances but also on the time over which this has been allowed to occur. For example alfentanil has a short elimination half-life, a small steady state volume of distribution and small central and intercompartmental clearances. After an infusion of alfentanil, the small volume of distribution will tend to produce a more rapid decrease in plasma and effect site concentrations (and therefore more rapid recovery), but this will be offset by the lower clearance. The precise contributions of these opposing pharmacological characteristics, in particular the contribution of redistribution to the rate of decline and clinical offset, depend on the duration of the infusion. However, these factors are difficult to predict and their effects impossible to forecast based simply on the elimination half-life.
In order to quantify the relationship between plasma concentration and predicted time for recovery after infusions of varying duration, Shafer and Varvel developed a computer-simulated model incorporating the known pharmacokinetic parameters and pharmacodynamic effects of different opioids (Shafer & Varvel 1991). They demonstrated that plasma and effect site concentrations (and consequently clinical recovery after an infusion) could not be predicted from elimination half-life alone. Subsequently, Hughes and colleagues used computer models to predict the decrease in plasma concentrations after an infusion of intravenous anaesthetic drugs (Hughes et al. 1992). They showed that for infusions of intravenous opioids (alfentanil, fentanyl or sufentanil) or hypnotic anaesthetic drugs (thiopental, propofol or midazolam), the decrease in plasma and effect site concentrations was unrelated to elimination half-life, confirming its limitations as a predictor of offset of clinical effect. Plasma concentrations were related to the rate of equilibration between compartments, the size of these compartments, and the duration of the infusion. They also defined a new concept, the context-sensitive half-time (CSHT). This is the time for a 50% decrease in plasma concentrations after termination of an infusion designed to maintain steady state plasma concentrations, as a function of the duration of the infusion. The CSHT varied significantly for the different drugs modeled, but in all cases increased with increasing duration of drug infusion. Because of the polyexponential pharmacokinetics of intravenous anaesthetic drugs, the elimination half-life consistently exceeds the context-sensitive half-time (Schnider & Shafer 1995). The use of a 50% decrease in plasma concentrations was recognised as somewhat arbitrary, but CSHT was defined in this way to reflect the widely understood notion of half-life. Subsequently, Youngs and Shafer described which pharmacokinetic features (in terms of central and peripheral volumes of distribution and clearances) would be desirable to produce a rapid decrease in plasma opioid concentrations after infusions of varying duration (Youngs & Shafer 1994). They also defined the decrease in plasma concentrations in terms of ‘decrement times’, whereby 50% decrement times equates to CSHT. However, a decrement time greater or less than 50% may be more important in terms of offset of clinical effect (Shafer & Stanski 1992). It is important to recognize that the time to clinical recovery also depends on the potency and absolute concentrations of drug (Schraag et al. 1998a) and that the time for concentrations to decrease to a given level also depends on the dose administered. Furthermore, the action of most relevant drugs is within the CNS rather than the plasma, and these terms are only strictly applicable if plasma drug concentrations
have reached steady state. However, these concepts were important and have helped illustrate the advantages of remifentanil over previously available opioids. The concept of CSHT has subsequently been validated and the predicted CSHTs for remifentanil and alfentanil shown to be very close to measured values (Glass et al. 1993a, Kapila et al. 1995).
5.5 Pharmacology of remifentanil

5.5.1 Physicochemical characteristics of remifentanil

Remifentanil is the hydrochloride salt of 3-[4-methoxycarbonyl-4-[(1-oxopropyl)-phenylamino]-l-piperidine] propanoic acid methyl ester. The molecular formula of remifentanil hydrochloride is \( \text{C}_{20}\text{H}_{28}\text{N}_{2}\text{O}_{5} \cdot \text{HCl} \) and it has a molecular weight of 412.9 D. (Egan 1995). It has no chiral centre and exists as a single form. Remifentanil is a 4-anilidopiperidine derivative with a similar molecular structure to other piperidine-derived opioids, for example fentanyl and alfentanil (Figure 5.3). It was specifically designed as a mu opioid receptor agonist which would undergo ester hydrolysis \textit{in vivo} to produce a metabolite with much lower affinity for the mu receptor, and thereby lose its pharmacological activity (Feldman \textit{et al.} 1991). Opioid binding studies in guinea pig ileum and mouse vas deferens confirmed the high affinity of remifentanil for mu opioid receptors (EC\(_{50}\) for inhibition of electrically evoked contractions = 2.4 nM), with a similar potency to fentanyl (EC\(_{50}\) = 1.8 nM), and approximately eight times more potent than alfentanil (EC\(_{50}\) = 20.1 nM). Opioid receptor binding by remifentanil is competitively inhibited by naloxone, but is unaffected by nor-binaltorphimine (a selective kappa antagonist) or by ICI 174864 (a selective delta opioid antagonist) (James \textit{et al.} 1991). In ligand binding studies, the high affinity of remifentanil for mu opioid receptors was confirmed (EC\(_{50}\) = 2.6 nM), compared with delta receptors (EC\(_{50}\) = 66 nM), and kappa receptors (EC\(_{50}\) = 6.1 mcM) (Glaxo Wellcome 1996). This suggests a mu:delta selectivity ratio of approximately 25:1 and a mu:kappa selectivity ratio of approximately 300:1. The reversal of the effects of remifentanil by naloxone has been confirmed \textit{in vivo} in animals (James \textit{et al.} 1992) and humans (Amin \textit{et al.} 1995). Remifentanil does not bind significantly to any non-opioid receptors (James \textit{et al.} 1991).
Figure 5.3 Molecular structures of remifentanil, alfentanil and fentanyl

 Alfentanil

 Fentanyl

 Remifentanil

*The ester group of remifentanil susceptible to hydrolysis by esterases is highlighted*
Remifentanil is supplied as a white lyophilised powder of remifentanil hydrochloride that must be reconstituted and diluted in water or 5% dextrose before administration, in a formulation that also contains glycine as a buffer. Once reconstituted, it has a pH of ± 3.0 and a pKa of 7.07. It undergoes spontaneous degradation, but at a pH < 4 is stable in solution for 24 hours. It is lipid-soluble, with an octanol/water partition coefficient at pH 7.4 of 17.9. This is similar to alfentanil, which has a partition coefficient of 13.14. Remifentanil is approximately 70% bound to plasma proteins, predominantly \( \alpha_1 \) acid glycoprotein (Glaxo Wellcome 1996).

### 5.5.2 Metabolism of remifentanil

The propanoic methyl ester group on its piperidine nitrogen makes remifentanil vulnerable to hydrolysis in vivo by non-specific blood and tissue esterases. These enzymes are widely distributed in the tissues, but most remifentanil is metabolised in the circulation. However, the plasma half-life of remifentanil is much shorter when measured in vivo than in vitro, indicating that it is metabolised in other tissues as well as in blood (Glaxo Wellcome 1996). Approximately 12-15% of the total metabolism of remifentanil (administered as an infusion for 40 min) in dogs occurred within muscle and intestines, muscle predominating as a result of its relatively high blood flow. Less than 3% of the metabolism of remifentanil occurs in the liver and kidneys (Chism & Rickert 1996). Remifentanil is not metabolised significantly or sequestered in the lungs (Duthie et al. 1997).

Metabolism by de-esterification produces a carboxylic acid metabolite, remifentanil acid, also described as GR-90921 (molecular weight 362.5), which is excreted via the kidneys. This is the predominant pathway and accounts for over 90% of total remifentanil metabolism. The other metabolic pathway for the metabolism of remifentanil is by n-dealkylation to GR-94219 (Figure 5.5), which accounts for less than 2%. GR-90921 is detectable in plasma and urine after remifentanil infusion (Glass et al. 1993a, Westmoreland et al. 1993). GR-90921 also binds to \( \mu \), delta and kappa receptors, but with much lower affinity, and the potency of GR-90921 is approximately 4600 times less than remifentanil as determined by the EEG effects in dogs (Hoke et al. 1997a). The elimination half-life of GR-90921 is prolonged (88-137 minutes) compared with remifentanil (10-21 minutes). GR-90921 accumulates in patients with renal failure, but
because of its low potency it has no pharmacological effects, even after a simulated remifentanil infusion of 12 hours (Hoke et al. 1997b).

Figure 5.4 Metabolism of remifentanil

The predominant metabolic pathway is by de-esterification by non-specific plasma and tissue esterases to produce a carboxylic acid metabolite, remifentanil acid (GR-90921) which has $1/300$ to $1/1000^{th}$ of the potency of remifentanil, and is excreted by the kidneys. A minor pathway is by $N$-dealkylation producing GR-94219.
5.5.2.1 Effect of abnormal cholinesterase activity

In vitro studies showed that the hydrolysis of remifentanil is associated with red cell esterase enzymes and is not mediated by pseudocholinesterase, acetylcholinesterase or carbonic anhydrase (Selinger et al. 1995). Stiller and colleagues reported that the in vitro hydrolysis of remifentanil was similar in blood from patients with known pseudocholinesterase deficiency and that from normal individuals (Stiller et al. 1995). It is theoretically possible that the metabolism of remifentanil could be affected by other drugs metabolised by similar esterase enzymes, for example the beta-blocker esmolol (which is metabolised by red cell esterases). However, Haidar and colleagues showed that the metabolism of both remifentanil and esmolol were unaffected by the co-administration of both drugs in rats (Haidar et al. 1997a, 1997b). The metabolism of remifentanil is also unaffected by neostigmine or other inhibitors of plasma cholinesterase (Glaxo Wellcome 1996) or by succinyl choline (Schuster et al. 1992). A recent case report has confirmed that the metabolism of remifentanil was unaffected in a patient undergoing routine ophthalmic surgery who had unsuspected plasma pseudocholinesterase deficiency, and who received propofol, suxamethonium and remifentanil 0.1-0.2 mcg kg\(^{-1}\) min\(^{-1}\) (Manullang & Egan 1999). At the end of surgery, the patient recovered consciousness rapidly but showed clinical features of persisting neuromuscular blockade, which was confirmed using a nerve stimulator. Sedation was therefore recommenced with midazolam and remifentanil 0.2 mcg kg\(^{-1}\) min\(^{-1}\) for a further 4 h, at which the remifentanil was discontinued and she recovered consciousness. By this time, the effects of the neuromuscular blockade had subsided and she was extubated uneventfully. At both times, the recovery profile after discontinuation of the remifentanil infusion was rapid and typical. Postoperative testing showed a dibucaine number of 16 (normal >75) and plasma pseudocholinesterase concentrations of 1088 u l\(^{-1}\) (normal range 12,800 – 52,000), indicating a homozygous state for pseudocholinesterase deficiency.
5.5.3 Toxicological considerations

In animal models, using up to 2500 times the maximum recommended bolus dose in humans, toxicological studies found effects consistent with that expected from a typical mu opioid receptor agonist e.g. respiratory depression. The only consistent finding associated with the administration of remifentanil in doses of > 0.3 mg kg$^{-1}$ has been reversible micro-haemorrhages in the brains of dogs. These micro-haemorrhages were not found when ventilatory support was available and are considered to be caused by hypoxaemia consequent on respiratory depression (Glaxo Wellcome 1996). There is no evidence of genetic toxicity with remifentanil at doses up to 2000 times greater than clinically relevant plasma concentrations. No studies investigating possible carcinogenicity have been performed because the chemical structure has no features associated with possible carcinogenicity and the duration of administration and action is short. In common with other mu agonists, long term exposure to remifentanil over 4-10 weeks caused a reduction in male fertility in rats, thought to be mediated by an indirect hormonal mechanism. However, it has no effects on female fertility in animals (Glaxo Wellcome 1996). In human studies, remifentanil had no effects on in-vitro fertilisation when used for general anaesthesia during oocyte retrieval (Hammadeh et al. 1999).

Remifentanil is formulated in glycine as a chemical buffer. Glycine is also an inhibitory neurotransmitter in the CNS. In dogs, daily intrathecal administration of the buffer (containing glycine but no remifentanil) caused pain, agitation and limb dysfunction. These signs were masked when a remifentanil-containing formulation was administered, perhaps because of the analgesic actions of remifentanil. However, these data confirm the potential neurotoxicity of the current formulation of remifentanil and its administration by the spinal or epidural routes is contra-indicated.
5.5.4 Pharmacokinetic Characteristics Of Remifentanil

The rapid metabolism of remifentanil is reflected in its calculated pharmacokinetic parameters. These have been described using two or three compartment models (Glass et al. 1993a, Egan et al. 1993, Westmoreland et al. 1993). Both models comprise a small well-perfused central compartment and include one or two peripheral compartments through which distribution occurs more slowly. Both describe accurately the kinetics of remifentanil after brief infusions (up to 20 minutes) although the two compartment model underestimates remifentanil concentrations after this time and the three compartment model is more accurate (Egan et al. 1993). Early studies confirmed it to have a rapid onset, small volume of distribution, rapid redistribution and clearance with a terminal elimination half-life of 10-21 min. Time to peak effect after a bolus is approximately 1.2 min and its $t_{1/2} k_{e0}$ is 1.0-1.6 minutes (Egan et al. 1996a, Glass et al. 1993, Westmoreland et al.1993), similar to alfentanil (Glass 1998). Distribution half life is 0.8-0.9 min (Glass et al. 1993a, Egan et al. 1993). Central compartment volume of distribution ($V_{DC}$) is 100-150 ml kg$^{-1}$. Volume of distribution at steady state ($V_{Dss}$) is approximately 0.3-0.4 ml kg$^{-1}$, and is also independent of dose over a wide range, (up to 30 mcg kg$^{-1}$) (Egan et al. 1993, Westmoreland et al. 1993, Glass et al. 1993). Clearance, calculated from data obtained after a 20 min infusion or bolus administration in healthy adult male volunteers, is approximately 40 ml kg$^{-1}$ min$^{-1}$ (Egan et al. 1993, Glass et al. 1993). Similar values were obtained after a 1 min infusion in patients undergoing elective surgery (Westmoreland et al. 1993). Clearance is also independent of age, weight and dose. These values for clearance exceed hepatic blood flow and confirm the widespread extra-hepatic metabolism of remifentanil. Clearance is reduced slightly during hypothermic cardiopulmonary bypass, but returns to normal values on rewarming, and the decrease has been attributed to the effect of temperature on blood and tissue esterase activity (Russell et al. 1997). The comparative pharmacokinetic parameters of remifentanil, fentanyl and alfentanil are summarised in Table 5.4.
Table 5.4  Pharmacokinetic parameters of remifentanil, alfentanil, and fentanyl

<table>
<thead>
<tr>
<th></th>
<th>Alfentanil</th>
<th>Fentanyl</th>
<th>Remifentanil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKa</td>
<td>6.5</td>
<td>8.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Ionisation at body pH (%)</td>
<td>11</td>
<td>91</td>
<td>10-21</td>
</tr>
<tr>
<td>VDC (l kg(^{-1}))</td>
<td>0.1-0.4</td>
<td>0.5-1.0</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>VDSS (l kg(^{-1}))</td>
<td>0.25-0.75</td>
<td>3-5</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Clearance (ml min(^{-1}) kg(^{-1}))</td>
<td>3-8</td>
<td>10-20</td>
<td>40-60</td>
</tr>
<tr>
<td>Elimination half-life (min)</td>
<td>60-120</td>
<td>180-300</td>
<td>10-20</td>
</tr>
<tr>
<td>t(<em>{1/2}) (k</em>{eo}) (min)</td>
<td>0.9-1.2</td>
<td>4-5</td>
<td>1.0-1.2</td>
</tr>
<tr>
<td>Time to peak effect (min)</td>
<td>1.0-1.3</td>
<td>3.8</td>
<td>1.0-1.3</td>
</tr>
<tr>
<td>CSHT after 60 min infusion (min)</td>
<td>34</td>
<td>38</td>
<td>3.7</td>
</tr>
<tr>
<td>CSHT after 120 min infusion (min)</td>
<td>46</td>
<td>75</td>
<td>3.7</td>
</tr>
<tr>
<td>CSHT after 180 min infusion (min)</td>
<td>58</td>
<td>262</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Adapted from Glass et al. (1999)

\(VDC\) = volume of distribution of the central compartment

\(VDSS\) = volume of distribution at steady state

\(t_{1/2} \ k_{eo}\) = equilibration constant half-time between plasma and effector site

\(CSHT\) = modelled context-sensitive half-time after infusions of varying duration

Terminal elimination half-life \((t_{1/2p})\) is 10-21 min, although there is a slower elimination phase when very large doses are administered. Mean residence time is 5-10 min (Egan et al. 1993, Westmoreland et al. 1993) and its measured context-sensitive half-time after a 3 h infusion was 3.65 min (Kapila et al. 1995). Computer simulations also predict that the time for its concentration to decrease by 80% is less than 15 min for any length of infusion (Glass et al. 1993a). Therefore the context sensitive decrement time for remifentanil is independent of the duration of the infusion. This feature is unique among opioids and other anaesthetic drugs, and it has allowed remifentanil to be used in a different manner to
other opioids during anaesthesia. In order to attenuate autonomic and potentially adverse cardiovascular responses during anaesthesia with fentanyl or sufentanil, relatively high doses are required. This meant that clinical recovery at the end of anaesthesia was delayed. The same is true for alfentanil if infusions are used (Shafer & Varvel 1991, Hughes et al. 1992, Glass 1998). Conversely, owing to its unique pharmacokinetics, it was predicted that relatively high doses of remifentanil could be administered, without the risk of prolonged postoperative recovery. In addition, the rapid onset and offset meant that it could in theory be titrated to effect (Egan et al. 1993, Glass et al. 1993). The exploration of these possible advantages was a major stimulus to the research detailed in Chapters 7-12. However, at this time, most of the proposed advantages were theoretical.

Confirmation of the rapid offset of effect, as predicted by its short context-sensitive half time, came from Kapila and colleagues. They administered equipotent infusions of remifentanil and alfentanil, titrated to depression of minute ventilation as a measure of drug effect. Plasma samples for drug concentration were taken during and after drug infusion. After 3 h, the opioid infusion was stopped, the recovery of minute ventilation and decrease in blood drug concentration was plotted, and the time for a 50\% change was determined. Mean measured context-sensitive half-time for remifentanil was 3.2 min and its pharmacodynamic offset was 5.4 min. The corresponding values for alfentanil were 47.3 and 54.0 min respectively. Full recovery of respiratory drive had occurred within 15 min for remifentanil but was incomplete after 45 min for alfentanil (Kapila et al. 1995). These results confirmed both the value of the context-sensitive half-time in describing drug offset, and also the rapid offset of clinical effect of remifentanil predicted by its pharmacokinetic characteristics. The rapid offset of effect were confirmed in patients undergoing surgery who received intraoperative infusions of remifentanil at rates between 0.0125-1.0 mcg kg\(^{-1}\) min\(^{-1}\), and in whom times to recovery were similar irrespective of remifentanil infusion rate (Dershwitz et al. 1995).
5.5.4.1 Effects of impaired organ function

The metabolism of remifentanil is independent of organ function. The pharmacokinetics of remifentanil during and after a 4 hour infusion were studied in patients with chronic stable severe liver disease awaiting hepatic transplantation. Patients were recruited on the basis of decreased serum albumin concentrations and prolonged prothrombin times; all had evidence of portal hypertension. Pharmacokinetic parameters were similar to those of volunteers acting as matched controls, although the patients with liver disease were more sensitive to the respiratory depressant effects of remifentanil, requiring lower concentrations to produce a 50% suppression of minute ventilation (Dershwitz et al. 1996). The pharmacokinetics of remifentanil were also unaltered in patients undergoing liver transplantation and clearance during the anhepatic phase was similar to that of healthy adults (Navapurkar 1998).

In volunteers with chronic renal failure who received either low dose (0.0125 mcg kg\(^{-1}\) min\(^{-1}\) for 1 h followed by 0.025 mcg kg\(^{-1}\) min\(^{-1}\) for 3 h) or high dose (0.025 mcg kg\(^{-1}\) min\(^{-1}\) for 1 h followed by 0.05 mcg kg\(^{-1}\) min\(^{-1}\) for 3 h) remifentanil, plasma remifentanil concentrations, clearance volume of distribution and elimination half-life were similar to values obtained in age and weight matched controls. No increased pharmacodynamic sensitivity as assessed by depression of minute ventilation was observed. However, the elimination half-life of the remifentanil metabolite GR-90291 was more than 26 h in patients with renal failure compared with 1.5 h in the control subjects. Pharmacokinetic simulations predicted that the steady-state concentration of GR-90291 at the end of a 12-h remifentanil infusion is likely to be more than 25 times higher in persons with renal failure compared with control subjects (Hoke et al. 1997b). However, as GR-90921 is approximately 4,600 times less potent than remifentanil (Hoke et al. 1997a), its accumulation is unlikely to produce significant opioid effects. Approximately 35% of plasma GR-90921 is extracted by haemodialysis (Hoke et al. 1997b).
5.5.4.2. Effect of age and gender

There are very few data describing the pharmacokinetics of remifentanil in infants or children but the available data suggest that they are similar to those in adults when adjusted according to body weight (Davis et al. 1995, 1997). However, a number of clinical studies have been performed in several hundred children undergoing surgery, using similar doses and regimens to adults, with no important pharmacodynamic effects being observed (Rusch et al. 1999, Prys-Roberts et al. 2000, Chiaretti et al. 2000). When used as an adjunct to sedate children aged 2–12 years old during painful procedures, a high incidence of respiratory depression was observed (Litman 1999), similar to that seen with adults (Litman 2000). Conversely, it has been used successfully in children undergoing fibre-optic bronchoscopy (Reyle-Hahn et al. 2000).

In elderly patients, the VDc of remifentanil is reduced, which will result in higher plasma concentrations after a given bolus dose compared with younger patients. This is balanced by an increased $t_{1/2} k_{e0}$, so that the onset of drug effect is slower. The EC$_{50}$ as determined by effects on EEG spectral edge is decreased by approximately 50% in the elderly and clearance is decreased (Minto et al. 1997a). Therefore dose requirements are reduced, similar to other opioids (Bailey et al. 1985). It is recommended that initial bolus doses and starting infusion rates of remifentanil are reduced by approximately 50% in the elderly and subsequent dosing titrated to individual requirements. However, 20%, 50%, and 80% decrement times after prolonged administration are rapid and little affected by age (Minto 1997b).

Although one study suggested that plasma concentrations required to prevent movement during surgery were higher in females (Drover & Lemmens 1998), type of surgery was not standardised and other volunteer studies have found no effect of gender on the pharmacokinetics or pharmacodynamics of remifentanil (Minto et al. 1997a).
5.5.4.3 Effect of body size

A pharmacokinetic study in obese patients undergoing abdominal surgery who received a bolus dose of 7.5-10 mcg kg\(^{-1}\) body weight found that absolute values for clearance and volumes of distribution were similar to those of non-obese age-matched patients. However, when corrected for body weight, clearance, \(V_{Dss}\) and \(V_{De}\) were lower in the obese. Plasma remifentanil concentrations were higher, and hypotension and bradycardia were observed more frequently in the obese patients (Egan et al. 1998). Doses in the obese patient should therefore be based on lean body mass rather than total body weight.

5.5.4.4 Placental transfer

Remifentanil crosses the placenta but has little or no effect on the foetus or neonate. Kan and colleagues evaluated the placental transfer and neonatal effects of remifentanil in 19 parturients undergoing elective caesarean section. All received epidural anaesthesia and an intravenous infusion of remifentanil 0.1 mcg kg\(^{-1}\) min\(^{-1}\), and women and babies were observed for 24 h after surgery. Umbilical venous remifentanil concentrations were 56% of maternal concentrations and umbilical arterial concentrations were 123% of maternal placental vein concentrations. Sedation and respiratory depression were noted in the mothers but no effects on the neonates were found. They concluded that remifentanil crosses the placenta but appears to be rapidly metabolised, redistributed or both (Kan et al. 1998). It has also been shown that remifentanil has no effects on in-vitro fertilisation when used for general anaesthesia during oocyte retrieval (Hammadeh et al. 1999).
5.6 Pharmacodynamic characteristics of remifentanil

In pharmacodynamic terms, remifentanil is a typical mu opioid receptor agonist, and its effects are similar to those of other opioids such as morphine or fentanyl (Table 5.2)

5.6.1 Potency

The potency of an opioid is often assessed by comparing its potency after a single bolus administration with that of morphine. However, because of pharmacokinetic differences between drugs, potency depends on the mode of administration (e.g. bolus or intravenous infusion). Opioid potency would ideally be compared by analgesic effects but these are difficult to assess objectively. Other available measures include the ability to induce loss of consciousness or prevent movement on skin incision (when combined with nitrous oxide), reduction of the minimum alveolar anaesthetic concentration (MAC) of a volatile anaesthetic, ability to suppress the spectral edge of the EEG, or cause a particular degree of respiratory depression. All these techniques have been used to assess the potency of remifentanil (Glass et al. 1999).

Early studies in animals showed that remifentanil inhibited electrically-evoked contractions in the guinea pig ileum, rat and mouse vas deferens (James et al. 1991) by an action at mu opioid receptors. Its EC\(_{50}\) in a guinea pig ileum model was 2.4 nM, similar to fentanyl (EC\(_{50}\) = 1.8 nM) and it was approximately eight times more potent than alfentanil (EC\(_{50}\) = 20.1). The plasma concentration required to decrease the MAC of enflurane by 50% in dogs was 7.4 ng ml\(^{-1}\), similar to fentanyl (5.5 ng ml\(^{-1}\)) (Salmenpera et al. 1992). Using the rat tail-withdrawal technique, the potency of remifentanil was similar to fentanyl and alfentanil, although the duration of action was noted to be much shorter (Feldman et al. 1991).

Remifentanil is a potent analgesic, similar to other mu opioid receptor agonists. In human volunteers, when the analgesic effects of a single bolus dose of each drug were compared using a spring-loaded rod at the tibia and sternum, remifentanil was 22 times more potent than alfentanil. In the same study, using the maximal increase in arterial carbon dioxide
tensions as a measure of respiratory depression, remifentanil was 23 times more potent than alfentanil (Glass et al. 1993a). During a 3 h infusion in volunteers, when opioid infusion rates were titrated to produce a 40-70% depression of minute ventilation when breathing 7.5% carbon dioxide, the relative potencies of remifentanil and alfentanil were calculated at 10-20:1 (Glass et al. 1992, Kapila et al. 1995). In a similar study, using a target-controlled infusion device to produce equivalent respiratory depression, remifentanil was found to be 32 times more potent than alfentanil based on whole blood concentrations (Glass et al. 1993b). EC₅₀ values for depression of ventilation were calculated as 1.17 ng ml⁻¹ for remifentanil and 49.4 ng ml⁻¹ for alfentanil, suggesting a potency ratio of 40:1 (Glass et al. 1999b).

The ED₅₀ to induce loss of consciousness at induction of anaesthesia was calculated as 9.5 mcg kg⁻¹ for remifentanil and 154 mcg kg⁻¹ for alfentanil (Joshi et al. 1993). Subsequent analysis of data from this study revised these values for ED₅₀ of remifentanil and alfentanil to 12 mcg kg⁻¹ and 176 mcg kg⁻¹ respectively, and calculated the median effective whole blood concentration (EC₅₀) to be 53.8 ng ml⁻¹ and 1012 ng ml⁻¹ for remifentanil and alfentanil respectively (Jhaveri et al. 1997). These data suggest relative potencies of 15-20: 1. However, in this study, intravenous doses of 20 mcg kg⁻¹ remifentanil did not cause loss of consciousness in all patients, confirming that remifentanil is an analgesic but does not produce anaesthesia. In this respect it is similar to other opioids (Bailey et al. 1985). In a study of the effect of remifentanil and alfentanil infusions on movement at a surgical incision, remifentanil 0.04 mcg kg⁻¹ min⁻¹ and alfentanil 0.75 mcg kg⁻¹ min⁻¹ had similar effects, suggesting a potency ratio of 20:1 (Randel et al. 1994).

Other studies have compared the potency of opioids in terms of their ability to suppress the spectral edge frequency of the EEG. The plasma concentration of opioid necessary to produce a 50% decrease in spectral edge frequency (EC₅₀) can then be derived. Using this approach, Egan and colleagues found remifentanil to be 16-19 times more potent than alfentanil after an infusion lasting 10-14 minutes in healthy volunteers (Egan et al. 1994, 1996a). Although the EC₅₀ values which produce EEG suppression of opioids are much higher than their analgesic EC₅₀ values and the relationship between them is uncertain, EEG EC₅₀ values have correlated with other clinical measures of opioid potency (Egan et al. 1996b).
The ability of opioids to reduce the MAC of the volatile anaesthetic agent isoflurane is also used as a comparative measure of opioid potency. Lang and colleagues found that the whole blood concentration of remifentanil required to reduce the MAC of isoflurane by 50% was 1.37 ng ml$^{-1}$ (Lang et al. 1996). Previous values for plasma concentrations of fentanyl were 1.67 ng ml$^{-1}$ and 28.8 ng ml$^{-1}$ for alfentanil, suggesting that remifentanil is similar in potency to fentanyl and approximately 30-40 times more potent than alfentanil when whole blood concentrations were compared (Glass et al. 1999a).

In summary, the potency of remifentanil relative to that of other opioids depends on the study methodology. Ideally, analgesic effects would be compared, but this is highly variable between individuals and is difficult for remifentanil because of its short duration of action. The surrogate measures discussed above all have limitations, and although it is assumed that relative values of EC$_{50}$ derived by different methods (ie effects on respiration, MAC reduction of isoflurane, or EEG) are equivalent, there is little evidence to support this. All the available data suggest that remifentanil is approximately equipotent to fentanyl. When used in doses with a ratio of approximately 20:1, remifentanil and alfentanil have been shown to have similar effects on consciousness, EEG spectral edge, movement at surgical incision, analgesia and respiratory depression in volunteers. When titrated to a similar pharmacodynamic endpoint (e.g. respiratory depression, or MAC reduction of isoflurane) and whole blood concentrations have been measured, remifentanil is approximately 20-40 times more potent than alfentanil (in terms of whole blood concentrations).

5.6.2 Cardiovascular effects

Early studies suggested that remifentanil would cause similar cardiovascular effects to other fentanyl derivatives. James and colleagues found that remifentanil produced dose-dependent decreases in arterial pressure, heart rate (HR) and cardiac output in dogs (James et al. 1992). In patients undergoing elective surgery, ascending repeated doses of remifentanil up to 3.75 mcg kg$^{-1}$ alone (or up to 20 mcg kg$^{-1}$ if preceded by glycopyrrolate 0.3-0.4 mg), produced dose-dependent decreases in arterial pressure and HR. In those not receiving glycopyrrolate, a marked reduction in systolic arterial pressure (mean 76 mm Hg) was observed after remifentanil 3.75 mcg kg$^{-1}$. In those who received pre-treatment
with glycopyrrolate, the maximum decrease occurred after the first dose (Pitts et al. 1992). Boluses of remifentanil administered over 1 min to volunteers anaesthetised with nitrous oxide 66% and isoflurane (mean concentration 0.5-0.9%), produced decreases in arterial pressure and heart rate of 20-30%. These changes were independent of remifentanil dose between 2 and 30 mcg kg\(^{-1}\) (Sebel et al. 1995) and were reversed by intravenous ephedrine 10 mg. The authors noted that the incidence of significant bradycardia might have been greater, had the patients not received intravenous glycopyrrolate. In contrast, three studies investigating the pharmacokinetic and EEG effects of remifentanil in healthy adult volunteers found no 'untoward haemodynamic events' (defined as severe bradycardia, tachycardia or hypotension) when remifentanil was administered alone as an infusion of up to 8 mcg kg\(^{-1}\) min\(^{-1}\) for 20 min (Egan et al. 1993, 1996a, Minto et al. 1997a). However, all volunteers also received glycopyrrolate 0.2 mg and pancuronium 0.5 mg, which would have attenuated any cardiovascular depressant effects, and the actual cardiovascular data (or the definitions of an 'untoward' event) were not presented. In a study of the pharmacology and analgesic effects of remifentanil and alfentanil in awake volunteers, boluses of remifentanil of 0.25 to 2.0 mcg kg\(^{-1}\) administered over 60 s produced a transient increase in heart rate and arterial pressure (of 5-10%) after 2-3 min (Glass et al. 1993a). These were attributed to anxiety but the volunteers were also receiving painful stimuli, and significant respiratory depression with hypoxaemia and hypercapnia were noted. These factors may have contributed to the increases in HR and arterial pressure. Remifentanil in doses of up to 30 mcg kg\(^{-1}\) does not cause histamine release (Sebel et al. 1995).

The aetiology of the cardiovascular effects of fentanyl-derived opioids is not fully established (see Chapter 3) but the decrease in HR usually observed may result from a centrally-mediated increase in vagal activity (Reitan et al. 1978), a direct effect on the sino-atrial node (Bovill et al. 1984) or from a reduction in central sympathetic tone (Flacke et al. 1983, 1985). Fentanyl derivatives have minimal effects on peripheral vascular tone, compared with the reduction in systemic vascular resistance seen with morphine (Chapter 2). In a recent study, Shinohara and colleagues administered remifentanil in doses of 1-5 mcg kg\(^{-1}\) to both intact and baroreceptor-denervated rabbits (who had undergone bilateral denervation of the carotid sinus, aortic and vagus nerves). In the intact animals, MAP and HR decreased rapidly and briefly (for < 60 s) after remifentanil injection, associated with a reflex increase in renal sympathetic nerve
activity (Shinohara et al. 2000). In the baroreceptor-denervated animals, remifentanil 5 mcg kg\(^{-1}\) caused a transient increase in renal sympathetic nerve activity, but HR and MAP remained initially unchanged before decreasing gradually; these changes were abolished by naloxone. These results suggest that the acute decreases in HR and MAP in the intact animals were mediated by a central vagal action. The gradual decreases in MAP and HR in the denervated animals, without a decrease in sympathetic activity, suggests a direct peripheral action mediated by mu opioid receptors. Further studies are required to confirm these findings.

Lee and colleagues found that remifentanil did not decrease rat cardiac myocyte contractility in vitro, and at higher doses contractility was increased with no associated changes in excitability (Lee et al. 1998). The clinical significance of this possible positive inotropic effect of remifentanil is unclear and cardiovascular depression has been reported in recent clinical studies. Opioids have also been shown to have a negative inotropic effect in isolated cardiac muscle preparations, though this effect is less with fentanyl derivatives than with morphine. Remifentanil, as a slow bolus of 1.0 mcg kg\(^{-1}\) over 5 min or as an infusion of 0.5-2.0 mcg kg\(^{-1}\) min\(^{-1}\), caused bradycardia and hypotension in patients undergoing coronary artery bypass surgery (Elliott et al. 2000, Kazmaier et al. 2000). Both of these studies were performed in patients with good cardiac function (left ventricular ejection fraction >40%, normal cardiac valves and no evidence of left main stem coronary artery stenosis). Remifentanil infusion 2.0 mcg kg\(^{-1}\) min\(^{-1}\) caused a 30% decrease in arterial pressure as a consequence of decreased heart rate (13%) and stroke volume (14%). Plasma catecholamine concentrations were also decreased by 56-63%. Myocardial oxygen consumption, myocardial blood flow and coronary perfusion pressure all decreased, but there were no electrocardiographic or metabolic signs of myocardial ischaemia (Kazmaier et al. 2000). In another study, remifentanil (0.5 mcg kg\(^{-1}\) bolus over 90 s followed by a 0.025 mcg kg\(^{-1}\) min\(^{-1}\) infusion) caused significant bradycardia which was associated with myocardial ischaemia in some patients, and led to cardiac asystole in one patient after laryngoscopy (Wang et al. 1998, 1999). However as the authors noted, all the patients in the latter study were taking beta blocking drugs, and the patient who developed asystole was also receiving diltiazem. Conversely, other early studies failed to show any major effects of remifentanil on heart rate, arterial pressure or cardiac output in healthy patients (Sebel et al. 1995, Glaxo Wellcome 1996, Minto et al. 1997a).
Other early reports suggested that remifentanil would effectively attenuate the autonomic and cardiovascular responses to skin incision or trocar insertion during surgery, but were published only in abstract form or remained unpublished. Further studies were reported to show that the cardiovascular response to tracheal intubation was attenuated by remifentanil in doses of 1.0 mcg kg\(^{-1}\) and that the incidence of patients responding to intubation was lower in patients who had received a bolus of remifentanil 1.0 mcg kg\(^{-1}\) followed by an infusion of 0.4 or 0.5 mcg kg\(^{-1}\) min\(^{-1}\) than in patients who had received alfentanil 20 or 25 mcg kg\(^{-1}\) followed by an infusion of 1.0 or 2.0 mcg kg\(^{-1}\) min\(^{-1}\) (Glaxo Wellcome data on file, 1995). However, these data also remain unpublished. Another study sponsored by the manufacturers reported that remifentanil (1.0 mcg kg\(^{-1}\) bolus followed by a 1.0 mcg kg\(^{-1}\) min\(^{-1}\) infusion) abolished the cardiovascular response to laryngoscopy and tracheal intubation in 94% of patients studied, compared with 79% of patients who received a lower dose of remifentanil (1.0 mcg kg\(^{-1}\) bolus followed by a 0.5 mcg kg\(^{-1}\) min\(^{-1}\) infusion) (Hogue et al. 1996). The mean increases in systolic pressure at intubation were approximately 15 mm Hg and 25 mm Hg in the two groups respectively, and despite intravenous fluid preloading, hypotension occurred in 10-15% of patients. Heart rate and arterial pressure both decreased by 10-40% after induction of anaesthesia, but bradycardia requiring escape medication (defined as a heart rate of < 40 min\(^{-1}\) for > 1 min) and other details of the study were not reported.

The recommended dose of remifentanil at induction of anaesthesia was a bolus (over at least 30 seconds) of 1.0 mcg kg\(^{-1}\) followed by an infusion of 0.5-1.0 mcg kg\(^{-1}\) min\(^{-1}\). The dose recommended to provide analgesia and attenuate autonomic responses to a brief painful procedure was a bolus of 1 mcg kg\(^{-1}\). This was recommended to provide intense analgesia after 1 min, and lasting for 1-3 min (Glaxo Wellcome 1996). However, there were no independent data to corroborate these recommendations.

5.6.3 Respiratory Effects

All opioids cause dose-dependent depression of respiration, via effects on the respiratory centre in the medulla oblongata. The primary effect is a reduction in the sensitivity of the respiratory centre to carbon dioxide, so that respiratory rate decreases initially more than tidal volume, leading to dose-dependent apnoea. Sensitivity to carbon dioxide is
decreased, and both end-tidal and arterial CO₂ are increased (Glass et al. 1993a). The ventilatory response to hypoxia is also depressed (Amin et al. 1995). The effects of remifentanil on respiration are identical to those of other opioids but occur more rapidly and are briefer (Babenco et al. 2000). The degree of respiratory depression is dependent on age, general medical condition and the presence of pain or other stimuli. After a bolus of 0.5 mcg kg⁻¹, the maximum effect on carbon dioxide response occurred at 2 min, with an offset half-time of 6.2 min, and a return to baseline after approximately 10 min (Babenco et al. 2000). This onset time is slightly longer than time to onset of EEG effect after an infusion of 3 mcg kg⁻¹ min⁻¹ (Egan et al. 1996a), which may reflect different CNS responsiveness to different opioid effects, or be related to the different doses used. In another study in volunteers, the maximum depression of arterial PaCO₂ occurred 5 min after a bolus of remifentanil, returning to baseline at 10 min (Glass et al. 1993a). After a 3 h infusion, the time for 50% recovery of ventilation was 5.4 min (Kapila et al. 1995). Respiratory depression may be marked and apnoea may occur when bolus doses are administered, particularly after surgery (Bowdle et al. 1996, Schuttler et al. 1997). The degree of depression of minute ventilation in the presence of additional inspired CO₂ has been used as a marker in studies comparing the potency of remifentanil relative with other opioids (Glass et al. 1992, Kapila et al. 1995, Glass et al. 1999b). In common with other opioids, respiratory depression caused by remifentanil is reversed by naloxone (Amin et al. 1995).

5.6.4 Central nervous system effects

Remifentanil has minimal effects on intracranial pressure and cerebral perfusion pressure, similar to other opioids (Warner et al. 1996, Guy et al. 1997). These characteristics, together with its rapid offset of effect after a prolonged infusion, are desirable for neuroanaesthetic practice.
5.6.5 Other effects

Like other opioids, remifentanil causes dose-dependent muscle rigidity (Egan et al. 1993, Drover & Lemmens 1993, Jhaver et al. 1997). Nausea and vomiting occurred frequently during studies when remifentanil was administered as an infusion to awake volunteers (Egan et al. 1993), women in labour (Olufabi et al. 2000) or patients after surgery, with an incidence of nausea of up to 26% (Schraag et al. 1998b). This is expected from its opioid effects. In patients receiving remifentanil during surgery, the incidence is lower, and comparable to situations when other opioids are used (Cartwright et al. 1997).

5.6.6 Use in parturients

Jones and colleagues suggested that the rapid onset and offset of action of remifentanil would be suitable for intermittent bolus administration to provide analgesia during uterine contractions (Jones et al. 1999). They reported the use of patient-controlled intravenous remifentanil to provide analgesia during labour for three thrombocytopaenic women. The most successful regimen comprised a patient-demand bolus of 0.5 mcg kg\(^{-1}\) with a lockout period of 2-3 min. Analgesia was reported to be excellent, with a range of consumption of 426-1050 mcg h\(^{-1}\). Apart from one episode of maternal sedation and foetal heart rate decelerations resulting from an excessive demand bolus, mothers and neonates tolerated the remifentanil without sequelae. Subsequently, Olufolabi and colleagues formally investigated the use of remifentanil as patient-controlled analgesia in labour, in boluses of up to 0.5 mcg kg\(^{-1}\) with a lockout period of 2 min (Olufabi et al. 2000). They found an initial decrease in pain but the study was abandoned after 4 patients had been enrolled because all experienced increasing pain, unresponsive to increasing remifentanil boluses. Three experienced hypoxaemia, nausea and vomiting, and all complained of facial pruritis. The lack of analgesic effect was attributed to either acute tolerance, or the resistance of labour pain to opioids. It also demonstrates the difficulty of titrating analgesia with remifentanil without the production of opioid-related adverse effects. However, another group recently reported adequate analgesia for uterine contractions with minimal adverse effects in 6 patients by using a remifentanil bolus of 25 mcg kg\(^{-1}\) combined with a background infusion of remifentanil 0.05 mcg kg\(^{-1}\) min\(^{-1}\) (Roelants et al. 2001). Clearly, further studies are required to establish the efficacy and safety of remifentanil in this setting.
5.6.7 Pharmacodynamic Interactions

Remifentanil reduces the MAC of volatile and intravenous anaesthetic agents, similar to other opioids (McEwan et al. 1993). Lang and colleagues showed that at a calculated whole blood remifentanil concentration of approximately 1.37 ng ml\(^{-1}\), the MAC of isoflurane was reduced by 50\% (Lang et al. 1996). However, even at concentrations of 32 ng ml\(^{-1}\) movement on surgical incision still occurred, and the maximal MAC reduction (85\%) was achieved at a remifentanil concentration of 8 - 12 ng ml\(^{-1}\). Similarly, Jhaveri and colleagues administered remifentanil and alfentanil in ascending doses but were unable to produce loss of consciousness reliably up to a remifentanil dose of 20 mcg kg\(^{-1}\). The median effective dose (ED\(_{50}\)) was calculated to be 12 mcg kg\(^{-1}\), and the EC\(_{50}\) 53.8 ng ml\(^{-1}\), but the authors emphasised that for use during anaesthesia, supplementation with a hypnotic drug (e.g. propofol or a volatile anaesthetic agent) is still required (Jhaver et al. 1997).

Remifentanil augments the hypnotic effects of propofol. When administered in conjunction with propofol, with both drugs delivered via a target controlled infusion device and titrated to depression of bispectral index, the effects of remifentanil and propofol were synergistic (Ropcke et al. 2001). Mertens and colleagues also investigated the relationship between remifentanil and propofol by assessing response to skin incision in patients undergoing abdominal surgery. Plasma concentrations of both drugs were measured and a synergistic action of remifentanil and propofol was demonstrated, although low concentrations of propofol (< 2 mcg ml\(^{-1}\)) were not used (Mertens et al. 2001). Conversely, another recent study using EEG as a measure of hypnotic found the interaction between propofol and remifentanil to be additive (Fechner et al. 2001) and further data are required.
5.7 Summary and aims of this thesis

The data reviewed in Chapters 2-4 have shown that opioid peptides are intimately involved in the autonomic nervous system and, physiological cardiovascular control mechanisms. Opioids attenuate the cardiovascular and autonomic responses to noxious stressful stimuli, such as those occurring during surgery, laryngoscopy and tracheal intubation. In terms of its pharmacodynamic effects, remifentanil is similar to other opioids, but it has pharmacokinetic properties, with a rapid onset and offset of effect, which is independent of the duration of infusion. It is the only opioid to have a context-sensitive half-time which does not increase during prolonged infusions, and therefore its effects could be expected to disappear in a predictable manner after stopping an infusion. This pharmacological profile suggested that it would be ideal for the attenuation of noxious stimuli during surgery but with the advantage of being titratable as its effects would not be prolonged, and it was therefore marketed as such. However, when the studies that form part of this thesis were designed, there were no independent data to corroborate these claims.

The cardiovascular responses to laryngoscopy and tracheal intubation are relatively reproducible, and investigation of the effects of remifentanil on these responses could serve as a model for its effects in other clinical situations. This thesis describes the studies performed to investigate the effects of remifentanil on cardiovascular responses during anaesthesia. The aim of Study 1 was to investigate independently the effect of remifentanil on cardiovascular dynamics at induction of anaesthesia, and the other studies ensued.
CHAPTER 6: STUDY METHODOLOGY

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STUDY METHODOLOGY

This chapter contains a description of the study methodology, methods of measurement, power calculations and methods of statistical analysis used in the studies detailed in this thesis. Although the details of methods and doses of drugs administered for each individual study are stated in Chapters 7-12, many of the methods (for example measurement techniques) were common to all the studies and are therefore described together. All aspects of study design, ethical submission, data interpretation, and statistical analysis for each study were performed by myself. In addition, I recruited many of the patients into each study and was responsible for the preparation, submission and revision of each published manuscript arising from the studies described in Chapters 7-12. However, I am grateful for the assistance of my colleagues mentioned on page iii of this thesis who assisted with patient recruitment, data collection, and the presentation of data to national meetings in the U.K.

6.1 Ethical considerations

Each study was given ethical approval by the Leicestershire Research Ethical Committee (see Appendix 1) and approved by the Leicester Royal Infirmary Research and Development Department for indemnification by the Leicester Royal Infirmary NHS Trust. All patients included in the studies were presenting for elective surgery. They were interviewed as far as possible before the time of surgery (usually the preceding day) and the nature of the study explained. Patients were informed that they could withdraw from the study at any time, and written, informed consent was obtained before formal inclusion into any of the studies. Verbal assent was also obtained from any surgeons and anaesthetists responsible for the patients care.
6.2 Patient selection and recruitment

6.2.1 Criteria for patient inclusion

All patients were adults presenting for elective surgery (predominantly gynaecological and general surgery), for which the standard accepted anaesthetic technique involved tracheal intubation. Studies of the cardiovascular responses to laryngoscopy and tracheal intubation in healthy adults (Studies 1, 3 and 4) included predominantly adult female patients. Male patients were not specifically excluded, but healthy females comprised a larger number of the potentially eligible patients. The study of emergence from anaesthesia and tracheal extubation (Study 2) specifically included patients undergoing a single surgical procedure (elective diagnostic gynaecological laparoscopy) and all patients were female. Studies 5 (treated hypertensive) and 6 (elderly normotensive) comprised specific patient groups, which are described in more detail in the relevant chapters (Chapters 7-12). The American Society of Anesthesiologists (ASA) grading system for physical status (American Society of Anesthesiologists, 1963) was used to assess patients' suitability for inclusion into the studies. In Studies 1 to 4, only patients of ASA grade 1 and 2 were included. Study 5 included patients of ASA grades 2 and 3, and Study 6 included patients of ASA grades 1 to 3.

6.2.2 Specific patient exclusion criteria

No patients had significant cardiac or respiratory disease, or were anticipated to present difficulties with airway maintenance, laryngoscopy or tracheal intubation. Other exclusion criteria were: a history of hiatus hernia or gastro-oesophageal reflux (which would have mandated a rapid sequence technique for induction of anaesthesia), obesity (defined as a body mass index > 30 kg m\(^{-2}\) and posing potential problems with airway maintenance and tracheal intubation). No patient in any study was taking opioids, or had known medical contra-indications to any of the drugs used in each study. With the exception of the study investigating remifentanil in hypertensive patients (Study 5) where patients receiving long-term medication for arterial hypertension were specifically recruited, no patients receiving antihypertensive or other vasoactive drugs were included. In the studies in hypertensive (Study 5) and elderly patients (Study 6) where ECG
recordings were analysed for evidence of myocardial ischaemia, those with a cardiac pacemaker or ECG evidence of heart block were also excluded.

6.2.3 Study duration

For all the studies at induction of anaesthesia (Studies 1 and 3-6), the study duration was defined as being from the start of baseline measurements before the start of anaesthesia up to 5 minutes after laryngoscopy and tracheal intubation; measurements and observations terminated at this time point. Surgery was then permitted to proceed and the conduct of subsequent anaesthesia, including the appropriate administration of other opioids or drugs with cardiovascular effects, was at the discretion of the responsible anaesthetist. Study 2 was performed at the end of anaesthesia and surgery, and observations also continued into the recovery room. Therefore the surgical procedure and the whole conduct of anaesthesia were standardised. Further aspects of the study methodology are detailed in Chapters 7-12.

6.3 Randomisation and blinding

In all studies, randomisation was performed using a sealed envelope technique. After allocating groups in equal numbers inside a sealed envelope, the envelopes were randomly shuffled by two investigators before being numbered sequentially. Each study was completed within a 3-4 month period, and randomisation was not stratified. Randomisation envelopes contained detailed instructions of the study drug and its method of dilution (see below). They were opened immediately before each study episode and study drug syringes prepared by an anaesthetist in an adjacent theatre, so that in all cases the investigators were blinded as to the identity of the study drug.
6.4 Measurement of cardiovascular variables

Arterial pressure was measured non-invasively in all studies using a Cardiocap™ II (Datex Division, Instrumentarium Corporation, Helsinki, Finland) and heart rate (HR) recorded from the conventional 3 lead electrocardiograph trace displayed on the Cardiocap™ II. The Cardiocap™ II incorporates a non-invasive automatic oscillometric monitor, and measures systolic, mean and diastolic arterial pressures (SAP, MAP and DAP respectively). Non-invasive oscillometers are microprocessor-controlled devices which measure the fluctuations in pressure in an occluding cuff via a pressure transducer. The cuff is inflated via an air pump, and cuff pressure is held constant whilst the pressure oscillations are sensed; the cuff is then deflated sequentially over the range of detected oscillations. The point of maximum pressure oscillations corresponds to MAP. Systolic and diastolic pressures are measured according to the appearance and disappearance of the sudden pressure oscillations with the exact recording point determined by algorithms. The pressure transducer output is digitalised by the microprocessor, which evaluates the signal over several cardiac cycles and incorporates further algorithms to minimise errors caused by marked motion artefact or respiratory variation. The use of oscillometers has some limitations, but they cause minimal morbidity, are considered the most accurate method of non-invasive arterial pressure measurement (Mark et al. 2000) and they avoid the inaccuracies associated with fluid-filled transducers used for direct arterial pressure measurement (Murphy & Vender 2001). Measurements of arterial pressure obtained using an oscillometer correlate well with direct intra-arterial measurements of MAP and DAP, although SAP is often underestimated by 6-8 mm Hg overall. The underestimation of SAP is greater at higher absolute pressures (Murphy & Vender 2001). Accuracy of the Cardiocap™ II is quoted by the manufacturers as within 3 mm Hg or 2 % (whichever is greater), with a mean (SD) correlation with direct intra-arterial measurements of within 5 (8) mm Hg, between pressures of 25 to 260 mm Hg. Its typical measurement time is 25 s, with measurement intervals of 1–60 min (Datex 1994).

The oscillometer is the standard current method of perioperative arterial pressure monitoring, and although not exclusively, many investigators have demonstrated a close correlation between oscillometric and direct arterial pressure measurement (Mark et al. 2000). Oscillometry is an accepted technique used in previous studies of the

The electrocardiograph calculates heart rate over 10 seconds (s) with a resolution of 1 beat min⁻¹ and an accuracy of ±1 % over the range 30-250 beats min⁻¹ (Datex 1994). Peripheral oxygen saturation in all studies was also monitored using the pulse oximeter incorporated within the Cardiocap™ II, which calculates the functional oxygen saturation of haemoglobin by measuring the ratio of light absorbed at 660 nm and 940 nm transmitted from two light-emitting diodes, and detected by a photodiode. The signal is averaged every 5 s and accuracy is quoted as ±2 % for 68 % of readings, when oxygen saturation is > 80 % (Datex 1994). End-expired carbon dioxide tensions were measured by infrared absorption spectrophotometry using a Capnomac Ultima™ II (Datex Division, Instrumentarium Corporation, Helsinki, Finland). Accuracy of this device is quoted as being within < 0.2 vol % or < 1.5 mm Hg within the range of carbon dioxide tensions of 0-10 % (Datex 1991).

In all studies, the mean values of three consecutive arterial pressure and heart rate measurements taken at one-minute intervals before induction of anaesthesia were calculated and termed the 'baseline' values for each patient. These baseline values were used as a reference point for the requirement for escape medication, for statistical comparison of subsequent changes in heart rate and arterial pressure, and for estimating the power of Studies 1 and 2 (see below). In the study of emergence and tracheal extubation (Study 2), arterial pressure during surgery was maintained within 10% of baseline values.
6.5 Blood sampling and measurement of plasma catecholamines

In study 4, blood samples were taken from each patient for determination of plasma catecholamine concentrations. An 18G intravenous cannula, dedicated to blood sampling, was inserted into a vein on the antecubital fossa in the opposite arm to the cannula used for administration of anaesthetic and study drugs. At each of the sampling times, 10 ml blood was withdrawn from the cannula into a lithium heparin impregnated plastic sampling tube containing heparin 15 i.u. ml⁻¹, (Monovette®, Sarstedt, Germany) and was placed immediately in ice. The cannula was flushed with 0.9% saline to prevent clotting of the cannula between samples, and blood 5 ml withdrawn and discarded before each sample was taken to ensure that the sample contained none of the saline flush. Blood samples were separated by centrifugation at 3000 rpm for 5 min at a temperature of 4 °C in a Heraeus Labofuge B centrifuge (Phillip Harris Scientific, Lichfield, U.K.) within 20 min of being sampled. The supernatant plasma was transferred to plastic containers and frozen at −70 °C, pending analysis for catecholamine concentrations.

Plasma catecholamine concentrations were determined by high performance liquid chromatography (HPLC) using electrochemical detection, by Mr Jim Strupish, Chief Laboratory Technician, in the University Department of Anaesthesia, Critical Care and Pain Management, Leicester Royal Infirmary. All analyses were performed between June and August 2000, using the methodology described below. This methodology was used in previous published studies from our own laboratory (Thompson et al. 1997), and is described below.

6.5.1 Analysis of plasma catecholamine concentrations

Principles of extraction, separation and detection

Plasma catecholamine concentrations were measured by high performance liquid chromatography (HPLC) (Pryde & Gilbert 1979). Under alkaline conditions, catecholamines bind strongly to alumina. Once the catecholamines are immobilised, the bulk of the plasma matrix, which would interfere with the separation and detection
process, can be removed by washing with water. On subsequent acidification the catecholamines are displaced into the liquid phase ready for analysis. The catecholamines in the sample must be separated before they can be quantified. This is achieved by pumping the initial mixture through a HPLC column containing silica spheres which have a hydrophobic coating of hydrocarbon molecules. Each chemical will have characteristic elution time depending on the degree to which it interacts with the stationary hydrocarbon phase and the moving phase (i.e. the buffer). The composition of the buffer has a dramatic effect on the elution time, so by manipulating the ionic strength, pH, and organic components (methanol and in particular sodium octyl sulphonate) of the buffer, separation of catecholamines from each other and interfering species can be effected (Anton & Sayre 1962).

Once separated by HPLC, catecholamine concentrations can be quantified by an electrochemical method, because they can be induced to oxidise on the surface of a graphite electrode if a sufficient electrical potential is applied. The products are a quinone molecule, two free electrons and hydrogen ions. The electrons migrate into the graphite electrode causing a change in current proportional to the mass of the catecholamine oxidised. The current can be measured using a dual electrode analytical cell. In practice the coulochem detector used comprises three such electrodes in series. The first oxidises the catecholamines, the second acts as a screen, operating at low electrical potential to reduce back any interfering molecules and the final electrode, at high negative potential, reduces the catecholamines. It is therefore the current of reduction that actually gives the mass of catecholamine in this procedure. This somewhat complex method gives greater selectivity for catecholamines than purely oxidative methods (Pryde & Gilbert 1979).

**Extraction procedure**

Frozen plasma was rapidly thawed and a 1 ml aliquot placed in a 5 ml polystyrene test tube containing 5 pmol internal standard, 1 ml Tris/EDTA buffer (comprising 12 g Tris HCl (Sigma, Poole, U.K.), 1 g EDTA (Sigma, Poole, U.K.) in 100 ml electrochemical grade water (Fisher Scientific, Loughborough, U.K.) and adjusted to pH of 8.2 with sodium hydroxide (Fisher Scientific, Loughborough, U.K.). Acid-washed
chromatography grade alumina 30 mg (Sigma, Poole, U.K.) was added and the samples mixed for 15 min using a Spiramix blood mixer (A.J.Cope, London, U.K.). The alumina was then allowed to settle. The liquid phase was removed and the alumina washed 3 times with 4 ml of ice-cold electrochemical grade water. After the final wash, the remaining water was removed by aspiration and 125 mcl of 0.1 M perchloric acid (Aldrich, Poole, U.K.) added. Tubes were gently agitated and placed on ice for a minimum of 5 min. The acid phase was removed and 100 mcl was injected onto the HPLC column.

**HPLC conditions and method of detection**

The HPLC system comprised a Spectrophysics SP8800 HPLC pump (Thermohypersyl Runcorn, U.K.) operating at 1.25 ml min⁻¹ (giving a pressure of approximately 3000 psi) and using a 5 mcC 18 Primesphere column of internal diameter 150 mm x 4.6 mm (Phenomenex, Macclesfield U.K.). Samples were introduced into the system via a rheodyne model 7125 manual injection valve (Fisher Scientific, Loughborough, U.K.). The mobile phase used was ‘CAT-A-PHASE II’ (ESA, St.Ives, U.K.). Retention time of the catecholamines was adjusted by the addition of sodium octyl sulphonate (Sigma, Poole, U.K.) to give adequate separation of norepinephrine from the solvent front (typically 6 min). The buffer was continuously recycled to increase the stability of both baseline noise and retention times. Detection was by a Coullochem electrochemical detector model S100A (ESA) fitted with a model 5020 guard cell operating at +0.25 V. This was followed by a model 5011 dual electrode analytical cell. The first electrode was set at +0.10 V and the second at -0.22 V. The subsequent signal was recorded in a Shimadzu C-RIB set at 250 mV full-scale deflection, with a chart speed of 2.5 mm min⁻¹. The height of the recorded signal is proportional to the mass of catecholamine present.

**Calculation of plasma catecholamine concentrations**

Plasma catecholamine concentrations were calculated by comparing the height of deflection of each peak on the chart recorded with the height of peaks produced by known concentrations of norepinephrine and epinephrine (the calibration standard), analysed via HPLC at the same time as the patients’ samples. Efficiency of extraction by HPLC varies
according to the protein and lipid content of the samples (Pryde & Gilbert 1979). This was corrected by the use of internal standards. An internal standard of a synthetic catecholamine, dihydroxybenzylamine (DHB) (Sigma, Poole, U.K.) was added to both calibration standards and patient samples prior to extraction. Comparison of the peak heights for DHB between the calibration standard and the patients sample allows the final catecholamine concentrations to be corrected for any variability in the extraction procedure. A typical calibration standard, and a patient sample showing plasma norepinephrine, epinephrine, and DHB as the internal standard are shown in Figures 6.1 and 6.2.

**Preparation of calibration and internal standards**

Calibration standards were prepared by dissolving 10 mg quantities of norepinephrine, epinephrine and DHB in HPLC grade water to give a concentration of 1 micromole ml\(^{-1}\). This solution was immediately diluted in 0.1 M perchloric acid to give 1 n mol ml\(^{-1}\) (acid conditions prevent the spontaneous oxidation of catecholamines). Calibration standards in 1 ml aliquots were stored in polystyrene tubes at -70°C. Immediately before use, calibration standards were thawed, diluted to 50 p mol ml\(^{-1}\) (5 p mol per 100 microlitre injection) with 0.1M perchloric acid. Internal standards (containing DHB) were added to the patient samples and samples were analysed in batches of five (i.e. corresponding to one individual patient), along with a calibration standard. After extraction, the purified samples were injected onto the HPLC column, and the subsequent signal recorded on the chart recorder. The plasma catecholamine concentrations in each sample were then calculated, as described above.
Figure 6.1

HPLC from calibration standard showing peaks corresponding to norepinephrine (NE), epinephrine (Epi) and internal standard (DHB). Time of sample injection onto the HPLC column is shown by the arrow.

Figure 6.2

HPLC from sample patient showing peaks corresponding to norepinephrine (NE), epinephrine (Epi) and internal standard (DHB). Time of sample injection onto the HPLC column is shown by the arrow.
**Limits of detection and coefficients of variation**

The lower limit of detection was defined as 0.2 p mol, approximately three fold above the baseline noise, although in some individual samples where baseline noise and drift were low, the lower limit of detection was 0.12 p mol. The inter-assay coefficient of variation is the variation between successive internal standards within an analysis batch, calculated by dividing the standard deviation of the measured values by the mean value, and expressed as a percentage. The intra-assay coefficient of variation is the variation between internal standards from different batches (i.e. analyses on different days) using the same formula. In the analyses performed in study 4, the inter-assay coefficients of variation were 2.51% and 3.14% at 5 pmol ml⁻¹ for norepinephrine and epinephrine respectively. The intra-assay coefficients of variation were 3.96% and 4.50% at 5 pmol ml⁻¹ for norepinephrine and epinephrine respectively.

### 6.6 Methods of assessment of categorical variables

#### 6.6.1 Study 2

In study 2, which examined the cardiovascular responses to emergence from anaesthesia and tracheal extubation, a number of ordinal categorical rating scores were employed to assess the incidence of coughing or gagging at tracheal extubation and the occurrence of potential postoperative adverse effects (excessive sedation, nausea, vomiting, respiratory depression). The incidence of coughing or gagging at extubation was noted and scored on a four point categorical scale (1 = none, 2 = minimal, 3 = moderate, 4 = severe). Sedation was assessed by a three point categorical rating score (1 = alert and responsive, 2 = drowsy but responsive to verbal command, 3 = drowsy and unresponsive to verbal command). These scales were similar to those used previously by Nishina and colleagues (Nishina *et al.* 1996, Mikawa *et al.* 1996a). Respiratory rate was calculated by nurses on arrival in the recovery area and at 15-min intervals thereafter. For the purposes of the study, respiratory depression was defined as a respiratory rate < 8 min⁻¹. Recovery room nurses also determined the incidence of nausea and vomiting, and fitness for discharge to the postoperative ward. The incidence of nausea and vomiting were noted as a binary
variable (as present or absent). Fitness for discharge was assessed by the conventional clinical criteria used at the Leicester Royal Infirmary. These were that the patient was awake (sedation score =1) had no difficulties with maintenance of their airway, postoperative pain was controlled, and there was no evidence of nausea, vomiting or other adverse effects. The time from arrival in the postoperative recovery room until the patient was judged fit for transfer by the recovery room nurses was recorded.

6.6.2 View at laryngoscopy

In studies 1 and 3-6, the investigator performing laryngoscopy and tracheal intubation rated the best view of the patients vocal cords at laryngoscopy according to the four-point rating scale described by Cormack and Lehane (1984). The duration of laryngoscopy was defined as the time from insertion of the laryngoscope until tracheal cuff inflation, and was measured using a stopwatch.
6.7 Equipment used for administration of study drugs

6.7.1 Drug dilutions

Study drug bolus

In all studies, the remifentanil solution was constituted by dissolving 1 mg remifentanil (Glaxo Wellcome, Stockley Park West, Uxbridge, U.K.) into 50 ml 0.9% saline to give a 20 mcg ml\(^{-1}\) solution. This solution was then used to make the bolus by withdrawing the appropriate volume (determined by the patient's body weight) using a 1 ml syringe graduated in 0.1 ml increments (Appendix 2). This volume of remifentanil 20 mcg ml\(^{-1}\) solution was then further diluted to 10 ml with 0.9% saline for bolus administration, the exact concentration depending on the patient's body weight. In Studies 1, 2 and 4 (where remifentanil was being compared with placebo), the 10ml syringe contained either 1.0 mcg kg\(^{-1}\) remifentanil in saline, or saline without remifentanil. In Study 3 (when different doses of remifentanil were being compared), the 10 ml syringe contained either remifentanil 1.0 mcg kg\(^{-1}\) or remifentanil 0.5 mcg kg\(^{-1}\). In Studies 6 and 7, (when the effects of remifentanil were compared with those of alfentanil), the 10 ml syringe contained either remifentanil 0.5 mcg kg\(^{-1}\) or alfentanil 10 mcg kg\(^{-1}\). For Studies 6 and 7, alfentanil 10 mcg kg\(^{-1}\) was withdrawn using a 1 ml syringe and diluted into a total volume of 10 ml using normal saline. In all studies these 10 ml boluses were administered manually over 30 seconds.

Glycopyrrolate

In all studies examining cardiovascular responses at induction of anaesthesia (Studies 1 and 3-6), patients received intravenous glycopyrronium bromide ('glycopyrrolate'). This was administered from a 2 ml syringe as a slow bolus of 200 mcg (undiluted). In Studies 1 and 3 an equivalent volume of saline (i.e. 1.0 ml) was administered to those patients in the appropriate groups.
6.7.2 Infusions

For all studies except Study 2, a remifentanil bolus/infusion technique was used. The remifentanil infusion was administered from the 50 ml syringe containing remifentanil 20 mcg ml\(^{-1}\) using a Graseby 3400 anaesthesia syringe pump (Graseby Medical Ltd, Watford, U.K.). The rate of the infusion was determined by entering the patient’s weight and concentration of remifentanil into the infusion pump, which automatically calculated the corresponding infusion rate. The accuracy of the Graseby infusion pump is quoted as ±2\% when a ‘perfect’ syringe is used (Graseby Medical 1994). In Studies 5 and 6, the infusion regimen was modified, because patients were at higher risk from adverse effects associated with remifentanil and a lower infusion rate was desirable. The precise regimen was calculated from the pharmacokinetic modelling software ‘Ultiva Trainer’ version 1.0 (Engbers et al. 1999) to produce stable plasma and effect site remifentanil concentrations within 2-3 min. The plasma and effect site concentrations predicted by this model for the regimens in the studies performed at induction of anaesthesia (Studies 1 and 3-6) are shown in Appendix 3.
6.8 Data collection, storage and display

Data for all studies were collected by the author and co-workers manually and entered onto SPSS (Statistical Package for the Social Sciences, SPSS Inc.) spreadsheets, on computers in the University Department of Anaesthesia, Critical Care and Pain Management, Leicester Royal Infirmary. Data from Studies 1, 2 and 3 were entered into SPSS for Windows release 6.0 (1993) files. Data from Studies 4, 5 and 6 were entered into SPSS for Windows release 9.0. files (1998), owing to upgrading of departmental computer software. There are differences in the statistical analyses available between the versions of SPSS, particularly in the analysis of repeated measures, and the analyses presented in this thesis were all performed on the later software. These differences are described in section 6.11.4 below.

Data display
Data were exported into GraphPad Prism (GraphPad Software Inc., Version 2.0, 1995) for graphical display.

6.9 Criteria for escape medication

In all studies, the criteria for administration of escape medication were based on aberrations from predetermined physiological values. In the studies of healthy young adult patients (studies 1, 3 and 4), significant hypotension was defined as a systolic arterial pressure (SAP) < 80 mm Hg for > 60 s and was treated with intravenous ephedrine 3 mg increments. If hypotension was accompanied by a HR < 50 min⁻¹, intravenous atropine 300 mcg increments were administered. Bradycardia was defined as HR < 45 min⁻¹ for more than 60 s and was treated with intravenous atropine 300 mcg increments. Hypertension was defined as SAP > 200 mm Hg for > 60 s and tachycardia was defined as a HR > 130 min⁻¹; both tachycardia and hypertension were treated with an increase in the inspired isoflurane concentration by increments of 0.5%. The study of cardiovascular responses at tracheal extubation (Study 2) was performed at the end of anaesthesia and time to emergence was one of the outcome measures. Deepening anaesthesia by increasing the inspired isoflurane concentration in response to tachycardia and hypertension would therefore have been inappropriate. The definitions of tachycardia
and hypertension were unchanged, but specific antihypertensive medications were used. These were: hydralazine 5mg increments for hypertension and esmolol 0.5mg increments for tachycardia.

Study 5 involved patients on long-term treatment for hypertension, whose upper and lower limits of cerebral, renal and coronary autoregulation are increased (Guyton & Hall 2000), and who are at increased risk of hypertension associated with laryngoscopy and tracheal intubation (Prys-Roberts et al. 1971a, 1971b, Low et al. 1986). The threshold for the definition of hypotension in study 5 was therefore increased to SAP < 100 mm Hg, or a decrease of SAP > 30 % compared with baseline, for > 60 s. Study 6 specifically involved elderly patients, who are at greater risk of both rapid alterations in arterial pressure at induction of anaesthesia (Krechel 1994) and of the complications arising from these swings (Kovac 1996). The definition of hypotension was broadened to include a decrease in SAP < 80 mm Hg or a decrease of > 30 % compared with baseline, for > 60 s. The definition of hypertension in Studies 5 and 6 was expanded to include an increase in SAP of > 30 % more than baseline values in addition to an absolute SAP > 200 mmHg for > 60 s. However, the escape medications used in studies 5 and 6 were identical to the earlier studies.
6.10 Study Power and Sample Size Calculations

The power of a study is a measure of the likelihood of a statistical test to detect a specified difference between two variables. If \( \beta \) is the probability of making a Type II error i.e. failing to demonstrate a statistically significant difference between groups where one exists, the power of a study is the probability of not making a Type II error, and can be defined as power = \( (1 - \beta) \) (Bourke et al. 1985). Power calculations were performed using the method suggested by Armitage and Berry (Armitage & Berry 1994). For the studies comparing the effects of remifentanil with placebo at laryngoscopy and tracheal intubation (Studies 1 and 4), a study power of 80% (\( \beta = 0.2 \)) was considered acceptable. However, for the studies comparing the effects of different doses of remifentanil (Study 3) or comparing remifentanil with alfentanil, (Studies 5 and 6), it was reasoned that a study power of 90% was more appropriate, to decrease the chances of a Type II error. The absolute cardiovascular changes at emergence from anaesthesia and tracheal extubation are less than those at laryngoscopy and tracheal intubation, and any possible differences between study groups likely to be smaller. Because it was important to be able to detect a significant potential effect of remifentanil in these circumstances, a study power of 90% was selected for Study 2.

6.10.1 Study 1

When the first study was designed, no independent published data were available on the effects of remifentanil on the cardiovascular response to laryngoscopy and tracheal intubation. The manufacturers data were unpublished, and were obtained using different doses of remifentanil (Glaxo Wellcome 1996). Historical data were therefore used to assess the size and distribution (standard deviation) of the typical response, and to estimate what would be considered a clinically useful effect of remifentanil on this response (Altman 1991). Previous studies had suggested that the cardiovascular response to laryngoscopy and tracheal intubation in untreated individuals typically comprises an increase in HR of 15-30 beats min\(^{-1}\), and an increase in arterial pressure of 25-50 mm Hg. It reaches a peak 1-2 min after laryngoscopy and intubation and persists for 5-10 min (King et al. 1951, Stoelting 1979, Russell et al. 1981, Dahlgren & Messeter 1981, Derbyshire et al. 1983, Crawford et al. 1987, Lindgren et al. 1993). It was hypothesised
that a 50% reduction in these responses by remifentanil would constitute a clinically useful effect. If the typical response to laryngoscopy and intubation is estimated as an increase in SAP of 40 mm Hg, 10 patients per group were required to detect a difference of 20 mm Hg between the remifentanil and saline groups, and between the remifentanil/glycopyrrolate and saline/glycopyrrolate groups, with a study power of 80% ($\alpha = 0.05$, $\beta = 0.2$). Post-hoc power calculations confirmed that the sample size was adequate and are detailed in Chapter 7.

6.10.2 Study 2

The cardiovascular response to emergence from anaesthesia and tracheal extubation is quantitatively less than for laryngoscopy and intubation, with previous studies demonstrating increases in HR and arterial pressure of 12-20 beats min$^{-1}$ and 20-40 mm Hg (Lowrie et al. 1992, Bidwai et al. 1979, O’Dwyer et al. 1993, Miller et al. 1995). Others defined a significant pressor response as an increase in SAP $> 20\%$ of baseline or an increase in HR $> 20\%$ of baseline (Dyson et al. 1990). However, there were no data relating to the use of remifentanil. Based on previous data, (Lowrie et al. 1992, Bidwai et al. 1979, O’Dwyer 1993), I calculated that 20 patients per group would have 90% power to demonstrate a difference of 20 mmHg in MAP and 15 beats min$^{-1}$ in HR ($\alpha = 0.05$, $\beta = 0.1$).

6.10.3 Study 3

Power calculations were based on data from Study 1, and showed 20 patients per group would detect a difference of 15 mm Hg in MAP or of 15 beats min$^{-1}$ in HR between groups, ($\alpha = 0.05$, $\beta = 0.1$), or a reduction in the incidence of bradycardia or hypotension to 10% ($\alpha = 0.05$, $\beta = 0.2$).

6.10.4 Study 4

Power calculations were based on previous investigations of the plasma catecholamine response to laryngoscopy and tracheal intubation (Shribman et al. 1987, Scheinin et al.
Based on these data, I calculated that 19 patients per group would detect a 30% difference between groups in plasma norepinephrine concentrations ($\alpha = 0.05$, $\beta = 0.2$).

### 6.10.5 Study 5

Power calculations were based on data from Study 1, as there are no recent published data relating to this patient population. This showed that 20 patients per group would detect a difference of 15 mm Hg in MAP or of 15 beats min$^{-1}$ in HR after intubation ($\alpha = 0.05$, $\beta = 0.1$).

### 6.10.6 Study 6

Power calculations based on previous data in elderly patients (Kirby et al. 1988, Sweeney et al. 1989) suggested that 20 patients per group would detect a 15% difference in HR between the groups after intubation ($\alpha = 0.05$, $\beta = 0.1$) or a 15% difference in SAP ($\alpha = 0.05$, $\beta = 0.2$). This approximates to differences of of 13 beats min$^{-1}$ in HR or 22 mm Hg in SAP.

### 6.11 Data Analysis

#### 6.11.1 Data distribution

The distribution of data was analysed using the one sample Kolmogorov-Smirnov goodness of fit test to determine the normality of its distribution. Appropriate non-parametric or parametric tests were then performed.

#### 6.11.2 Patient characteristics

Patient height, weight, baseline cardiovascular variables and age were compared using Student’s t-test in all studies except Study 3, when the three groups were compared using one-way analysis of variance.
6.11.3 Categorical variables

Ordered categorical variables were compared using Chi squared tests with Yates' correction if appropriate. When small numbers were present in each group, Fisher's exact test was performed. In Study 2, Mann-Whitney tests were performed to compare times to tracheal extubation. In Study 5, Spearman correlation co-efficients were used to examine the relationship between intubation difficulties and grade of anaesthetist.

6.11.4 Analysis of repeated measures

Arterial pressure and heart rate data in studies 1, 2, 3 were treated as repeated measures for statistical analysis, and were originally analysed using multivariate analysis of variance for repeated measures (MANOVA) (with treatment group and time as the between-and within-group factors) using SPSS for Windows release 6.0, 1993. Differences at each time point within and between groups were confirmed using paired and unpaired t-tests as appropriate with Bonferroni corrections (Puri 1996). These data were published using these methods of analysis (Thompson et al. 1998, Shajar et al. 1999, Hall et al. 2000). However, later versions of SPSS (releases 8.0 and 9.0) replaced MANOVA with general linear model analysis of variance. The University of Leicester replaced their SPSS software with release 8.0 and subsequently with release 9.0. By the time Studies 4, 5 and 6 were completed the University's software licence for release 6.0 had expired, so the statistical methods used in earlier studies where unavailable. These data were therefore analysed using general linear model analysis of variance for repeated measures (with treatment group and time as between- and within-group factors, and Bonferroni adjustment of the confidence intervals to adjust for multiple comparisons of each parameter) using SPSS for Windows release 9.0. Mauchly's test of sphericity was applied and when significant, F values were adjusted by Greenhouse-Geisser epsilon values (Kinnear & Gray 1994). Where general linear model analysis of variance suggested a significant effect over time, within-group comparisons at each time point were made by pairwise comparisons of the estimated marginal means. Significant between-group comparisons suggested by comparisons of the estimated marginal means were confirmed by examining parameter estimates at each time point. Having consulted professional statisticians (Drs Andrew Lloyd and Dr Nicky Spiers), it was clear that the latter approach was preferable to the older MANOVA. The data from Studies 1-3 were
therefore re-analysed, and all the data contained in this thesis have been analysed using general linear model analysis of variance for repeated measures as described above, and are presented as such.

This re-analysis has led to very minor discrepancies in the timings of statistically significant differences between groups in Studies 2 and 3. In Study 2, HR was significantly lower in Group S for 3 min after extubation, compared with 4 min when initially analysed (Shajar et al. 1999). In Study 3, HR was significantly lower in Group 3 for 3 min after induction of anaesthesia compared with 1 min in the initial analysis (Hall et al. 2000). In addition, absolute p values at certain time points differ in this thesis from those previously published. This is because the p values for pairwise comparisons of the estimated marginal means (for within-group comparisons) and parameter estimates at each time point (for between-group comparisons) have been presented. These may differ from, and are generally lower than the p values obtained from analysis of overall effects over time, but are preferable (Dr A. Lloyd, personal communication). Also, where p values varied within a group over a short period of time, the higher values of p have been presented in this thesis for clarity. For example, in Group S, Study 1 the absolute p values for the increase in SAP after intubation were p<0.005 at 1-3 min, and p<0.05 at 4 min after intubation, although these have been summarised in Figure 7.1 as p<0.05 for 4 min. However, these discrepancies in timings of, and values of p compared with the data previously published are minimal and overall have had no effect on the interpretation of the data from any of the studies.
CHAPTER 7. THE EFFECT OF REMIFENTANIL ON THE CARDIOVASCULAR RESPONSES TO LARYNGOSCOPY AND TRACHEAL INTUBATION (STUDY 1).

7.1 Introduction
7.2 Study design
7.3 Results
7.4 Discussion
7.5 Conclusions
7.6 Figures
7.1 Introduction

The cardiovascular responses to laryngoscopy and tracheal intubation and the potential consequences have been discussed in detail in Chapter 4. It is common clinical practice to attempt to attenuate these responses, particularly in those at risk. Although several methods had been described, they all have disadvantages (Kovac 1996). The pharmacokinetics of remifentanil, with its rapid onset and short duration of action, seemed to be ideal for the attenuation of responses to brief but noxious stimuli, but no independent published data were available. We therefore studied the effects of remifentanil on cardiovascular responses to laryngoscopy and tracheal intubation. In view of the reported association of remifentanil with bradycardia, (Sebel et al. 1995), the effect of concurrent administration of glycopyrrolate was also examined.

The results of this study were presented to the 8th International Symposium on Pain, Anaesthesia and Endocrinology in September 1997, and published in abstract form in the British Journal of Anaesthesia (Hall et al. 1998). The full results were also published in the British Journal of Anaesthesia (Thompson et al. 1998).

7.2 Study design

Following approval by the Leicestershire Research Ethics Committee, the informed consent of 40 females (of ASA grades 1 and 2), aged 18-48 years, was obtained. All were scheduled to undergo elective surgery for which laryngoscopy and tracheal intubation was routine. Criteria for exclusion and a full description of the methodology and methods of statistical analysis are given in Chapter 6.

Patients were randomly allocated to one of four treatment groups in a double-blind fashion. These were:

1. saline bolus followed by remifentanil (1 mcg kg⁻¹ bolus given over 30 s, followed by an infusion at 0.5 mcg kg⁻¹ min⁻¹) (Group R)
2. saline placebo bolus followed by saline bolus infusion (Group S)
3. glycopyrrolate (200 mcg) followed by remifentanil (1 mcg kg⁻¹ bolus given over 30 s, followed by an infusion at 0.5 mcg kg⁻¹ min⁻¹) (Group GR)
4. glycopyrrolate (200 mcg) followed by saline infusion (Group GS)

After placement of intravenous cannulae for administration of anaesthetic drugs and the study drug, measurements of HR and arterial pressure (SAP, MAP and DAP) were taken as described in Chapter 6. Patients' lungs were pre-oxygenated for 3 min, and the mean of 3 readings of MAP taken at 1 min intervals during this time defined as the baseline values for each patient. The study medication was then administered. This was followed by induction of general anaesthesia using a standard technique comprising intravenous propofol (0.5 mg kg\(^{-1}\) bolus followed by 10 mg every 10 s titrated to loss of verbal contact), vecuronium (0.1 mg kg\(^{-1}\)) and inhaled isoflurane 1% with nitrous oxide 66% in oxygen 33%. Manual ventilation of the lungs was performed before intubation and mechanical ventilation performed thereafter. The tidal volume was set to 10 ml kg\(^{-1}\), and minute volume adjusted to maintain target end-tidal CO\(_2\) tension of 4.0-4.5 kPa. Laryngoscopy and tracheal intubation was performed 3 min after induction of anaesthesia; HR and arterial pressure were measured every min from the start of induction to 5 min after tracheal intubation.

7.3 Results

Patient data are shown in Table 7.1. There were no differences between the groups in age, weight, or baseline heart rate and arterial pressure. Laryngoscopy was prolonged (defined as duration > 20 s) in two patients in Group GR, but mean duration of laryngoscopy, and grade of intubation were similar between the groups. The dose of propofol required at induction of anaesthesia was significantly lower in the two remifentanil groups (Groups R and GR) compared with the non-remifentanil groups (Groups S and GS). Changes in arterial pressure and heart rate are shown in Figures 7.1-7.4.
Table 7.1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group R</th>
<th>Group S</th>
<th>Group GR</th>
<th>Group GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32.1 (20-44)</td>
<td>29.7 (21-39)</td>
<td>31.9 (18-48)</td>
<td>30.3 (20-44)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.9 (12.9)</td>
<td>61.5 (10.6)</td>
<td>63.2 (9.6)</td>
<td>62.2 (10.2)</td>
</tr>
<tr>
<td>Baseline SAP (mm Hg)</td>
<td>123.1 (8.7)</td>
<td>122.9 (9.8)</td>
<td>131.5 (21.6)</td>
<td>129.3 (9.5)</td>
</tr>
<tr>
<td>Baseline MAP (mm Hg)</td>
<td>88.7 (6.9)</td>
<td>88.8 (7.4)</td>
<td>95.0 (14.6)</td>
<td>94.0 (8.8)</td>
</tr>
<tr>
<td>Baseline DAP (mm Hg)</td>
<td>72.8 (5.5)</td>
<td>77.0 (5.2)</td>
<td>80.4 (10.8)</td>
<td>80.8 (5.0)</td>
</tr>
<tr>
<td>Baseline HR (min⁻¹)</td>
<td>83.7 (16.6)</td>
<td>78.2 (11.4)</td>
<td>89.8 (16.9)</td>
<td>81.4 (9.9)</td>
</tr>
<tr>
<td>Dose of propofol (mg)</td>
<td>108.0 (32.6)</td>
<td>139 (56.9)</td>
<td>94.5 (10.1)</td>
<td>136 (39.8)</td>
</tr>
<tr>
<td>Dose of propofol (mg kg⁻¹)</td>
<td>1.81 (0.74)*</td>
<td>2.29 (0.95)</td>
<td>1.52 (0.26)*</td>
<td>2.17 (0.50)</td>
</tr>
<tr>
<td>Duration of laryngoscopy (s)</td>
<td>10.1 (4.6)</td>
<td>10.0 (3.4)</td>
<td>17.7 (14.3)</td>
<td>10.9 (4.3)</td>
</tr>
<tr>
<td>View at laryngoscopy (1/2)</td>
<td>7/3</td>
<td>10/0</td>
<td>8/2</td>
<td>7/3</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD or range). \( n = 10 \) in each group. Baseline arterial pressures and HR are the mean of 3 values taken immediately before induction of anaesthesia. Dose of propofol is expressed as absolute dose and dose per kg body weight. * \( p<0.05 \), Groups R and GR compared with Groups S and GS.

Arterial pressure and HR decreased in all groups following induction of anaesthesia (\( p<0.01 \)). Arterial pressure was lower in the remifentanil groups (groups R and GR) than the non-remifentanil groups (Groups S and GS) before intubation (Figures 7.1-7.3). Arterial pressure increased significantly in all groups after laryngoscopy and tracheal intubation but was significantly greater in Groups S and GS than Groups R and GR (\( p<0.05 \)). The mean increases in MAP at intubation were: 39.9 mmHg (Group S), 42.0 mmHg (Group GS), 8.1 mmHg (Group R) and 11.3 mmHg (Group GR) respectively; arterial pressure remained below pre-induction values in Groups R and GR. The mean difference in MAP between Groups R and S one min after intubation was 31.8 mm Hg, (with 95% confidence intervals for the difference of 44.7-18.9 mm Hg). The corresponding mean (95% confidence intervals) difference in MAP between Groups GR and GS was 30.7 mm Hg (40.8-20.6 mm Hg). There were no significant differences in arterial pressure between Groups R and GR, or between Groups S and GS before and
after intubation. HR decreased in all groups following induction of anaesthesia and was significantly lower in Group R immediately before intubation compared with the other 3 groups, and pre-induction values (Figure 7.4). HR increased after intubation in Groups S and SG compared with pre-intubation values and was significantly greater 1 min after intubation in Groups S and GS compared with Groups R and GR. There were no significant differences in HR immediately before and after intubation in Groups R and RG. Five patients in Group R and 1 in Group GR displayed bradycardia (HR < 45 min⁻¹) and/or hypotension (SAP < 80 mm Hg) requiring escape medication. In all cases, it responded to intravenous atropine or ephedrine increments.

**Adverse events**

One patient in Group S required an increase in the inspired isoflurane concentration to treat tachycardia (HR 130-135 min⁻¹ from 3 to 4 min after intubation). Three patients in Group S had transient SAP readings > 160 mm Hg but hypertension requiring escape medication was not observed.

**7.4 Discussion**

In this study, the cardiovascular response to laryngoscopy and tracheal intubation was significantly attenuated in patients receiving remifentanil (1 mcg kg⁻¹ bolus given over 30 s, followed by a 0.5 mcg kg⁻¹ min⁻¹ infusion) at induction of anaesthesia. In fact, MAP decreased in all four groups after induction of anaesthesia. This decrease was greatest in Group R and was associated with bradycardia. Both hypotension and bradycardia were less marked in patients receiving remifentanil and glycopyrrolate (Group GR). However, clinically relevant hypotension requiring escape medication was observed in 5 patients in Group R but only 1 from Group GR, so that the protective effect of glycopyrrolate against hypotension is probably greater than that recorded. Laryngoscopy and tracheal intubation had no significant effect on HR in Groups R and GR, but arterial pressure increased significantly in all 4 groups. However, in patients receiving remifentanil, (Groups R and GR), this increase was minimal (mean increases 8.1 mm Hg and 11.3 mm Hg) and did not exceed baseline pre-induction values. We therefore concluded that remifentanil in this dose regimen attenuated the cardiovascular response to laryngoscopy and tracheal
intubation. The size of the pressor response observed in those patients who did not receive remifentanil in this study was similar to that previously reported (Russell et al. 1981, Derbyshire et al. 1983, Crawford et al. 1987, Ebert & Kampine 1989, Brossy et al. 1994, Saarnivaara & Klemola 1991), although some workers have reported increases in arterial pressure of up to 70 mmHg in similar groups of control patients (Dahlgren & Messeter 1981, Korpinen et al. 1982, Martin et al. 1982, Singh et al. 1995).

Bradycardia and hypotension associated with remifentanil had been alluded to in three studies supported by the manufacturers Glaxo Wellcome, but the details published were sparse. Schüttler and colleagues used a similar dose regimen of remifentanil in a multicentre study comparing perioperative anaesthesia with remifentanil or alfentanil (Schuttler et al. 1997). They reported that 53% of patients had a significant hypotensive episode intra-operatively and 4% had a significant bradycardia. All patients received either atropine or glycopyrrolate and pre-hydration with a crystalloid solution (5 ml kg⁻¹) before induction of anaesthesia. However, the timing of the episodes of hypotension and bradycardia were not stated, and the study was performed in patients undergoing major abdominal surgery, so the aetiology of these episodes is not clear. Hogue and colleagues reported an incidence of hypotension and bradycardia on induction of anaesthesia of 10% and 7% respectively with a remifentanil dose regimen of 1 mcg kg⁻¹ bolus followed by a 0.5 mcg kg⁻¹ min⁻¹ infusion, identical to this study (Hogue et al. 1996). Philip and colleagues also investigated this regimen in patients undergoing gynaecological laparoscopic surgery, and reported a 17% incidence of hypotension or bradycardia throughout the operative period, though the precise timing in relation to induction of anaesthesia were not stated (Philip et al. 1997). The results of this study are discussed further in Chapter 13.
7.5 Conclusions

These were the first independent published data recording the effect of remifentanil on the cardiovascular response to laryngoscopy and tracheal intubation. Remifentanil (1 mcg kg\(^{-1}\) bolus given over 30 s, followed by a 0.5 mcg kg\(^{-1}\) min\(^{-1}\) infusion) effectively attenuated the increase in HR and arterial pressure that occurred in the control groups (Groups S and GS). I therefore decided to investigate the effect of remifentanil on the cardiovascular response to emergence from anaesthesia and tracheal extubation (Chapter 8). In view of the high incidence of bradycardia and hypotension in this study (half of the patients who received remifentanil without glycopyrrolate required rescue medication), I also decided to investigate the effects of a lower dose of remifentanil at laryngoscopy and tracheal intubation (Chapter 9).
Figure 7.1

Mean (SEM) systolic pressure (SAP) in Groups R (remifentanil -○-), RG (remifentanil/glycopyrrolate -■-), S (saline -○-), and SG (saline/glycopyrrolate -●-). Baseline values are represented by t=0; the timings of induction of anaesthesia and of laryngoscopy and tracheal intubation are shown by arrows.

*  p<0.05 compared with pre-intubation values
†  p<0.05 compared with baseline values before induction
‡  p<0.05 between remifentanil groups (R and RG) and saline groups (S and SG)

SAP decreased significantly after induction of anaesthesia in Groups R and RG and remained stable thereafter, with no significant change at laryngoscopy and tracheal intubation. SAP was significantly lower in Groups R and RG compared with Groups S and SG both before and after intubation. SAP decreased in Group SG from 2 min after induction, and increased significantly for 4 min after intubation. SAP was stable in Group S after induction but increased significantly for 1 min after intubation.
Figure 7.2
Mean (SEM) MAP in groups R (remifentanil - □-), RG (remifentanil/glycopyrrole - ■-), S (saline -○-), and SG (saline/glycopyrrole -●-)

* p<0.05 compared with pre-intubation values
† p<0.05 compared with baseline values before induction
‡ p<0.05 between remifentanil groups (R and RG) and saline groups (S and SG)

MAP decreased significantly after induction of anaesthesia in Groups R and RG, and remained stable thereafter, with no significant change at laryngoscopy and tracheal intubation. MAP was significantly lower in Groups R and RG compared with Groups S and SG both before and after intubation. MAP decreased in Group SG from 2 min after induction and increased significantly for 4 min after intubation. MAP was stable in Group S after induction but increased significantly for 2 min after intubation.
Figure 7.3
Mean (SEM) diastolic pressure (DAP) in Groups R (remifentanil -□-), RG (remifentanil/glycopyrrolate -■-), S (saline -○-), and SG (saline/glycopyrrolate -●-)

* p<0.05 compared with pre-intubation values
† p<0.05 compared with baseline values before induction
‡ p<0.05 between remifentanil groups (R and RG) and saline groups (S and SG)

DAP decreased significantly after induction of anaesthesia in Groups R and RG, and remained stable thereafter, with no significant change at laryngoscopy and tracheal intubation. DAP was significantly lower in Groups R and RG compared with Groups S and SG both before and after intubation. DAP decreased in Group SG from 2 min after induction and increased significantly for 4 min after intubation. DAP was stable in Group S after induction, but increased significantly for 2 min after intubation.
Figure 7.4

Mean (SEM) heart rate (HR) in Groups R (remifentanil -□-), RG (remifentanil/glycopyrrolate -■-), S (saline -○-), and SG (saline/glycopyrrolate -●-).

* p<0.05 compared with pre-intubation values
† p<0.05 compared with baseline values before induction
‡ p<0.05 between remifentanil groups (R and RG) and saline groups (S and SG)

HR decreased after induction of anaesthesia in Group R, and was significantly lower in Groups R and RG compared with Groups S and SG from immediately after induction to the end of the study. HR did not change at laryngoscopy and intubation in Groups R and RG. HR in Groups S and SG increased significantly for 2 min after intubation in Group S and for 4 min after intubation in Group SG.
CHAPTER 8. THE EFFECT OF REMIFENTANIL ON THE CARDIOVASCULAR RESPONSES TO EMERGENCE FROM ANAESTHESIA AND TRACHEAL EXTUBATION (STUDY 2).

8.1 Introduction
8.2 Study design
8.3 Results
8.4 Discussion
8.5 Conclusions
8.6 Figures
8.1 Introduction

Although fewer data are available, the cardiovascular changes at extubation are quantitatively smaller than those at tracheal intubation, although they may be clinically significant (Bidwai et al. 1979, O’Dwyer et al. 1993). They are also associated with increases in plasma catecholamine concentrations (Lowrie et al. 1992) and may cause myocardial ischaemia (Edwards et al. 1994). Many of the methods used to attenuate the responses at induction of anaesthesia, laryngoscopy and tracheal intubation are not suitable for use at the end of anaesthesia as they cause an increase in the depth of anaesthesia and therefore delay recovery (Miller et al. 1995). The ideal agent for this purpose would allow the rapid return of protective reflexes and avoid residual hypotension, respiratory depression or sedation. The pharmacokinetic properties of remifentanil result in a rapid onset and offset of action and it therefore seemed suitable for use at emergence from anaesthesia. Having established that remifentanil effectively attenuated the cardiovascular responses to laryngoscopy and tracheal intubation in healthy patients (study 1), the aim of this study was to assess the effect of remifentanil upon the cardiovascular responses to, and timing of, emergence from anaesthesia and tracheal extubation.

The results of this study were presented to the Anaesthetic Research Society in March 1998 and the abstract published in the British Journal of Anaesthesia (Shajar et al. 1998). The full paper was also published in the British Journal of Anaesthesia (Shajar et al. 1999).

8.2 Study design

Following approval by the Leicestershire Research Ethics Committee and informed consent, 40 healthy females (ASA grades 1 and 2), aged 18-50 years, presenting for elective diagnostic gynaecological laparoscopy, were recruited to the study. Criteria for exclusion and a full description of the methodology and methods of statistical analysis are given in Chapter 6. Patients were divided into two treatment groups in a double-blind randomised fashion.
All patients received a standard general anaesthetic comprising intravenous propofol to loss of verbal contact (approximately 2-3 mg kg\(^{-1}\)), fentanyl 1.5 mcg kg\(^{-1}\) and vecuronium 0.1 mg kg\(^{-1}\) to facilitate tracheal intubation. After induction of anaesthesia all patients received rectal diclofenac 100 mg. Anaesthesia was maintained with isoflurane 0.5-2.0% and 66% nitrous oxide in oxygen, and the patients’ lungs ventilated mechanically (Blease 8200S, Blease Medical Ltd, Chesham, Buckinghamshire, England) with a tidal volume of 10ml kg\(^{-1}\). End tidal carbon dioxide was maintained to a target value of 4.5 kPa, by adjustment of ventilatory rate and was measured using a Capnomac Ultima™ II (Datex Division, Instrumentarium Corporation, Helsinki, Finland). Baseline MAP was taken from the mean of 3 resting values in the anaesthetic room before any instrumentation. MAP during surgery was controlled to within 10% of the resting preoperative baseline value by titration of inspired isoflurane concentration.

At the end of surgery the wounds were infiltrated with bupivacaine 0.5% 10 ml. The time at last suture was designated ‘time zero’. At this point, the patient received either remifentanil 1 mcg kg\(^{-1}\) (Group R) or an equivalent volume of saline (Group S) over 30 s. This was followed by neostigmine 2.5 mg with glycopyrrolate 0.5 mg to reverse any residual neuromuscular blockade. Mechanical ventilation was continued with 100% oxygen and after two minutes, oropharyngeal suction was performed using a Yankeur device. Tracheal extubation was only performed when the patients were able to open their eyes and squeeze an investigator’s hand on command. Extubation was accomplished in a standardised manner: the patient was asked to open their mouth, the tracheal tube cuff was then deflated gently and the tracheal tube removed immediately. Further oropharyngeal suction, airway manipulation or pressure on the reservoir bag of the breathing circuit were all avoided as these could potentially confound the cardiovascular response to tracheal extubation. Extubation time was defined as time between study drug administration (time zero) and extubation. Observations of HR, SAP, MAP and DAP and SpO\(_2\) were recorded every minute from time zero to 5 min after extubation. The quality of extubation was assessed by the investigators according to the incidence and severity of coughing or gagging at tracheal extubation, and was scored on a four point categorical scale (0 = none, 1 = minimal, 2 = moderate, 3 = severe). Sedation score, respiratory rate, incidence of nausea and vomiting and fitness for discharge to the postoperative surgical ward were determined by nurses on arrival in the recovery room and at 15 minute intervals thereafter.
8.3 Results

Patient data are shown in Table 8.1. There were no differences between the groups in age, weight, or baseline HR and arterial pressure. The doses of propofol and fentanyl at induction of anaesthesia and the duration of surgery were also similar. The time between administration of the study drug and the time at which tracheal extubation was possible was significantly longer in Group R ($p<0.001$) and end-tidal isoflurane concentrations at tracheal extubation were correspondingly lower in Group R, although this difference was not statistically significant ($p = 0.063$).

<table>
<thead>
<tr>
<th>Table 8.1 Patient characteristics</th>
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<tbody>
<tr>
<td><strong>Group R</strong></td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Baseline SAP (mmHg)</td>
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<tr>
<td>Baseline MAP (mmHg)</td>
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<tr>
<td>Baseline DAP (mmHg)</td>
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<tr>
<td>HR (min$^{-1}$)</td>
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<tr>
<td>Propofol dose (mg)</td>
</tr>
<tr>
<td>Propofol dose (mg kg$^{-1}$)</td>
</tr>
<tr>
<td>Fentanyl dose (mcg)</td>
</tr>
<tr>
<td>End-tidal isoflurane (%)</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
</tr>
<tr>
<td>Time to extubation (min)</td>
</tr>
<tr>
<td>Time to discharge (min)</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD or range). All patients were female. $n = 20$ in each group. Baseline arterial pressures and HR are the mean of 3 values taken immediately before induction of anaesthesia. Dose of propofol at induction of anaesthesia are expressed both as absolute dose and dose according to body weight. Time to extubation refers to the time between administration of study drug (at last suture) and tracheal extubation. Time to discharge refers to the time from last suture to time of readiness for discharge from the postoperative recovery room. End-tidal isoflurane concentration was measured immediately before tracheal extubation. * $P<0.001$ between groups.
Arterial pressures and heart rate are shown in Figures 8.1-8.4. There were no significant differences in baseline arterial pressure (SAP, MAP and DAP) and HR between the groups before or at the end of surgery (Table 8.1, Figures 8.1-8.4). Mean HR and arterial pressure (SAP, MAP and DAP) increased in Group S after the end of surgery for several minutes after tracheal extubation. In contrast, HR and arterial pressure decreased slightly after the end of surgery before increasing slightly after tracheal extubation in Group R. However, values in Group R after extubation were not significantly above baseline. HR and arterial pressures were significantly higher in Group S from pre-extubation until 3 min after extubation (Figures 8.1-8.4).

The quality of extubation and sedation scores and incidence of adverse events are shown in Tables 8.2-8.4. Mean time to extubation was significantly longer in Group R (p<0.001). Coughing or gagging at tracheal extubation was rated as ‘none or minimal’ in 11 patients in Group R and 9 patients in Group S (p=0.177). The incidence of drowsiness (sedation score 2 or 3) on arrival in the recovery room was 4/20 in Group R compared with 9/20 in Group S (p=0.085). At 15 minutes after arrival in the recovery room, 5 patients in Group R (2 of whom had received parenteral morphine in the interim) and 2 patients in Group S were drowsy. However, times to discharge from the recovery room were similar.
Adverse events

Escape medication (intraoperative bradycardia requiring atropine) was administered to 2 patients in Group R and 1 patient in Group S. However, no patients required escape medication for hypertension, hypotension, tachycardia or bradycardia at emergence from anaesthesia and tracheal extubation. One patient in each group was deemed to require a second dose of neostigmine because of incomplete reversal of neuromuscular blockade. Postoperative anti-emetic medication was required by 1 patient in Group R and 2 patients in Group S; 2 patients in Group R and 1 in Group S received parenteral morphine in the recovery room for postoperative pain (Table 8.4).

8.4 Discussion

Remifentanil 1 mcg kg\(^{-1}\) when given as a slow intravenous bolus at the end of surgery attenuated the increase in MAP and HR associated with emergence from anaesthesia and tracheal extubation. The increase in MAP and HR at tracheal extubation in the control group was consistent with previous data (Lowrie et al. 1992, Miller et al. 1995). The time at which extubation was possible was delayed after remifentanil, although this delay (3.2 minutes) is not considered to be clinically significant. The greater tendency towards sedation may reflect the shorter time between the end of surgery and arrival in the recovery room in the saline group, although end-tidal concentrations of isoflurane were similar at extubation. There were no incidences of bradycardia or hypotension associated with the administration remifentanil, though this had been previously reported at induction of anaesthesia and intubation (Sebel et al. 1995), and occurred in study 1. This may be because glycopyrrolate was co-administered with neostigmine, and also the effect of diminishing anaesthetic concentration at emergence from anaesthesia. In contrast, patients in study 1 also received propofol, which is known to cause bradycardia, particularly when co-administered with opioids (Egan & Brockutne 1991, Hiller & Saarnivaara 1992, Vuyk et al. 1996). The incidence of severe coughing or gagging at extubation was 15% in the control group and zero in Group R, but between-group differences in overall quality of extubation were not statistically significant.
8.5 Conclusions

The conclusions from this study were that a remifentanil bolus of 1 mcg kg\textsuperscript{-1} could usefully obtund the cardiovascular responses to emergence from anaesthesia and tracheal extubation without compromising clinical recovery. Although this study was performed in healthy patients, the logical extrapolation from these data is that this technique might be useful before tracheal extubation, endotracheal toilet or physiotherapy in Intensive Care patients at risk from neurological or cardiovascular disease. It might also be suitable for the attenuation of responses to other brief but noxious stimuli, in patients undergoing short procedures under anaesthesia, for example manipulation of fractures or electroconvulsive therapy. These findings are discussed further in Chapter 13.
Mean (SEM) systolic and diastolic pressures in remifentanil (SAP - ■-, DAP - ▼-) and saline (SAP - □-, DAP - ▼-), groups at the end of surgery, immediately before, and for 5 min after tracheal extubation. The precise duration between the end of surgery, and extubation varied between individuals.

* p<0.05 compared with end of surgery
† p<0.05 compared with pre-extubation values
‡ p<0.05 between groups

SAP and DAP increased in Group S after the end of surgery and remained significantly higher than pre-extubation values for the first 1-2 min after tracheal extubation. DAP and SAP decreased after the end of surgery in Group R (p< 0.05 for DAP, p= 0.63 for SAP at extubation compared with end of surgery), and then increased significantly after extubation compared with immediate pre-extubation values. However, values after extubation were not significantly above baseline. SAP and DAP were significantly higher in Group S from pre-extubation to 3 min after extubation.
Figure 8.2

Mean (SEM) MAP in remifentanil (■) and saline (○) groups at the end of surgery, immediately before, and for 5 min after tracheal extubation.

* p<0.05 compared with end of surgery
† p<0.05 compared with pre-extubation values
‡ p<0.05 between groups

MAP increased in Group S after the end of surgery and remained significantly higher for the first 1-2 min after tracheal extubation. MAP decreased significantly after the end of surgery in Group R and then increased after extubation. However, values after extubation were not significantly above baseline. MAP was significantly higher in Group S from pre-extubation to 3 min after extubation.
Figure 8.3

Mean (SEM) heart rate in remifentanil (-■-) and saline (-○-) groups at the end of surgery, immediately before and for 5 minutes after tracheal extubation.

* p<0.05 compared with end of surgery

† p<0.05 between groups

Mean HR increased in Group S after the end of surgery and remained significantly higher for the first 3 min after tracheal extubation. HR decreased slightly after the end of surgery and increased slightly after tracheal extubation in Group R but changes were not significantly significant. HR was significantly higher in Group S from pre-extubation to 1 min after extubation.
CHAPTER 9. COMPARISON OF THE EFFECTS OF DIFFERENT DOSES OF REMIFENTANIL ON THE CARDIOVASCULAR RESPONSES TO LARYNGOSCOPY AND TRACHEAL INTUBATION (STUDY 3).

9.1 Introduction
9.2 Study design
9.3 Results
9.4 Discussion
9.5 Conclusions
9.6 Figures
9.1 Introduction

In Study 1, it was established that remifentanil attenuated the increase in heart rate and arterial pressure at laryngoscopy and tracheal intubation in healthy adults. The dose used in Study 1 was that recommended by the manufacturers i.e. 1 mcg kg\(^{-1}\) followed by an infusion of 0.25-1.0 mcg kg\(^{-1}\) min\(^{-1}\) (Glaxo Wellcome 1996). However, bradycardia and hypotension occurred in 5 out of 10 patients who did not receive pre-treatment with glycopyrrolate and so it was postulated that a lower dose of remifentanil might be effective, without these adverse effects. Although bradycardia had been reported previously (Sebel et al. 1995), there were no other data available regarding the use of remifentanil without glycopyrrolate. Since the pharmacodynamic effects of opioids are dose-dependent, it was also possible that glycopyrrolate pre-treatment might be unnecessary when using a lower dose of remifentanil. Study 3 was therefore designed to compare the haemodynamic response to laryngoscopy and tracheal intubation in patients receiving the previously recommended remifentanil regimen (with glycopyrrolate) as used in Study 1, or half this dose regimen, both with and without glycopyrrolate pre-treatment.

The results of this study were presented to the Anaesthetic Research Society, University of Dundee, in July 1998 and the abstract published in the British Journal of Anaesthesia (Leslie et al. 1998). The full paper was also published in the British Journal of Anaesthesia (Hall et al. 2000).

9.2 Study design

Following approval by the Leicestershire Research Ethics Committee, the informed consent of 60 female patients (of ASA grades 1 and 2) undergoing elective surgery was obtained. The exclusion criteria were identical to those applied in Studies 1 and 2 and full details of the methodology are given in Chapter 6.

Patients were assigned to one of three groups in a randomised double-blind fashion. These consisted of: glycopyrrolate 200 mcg iv, followed by a remifentanil bolus of 1 mcg kg\(^{-1}\) given over 30 s and a subsequent remifentanil infusion of 0.5 mcg kg\(^{-1}\) min\(^{-1}\) (Group...
1); remifentanil 0.5 mcg kg\(^{-1}\) given over 30 s and a remifentanil 0.25 mcg kg\(^{-1}\) min\(^{-1}\) infusion preceded by either glycopyrrolate 200 mcg (Group 2) or saline (Group 3). An independent anaesthetist prepared all study drugs; the investigators were blinded to their identity. Immediately after the remifentanil bolus, anaesthesia was induced with propofol (0.5 mg kg\(^{-1}\) followed by 10 mg every 10 s to loss of verbal contact), and rocuronium 0.6 mg kg\(^{-1}\). Patients’ lungs were ventilated manually with isoflurane 1% with N\(_2\)O 66% in O\(_2\) 33% until intubation, and mechanically thereafter (tidal volume 10 ml kg\(^{-1}\), end-tidal CO\(_2\) 4.0-4.5 kPa) (Blease Medical Ltd, Chesham, Buckinghamshire, England). Laryngoscopy and tracheal intubation were performed three minutes after induction of anaesthesia. HR and arterial pressure (SAP, MAP and DAP) were recorded at 1 min intervals from before induction of anaesthesia to 5 min after intubation. The methodology is further described in Chapter 6.

9.3 Results

Patient characteristics and baseline data were similar in the three groups and are shown in Table 9.1. Grade of view at laryngoscopy is shown in Table 9.2. Cardiovascular data are shown in figures 9.1-9.4. HR decreased in Group 3 after induction of anaesthesia and remained lower after tracheal intubation compared with the other groups (p<0.05). MAP increased by 11-16 mm Hg and mean HR increased by 6-11 beats min\(^{-1}\) after intubation, but there were no other statistically significant within- or between-group differences in arterial pressure (SAP, MAP and DAP) at any time point.

Adverse events

Hypotension requiring a decrease in the inspired concentration of isoflurane occurred in two patients in Groups 1 & 2 and three patients in Group 3. One patient in Group 2 developed bradycardia, requiring atropine. There were no incidences of tachycardia or hypertension; the maximum SAP after intubation in all groups was 155 mm Hg (one patient in group 2).
Table 9.1 Patient characteristics and baseline data

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34.4 (21-45)</td>
<td>35.3 (24-56)</td>
<td>32.1 (20-48)</td>
</tr>
<tr>
<td>Weight</td>
<td>67.6 (11.6)</td>
<td>69.2 (15.3)</td>
<td>65.9 (15.7)</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>125.2 (13.0)</td>
<td>127.2 (15.5)</td>
<td>123.3 (12.8)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>87.8 (10.5)</td>
<td>91.4 (14.2)</td>
<td>87.7 (9.9)</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>72.4 (8.9)</td>
<td>75.6 (10.6)</td>
<td>76.3 (9.6)</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>81.7 (13.4)</td>
<td>85.5 (12.5)</td>
<td>77.0 (13.4)</td>
</tr>
<tr>
<td>Propofol (mg)</td>
<td>133.5 (26.0)</td>
<td>137.9 (32.6)</td>
<td>131.5 (34.8)</td>
</tr>
<tr>
<td>Propofol (mg kg⁻¹)</td>
<td>1.97 (.46)</td>
<td>2.01 (.43)</td>
<td>2.04 (.54)</td>
</tr>
<tr>
<td>Duration of laryngoscopy (s)</td>
<td>17.2 (13.0)</td>
<td>19.2 (10.9)</td>
<td>11.8 (6.2)</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD or range). n = 20 in each group. Baseline arterial pressures and HR are the mean of 3 values taken immediately before induction of anaesthesia. There were no significant differences between the groups. Mean duration of laryngoscopy was slightly shorter in Group 3, but the differences were not statistically significant (p=0.093). Laryngoscopy was prolonged (> 20 s) in five patients in Groups 1 and 3, and in two patients in Group 3.

Table 9.2 Grade of view at laryngoscopy

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 view</td>
<td>11</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Grade 2 view</td>
<td>8</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Grade 3 view</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Data expressed as numbers. The differences between groups in grade of view at laryngoscopy were not statistically significant (p=0.068).
9.4 Discussion

There was no difference between a bolus dose of remifentanil 0.5 mcg kg\(^{-1}\) followed by an infusion of 0.25mcg kg\(^{-1}\) min\(^{-1}\) and double these doses in attenuating the potential cardiovascular responses to laryngoscopy and tracheal intubation. Power calculations for this study were based on data from Study 1 (Chapter 7), and showed 20 patients per group would detect a difference of 15 mm Hg in MAP or of 15 beats min\(^{-1}\) in HR between groups, (\(\alpha=0.05, \beta=0.1\)), or a reduction in the incidence of bradycardia or hypotension to 10\% (\(\alpha=0.05, \beta=0.2\)). Post-hoc analysis demonstrated that the sample size was adequate: the mean difference in MAP at the time of the maximum predicted response (1 min after intubation) between Groups 2 and 1 was 1.8 mm Hg, with 95\% confidence intervals of the difference of -8.8 to 12.5 mm Hg. Corresponding differences between Groups 2 and 3 were 3.5 mm Hg (95\% confidence intervals -7.1 to 14.1 mm Hg).

HR decreased after induction of anaesthesia in patients who did not receive glycopyrrolate, and remained significantly lower compared with the other two groups. However, the only episode of bradycardia fulfilling criteria for escape medication occurred in a patient who received glycopyrrolate at induction (Group 2). In contrast to Study 1, the incidence of bradycardia and hypotension was low. Hypertension and tachycardia were not observed; the cardiovascular response to laryngoscopy and tracheal intubation was effectively attenuated in all patients in all three groups.

Other data have suggested that larger bolus doses or remifentanil are required to attenuate the cardiovascular response to laryngoscopy and tracheal intubation (O'Hare et al. 1999, Barclay & Kluger 2000). However, McAtamney and colleagues found that the effects of remifentanil 0.5 mcg kg\(^{-1}\) and 1.0 mcg kg\(^{-1}\) on heart rate and systolic pressure were similar when administered 1 min before laryngoscopy (McAtamney et al. 1998). Guignard and colleagues found cardiovascular responses of very similar magnitude to this study after the infusion of remifentanil to a target concentration of 2 and 4 ng ml\(^{-1}\), (Guignard et al. 2000). These values correspond closely to the calculated remifentanil concentrations obtained using the dosage regimens employed in this study (Appendix 1). Despite differences in methodology, these results confirm the suggestion that remifentanil
0.5 mcg kg\(^{-1}\) is as effective as a dose of 1.0 mcg kg\(^{-1}\) in attenuating the pressor response to laryngoscopy and tracheal intubation. These findings are discussed further in Chapter 13.

9.5 Conclusions

In this study, only slight changes in HR and arterial pressure were observed after laryngoscopy and tracheal intubation when remifentanil (0.5 mcg kg\(^{-1}\) iv given over 30 s followed by a 0.25 mcg kg\(^{-1}\) min\(^{-1}\) infusion) was used as part of a balanced anaesthetic technique at induction of anaesthesia, in healthy adults. Adverse effects were uncommon. The results of this study confirmed that lower doses of remifentanil than previously recommended would be effective in attenuating arterial pressure and heart rate responses to laryngoscopy and tracheal intubation, with a lower incidence of adverse effects. However, although this dose regimen was effective in attenuating the end-organ responses, the effects on plasma catecholamine concentrations were unknown, and no data were available. This was investigated in Study 4 (Chapter 10).
Figure 9.1
Mean (SEM) systolic and diastolic pressures in groups 1 (SAP -□-, DAP -○-), 2 (SAP -▼-, DAP -▲-), and 3 (SAP -●-, DAP -♦-). Baseline values are represented by t=0; the timing of induction of anaesthesia and of laryngoscopy and tracheal intubation are shown by arrows.

* p<0.05 compared with baseline values before induction of anaesthesia

† p<0.05 compared with pre-intubation values

SAP and DAP decreased in all groups after induction of anaesthesia and remained below baseline values throughout the study. SAP increased slightly after intubation for 2 min in Group 2 only. DAP increased slightly after intubation for 1 min in Groups 1 and 3 and for 2 min in Group 2 (p<0.05). There were no significant differences in SAP or DAP between the groups at any time point.
Figure 9.2

Mean (SEM) MAP in groups 1 (SAP -□-, DAP -●-), 2 (SAP -▼-, DAP -▲-), and 3 (SAP -○-, DAP -♦-). Baseline values are represented by t=0; the timing of induction of anaesthesia and of laryngoscopy and tracheal intubation are shown by arrows.

* p<0.05 compared with baseline values before induction

† p<0.05 compared with pre-intubation values

MAP decreased after induction of anaesthesia and remained below baseline values throughout the study (p<0.05) in all groups. MAP increased slightly after intubation for 1 min in Groups 1 and 3 and for 2 min in Group 2 (p<0.05). There were no significant between-group differences in MAP at any time point.

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Figure 9.3

Mean (SEM) heart rate in groups 1 (SAP -□-, DAP -○-), 2 (SAP -▼-, DAP -▲-) and 3 (SAP -●-, DAP -◆-). Baseline values are represented by t=0; the timing of induction of anaesthesia and of laryngoscopy and tracheal intubation are shown by arrows.

* p<0.05 compared with baseline values before induction
† p<0.05 compared with pre-intubation values
‡ p<0.05 compared with Groups 1 and 2

HR was stable in all groups with no significant changes after induction of anaesthesia or intubation. HR in Group 3 decreased after intubation in Group 3 to below baseline values, and was significantly lower compared with Groups 1 and 2 both before and after tracheal intubation.
CHAPTER 10. THE EFFECTS OF REMIFENTANIL ON THE PLASMA CATECHOLAMINE RESPONSE TO LARYNGOSCOPY AND TRACHEAL INTUBATION (STUDY 4).

10.1 Introduction

10.2 Study design

10.3 Results

10.4 Discussion

10.5 Conclusions

10.6 Figures
10.1 Introduction

In Studies 1 and 3 it was established that remifentanil attenuated the cardiovascular responses to laryngoscopy and tracheal intubation in healthy patients. These responses are associated with increases in plasma epinephrine and norepinephrine concentrations (Shribman et al. 1987, Derbyshire et al. 1987, Miller et al. 1993). As discussed in Chapter 4, although several types of drugs or manoeuvres will attenuate changes in heart rate and arterial pressure, many of these do not affect the associated plasma catecholamine response. Indeed, beta blocking drugs may actually exaggerate the increases in plasma catecholamine concentrations caused by laryngoscopy and tracheal intubation (Thompson et al. 1997, Maguire et al. 2001). Other opioids attenuate the increase in plasma catecholamines associated with surgery (Stanley et al. 1980) and with laryngoscopy and tracheal intubation (Crawford et al. 1987, Scheinin et al. 1989) but the effects of remifentanil were not known. Study 4 was therefore designed to investigate the effects of a remifentanil bolus/infusion regimen on cardiovascular and plasma catecholamine responses to induction of anaesthesia and laryngoscopy and tracheal intubation.

The results of this study were presented at the Anaesthetic Research Society Meeting, Newcastle, March 2001 and published in abstract form in the British Journal of Anaesthesia (Gregg et al. 2001).

10.2 Methods

Following approval by the Leicestershire Research Ethics Committee, and written informed consent, 40 ASA I-II female patients were studied. Inclusion and exclusion criteria are described in Chapter 6. Patients were divided into two groups in a double-blind randomised fashion to receive either intravenous remifentanil (0.5 mcg kg\(^{-1}\) bolus over 30 s, followed by remifentanil 0.25 mcg kg\(^{-1}\) min\(^{-1}\) infusion) (Group R) or a saline placebo bolus and infusion (Group S). Anaesthesia was then induced with propofol (0.5 mg kg\(^{-1}\) followed by 10 mg every 10 s until loss of verbal contact) and rocuronium 0.6 mg kg\(^{-1}\) to produce neuromuscular blockade. Patients' lungs were ventilated manually with
1% isoflurane, 66% nitrous oxide and 33% oxygen until laryngoscopy and tracheal intubation and mechanically thereafter (tidal volume 10 ml kg\(^{-1}\), maintaining end-tidal CO\(_2\) 4.0-4.5 kPa) (Blease Medical Ltd, Chesham, Buckinghamshire, England). Laryngoscopy and tracheal intubation were performed 3 min after induction of anaesthesia. Arterial pressure and HR were measured as previously described at 1 min intervals from before induction of anaesthesia to 5 min after intubation.

Blood from an ante-cubital fossa vein was sampled via a pre-inserted cannula into a lithium-heparin tube. Samples were taken immediately before and 1.5 min after induction of anaesthesia, and 1, 3 and 5 min after intubation. All the sample tubes were transported in ice, centrifuged within 15 min at 3000 rpm for 5 min, then stored at -70°C. Plasma epinephrine and norepinephrine concentrations were assayed using reverse-phase high-pressure liquid chromatography with electrochemical detection. Further details of the assays of plasma catecholamine concentrations, study methodology and statistical analysis are described in Chapter 6.

### 10.3 Results

One patient in Group R was excluded because of difficulty obtaining blood samples during the study, so data from 19 patients in Group R and 20 patients from Group S were analysed. Baseline observations, age, height, weight and grade of intubation were comparable in both groups (Table 10.1). The mean dose of propofol required to induce anaesthesia was significantly lower in group R compared with group S (Table 10.1). The lower limit of detection of inter- and intra-assay coefficients of variation for were 2.51% and 3.96% for norepinephrine, and 3.14% and 4.50% for epinephrine respectively. Changes in HR and arterial pressure over time are shown in Figures 10.1-10.3.

Baseline SAP was significantly higher in Group S, but MAP, DAP and HR were similar. Arterial pressure (SAP, MAP and DAP) decreased after induction of anaesthesia in both groups. There was no change in arterial pressure at laryngoscopy and tracheal intubation in Group R and arterial pressure remained below baseline values throughout the study period. Arterial pressure increased significantly for 1-2 min after intubation in Group S, but decreased below baseline values by 4-5 min after intubation. HR remained stable in Group R throughout the study, but increased in Group S after induction of anaesthesia and
increased further after laryngoscopy and tracheal intubation. HR and arterial pressure were significantly higher after intubation in Group S compared with Group R.

Changes in plasma epinephrine and norepinephrine concentrations are shown in Figures 10.4 and 10.5. Plasma epinephrine concentrations decreased after induction of anaesthesia, and remained below baseline values throughout the study, with no response to laryngoscopy and tracheal intubation. Plasma norepinephrine concentrations decreased slightly in both groups after induction of anaesthesia (p=ns), but increased in Group S after intubation, and were significantly higher than in Group R.

Table 10.1 Patient characteristics and baseline data

<table>
<thead>
<tr>
<th></th>
<th>Group R</th>
<th>Group S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41 (14.8)</td>
<td>37 (10.7)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.63 (0.06)</td>
<td>1.63 (0.07)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.1 (8.9)</td>
<td>60.4 (9.7)</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>81.9 (16.3)</td>
<td>78.0 (14.6)</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>133.8* (19.1)</td>
<td>121.5 (18.0)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>94.9 (12.3)</td>
<td>87.8 (14.4)</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>76.3 (10.9)</td>
<td>72.1 (14.3)</td>
</tr>
<tr>
<td>Propofol dose (mg)</td>
<td>117 * (27)</td>
<td>153 (30)</td>
</tr>
<tr>
<td>Propofol dose (mg kg⁻¹)</td>
<td>2.60 * (0.59)</td>
<td>1.88 (0.58)</td>
</tr>
<tr>
<td>View at laryngoscopy (1/2/3)</td>
<td>14 /5</td>
<td>16/3/1</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD or range) or number. n = 19 in Group R and n = 20 in Group S. Baseline HR and arterial pressures are the mean of 3 values taken immediately before induction of anaesthesia. *p<0.05 between groups.

Adverse events

Transient hypotension (isolated SAP reading < 80 mmHg) was observed in one patient in Group S and two patients in Group R required escape medication to treat hypotension (SAP < 80 mm Hg for > 1 min). Transient hypertension (SAP > 180 mm Hg for < 1 min) was noted in one patient in Group S but no patients in either group required escape medication for tachycardia, bradycardia or hypertension.
In this study, remifentanil (bolus 0.5 mcg kg\(^{-1}\) followed by an infusion of 0.25 mcg kg\(^{-1}\) min\(^{-1}\)) prevented the cardiovascular and plasma norepinephrine response to laryngoscopy and tracheal intubation. Plasma norepinephrine concentrations decreased in both groups after induction of anaesthesia, and increased after laryngoscopy and tracheal intubation in Group S. Plasma epinephrine concentrations decreased after induction of anaesthesia and remained below baseline throughout. Arterial pressure and HR increased by approximately 25-30 mm Hg and 15 min\(^{-1}\) respectively after intubation in Group S. These increases are slightly less than those observed in the control group in Study 1, although they are consistent with previous studies (Crawford et al. 1987, Achola et al. 1988, Scheinin et al. 1989). However, a number of other investigators have demonstrated pressor responses of greater magnitude than observed here (Dahlgren & Messeter 1981, Derbyshire et al. 1983, Korpinen et al. 1995). The effects of remifentanil on the catecholamine response to laryngoscopy and tracheal intubation were as predicted, and confirm the effects of other opioids (Mustola et al. 1995, Derbyshire et al. 1987, Shribman et al. 1987). Increases in arterial pressure, HR and plasma catecholamine concentrations in this study were short-lived and absolute values exceeded baseline for only 2-3 min after intubation. The magnitude of these changes in Group S was less than anticipated; and is small in comparison with previous work (Dahlgren & Messeter 1981, Derbyshire et al. 1983, Korpinen et al. 1995). The factors affecting the size of the pressor response to laryngoscopy and tracheal intubation include patient characteristics, co-existing disease and medications, the anaesthetic drugs and precise technique used at induction of anaesthesia, and the laryngoscopic technique of the investigators. These factors are detailed in Chapter 4. The results of this study, in particular the possible explanations for the relatively small increase in arterial pressure, heart rate and plasma catecholamine concentrations observed in both groups, are discussed in more detail in Chapter 13.
10.5 Conclusions

The results of this study confirmed that remifentanil (0.5 mcg kg\(^{-1}\) followed by an infusion of 0.25 mcg kg\(^{-1}\) min\(^{-1}\)) attenuates the cardiovascular and plasma catecholamine responses to laryngoscopy and intubation in healthy patients. The patient group of most interest are those at high risk of exaggerated pressor responses or those at increased risk of morbidity resulting from these responses. However, there were no published data relating to the effects of remifentanil in these patient groups. Having established the therapeutic efficacy of remifentanil and discovered some of its associated adverse effects, it was now appropriate to investigate the effects of remifentanil in higher-risk patients. I therefore chose to study the cardiovascular effects of remifentanil at induction of anaesthesia in treated hypertensive patients (Study 5) and in normotensive elderly patients (Study 6).
Figure 10.1

Mean (SEM) systolic and diastolic pressures in remifentanil (SAP - ■-, DAP-▼-) and saline (SAP-□-, DAP -▼-) groups. Baseline values are represented by t=0; the timing of induction of anaesthesia and laryngoscopy and tracheal intubation are shown by arrows.

* p<0.05 between groups

† p<0.05 compared with pre-intubation values

SAP and DAP decreased in both groups after induction of anaesthesia. In Group R, SAP and DAP remained below baseline values throughout the study, with no significant changes at intubation. In Group S, SAP and DAP increased significantly after intubation but had returned to below baseline values by 5 min after intubation. Both SAP and DAP were significantly lower in Group R from before intubation up to the end of the study.
MAP decreased in both groups after induction of anaesthesia, and remained below baseline values throughout the study in Group R, with no significant changes at intubation. In Group S, MAP increased significantly after intubation but had returned to below baseline values by 4 min after intubation. MAP was significantly lower in Group R from before intubation up to the end of the study.
Figure 10.3

Mean (SEM) heart rate in remifentanil (▼-) and saline (□-) groups

* p<0.05 between groups

† p<0.05 compared with pre-intubation values

HR increased in Group S after induction of anaesthesia and increased further after intubation. In Group R, HR decreased slightly after induction of anaesthesia and remained below baseline throughout the study, with no significant changes at intubation. HR was significantly lower in Group R from before intubation up to the end of the study.
Figure 10.4

Mean (SEM) plasma norepinephrine concentrations in remifentanil (••••) and saline (△△△△) groups

† p<0.05 between groups and compared with pre-intubation values

* p<0.05 compared with baseline

Plasma norepinephrine concentrations decreased in both groups after induction of anaesthesia although changes were not statistically significant. Norepinephrine concentrations increased significantly after intubation in Group S but remained stable in Group R and were below baseline values at the end of the study.
Figure 10.5

Mean (SEM) plasma epinephrine concentrations in remifentanil (-●-) and saline (-Δ-) groups

* p<0.05 compared with baseline

Plasma epinephrine concentrations decreased in both groups after induction of anaesthesia and remained below baseline values throughout the study, with no significant response to tracheal intubation. There was no difference between the groups at any time point.
CHAPTER 11. THE EFFECT OF REMIFENTANIL ON THE CARDIOVASCULAR RESPONSE TO LARYNGOSCOPY AND TRACHEAL INTUBATION IN HYPTERTENSIVE PATIENTS (STUDY 5).

11.1 Introduction
11.2 Study design
11.3 Results
11.4 Discussion
11.5 Conclusions
11.6 Figures
11.1 Introduction

In Studies 1-4 it was established that remifentanil effectively attenuated the cardiovascular and catecholamine responses to laryngoscopy and tracheal intubation in healthy young adult patients. However, the populations at risk from these responses are primarily those with cardiovascular or cerebrovascular disease, but also patients with both treated and untreated essential hypertension, who display both exaggerated responses (Prys-Roberts et al. 1971a, 1971b, Low et al. 1986) and have a greater incidence of complications resulting from these responses (Steen et al. 1978, Stone et al. 1988a, Kleinman et al. 1986, Moffitt et al. 1985). For various reasons (Thomson 1989), few studies of the haemodynamic response to intubation have been carried out in hypertensive patients or those at risk of developing myocardial ischaemia and none had used remifentanil. In contrast to Studies 1-4, it was considered unethical to use a placebo control group, and I decided to compare remifentanil with a standard treatment, alfentanil. Alfentanil is an opioid with a rapid onset of effect, but its duration of action is longer than remifentanil, and its pharmacokinetics less predictable (Glass et al. 1993b). Study 6 was therefore designed to compare the relative effects of remifentanil and alfentanil in modifying the haemodynamic response to intubation in patients receiving long-term treatment (>6 months) for hypertension.

The results of this study were presented to the Anaesthetic Research Society, University of Edinburgh, in November 1999 and the abstract published in the British Journal of Anaesthesia (Maguire et al. 2000). The full paper was published in the British Journal of Anaesthesia (Maguire et al. 2001).

11.2 Study design

Following approval by the Leicestershire Research Ethics Committee and informed consent of 40 ASA II-III patients aged 33-78 years, receiving long-term treatment (>6 months) for hypertension and undergoing elective surgery requiring tracheal intubation were recruited. Criteria for exclusion and escape medications are described in more detail in Chapter 6. Patients were randomised in a double-blind fashion into two treatment groups to receive either remifentanil (Group R) or alfentanil (Group A).
Patients were unpremedicated and received their usual antihypertensive drugs on the day of surgery. All patients received intravenous Hartmanns solution 5 ml kg\(^{-1}\) over 5-10 min before induction of anaesthesia. Patient's lungs were pre-oxygenated for 3 min and glycopyrrrolate 200 mcg was administered iv followed by the study drug over 30 seconds and by an infusion, as described below. At induction of anaesthesia, Group R (n = 20) received a bolus of remifentanil of 0.5 mcg kg\(^{-1}\) over 30 s followed by an infusion of remifentanil at 0.1 mcg kg\(^{-1}\) min\(^{-1}\). Group A (n = 20) received a bolus of alfentanil 10 mcg kg\(^{-1}\) over 30 s followed by an infusion of saline at the same rate (in ml h\(^{-1}\)) as Group R. Infusions of remifentanil (Group R) and saline (Group A) were continued throughout the study period. Immediately after the study drug, a standard general anaesthetic was administered, comprising propofol 0.5 mg kg\(^{-1}\) followed by 10 mg every 10 s until loss of verbal contact, and rocuronium 0.6 mg kg\(^{-1}\) to produce neuromuscular blockade. Patients’ lungs were ventilated manually to an end-tidal carbon dioxide tension of 4.0-4.5 kPa with isoflurane 1% and nitrous oxide 66% in oxygen until laryngoscopy and tracheal intubation, and mechanically thereafter (tidal volume 10 ml kg\(^{-1}\)) (Blease Medical Ltd, Chesham, Buckinghamshire, England). Following establishment of neuromuscular blockade, confirmed with a nerve stimulator (Fisher Paykell NS272, Fisher Paykell Electronics Ltd, Auckland, New Zealand), laryngoscopy and tracheal intubation were performed, 3 min after induction of anaesthesia. Heart rate (HR), systolic (SAP), mean (MAP) and diastolic arterial pressure (DAP) were measured non-invasively (as described in Chapter 6) at one min intervals from pre-oxygenation to five min after intubation. The duration of laryngoscopy and any difficulties in laryngoscopy or tracheal intubation were noted. Power calculations were based on data from study 1, as there are no recent published data relating to this patient population. This showed that 20 patients per group would detect a difference of 15 mm Hg in MAP or of 15 beats min\(^{-1}\) in HR after intubation (α=0.05, β=0.1). Further details of the methodology including criteria for administration of escape medication, and methods of statistical analysis are described in Chapter 6.

11.3 Results

Patient characteristics, baseline haemodynamic variables (SAP, DAP, MAP, HR) and antihypertensive medication were similar (Tables 11.1-11.2). There were significant
changes over time in SAP, DAP, MAP and HR (p<0.001) but no difference at any time point between groups (Figure 11.1-11.4). Arterial pressure (SAP, DAP, MAP) decreased significantly following induction of anaesthesia in both groups (p<0.05 within groups). Changes in HR after induction of anaesthesia were minimal, but there was a significant increase in HR following intubation in both groups (p<0.05) (Figure 11.1). Mean maximum HR occurred 1 min after intubation in both groups, and was 87 min\(^{-1}\) (12 min\(^{-1}\) above baseline, p=0.065) in the remifentanil group, and 89 min\(^{-1}\) (15 min\(^{-1}\) above baseline, p<0.05) in the alfentanil group. Arterial pressure (SAP, MAP and DAP) increased significantly after intubation in both groups (p<0.05 compared with pre-intubation) (Figures 11.2-11.4). The increase in arterial pressure was sustained for longer (4-5 min) in the alfentanil group than in the remifentanil group (2 min). The greatest mean increase in SAP occurred two min after intubation in both groups, (35 mm Hg, and 41 mm Hg greater than pre-intubation values in groups R and A respectively). However, arterial pressure in both groups remained below baseline pre-induction values throughout, and was significantly lower compared with baseline towards the end of the study period (p<0.05 from 4-5 min after intubation) (Figures 11.2-11.4).

**Adverse events**

Seven patients in Group R and four in Group A required ephedrine 3-9 mg to treat hypotension (SAP < 100 mm Hg). Marked hypotension, (defined as SAP < 80 mm Hg for > 1 min) occurred in three patients in the remifentanil group and two in the alfentanil group. There was no clear relationship between type of antihypertensive medication and requirement for escape medication (Table 11.2) and data from all patients, including those who required escape medication, were analysed. Three patients in Group A required an increase in the inspired concentration of isoflurane to treat hypertension (increase in SAP of > 30% more than baseline values), but SAP > 200 mm Hg occurred in only one patient (Group A). No patient required treatment for bradycardia. Transient ST segment depression associated with tachycardia (HR 100-105 min\(^{-1}\)) occurred in one patient in Group R following intubation. This resolved spontaneously within three minutes without specific treatment. No other ST segment changes were observed.
Table 11.1  Patient characteristics and baseline data

<table>
<thead>
<tr>
<th></th>
<th>Group R</th>
<th>Group A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 (33-78)</td>
<td>63 (49-78)</td>
</tr>
<tr>
<td>Sex M:F</td>
<td>10 : 10</td>
<td>10 : 10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.2 (14.7)</td>
<td>77.0 (11.9)</td>
</tr>
<tr>
<td>Propofol dose (mg)</td>
<td>95.3 (26.3)</td>
<td>95.3 (24.8)</td>
</tr>
<tr>
<td>Propofol dose (mg kg⁻¹)</td>
<td>1.27 (0.28)</td>
<td>1.24 (0.29)</td>
</tr>
<tr>
<td>Baseline SAP (mmHg)</td>
<td>159 (26)</td>
<td>162 (20)</td>
</tr>
<tr>
<td>Baseline MAP (mmHg)</td>
<td>106 (19)</td>
<td>111 (15)</td>
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<tr>
<td>Baseline DAP (mmHg)</td>
<td>85 (15)</td>
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<td>Duration of laryngoscopy (sec)</td>
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<td>View at laryngoscopy (1/2/3)</td>
<td>15/4/1</td>
<td>13/5/2</td>
</tr>
<tr>
<td>Grade of anaesthetist intubating (Consultant/SpR/SHO)</td>
<td>6/13/1</td>
<td>3/16/1</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD, or range), or number. n = 20 in each group. Baseline arterial pressures and HR are the mean of 3 values taken immediately before the start of the study. There were no significant differences between the two groups.
Table 11.2 Concurrent antihypertensive medication, and requirements for escape medication, according to treatment group

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<th>Group R</th>
<th>Group A</th>
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<tbody>
<tr>
<td>Diuretic</td>
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<td>2 (1)</td>
</tr>
<tr>
<td>Beta-blocker</td>
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<td>4 (1)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>3 (2)</td>
<td>1</td>
</tr>
<tr>
<td>Calcium Channel blocker</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Combination therapy</td>
<td>10 (4)</td>
<td>10 (2)</td>
</tr>
</tbody>
</table>

Figures in brackets refer to numbers of patients who required ephedrine to treat hypotension (SAP < 100 mmHg)

11.4 Discussion

This study showed that the effect of a bolus dose of remifentanil 0.5 mcg kg\(^{-1}\) followed by a 0.1 mcg kg\(^{-1}\) min\(^{-1}\) infusion was similar to that of a 10 mcg kg\(^{-1}\) bolus of alfentanil at induction of anaesthesia, laryngoscopy and tracheal intubation in treated hypertensive patients. HR increased above baseline values after intubation in both groups but increases in HR and arterial pressure were not considered clinically significant, and arterial pressure remained below baseline values. The increase in HR above pre-intubation values was sustained for longer in the alfentanil group. This is probably because remifentanil was administered by intravenous infusion whereas after only a single bolus dose, plasma and effect site alfentanil concentrations would have been declining by this time (Shafer & Varvel 1991).

Mean decreases in SAP after induction of anaesthesia were 55-60 mm Hg in this study, although decreases in MAP and DAP were less. These decreases are greater than in studies 1, 3 and 4, which were performed in healthy young adults, and occurred despite intravenous fluid preloading and pre-treatment with glycopyrrolate. In most cases the hypotension was moderate (i.e. SAP > 80 mm Hg), and responded well to small doses of ephedrine. In contrast to Studies 1 and 3, no patient required treatment for bradycardia.
The incidence of hypotension confirmed that cardiovascular responses to induction of anaesthesia are exaggerated in hypertensive patients. The absolute increases in arterial pressure at intubation were also greater than in Study 3, (mean SAP increased by 35-40 mm Hg compared with 10-15 mm Hg in Study 3) reflecting either the lower infusion regimen used (0.1 mcg kg\(^{-1}\) min\(^{-1}\)) or a greater cardiovascular response to laryngoscopy and tracheal intubation in hypertensive patients. However, the incidence of mild and marked hypotension observed in the present study implies that higher doses of either opioid would have been inappropriate (Crawford et al. 1987). The occurrence of transient ST depression despite treatment measures also confirms that this group is at risk of myocardial ischaemia. These issues are discussed in further detail in Chapter 13.

11.5 Conclusions

The results of this study showed that remifentanil was as effective to an established treatment, alfentanil, in attenuating the responses to laryngoscopy and tracheal intubation in patients receiving long-term treatment for hypertension, in lower doses than previously recommended. It also confirmed that hypertensive patients demonstrate exaggerated cardiovascular responses to induction of anaesthesia. Elderly patients are also at increased risk of marked cardiovascular responses to induction of anaesthesia and tracheal intubation, and I therefore decided to examine the effects of a similar remifentanil regimen in normotensive elderly patients.
Mean (SEM) systolic and diastolic pressures in remifentanil (SAP -■-, DAP-▼-) , and alfentanil (SAP-□-, DAP -▼-) groups  Baseline values are represented by t=0; the timing of induction of anaesthesia and of laryngoscopy and tracheal intubation are shown by arrows.

*  p<0.05 compared with baseline values

†  p<0.05 compared with pre-intubation

SAP and DAP decreased in both groups after induction of anaesthesia. SAP increased after intubation for 4 min in Group A and for 2 min in Group R but remained below baseline values throughout. DAP increased for 5 min in Group A and for 2 min in Group R. There were no significant differences between the groups at any time point during the study.
Figure 11.2

Mean (SEM) mean arterial pressure in remifentanil (-■-) and alfentanil (-○-) groups

* p<0.05 compared with baseline values
† p<0.05 compared with pre-intubation

MAP decreased in both groups after induction of anaesthesia. MAP increased after intubation for 3 min in Group A and for 2 min in Group R but remained below baseline values throughout. There were no significant differences between the groups at any time point during the study.
HR remained stable after induction of anaesthesia in both groups. HR increased after intubation for 5 min in Group A and for 2 min in Group R but HR in Group R was not significantly greater than baseline. There were no significant between-group differences at any time point during the study.
CHAPTER 12. COMPARISON OF THE EFFECTS OF REMIFENTANIL AND ALFENTANIL ON THE CARDIOVASCULAR RESPONSE TO TRACHEAL INTUBATION IN THE ELDERLY (STUDY 6).

12.1 Introduction

12.2 Study design

12.3 Results

12.4 Discussion

12.5 Conclusions

12.6 Figures
12.1 Introduction

In Study 5 it was established that remifentanil attenuated the cardiovascular response to laryngoscopy and tracheal intubation in patients receiving long-term treatment for hypertension. Elderly patients make up an increasing proportion of patients presenting for anaesthesia and surgery. In addition to diminished physiological reserve and alterations in autonomic function (Barnett et al. 1999, Barontini et al. 1997), they have an increased incidence of hypertension, coronary artery and cerebrovascular disease (Harris et al. 1985). The pharmacokinetic and pharmacodynamic effects of opioids and anaesthetic drugs are altered in the elderly (Scott & Stanski 1987, Minto et al. 1997a, Schnider et al. 1999). These factors may combine to make rapid fluctuations in blood pressure and heart rate during anaesthesia more likely in elderly patients (Pfeifer et al. 1983, Latson et al. 1994) with increased risk of resulting morbidity. However, there were few data regarding the haemodynamic responses to intubation in the elderly (Chung & Evans 1985) and none detailing the effects of remifentanil. The aim of this study was to assess the effects of remifentanil in modifying the haemodynamic response to intubation in elderly patients (aged > 65 years) and to compare it with alfentanil.

The results of this study have been accepted for publication as a full paper by the British Journal of Anaesthesia (Habib et al. 2002).

12.2 Study design

Following approval by the Leicestershire Research Ethics Committee, and written informed consent, 40 ASA I-III patients aged > 65 years, undergoing elective surgery requiring tracheal intubation were recruited. Criteria for exclusion and escape medications are described in more detail in Chapter 6.

Patients were allocated at random to two groups, (Group R = remifentanil, Group A = alfentanil), in a double-blind manner. No premedication was administered. All patients received intravenous Hartmanns solution 5 ml kg⁻¹ over 5-10 min before induction of anaesthesia. Routine monitoring was instigated; HR, SAP, MAP and DAP were measured as described in Chapter 6 and recorded at 1 min intervals throughout the study. All
patients received glycopyrrolate 0.2 mg iv. Patients in Group R (n = 20) then received a bolus of remifentanil (0.5 mcg kg\(^{-1}\) over 30 s) followed by an infusion of remifentanil 0.1 mcg kg\(^{-1}\) min\(^{-1}\). Group A (n = 20) received a bolus of alfentanil (10 mcg kg\(^{-1}\) over 30 s) followed by an infusion of saline. Infusions of remifentanil (Group R) and saline (Group A) were continued throughout the study. Immediately after the administration of the study drug a standard general anaesthetic was administered, comprising propofol 0.5 mg kg\(^{-1}\) followed by 10 mg every 10 s until loss of verbal contact and rocuronium 0.6 mg kg\(^{-1}\) to produce neuromuscular blockade. Patients’ lungs were ventilated manually with isoflurane 1% and nitrous oxide 66% in oxygen, to an end-tidal carbon dioxide tension of 4.0-4.5 kPa until intubation and mechanically thereafter (tidal volume 10 ml kg\(^{-1}\), Blease Medical Ltd, Chesham, Buckinghamshire, England). Establishment of neuromuscular blockade was confirmed with a peripheral nerve stimulator (Fisher Paykell NS272, Fisher Paykell Electronics Ltd, Auckland, New Zealand), and laryngoscopy and tracheal intubation were then performed 3 min after induction of anaesthesia. The duration of laryngoscopy, grade of anaesthetist and any difficulties in laryngoscopy or tracheal intubation were noted. Power calculations based on previous data in elderly patients (Kirby et al. 1988, Sweeney et al. 1989) suggested that 20 patients per group would detect a 15% difference in HR (\(\alpha = 0.05, \beta = 0.1\)) or a 15% difference in SAP between the groups after intubation (\(\alpha = 0.05, \beta = 0.2\)). This approximates to differences of 13 beats min\(^{-1}\) in HR or of 22 mm Hg in SAP. Further details of the methodology of this study are described in Chapter 6.

12.3 Results

One patient in Group R was withdrawn from the study owing to unanticipated difficulty in intubation (the duration of laryngoscopy was > 120 s). Since the cardiovascular responses to laryngoscopy and tracheal intubation are related to the duration of laryngoscopy and to the forces applied (Stoelting 1977, Bucx et al. 1992b, Hassan et al. 1991), data from this patient were excluded from the analysis. Data from 19 patients in Group R and 20 patients in Group A were analysed and patient details are shown in Table 12.1. Cardiovascular data are shown in Figures 12.1-12.3.
Table 12.1 Patient characteristics and baseline data

<table>
<thead>
<tr>
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</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>73.1 (65-83)</td>
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<tr>
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<td>11/9</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<td>69.8 (13.5)</td>
</tr>
<tr>
<td>ASA grade (1/2/3)</td>
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<td>7/12/1</td>
</tr>
<tr>
<td>Propofol dose (mg)</td>
<td>76.7 (25.0)</td>
<td>76.1 (23.9)</td>
</tr>
<tr>
<td>Propofol dose (mg kg⁻¹)</td>
<td>1.15 (0.27)</td>
<td>1.19 (0.35)</td>
</tr>
<tr>
<td>Baseline SAP (mm Hg)</td>
<td>158.6 (17.1)</td>
<td>153.4 (23.3)</td>
</tr>
<tr>
<td>Baseline MAP (mm Hg)</td>
<td>102.9 (11.2)</td>
<td>106.3 (15.1)</td>
</tr>
<tr>
<td>Baseline DAP (mm Hg)</td>
<td>78.9 (11.0)</td>
<td>82.7 (10.7)</td>
</tr>
<tr>
<td>Baseline HR (min⁻¹)</td>
<td>76.6 (13.9)</td>
<td>76.9 (9.6)</td>
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<tr>
<td>Duration of laryngoscopy (s)</td>
<td>14.7 (9.8)</td>
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<td>View at laryngoscopy (1/2/3)</td>
<td>12/6/1</td>
<td>17/2/1</td>
</tr>
<tr>
<td>Grade of anaesthetist intubating</td>
<td>3/12/4</td>
<td>2/16/2</td>
</tr>
<tr>
<td>(SHO/SpR/Consultant)</td>
<td></td>
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</tr>
</tbody>
</table>

Data expressed as mean (SD, or range), or number. n = 19 in Group R and n = 20 in Group A. Baseline arterial pressures and HR are the mean of 3 values taken immediately before induction of anaesthesia. There were no significant differences between the two groups.

Within-group changes in SAP, MAP, DAP and HR over time were statistically significant (p<0.001) but there were no differences between groups in SAP, MAP or HR at any time point. SAP, MAP and DAP decreased after induction of anaesthesia in both groups (p<0.05 compared with baseline values) (Figures 12.1-12.3) and increased for 3 min after intubation (p< 0.05 compared with pre-intubation) before decreasing gradually, but mean values of SAP and MAP remained below baseline throughout the study period. DAP remained below baseline values in the group R throughout but slightly exceeded baseline for 2 min after intubation in Group A (p=ns). DAP in Group R was significantly lower 4 to 5 min after intubation (p<0.05 compared with Group A) but there were no other significant differences in arterial pressure or HR throughout the study.
HR remained stable after induction of anaesthesia but increased after intubation in both
groups to exceed baseline values (p<0.05 compared with both baseline and with pre-
intubation). The increase in HR was sustained for longer in group A but differences in HR
between the groups were not statistically significant (Figure 12.3). Laryngoscopy was
prolonged (>20 s) in four patients in each group but mean duration of laryngoscopy was
similar in both groups. There were no differences between the groups in terms of duration
of, or difficulties with, intubation and no correlations between difficulties with intubation,
requirement for escape medication or grade of anaesthetist. Duration of intubation
correlated significantly with difficulty of intubation as indicated by Cormack and Lehane
grade of laryngoscopy (Spearmans rho = .645, p<0.001).

Adverse events
Four patients in Group R and three in Group A required ephedrine for hypotension (SAP
< 100 mmHg for > 1 min). However, nine patients in Group R and eight in Group A had
transient hypotension (isolated SAP reading of < 100 mmHg) which did not require
escape medication. Isolated single readings of SAP > 180 mm Hg) were noted in three
patients in Group R and four patients in Group A; one patient in Group R and two in
Group A received an increased inspired concentration of isoflurane to treat hypertension
(SAP > 180 mm Hg for > 60 s). There were no incidences of bradycardia or tachycardia
in either group and no arrhythmias, ST segment or other ECG changes were observed
during the study.

12.4 Discussion
This study showed that the cardiovascular effects of remifentanil (0.5 mcg kg⁻¹ over 30 s
followed by an infusion of 0.1 mcg kg⁻¹ min⁻¹) and alfentanil (10 mcg kg⁻¹ over 30 s) at
induction of anaesthesia and intubation were similar. Differences in DAP between 4-5
minutes after intubation are probably explained by the bolus/infusion regimen of
remifentanil compared with the single bolus of alfentanil, similar to the differences
observed in study 5. Transient hypotension (SAP < 100 mm Hg for < 60 seconds) after
induction of anaesthesia occurred in almost 50% of patients despite intravenous fluid
preloading and glycopyrrolate pre-treatment, although there were large variations
between individuals. This confirms that many elderly patients are susceptible to
exaggerated fluctuations in arterial pressure and HR at induction of anaesthesia (Pfeifer et al. 1983, Latson et al. 1994). However, observed hypotension in this study was mostly mild (SAP < 100 mmHg) and self-limiting. More marked hypotension (SAP < 80 mm Hg as a single reading) occurred in only three patients and requirements for escape medications were low in both groups.

There was also a large variation in response to laryngoscopy and tracheal intubation between individuals. Mean SAP increased by a 25-35 mm Hg and HR increased by approximately 20 min⁻¹ at laryngoscopy and tracheal intubation in both groups. The maximum HR and arterial pressure response occurred 2 min after laryngoscopy and tracheal intubation, as previously described. However, although the mean values of SAP at this time were 140.6 mm Hg and 143.5 mm Hg in Groups R and A respectively, individual values ranged from 100 to 193 mm Hg in Group R, and from 92 to 196 mm Hg in Group A. These findings suggest that the attenuation of cardiovascular responses was incomplete in some, with large variations between individual patients. However, the overall incidence and degree of hypotension is likely to have been greater had higher doses of remifentanil been used.

12.5 Conclusions

The conclusions from this study were that remifentanil and alfentanil had similar cardiovascular effects at induction of anaesthesia, laryngoscopy and tracheal intubation. Although observed increases in arterial pressure and HR at intubation were statistically significant, they were modest and clinically acceptable. The doses used here were lower than those previously shown to be effective in young adults but mild hypotension was noted in a number of patients. The results of this study and the implications are discussed further in Chapter 13.
Figure 12.1
Mean (SEM) systolic and diastolic pressures in remifentanil (SAP-■-, DAP-▼-) and alfentanil (SAP-□-, DAP-▼-) groups. Baseline values are represented by t=0; the timing of induction of anaesthesia, laryngoscopy and tracheal intubation are shown by arrows.

* p<0.05 compared with baseline values, both groups

† p<0.05 compared with pre-intubation, both groups

‡ p<0.05 between groups

SAP and DAP decreased in both groups after induction of anaesthesia, and increased after intubation for 3 min in both groups, but remained below baseline values throughout. Values were significantly below baseline from 4-5 min after intubation. DAP was significantly lower in group R compared with Group A from 4-5 minutes after intubation (p<0.05) but were no other significant differences between the groups during the study.
**Figure 12.2**

Mean (SEM) MAP in remifentanil (■) and alfentanil (○) groups

* p<0.05 compared with baseline values, both groups

† p<0.05 compared with pre-intubation, both groups

MAP decreased in both groups after induction of anaesthesia, and increased after intubation for 3 min in Group A and for 2 min in Group R but remained below baseline values throughout. There were no significant differences between the groups at any time point during the study.
Figure 12.3

Mean (SEM) HR in remifentanil (■) and alfentanil (○) groups.

* p<0.05 compared with baseline values

† p<0.05 compared with pre-intubation

HR remained stable after induction of anaesthesia in both groups, and increased after intubation for 4 min in Group A and for 1 min in Group R. There were no significant between-group differences at any time point during the study.
CHAPTER 13. DISCUSSION

13.1 Attenuation of the cardiovascular and sympathetic responses to laryngoscopy and tracheal intubation by remifentanil

13.2 Attenuation of the cardiovascular response to emergence from anaesthesia and tracheal extubation by remifentanil

13.3 Determination of an optimum dose of remifentanil

13.4 The use of remifentanil in higher risk patient groups

13.5 Clinical significance of the effects of remifentanil on cardiovascular response to laryngoscopy and tracheal intubation

13.6 Conclusions
The preceding sections have outlined the pharmacology of the opioid remifentanil, and its use in anaesthesia to attenuate the cardiovascular responses to laryngoscopy and tracheal intubation. The first pre-clinical data relating to remifentanil were published in the early 1990s, and it was introduced into clinical practice in the U.K. in 1996. At its introduction, it was recommended by the manufacturer as being a potent analgesic suitable to supplement other anaesthetic drugs during surgery, which could be given by intravenous bolus or infusion. Owing to its unique pharmacokinetic properties, it was suggested to be useful for the attenuation of autonomic responses to noxious stimuli. Furthermore, in contrast to the other available opioid analgesics, it was proposed that remifentanil could be administered in relatively high doses and titrated rapidly to the desired clinical effect without compromising recovery from anaesthesia, (Glaxo Wellcome 1996). These properties represented a significant potential advance in clinical anaesthesia, and its introduction into clinical practice was anticipated with interest by many editorial writers, reviewers and clinicians, including the author of this thesis (Rosow 1993, James 1994, Glass 1995, Thompson & Rowbotham 1996, Michelsen & Hug 1996).

The work presented in this thesis includes the first independent published corroboration of these potential advantages in several disparate groups of patients, and also documented some of the adverse effects associated with remifentanil. During the last five years, a number of other studies have confirmed these findings and the use of remifentanil during anaesthesia has since become an established technique. Further data supporting the concept that attenuation of adverse cardiovascular responses during anaesthesia and surgery reduces cardiovascular morbidity have been published. The purpose of this section is to discuss these issues, to interpret the findings of the studies detailed above and to outline possible future developments.
13.1 Attenuation of the cardiovascular and sympathetic responses to laryngoscopy and tracheal intubation by remifentanil

The data presented in Chapter 7 (Study 1) demonstrated that remifentanil effectively attenuated the increase in heart rate and arterial pressure caused by laryngoscopy and tracheal intubation in healthy patients. These findings were as anticipated, as alfentanil, which has a similar time to onset of clinical effect (Egan et al. 1996a) had previously been shown to attenuate these responses (Black et al. 1984, Crawford et al. 1987, Korpinen et al. 1995, Rout & Rocke et al. 1990, Scheinin et al. 1989). This effect of remifentanil was later confirmed by others (McAtamney et al. 1998, O'Hare et al. 1999, Alexander et al. 1999). McAtamney and colleagues found that HR and SAP increased significantly at laryngoscopy and tracheal intubation (by 15-20%) after remifentanil administered as a bolus of 1 mcg kg\(^{-1}\) after induction of anaesthesia. However, this dose was more effective than doses of 0.5 and 0.25 mcg kg\(^{-1}\) (McAtamney et al. 1998), where values were not significantly different from a saline control group. The same authors also compared three different bolus doses of remifentanil (0.5, 1.0 and 1.25 mcg kg\(^{-1}\)) during rapid-sequence induction of anaesthesia and found that 0.5 mcg kg\(^{-1}\) was ineffective in controlling the pressor response, whereas 1.25 mcg kg\(^{-1}\) was associated with hypotension (SAP less than 90 mm Hg) in seven out of twenty patients. Changes in HR and arterial pressure after intubation were minimal in the group receiving remifentanil 1.0 mcg kg\(^{-1}\) (O'Hare et al. 1999). Both these studies were performed in a similar patient group to those enrolled in Study 1, but used a bolus of remifentanil without a subsequent infusion, administered after intravenous thiopental for induction of anaesthesia. Heart rate increased after induction of anaesthesia and before laryngoscopy in all groups in both studies, and bradycardia was not reported. The apparently greater effect of remifentanil we found in Study 1 is explained by differences in the timing of drug administration, our use of propofol rather than thiopental for induction of anaesthesia and the use of a bolus dose of remifentanil without a subsequent infusion in these other studies. The absence of bradycardia is attributable to the use of thiopental, and the use of rocuronium for neuromuscular blockade. Propofol causes a decrease in heart rate, either alone (Tramer et al. 1997) or when administered in combination with other drugs (Jensen et al. 1995) and its cardiovascular effects at induction of anaesthesia are greater compared with thiopental (Mulier et al. 1991, Lindgren et al. 1993). Vecuronium may be associated with bradycardia (Inoue et al. 1988, McCoy 1988) because it has no direct cardiovascular
effects, and therefore does not oppose the negative chronotropic effects of other drugs or manoeuvres during anaesthesia (Hunter et al. 1987). The occurrence of bradycardia after remifentanil has subsequently been confirmed, particularly when it is co-administered with propofol (Elliott et al. 2000, Kazmaier et al. 2000).

Although the technique may have contributed to the occurrence of bradycardia, the use of a bolus/infusion regimen in the studies described in this thesis rapidly results in stable plasma and effect site remifentanil concentrations. This technique is also logical when remifentanil is to be continued intra-operatively. Remifentanil given before induction of anaesthesia decreases the subsequent dose of intravenous anaesthetic required, and propofol was titrated to loss of verbal contact, thereby minimising the required dose. The pressor response reaches a peak 1-2 min after laryngoscopy and intubation and usually subsides within 5-6 min, although tachycardia may persist for 10 min (Singh et al. 1995). The effect site half-life of a remifentanil bolus is 3.2 min, and the use of a bolus/infusion regimen to attenuate cardiovascular responses to laryngoscopy and tracheal intubation is therefore rational, as acknowledged by other authors (McAtamney et al. 1998). An earlier study using a total intravenous anaesthetic technique (comprising propofol 0.5-1.0 mg kg⁻¹ bolus followed by 75 mcg kg⁻¹ min⁻¹) reported that remifentanil (1.0 mcg kg⁻¹ bolus followed by a 1.0 mcg kg⁻¹ min⁻¹ infusion) attenuated the cardiovascular response to laryngoscopy and tracheal intubation more effectively than a lower dose of remifentanil (1.0 mcg kg⁻¹ bolus followed by a 0.5 mcg kg⁻¹ min⁻¹ infusion) (Hogue et al. 1996). The mean increases in SAP at intubation were approximately 15 mm Hg and 25 mmHg in the two groups respectively and despite intravenous fluid preloading, HR and arterial pressure both decreased by 10-40% after induction of anaesthesia. Hypotension (SAP < 80 mmHg for > 1 min) occurred in 10-15% of patients, but bradycardia requiring escape medication (HR < 40 min⁻¹ for > 1 min) was not reported. The incomplete attenuation of responses to intubation combined with the incidence of hypotension suggests that the regimen used in this study is not ideal.

The other main significant findings of Study 1 were that bradycardia (HR < 45 min⁻¹) or hypotension (systolic pressure < 80 mm Hg) occurred in 5 out of 10 patients in the remifentanil group. In all cases, it responded to intravenous atropine or ephedrine. Furthermore, intravenous glycopyrrolate 0.2 mg attenuated the decrease in HR caused by remifentanil, and only 1 patient in the remifentanil-glycopyrrolate group required escape
medication for hypotension or bradycardia. This protective effect of glycopyrrolate confirmed previous suggestions (Sebel et al. 1995), although no definitive data were available. Bradycardia and asystole have been associated with other mu opioid agonists fentanyl and alfentanil, particularly when co-administered with propofol (Egan & Brockutne 1991, Hiller & Saarnivaara 1992, Vuyk et al. 1996) and more recently with the combination of propofol and remifentanil (Altermatt & Munoz 2000, Elliott et al. 2000, Kazmaier et al. 2000). However, it had not previously been emphasised as a significant potential problem with remifentanil.

In Study 4, (Chapter 10) we also found that remifentanil attenuated the accompanying increase in plasma catecholamine concentrations in healthy young adult patients. Resting plasma epinephrine and norepinephrine concentrations in healthy individuals are approximately 0.1 ng ml\(^{-1}\) and 0.3 ng ml\(^{-1}\) respectively. Venous concentrations may be higher than those in arterial blood because of metabolism in the lungs, which takes up approximately 30% of venous catecholamines. Plasma catecholamine concentrations reflect sympathetic nervous system activity and increase in response to exercise, pain, surgery, or other stressful stimuli. Surgery causes a 2-5 fold increase, severe pain or hypovolaemia cause a 10-20 fold increase, and in shock, plasma concentrations may increase by up to 50 fold (Derbyshire & Smith 1984). Concentrations are increased in patients who are anxious immediately before induction of anaesthesia (Fell et al. 1985) Most epinephrine in the circulation is secreted by the adrenal medulla. Basal adrenal medullary epinephrine secretion is approximately 0.2 mcg kg\(^{-1}\) min\(^{-1}\) and basal secretion of norepinephrine is 0.05 mcg kg\(^{-1}\) min\(^{-1}\). The plasma half-lives of both catecholamines are approximately 1 min, as they are rapidly inactivated by neuronal and extra-neuronal re-uptake and metabolism (Langer & Hicks 1984). Stimulation of the sympathetic nerves to the adrenal medulla causes an increase in adrenal catecholamine secretion, of which approximately 80% is epinephrine. This is detectable in the plasma and causes widespread effects on organs or tissues that have little or no sympathetic nervous innervation, as described in Chapter 2. It also causes the effects of sympathetic stimulation to persist for 1-2 minutes after the original stimulus has diminished. The characteristic response to induction of anaesthesia is of a decrease in plasma epinephrine and norepinephrine concentrations (Derbyshire et al. 1987, Magnusson et al. 1983, Low et al. 1986, Scheinin et al. 1989, Chraemmer-Jorgensen et al. 1992). The potent stimulus of laryngoscopy and tracheal intubation causes an increase in sympathetic nervous system
activity (Ebert et al. 1990) and plasma catecholamine concentrations (Russell et al. 1981, Miller et al. 1993) which correlate with the accompanying increases in heart rate and arterial pressure (Derbyshire et al. 1983, 1984). Shribman and colleagues found that after laryngoscopy was maintained for 10 seconds, plasma norepinephrine and epinephrine concentrations increased significantly. There was no further increase when tracheal intubation was performed, implying that the majority of the stimulus was caused by laryngoscopy (Shribman et al. 1987). Conversely, others have found that tracheal intubation has an additional effect on arterial pressure and heart rate (Bucx 1992b). The increase in plasma catecholamine concentrations may be exaggerated by pre-existing hypertension (Low et al. 1986) and uraemia (Kirvela et al. 1995), and is related to the force and duration of laryngoscopy (Hassan et al. 1991). The decrease in plasma catecholamine concentrations after induction of anaesthesia is greater and the subsequent increase after tracheal intubation is also less when using propofol compared to thiopental (Lindgren et al. 1993, Mustola et al. 1995). This may explain why plasma epinephrine concentrations did not increase after intubation in Study 4, and were lower in the control group compared with previous studies (Derbyshire et al. 1983, 1987, Achola et al. 1988).

The catecholamine response to laryngoscopy and tracheal intubation is attenuated by alfentanil (Crawford et al. 1987, Scheinin et al. 1989), fentanyl (Chraemmer-Jorgensen et al. 1992) and increasing depth of anaesthesia (Turner et al. 1986). Topical lidocaine has no effect (Derbyshire et al. 1987). However, beta blockers, which attenuate the changes in arterial pressure and heart rate, may actually increase plasma catecholamine concentrations at laryngoscopy and tracheal intubation (Magnusson et al. 1983, Achola et al. 1988, Thompson et al. 1997, Maguire et al. 2001). This implies that although end-organ responses may be attenuated, underlying sympathetic nervous system activity is unaffected or even magnified by beta blockade. There is much current interest in the use of beta blocking drugs, after recent studies have shown that they improve outcome after major surgery in patients at high risk of cardiovascular disease (Mangano et al. 1996, Wallace et al. 1996, Poldermans et al. 1999). The rationale for using beta blockers perioperatively is that they reduce tachycardia and myocardial oxygen demand, both of which are associated with myocardial ischaemia, (Raby et al. 1999, Stone et al. 1988a, Warltier et al. 2000). However, increased sympathetic nervous activity and elevated norepinephrine concentrations cause vasoconstriction, increased myocardial oxygen demand and are associated with perioperative myocardial ischaemia (Backlund et al.

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In order to decrease perioperative cardiovascular responses in high-risk patients, it is therefore logical to employ measures that attenuate neurohumeral sympathetic nervous responses, rather than those which decrease end-organ effects alone.

13.2 Attenuation of the cardiovascular response to emergence from anaesthesia and tracheal extubation by remifentanil

In Study 2, we confirmed the hypothesis that remifentanil 1 mcg kg⁻¹ when given as a slow intravenous bolus at the end of surgery would attenuate the increase in MAP and HR associated with emergence from anaesthesia and tracheal extubation, without compromising clinical recovery. Tracheal extubation is associated with an increase in arterial pressure, HR, plasma catecholamine concentrations (Lowrie et al. 1992), intracranial pressure (Miller et al. 1995) and causes myocardial ischaemia in susceptible patients (Edwards et al. 1994). It was therefore inferred that this remifentanil regimen might be useful before tracheal extubation, endotracheal toilet or physiotherapy in surgical and Intensive Care patients at risk from neurological or cardiovascular disease, or for the attenuation of responses to other brief but noxious stimuli.

It had been anticipated that the incidence of coughing at extubation might be decreased by a bolus of remifentanil. Coughing causes an increase in intracranial pressure and the attenuation of coughing at tracheal extubation is desirable, particularly in patients with neurological disease (Miller et al. 1995). Although the incidence of ‘severe’ coughing or gagging was lower in the remifentanil group, the overall quality of extubation was not significantly different. This may be because the timing of extubation was delayed by remifentanil and the effect site concentrations would have been low (<1.0 ng ml⁻¹) (Appendix 3). This delay is predictable based on the pharmacokinetics of remifentanil and is not clinically important. The lack of difference may be also because of a Type II statistical error, or other variables that were not assessed (e.g. incidence of smoking). Alternatively, limitations in the method of assessment of coughing might be responsible, and a more objective measure of coughing or measurement of intracranial pressure might have been preferable. However, the primary goal of this study was examination of the cardiovascular effects of remifentanil at extubation; these measurements might be appropriate in further studies of higher risk groups.
Previous methods used to attenuate the pressor response to emergence from anaesthesia and tracheal extubation have included the use of beta blockers (Dyson et al. 1990, O’Dwyer et al. 1993), alfentanil (Fuhrman et al. 1992), fentanyl (Nishina et al. 1995a), local anaesthetics (Bidwai et al. 1979), and calcium channel blockers (Mikawa et al. 1996a, 1996b, Nishina et al. 1995b) or vasodilators (Nishina et al. 1996). Others have used combinations of these drugs (Mikawa et al. 1997, Nishina et al. 1997) or have avoided the issue by performing tracheal extubation under deep anaesthesia (Miller et al. 1995). These methods are not universally effective, and may cause hypotension, delayed recovery from anaesthesia and postoperative respiratory depression (Miller et al. 1995, Fuhrman et al. 1992). With the exception of opioids, these specific manoeuvres are also unlikely to attenuate increases in plasma catecholamine concentrations (Mikawa et al. 1996b). However, longer-acting opioids are more likely to compromise recovery. Delayed recovery is unlikely after a bolus of remifentanil, as shown by the tendency towards less drowsiness shortly after tracheal extubation in the remifentanil group in Study 2. Further studies are required to establish whether the use of a remifentanil bolus would attenuate the response to tracheal extubation or to other brief, noxious stimuli in higher risk patients.

13.3 Determination of an optimum dose of remifentanil

In Study 3 (Chapter 9), the cardiovascular effects of a bolus dose of remifentanil 0.5 mcg kg\(^{-1}\) followed by an infusion of 0.25 mcg kg\(^{-1}\) min\(^{-1}\) were similar to remifentanil 1.0 mcg kg\(^{-1}\) bolus and infusion of 0.5 mcg kg\(^{-1}\) min\(^{-1}\). Heart rate was significantly lower in patients who did not receive glycopyrrolate, but the incidence of bradycardia and hypotension was low, and all regimens effectively prevented tachycardia or hypertension after laryngoscopy and tracheal intubation. Following a change in hospital pharmacy policy, vecuronium was no longer available, and the neuromuscular blocking drug used in this study was rocuronium. Rocuronium has a mild vagolytic effect and is associated with a lower incidence of bradycardia than vecuronium (McCoy et al. 1993, Stevens et al. 1997) although values of mean MAP and HR immediately before laryngoscopy were similar in Studies 1 and 3, suggesting that the influence of the neuromuscular blocking drug on HR was moderate.
Other data have supported the findings that lower doses of remifentanil were effective in attenuating the cardiovascular response to laryngoscopy and tracheal intubation. McAtamney and colleagues found that when remifentanil 0.5 mcg kg$^{-1}$ was administered 1 min before laryngoscopy, HR and SAP increased at intubation by 30% and 16% respectively compared with baseline values and by approximately 20% and 10% compared with pre-intubation values. The increase in SAP was similar to a group receiving 1.0 mcg kg$^{-1}$ and returned to baseline values within 2 min of intubation (McAtamney et al. 1998). Guignard and colleagues found very similar cardiovascular responses to those observed in Study 3, both before and after intubation, when using an infusion of remifentanil to a target concentration of 2-4 ng ml$^{-1}$ (Guignard et al. 2000). The calculated remifentanil concentrations at 3-4 min after the start of the infusion using the dosage regimens in Study 3 were 3.6-4.0 ng ml$^{-1}$. These concentrations also correlate very closely with the values obtained 1 min after a bolus dose of remifentanil 1.0 mcg kg$^{-1}$ with no subsequent infusion (Appendix 3). This may explain the findings that during rapid-sequence induction of anaesthesia with thiopental 5-7 mg kg$^{-1}$ and succinylcholine 1.0 mg kg$^{-1}$, a bolus dose of remifentanil 1.0 mcg kg$^{-1}$ was more effective than remifentanil 0.5 mcg kg$^{-1}$ in attenuating the cardiovascular response to laryngoscopy and tracheal intubation, and produced less hypotension than remifentanil 1.25 mcg kg$^{-1}$ (O’Hare et al. 1999). Barclay and Kluger used a target-controlled infusion of propofol in combination with remifentanil and reported that a bolus doses of 2 mcg kg$^{-1}$ and 4 mcg kg$^{-1}$ completely blocked the pressor response; 1 mcg kg$^{-1}$ was partially effective (Barclay & Kruger 2000). However, in this latter study, SAP and HR decreased considerably after all doses of remifentanil (to approximately 90-95 mm Hg and 57-62 min$^{-1}$ respectively) throughout the study period. These values were significantly below baseline and although no escape medication was administered, published details were sparse. This approach increases the risks of hypotension and bradycardia and may therefore be inappropriate for clinical practice.

The differences between these studies may be related to the intravenous anaesthetic drug and regimen used, the timing of drug administration, or the use of a bolus of remifentanil. The rationale for the bolus/infusion regimen of remifentanil is discussed above. Calculated plasma and effect site concentrations decrease rapidly after a single bolus of remifentanil. The cardiovascular effects of laryngoscopy and tracheal intubation depend
on technique, experience, duration, and the forces applied (Hassan et al. 1991, Bucx et al. 1992c, 1995). The use of a bolus/infusion regimen of remifentanil becomes particularly important if cardiovascular responses are exaggerated following difficult or prolonged laryngoscopy.

Studies 1, 3 and 4 demonstrated that remifentanil is effective in attenuating the cardiovascular and plasma catecholamine responses to laryngoscopy and tracheal intubation in healthy adults. However, it was then important to examine the effects of remifentanil in higher risk groups. Ethical considerations dictated that studies in groups at risk of morbidity should be comparative, against an established effective therapy. Studies 5 and 6 therefore compared the effects of remifentanil with alfentanil in treated hypertensive (Study 5) and elderly normotensive (Study 6) patients.

13.4 The use of remifentanil in higher risk patient groups

In Study 5 (Chapter 11), we found that the cardiovascular effects of a bolus dose of remifentanil 0.5 mcg kg\(^{-1}\) followed by a 0.1 mcg kg\(^{-1}\) min\(^{-1}\) infusion was similar to that of a 10 mcg kg\(^{-1}\) bolus of alfentanil in treated hypertensive patients at induction of anaesthesia. Although arterial pressure increased following intubation in both groups, values remained below baseline. HR increased above baseline values after intubation in both groups but increases in HR and arterial pressure were not considered clinically significant. The increase in HR above pre-intubation values was sustained for longer in the alfentanil group, probably because the remifentanil was being administered by intravenous infusion whereas after only a single bolus dose, plasma and effect site alfentanil concentrations would have been declining (Shafer & Varvel 1991).

Increases in SAP, DAP, and MAP following intubation were greater in this study than in Studies 1 and 3, performed in healthy young adults. This could be related to the lower infusion regimen used in this study: plasma and effect site remifentanil concentrations at the time of intubation were 3.1-3.2 ng ml\(^{-1}\) compared with 4.0-4.4 ng ml\(^{-1}\) in Groups 2 & 3 in Study 3 (Appendix 3). However the incidence of hypotension, despite intravenous fluid loading and pre-treatment with glycopyrrolate, confirms that hypertensive patients demonstrate exaggerated cardiovascular responses to induction of anaesthesia and
tracheal intubation (Prys-Roberts et al. 1971a, 1971b, Low et al. 1986) and implies that higher doses of remifentanil would have been inappropriate. The occurrence of myocardial ischaemia confirms that hypertensive patients are at increased risk of incidence of complications resulting from these responses (Steen et al. 1978, Stone et al. 1988a, Kleinman et al. 1986, Moffitt et al. 1985).

Previous studies showed that a bolus dose of alfentanil 10-15 mcg kg\(^{-1}\) is effective in modifying the cardiovascular response to intubation in healthy adults (Crawford et al. 1987, Miller et al. 1993) and in elderly patients (Kirby et al. 1988). Higher doses were associated with bradycardia and hypotension (Crawford et al. 1987). The bolus dose of remifentanil (0.5 mcg kg\(^{-1}\)) was chosen to be equipotent with 10 mcg kg\(^{-1}\) alfentanil, based on previous pharmacokinetic and pharmacodynamic studies showing a comparative potency ratio of 20:1 (Egan et al. 1996b, Jhaveri et al. 1997). The infusion rate of remifentanil was lower than in Study 3 because both clearance and volume of distribution of remifentanil are reduced in the elderly, and the pharmacodynamic effects are greater (Glass et al. 1999a). The similarity in results between the two groups suggests that the bolus doses of remifentanil and alfentanil chosen were comparable.

Patients in Study 5 were receiving different types of antihypertensive medication, which was not controlled between groups. However, Sear and colleagues found no difference in the cardiovascular response to intubation in patients receiving different monotherapies for mild-moderate hypertension (Sear et al. 1994) and the distribution of type of antihypertensive medication between groups in Study 5 was similar. Furthermore, the aim of Study 5 was to establish whether remifentanil was as effective as alfentanil in a cohort of hypertensive patients, rather than examine the effects in those taking particular types of antihypertensive drugs. Although no firm conclusions can be made from a study of this size, escape medication for hypotension was required by patients taking beta blockers, ACE inhibitors, diuretics and combination therapy, with no clear association between type of antihypertensive treatment and hypotension after induction of anaesthesia.

The majority of the patients in Study 5 were elderly and it was possible that the effects of remifentanil were also dependent on the patients’ age as well as hypertensive status. This was examined in Study 6, which showed that the cardiovascular effects of remifentanil (0.5 mcg kg\(^{-1}\) over 30 s followed by an infusion of 0.1 mcg kg\(^{-1}\) min\(^{-1}\)) and alfentanil (10
mcg kg\(^{-1}\) over 30 s) at induction of anaesthesia and intubation were similar in normotensive elderly patients. Changes in mean HR, SAP, MAP and DAP were comparable to other data in the elderly (Kirby et al. 1988, Splinter & Cervenko 1989). Transient hypotension (SAP < 100 mm Hg for < 60 seconds) after induction of anaesthesia occurred in almost 50% of patients despite intravenous fluid preloading and glycopyrrolate pre-treatment. However, the hypotension was mostly mild (SAP < 100 mmHg) and self-limiting. Severe hypotension (SAP < 80 mm Hg as a single reading) occurred in only 3 patients and requirements for escape medications were low in both groups. Although studies in younger adults have used higher doses of alfentanil, 10 mcg kg\(^{-1}\) was shown to be optimal in elderly patients (Kirby et al. 1988). The elderly are more susceptible to the effects of opioids and intravenous anaesthetic agents because of alterations in cardiac output, autonomic function, pharmacokinetics and pharmacodynamics (Minto et al. 1997, Schnider et al. 1999); the incidence of hypotension would have been greater if higher drug doses had been used. Conversely, arterial pressure increased by 25-35 mm Hg and HR increased by approximately 20 min\(^{-1}\) at laryngoscopy and tracheal intubation in both groups, indicating that the attenuation of cardiovascular responses was incomplete, and three patients required escape medication for hypertension. As in Study 5, there was also a wide variation in individual responses, reflected by the occurrence of hypotension and hypertension in some patients. This confirms that the elderly are susceptible to exaggerated fluctuations in arterial pressure and HR at induction of anaesthesia (Pfeifer et al. 1983, Latson et al. 1994). Previous studies in the elderly have used thiopental for intravenous induction of anaesthesia, and despite careful titration of propofol to effect in Study 6, it might have contributed to the incidence of hypotension. Propofol may cause greater decreases in arterial pressure than other intravenous anaesthetic drugs (Harris et al. 1988) and the elderly are more sensitive to its depressant effects (Dundee et al. 1986, Schnider et al. 1999). However, propofol is used widely, and it has been suggested to be the preferred intravenous anaesthetic to attenuate cardiovascular responses to intubation (Kovac 1996).

Studies 5 and 6 demonstrated that remifentanil was at least as effective as alfentanil in attenuating the cardiovascular responses to laryngoscopy and tracheal intubation in normotensive elderly patients and in those on long-term treatment for hypertension. Although observed increases in arterial pressure and HR at intubation in both studies were statistically significant, they were modest and clinically acceptable. The variability in
response and occurrence of transient myocardial ischaemia also confirmed that cardiovascular responses may be exaggerated in these patients, and that they are at risk of associated complications.

13.5 Clinical significance of the effects of remifentanil on cardiovascular response to laryngoscopy and tracheal intubation

In common with other physiological responses, the cardiovascular responses to induction of anaesthesia, laryngoscopy and tracheal intubation vary between individuals. Factors contributing to this inherent variability include pre-existing disease, cardiovascular function, autonomic tone, laryngoscopic technique and the precise anaesthetic technique employed (Kovac 1996, Ng 1997). Because of these factors, the ‘holy grail’ of an anaesthetic regimen that produces perfectly stable haemodynamics may be an impossible target. Nevertheless, it provides a relatively reproducible model for noxious stimuli during anaesthesia and surgery, and is also a more potent stimulus than skin incision which has traditionally been used to compare the potency of anaesthetic drugs (Kazama et al. 1997). Laryngoscopy and tracheal intubation causes stimulation of predominantly vagal, but also trigeminal and glossopharyngeal afferent fibres which transmit impulses to the brain stem. There, they synapse with vagal and sympathetic nuclei in the nucleus tractus solitarius (NTS) and the nucleus ambiguus. These nuclei are in close association with nuclei involved with cardiovascular regulation and autonomic function, and laryngoscopy and tracheal intubation causes increased efferent sympathetic nervous activity. This results in a pressor response characterised by increased postganglionic sympathetic nervous activity, cardiovascular and adrenal medullary stimulation. Reflex activation of vagal efferent fibres may cause bradycardia in some circumstances but this is typically overwhelmed by the effects of sympathetic nervous stimulation. In some patients, the cardiovascular and autonomic responses to laryngoscopy and tracheal intubation are harmful per se, but the logical corollary is that a drug or technique that attenuate these responses will effectively attenuate similar responses to other noxious stimuli.

Some of the physiological actions of endogenous opioid peptides are well defined, whilst others remain to be clarified. However, they have widespread actions within the parts of
the central nervous system controlling cardiovascular and autonomic function, as well as direct and indirect actions on the heart and peripheral vasculature. Opioid drugs have potent effects on cardiovascular and autonomic function and the studies described here have established that remifentanil attenuates the cardiovascular effects of laryngoscopy and tracheal intubation in different groups of patients. Extrapolation from pharmacokinetic models suggests that a plasma or effect site remifentanil concentration of approximately 4-5 ng ml⁻¹ is required. The pharmacodynamic effects of opioids are dose-related and higher doses may increase the risks of hypotension and bradycardia. Although the experimental model of laryngoscopy and tracheal intubation is a relatively reproducible stimulus, the inherent variability in the cardiovascular response to induction of anaesthesia between individuals dictates that a perfect regimen may be impossible to attain. Although intravenous anaesthetic drugs are titrated to clinical effect (loss of consciousness), the administration of a predetermined bolus of drug to attenuate cardiovascular effects arising from a subsequent stimulus will always produce adverse effects in some patients. The effects of laryngoscopy and tracheal intubation are dynamic and begin to subside after 2-3 minutes. If hypotension or bradycardia were observed at this time in clinical practice, the infusion rate of remifentanil would be decreased. However, in the studies outlined in this thesis, almost all adverse cardiovascular events (hypotension or bradycardia) occurred before laryngoscopy and a decreasing infusion regimen would not have affected this. Conversely, opioids are known to enhance the cardiovascular effects of propofol and the administration of a bolus of remifentanil in conjunction with propofol may have contributed to the incidence of bradycardia and hypotension observed. It has been recommended that remifentanil boluses are avoided and future studies should investigate whether the use of a simple remifentanil infusion regimen (to achieve stable plasma and effect site concentrations) before induction of anaesthesia is associated with fewer adverse effects.
13.6 Conclusions

Remifentanil attenuates the cardiovascular and plasma catecholamine responses to laryngoscopy and tracheal intubation in healthy individuals. It also attenuates these cardiovascular responses in elderly and hypertensive patients, who are at increased risk both of cardiovascular disease and of complications related to laryngoscopy and tracheal intubation. It is also suitable for use during anaesthesia and surgery where the attenuation of cardiovascular and autonomic responses is desirable. Induction of anaesthesia is a period of potential cardiovascular instability, and remifentanil can cause bradycardia and hypotension if administered in the dose regimens previously recommended. Remifentanil administered as a bolus attenuates the cardiovascular responses to emergence from anaesthesia and tracheal extubation without compromising clinical recovery.

The clinical implications of the results of the studies detailed in this thesis extend beyond the experimental circumstances described. When introduced into clinical practice, it was suggested that remifentanil would be suitable to produce profound analgesia and attenuation of reflex cardiovascular responses for not only for brief but also prolonged noxious stimuli, such as those occurring during surgery (Egan 1995, Glass 1995, Thompson & Rowbotham 1996). In both types of situation, the unique pharmacokinetics of remifentanil would allow it to be administered in adequate doses without prolonged effects. These properties also had the theoretical advantage that it could be used to titrate flexibly the degree of intra-operative analgesia required dependent on a varying surgical stimulus. The studies reported in Chapters 7-12 and the subsequent clinical experience of myself and others have confirmed that remifentanil can be used in this way, and that the characteristics of remifentanil when used in clinical practice are as predicted from its pharmacokinetic and pharmacodynamic properties. In particular, it produces stable haemodynamic parameters and suppression of autonomic reflexes when used in conjunction with a balanced volatile anaesthetic technique for major abdominal, vascular, orthopaedic or cardiac surgical procedures, whilst still allowing rapid recovery at the end of surgery (Kovac et al. 1996, Howie et al. 1996, Song et al. 2000, Ahonen et al. 2000, Thompson J.P., unpublished observations). It has also been used to produce rapid alterations in the depth of intraoperative analgesia to facilitate neurological assessment during spinal surgery (Kimball-Jones et al. 1999). In addition remifentanil can be successfully titrated to a changing surgical stimulus, and in a slow bolus dose of 1-2 mcg
it effectively attenuates the cardiovascular responses to aortic cross-clamping (Thompson J.P., unpublished observations). The data contained in this thesis confirm that remifentanil can be used to attenuate the responses to brief but noxious stimuli in both low and high-risk patients, without significantly prolonging recovery from anaesthesia. It is likely anaesthetists will use remifentanil as part of a balanced anaesthetic technique for this purpose, and that the sparing effect of remifentanil on other concomitant volatile and intravenous anaesthetic drugs will allow a more rapid recovery at the end of surgery than is possible with previously available opioid drugs. Further work is required to establish the role of remifentanil in attenuating responses to other noxious stimuli and to ascertain whether the attenuation of noxious stimuli by remifentanil affects perioperative outcome. Other possible applications of remifentanil include its use in patients not undergoing surgery, although few data are currently available. Future studies should therefore address its place as an analgesic/sedative drug for use outside the operating room, for example in Intensive Care Units.
CHAPTER 14. APPENDICES

Appendix 1. Leicestershire Research Ethics Committee (LREC) reference numbers for the studies in this thesis

Study 1 (Chapter 7)  LREC reference number 4507
Study 2 (Chapter 8)  LREC reference number 4509
Study 3 (Chapter 9)  LREC reference number 4769
Study 4 (Chapter 10) LREC reference number 4854
Study 5 (Chapter 11) LREC reference number 5125
Study 6 (Chapter 12) LREC reference number 5124
Appendix 2. Volumes of remifentanil 20 mcg ml\(^{-1}\) added to make dilutions for study drug bolus of 1.0 mg kg\(^{-1}\), according to patients body weight

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Drug volumes were further diluted to 10 ml before administration over 30 s.
Appendix 3. Calculated plasma and effect site concentrations (in ng ml\(^{-1}\)) for the remifentanil bolus/infusion regimens used in each study.

Concentrations are based on the mean ages and weights from the patients in each study. ES = effect site.

### Table

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<td></td>
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<td>Blood</td>
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<td></td>
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<td>3.8</td>
</tr>
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<tr>
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<td>Blood</td>
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<td>ES</td>
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<tr>
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Variations in plasma and effect site concentrations with identical remifentanil bolus/infusion regimens are a consequence of differences in patient age and weight between studies.

In studies 1 and 3–6, anaesthesia was induced immediately after the administration of the remifentanil bolus, and laryngoscopy and tracheal intubation was performed 3–4 minutes after the remifentanil bolus. In study 2, which was performed at emergence from anaesthesia, tracheal extubation was performed at a variable time (mean 7.2 minutes) after the administration of remifentanil.

In studies 5 and 6, the bolus/infusion regime was designed to achieve steady plasma and effect site concentrations of remifentanil within 3 minutes.
Appendix 4. Publications arising from work detailed in this thesis

Papers


Abstracts


CHAPTER 15. REFERENCES


229


Ebert, T.J. (1990). Nitrous oxide produces differential effects on baroreflex control of heart rate and peripheral sympathetic nerve activity. Anesthesiology, 72, 16-22


232


of total intravenous anesthesia with remifentanil and propofol for elective inpatient surgery. *Anesthesia and Analgesia*, 83, 279-285


King, B.D., Harris, L.C., Greifenstein, F.E., Elder, J.D., & Dripps, R.D. (1951). Reflex circulatory responses to direct laryngoscopy and tracheal intubation performed under general anaesthesia. *Anesthesiology, 12*, 556-566


245


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inflammatory response correlate with organ failure in patients with multiple injuries. 
*Journal of Trauma, 42*, 446-454


Schultz, J.J., Hsu, A.K., Gross, G.J. (1997a). Ischemic preconditioning is mediated by a peripheral opioid receptor mechanism in the intact rat heart. *Journal of Molecular and Cellular Cardiology, 29*, 1355-1362

Schultz, J.J., Hsu, A.K., Gross, G.J. (1997b). Ischemic preconditioning and morphine-induced cardioprotection involve the delta opioid receptor in the intact rat heart. *Journal of Molecular and Cellular Cardiology, 29*, 2187-2195


262


Shapoval, L.N. The role of structures of the ventrolateral medulla in cardiovascular regulation. *Neurophysiology, 24*, 483-499


Song, D. Whitten, C.W., White, P.F. Remifentanil infusion facilitates early recovery for obese outpatients undergoing laparoscopic cholecystectomy. Anesthesia and Analgesia, 90, 1111-1113


lumen endobronchial intubation and the effect of esmolol. *Anaesthesia, 52,* 790-794

*Canadian Journal of Anaesthesia, 36,* 367-369

reflexes elicited by mechanical stimulation of the respiratory tract. *Journal of Physiology,
200,* 25-49

Lippincott Williams & Wilkins: Philadelphia 3-24

Causation, frequency and severity. *British Journal of Anaesthesia, 78,* 642-651

Trendelenberg, F. (1871). Beitrage zur den operationen au den Luftwegen Tamponade
der Trachea. *Arch J Klin Chir, 12,* 121

cardiovascular and plasma catecholamine responses to tracheal intubation. *British Journal
of Anaesthesia, 58,* 1365-1370

control of the sinus node. *Chest, 64,* 203-212

Urthaler, F.; Isobe, J.H.; James, T.N. (1975) Direct and vagally mediated chronotropic
effects of morphine studied by selective perfusion of the sinus node in awake dogs. *Chest,
68,* 222-228

Urthaler, F., Walker, A.A., James, T.N. (1976). Comparison of the inotropic action of
morphine and ketamine studies in canine cardiac muscle. *Journal of Thoracic and
Cardiovascular Surgery, 72,* 142-149

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