The α₁L-adrenoceptor is an alternative phenotype of the α₁A-adrenoceptor

Carl P. Nelson

Department of Cell Physiology & Pharmacology, University of Leicester, Henry Wellcome Building, Leicester, LE1 9HN, UK.

Summary

Despite over two decades of research, the molecular identity of the α₁L-adrenoceptor phenotype has remained elusive. Gray et al. (2008) present results in this issue of the journal that provide persuasive evidence that the in vivo α₁L-adrenoceptor phenotype requires the expression of the α₁A-adrenoceptor gene. They have shown that in mice lacking the functional α₁A-adrenoceptor gene, α₁L-mediated responses to noradrenaline in prostate smooth muscle are substantially attenuated. These findings support earlier evidence that the α₁L-adrenoceptor profile represents a functional phenotype of the α₁A-adrenoceptor gene product, but additional cell background-dependent factors must act in concert with the α₁A-adrenoceptor protein to determine whether an α₁L- or a classical α₁A-adrenoceptor profile is expressed. The challenge remains to establish the nature of these cellular factors and the mechanism(s) by which they influence G-protein coupled receptor pharmacology.

Keywords: α-adrenoceptor, G-protein coupled receptor, phenotypic pharmacology, cell background, knockout mice.
Abbreviations: BPH, benign prostatic hyperplasia; CHO, Chinese hamster ovary; GPCR, G-protein coupled receptor.
In the ‘post-genomic’ era, much attention has been focused on the study of the physiological roles and endogenous ligands for previously un-discovered GPCRs, identified from genomic sequencing (so-called ‘de-orphanisation’). In contrast, the α₁-adrenoceptor field has struggled with the opposite conundrum – a pharmacologically-defined receptor phenotype which has resisted molecular definition. Genes have been identified for three isoforms of the α₁-adrenoceptor (termed α₁A, α₁B and α₁D), but a fourth α₁-adrenoceptor phenotype, the α₁L-adrenoceptor, has until now been defined purely on the basis of a characteristically low affinity for a number of selective antagonists, including prazosin (Guimaraes & Moura, 2001). However, this phenotype is of physiological significance, as the α₁L-adrenoceptor profile has been identified in a variety of tissues, across a number of different species (see Guimaraes & Moura, 2001), where it regulates smooth muscle contractility in the vasculature and the lower urinary tract. It may also be of clinical relevance, as α₁-adrenoceptor antagonists such as tamsulosin are a front line therapy for benign prostatic hyperplasia (BPH), where they effectively and selectively relax prostatic smooth muscle, providing symptomatic relief for BPH patients (Milani & Djavan, 2005).

It has previously been proposed that the α₁L phenotype may represent an alternative conformational state of the α₁A-adrenoceptor gene product (Ford et al., 1997). When recombinantly expressed in CHO cells, the α₁A-adrenoceptor exhibited a classical α₁A-adrenoceptor profile in radioligand binding assays in membrane homogenates, but in [³H]-inositol phosphate accumulation assays in intact cells, a number of antagonists (including prazosin and 5-methylurapidil) displayed lower affinities, consistent with the pharmacological profile of the α₁L-adrenoceptor (Ford et al., 1997). In addition,
while the native α₁-adrenoceptor expressed in rat prostate smooth muscle exhibited an α₁A profile in membrane radioligand binding assays, the functional (contractile) phenotype in the same tissue was that of an α₁L-adrenoceptor (Hiraoka et al., 1999). These (and other) early studies therefore pointed to the α₁L-adrenoceptor being the functional manifestation of the α₁A-adrenoceptor gene product. However, the dependence upon assay conditions (i.e. functional assays in intact cells/tissues versus radioligand binding assays in membrane homogenates) of the observed phenotype, allied to the fact that functional α₁A profiles can be observed in some tissues (see Guimaraes & Moura 2001 and references therein) has confounded attempts to establish the relationship between the α₁A- and α₁L-adrenoceptors.

An analogous situation to the atypical pharmacological profile of the α₁L-adrenoceptor is that of the putative β₄-adrenoceptor, a phenotype defined by resistance to classical β-adrenoceptor antagonists and activation by so-called ‘non-conventional partial agonists’ (Kaumann, 1989). The molecular identity of this phenotype (as a novel ‘state’ of the β₁-adrenoceptor) was identified by the use of ‘knockout’ mice lacking combinations of β-adrenoceptors (see Granneman, 2001 and references therein). In this issue of the journal, Gray et al. (2008) apply a similar approach to provide the first definitive evidence that the manifestation of the α₁L-adrenoceptor phenotype (at least in mouse prostate smooth muscle) is dependent upon the expression of the α₁A-adrenoceptor gene product. Using a range of antagonists known to display selectivity between the α₁A- and α₁L-adrenoceptor profiles, the authors have previously characterised the noradrenaline-mediated contraction of mouse prostate smooth muscle as being mediated by an α₁L-adrenoceptor (Gray & Ventura, 2006). In the
present study, Gray and colleagues utilised ‘knockout’ mice lacking a functional $\alpha_{1A}$-adrenoceptor gene (Rokosh & Simpson, 2002), to investigate the role of this gene in the observed $\alpha_{1L}$ in vivo phenotype. They found that responses to noradrenaline were attenuated by approximately 80% in prostates from mice homozygous for the disrupted $\alpha_{1A}$-adrenoceptor gene, compared with wild-type mice, providing strong evidence that the expression of the $\alpha_{1L}$-adrenoceptor in mouse prostate smooth muscle requires the presence of a functional $\alpha_{1A}$-adrenoceptor gene (Gray et al., 2008).

In addition, the authors also examined contractile responses to electrical field stimulation, an experimental paradigm more closely resembling physiological stimulation. This contraction was partially inhibited by prazosin and the contraction to high frequency stimulation was approximately 30% smaller in mice lacking the functional $\alpha_{1A}$-adrenoceptor than in wild-type mice (Gray et al., 2008). Importantly, the residual contraction (most probably mediated by non-adrenergic, non-cholinergic transmitters) was insensitive to prazosin, indicating that all of the $\alpha_{1A/L}$-adrenoceptor-mediated contraction was lost in the absence of the $\alpha_{1A}$ gene. The case might have been strengthened if the authors had demonstrated that the adrenergic component of the electrical field-stimulated contraction was mediated by $\alpha_{1L}$-adrenoceptors, as the authors themselves acknowledge that the receptors mediating responses to nerve stimulation could differ from those mediating the response to exogenous noradrenaline. However, together with their findings with exogenously applied noradrenaline, these data provide the strongest evidence thus far that the $\alpha_{1A}$-adrenoceptor gene is essential for the generation of the $\alpha_{1L}$-adrenoceptor phenotype.
Providing that the dependence of the $\alpha_{1L}$ phenotype upon $\alpha_{1A}$-adrenoceptor gene expression is universally applicable (across all species/tissues where the $\alpha_{1L}$ phenotype has been identified), the next question to address is what determines whether an $\alpha_{1A}$-adrenoceptor exhibits an $\alpha_{1L}$- or a classical $\alpha_{1A}$-adrenoceptor phenotype? The fact that functional responses in certain tissues display a classical $\alpha_{1A}$-adrenoceptor profile (see Guimaraes & Moura, 2001) suggests that the $\alpha_{1L}$-phenotype is not simply the default functional profile of the $\alpha_{1A}$-adrenoceptor gene product, raising the possibility that tissue-dependent cellular factors may govern the observed phenotype (Nelson & Challiss, 2007). It is well established that the cellular environment can influence GPCR signalling and agonist pharmacology, but the traditional view that antagonist pharmacology is independent of the cellular context may also need to be re-evaluated (Nelson & Challiss, 2007).

Evidence has recently been presented that the intact cellular environment is important for the manifestation of the in vivo $\alpha_{1L}$-adrenoceptor phenotype (Morishima et al., 2007; 2008). These studies have shown that both $\alpha_{1A}$- and $\alpha_{1L}$-adrenoceptor populations can be distinguished in radioligand binding assays in intact tissue segments, but that upon tissue homogenization and membrane preparation, the $\alpha_{1L}$-adrenoceptors are either degraded or converted to $\alpha_{1A}$-adrenoceptors (Morishima et al., 2007; 2008). Clarification of what is happening to the $\alpha_{1L}$-adrenoceptor population upon its isolation in membrane homogenates might provide valuable clues as to the cellular factor(s) responsible for shaping the pharmacological profile of the $\alpha_{1A}$-adrenoceptor gene product. Numerous mechanisms for generating phenotypic pharmacological profiles of GPCRs have been identified (see Nelson & Challiss, 2007).
and references therein) and as our appreciation of the complexity of GPCR signalling advances, so does the list of possibilities. The identification of the $\alpha_{1L}$-adrenoceptor as an alternative phenotype of the $\alpha_{1A}$-adrenoceptor represents a significant advance in our understanding of this phenomenon and will hopefully provide a springboard for future progress in elucidating the mechanisms underlying these distinct phenotypes.
References


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