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The α_{1D}-adrenergic receptor: Cinderella or ugly stepsister

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Abstract

This *Perspective* focuses on the α_{1D}-adrenergic receptor (AR), the often neglected sibling of the α_1-AR family. This neglect is in part due to its poor cell-surface expression. However, it has recently been shown that dimerization of the α_{1D}-AR with either the α_{1B}-AR or the β_2-AR increases α_{1D}-AR cell-surface expression and in this issue of *Molecular Pharmacology,* Hague *et al.* demonstrate that dimerization of the α_{1D}-AR with the α_{1B}-AR not only leads to increased cell-surface expression but also results in the formation of a novel functional entity.

Adrenergic receptors (ARs) are critical mediators of sympathetic nervous system responses, particularly those involved in cardiovascular homeostasis, such as arteriolar vasoconstriction and cardiac contraction (Graham et al., 1996). Members of the class I or rhodopsin family of G-protein-coupled receptors (GPCRs), ARs consist of nine distinct proteins divided into three subfamilies (α_1-AR, α_2-AR and β-AR). Like other members of the GPCR superfamily, ARs function as ligand-activated molecular switches coupling catecholamine binding in the extracellular region of the binding pocket, which is formed by the juxtaposition of the seven transmembrane helical segments, to GTP/GDP exchange by the cognate G-protein interacting with their cytoplasmic loops. This topological schema, together with the hydrophilicity of their catecholamine agonists that precludes ready transit across the plasma membrane lipid bilayer, posits that for productive signaling ARs must be cell-surface expressed. And, in general, such localization has been found. A possible exception is the α_{1D}-AR, a subtype initially identified not on the basis of its pharmacological profile, but by molecular cloning of its cDNA from a rat hippocampus library (Perez et al., 1991). Indeed, lack of pharmacological data for this subtype initially lead to it being misidentified as the α_{1A}-AR (Lomasney et al., 1991), rather than being a novel previously uncharacterized subtype (Graham et al., 1996). Although highly conserved in species from fish to mammals, and showing 70.5 – 71.3% identity within its transmembrane domains with the corresponding residues of the α_{1A}- and α_{1B}-ARs and utilizing similar signaling pathways, the 560 amino acid α_{1D}-AR differs from its two 'siblings' by possessing an extra long (90-95 amino acids) N-terminus (Graham et al., 1996). This may contribute to its poor cell-surface expression although maturational processing by the entire family of GPCRs, which lack a leader sequence, remains poorly understood, with only rhodopsin among the class I family showing relatively robust membrane insertion.

Assembly of rhodopsin begins with the entry of the nascent polypeptide into the endoplasmic reticulum (Khorana, 1992). After high mannose glycosylation, the molecule folds, this involves formation and insertion of the helical segments into the membrane, albeit that this insertion is not coordinated. Subsequently, a structure is formed that includes a disulfide bond between cysteine residues in the juxtamembranous regions of the first and second extracellular loops that are highly conserved in virtually all GPCRs (Graham et al., 1996; Khorana, 1992). This allows alignment of the seven helical segments and establishment of critical interactions between adjacent helical residues, with formation of the mature ligand binding pocket, and concomitantly formation of a specific cytoplasmic domain tertiary structure. Similar paradigms apply for the maturational processing of the β_2-AR (Noda et al., 1994). Unfortunately, biogenesis of the α_{1D}-AR has been little

studied, although the contribution of its long N-terminus to its poor cell-surface expression is evident from studies showing that expression is markedly enhanced if its N-terminus is truncated, or with substitution of its N-terminus with that of the α_{1B}-AR (Fig. 1A) (Hague et al., 2004a; Pupo et al., 2003). Moreover, a reciprocal chimera in which the N-terminus of the α_{1B}-AR is substituted by that of the α_{1D}-AR, shows very poor expression (Hague et al., 2004a). Such poor cell-surface expression is also observed with other GPCRs, such as the calcitonin receptor-like receptor (CRLR), which require chaperone proteins called receptor activity modifying proteins (RAMPs) (McLatchie et al., 1998), not only for transport to the cell surface, but also for determining their pharmacology and gylcosylation state (Foord and Marshall, 1999).

In addition to the low cell-surface expression of the α_{1D}-AR, which has caused some to question if the receptor protein even exists in rat tissues (Yang et al., 1998; Yang et al., 1997), it has also been suggested by some to couple less efficiently to its intracellular signaling machinery than the α_{1A}- and α_{1B}-ARs (Garcia-Sainz and Villalobos-Molina, 2004), and in the case of the rat α_{1D}-AR to exhibit constitutive activity and basal phosphorylation; albeit that such receptor-phosphorylation can be enhanced by agonist-stimulation or phorbol ester treatment (Garcia-Sainz et al., 2001; Garcia-Sainz and Villalobos-Molina, 2004). Given that receptor-phosphorylation may be coupled to desensitization and internalization, the predominant localization of the α_{1D}-AR in intracellular vesicles may be due not to tardy biogenesis and sluggish membrane insertion, but to constitutive signaling activity that predisposes it to phosphorylation, desensitization, and internalization (Fig. 1B).

In support of the former scenario, however, Hague et al. (2004) recently demonstrated that not only does the α_{1B}-AR heterodimerize with the α_{1D}-AR, but co-expression with the α_{1B}-AR in a heterologous cell system causes quantitative translocation of the α_{1D}-AR to the cell surface (Fig. 1C), whereas co-expression with the α_{1A}-AR does not (Hague et al., 2004b). In this issue of *Molecular Pharmacology*, the same authors (Hague et al.) now extend these findings and present evidence that much like the interaction between the CRLR and its RAMP, the α_{1B}-AR acts as a chaperone to allow not only cell-surface expression of the α_{1D}-AR, but a novel pharmacological profile (Fig. 1C). However, unlike RAMPs, which themselves are pharmacologically inert, the α_{1B}-AR is active, and together with the α_{1D}-AR forms a novel functional entity that displays enhanced signaling activity. Interestingly, the same laboratory describing the effects here of co-expression of the α_{1B}-AR and α_{1D}-AR, has also recently reported that the β_2-AR can similarly chaperone the α_{1D}-AR to the cell surface, albeit that in this complex its high affinity for BMY 7378 is unaltered (Fig. 1C) (Uberti et al., 2005).

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So what is Hague et al.'s evidence for this elegant transformation from dormant ugly sister to uniquely active Cinderella, so gracefully shod by an α_{1B}-glass slipper? First they show that despite confocal microscopy and Western blotting data demonstrating cell-surface localization of the α_{1D}-AR when co-expressed with the α_{1B}-AR, a binding site consistent with an α_{1D}-pharmacolgy cannot be detected. From this they conclude that heterodimerization with the α_{1B}-AR although facilitating the cell-surface translocation of the α_{1D}-AR, nevertheless induces a conformational change in the receptor protein whereby it can no longer bind compounds, such as the α_{1D}-selective antagonist BMY 7378, with high affinity. Unfortunately few such subtype selective compounds are available and this has been a major factor in limiting our understanding of α_1-AR pharmacology. To obviate this issue, Hague and colleagues cleverly take advantage of a peptide, ρ-TIA, recently isolated from the venom of the predatory marine snail, *Conus tulipa*, by Rick Lewis and colleagues (Sharpe et al., 2001), which acts as an allosteric antagonist of α_1-ARs by binding to sites distinct from the receptors' ligand binding pockets (Sharpe et al., 2003). Using this peptide and an informative mutant that this group has previously shown allows selective recognition of the α_{1D}-AR (Chen et al., 2004), they demonstrate that despite loss of high affinity BMY 7378 binding when the α_{1B}-AR is co-expressed with the α_{1D}-AR, the latter is nonetheless present on the cell-surface presumably in the form of an α_{1B}/α_{1D} heterodimerized complex. Fortunately, the negative influence of the α_{1B}-AR on high affinity BMY 7378 binding by the α_{1D}-AR does not extend to an alteration in its recognition of ρ-TIA. They then show that even when the α_{1B}-AR is co-expressed with an N-terminally truncated α_{1D}-mutant, which spontaneously traffics to the cell surface but yet can heterodimerize with α_{1B}-AR (Uberti et al., 2003), a mixed population of cell surface binding sites appears that shows both high and low BMY 7378 affinity; an effect whose magnitude is dependent on the stoichiometry of the two interacting α_1-AR subtypes. Finally, Hague and colleagues provide evidence that co-expression of the two α_1-AR subtypes results in a unique complex that now signals with greater potency then either subtype alone, and additionally that this response is still seen even with co-expression of the α_{1D}-AR with a signaling incompetent α_{1B}-AR mutant.

While one can quibble with some aspects of these studies – for example, for technical reasons it was not possible to accurately quantitate the stoichiometry of the two α_1-AR subtypes when co-expressed, so a direct effect of one on the transcriptional or translational efficiency of the other cannot be completely excluded – overall the studies point to a unique, previously unrecognized receptor interaction that has implications for our understanding of the physiology regulated by these two receptor subtypes. For example, knockout studies in which the gene for one

or more α_1-AR subtypes has been inactivated have hinted at potential crosstalk between the various subtypes. Thus, in keeping with the present findings, it has been demonstrated that removal of the α_{1B}-AR leads to the appearance of binding sites with high affinity for BMY 7378 (Daly et al., 2002; Deighan et al., 2005; Hosoda et al., 2005). This suggests that the α_{1D}-AR is indeed expressed in the vasculature and contributes to maintenance of blood pressure – a contention that is also evident from the reduced blood pressure and impaired vasoconstrictor responses to norepeinephrine of α_{1D}-knock out mice (Tanoue et al., 2002) – but that its expression is masked by the coincident expression of the α_{1B}-AR. Of course this interpretation, and the increasingly robust evidence suggesting that endogenously expressed α_1-AR heterodimerize, is predicated on more than one α_1-AR subtype being expressed in a single cell; a contention supported by studies of various cell lines (Bockman et al., 2004; Esbenshade et al., 1993), albeit one that has not yet been definitively demonstrated using a single isolated cell.

So what then of the mechanisms by which the α_{1B}-AR so elegantly envelops the nascent α_{1D}-AR, enticing her to the cell surface where she dances so vigorously with intracellular signaling partners, and yet smothering her ability to recognize usual antagonist courtiers – well these questions, while at the heart of the ball, must surely await another evening.

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Figure 1: Going to the ball: Expression of the α_{1D}-adrenergic receptor (AR) on the cell membrane

The α_{1D}-AR is poorly expressed on the cell surface. It has been proposed that this is due to (A) the length of the α_{1D}-AR's N-terminus, as truncation of the N-terminus or substitution of it with the N-terminus of the α_{1B}-AR leads to increased cell surface expression, (B) a result of constitutive activity of the receptor leading to internalization of the receptor, or (C) the need for a chaperone in the form of either the α_{1B} or β_2-AR to facilitate cell surface expression. Dimerization of the α_{1D}-AR with the α_{1B}-AR results in a single pharmacological entity which has low affinity for the α_{1D}-AR selective antagonist BMY7378; in contrast, dimerization with the β_2-AR or monomeric expression of the α_{1D}-AR leads to a receptor with high affinity for BMY7378.