0026-895X/06/6901-1–4$20.00
MOLECULAR PHARMACOLOGY
Vol. 69, No. 1
20230/3075247
Mol Pharmacol 69:1–4, 2006
Printed in U.S.A.

PERSPECTIVE

The α_{1D}-Adrenergic Receptor: Cinderella or Ugly Stepsister

Angela M. Finch and Robert M. Graham

Molecular Cardiology Program, Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia

Received October 21, 2005; accepted October 21, 2005

ABSTRACT

This *Perspective* focuses on the α_{1D}-adrenergic receptor (AR), the often neglected sibling of the α_1-AR family. This neglect is due in part 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. (p. 45) 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. In general, such localization has been found. A possible exception is the α_{1D}-AR, a subtype initially identified not based on 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 led to its misidentification as the α_{1A}-AR (Lomasney et al., 1991), rather than a novel previously uncharacterized subtype (Graham et al., 1996). Although highly conserved in species from fish to mammals, showing 70.5 to 71.3% identity within its transmembrane domains with the corresponding residues of the α_{1A}- and α_{1B}-ARs and using similar signaling pathways, the 560-amino acid α_{1D}-AR differs from its two "siblings" in that it possesses 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, which involves formation and insertion of the helical segments into the membrane, although this insertion is not coordinated. Thereafter, 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 (Khorana, 1992; Graham et al., 1996). 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

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.105.020230.
Please see the related article on page 45.

ABBREVIATIONS: AR, adrenergic receptor; GPCR, G-protein-coupled receptor; RAMP, receptor activity-modifying protein; BMY-7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione.

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) (Pupo et al., 2003; Hague et al., 2004a). Moreover, a reciprocal chimera in which the N terminus of the α_{1B}-AR is substituted with 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, that 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 glycosylation state (Foord and Marshall, 1999).

In addition to the low cell-surface expression of the α_{1D}-AR, which has caused some to question whether the receptor protein even exists in rat tissues (Yang et al., 1997, 1998), 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, although 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 caused not by tardy biogenesis and sluggish membrane insertion but by constitutive signaling activity, which 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 also coexpression 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 coexpression with the α_{1A}-AR does not (Hague et al., 2004b). In this issue of *Molecular Pharmacology*, the same authors (Hague et al., 2006) now extend these findings and present evidence that, much like the interaction between the calcitonin receptor-like receptor and its RAMP, the α_{1B}-AR acts as a chaperone to allow not only cell-surface expression of the α_{1D}-AR but also a novel pharmacological profile (Fig. 1C). However, unlike RAMPs, which 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. It is noteworthy that the same laboratory describing the effects here of coexpression 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, although in this complex its high affinity for BMY-7378 is unaltered (Fig. 1C) (Uberti et al., 2005).

What evidence do Hague et al. (2006) supply 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 coexpressed with the α_{1B}-AR, a binding site consistent with an α_{1D}-AP pharmacology cannot be detected. They conclude therefore that

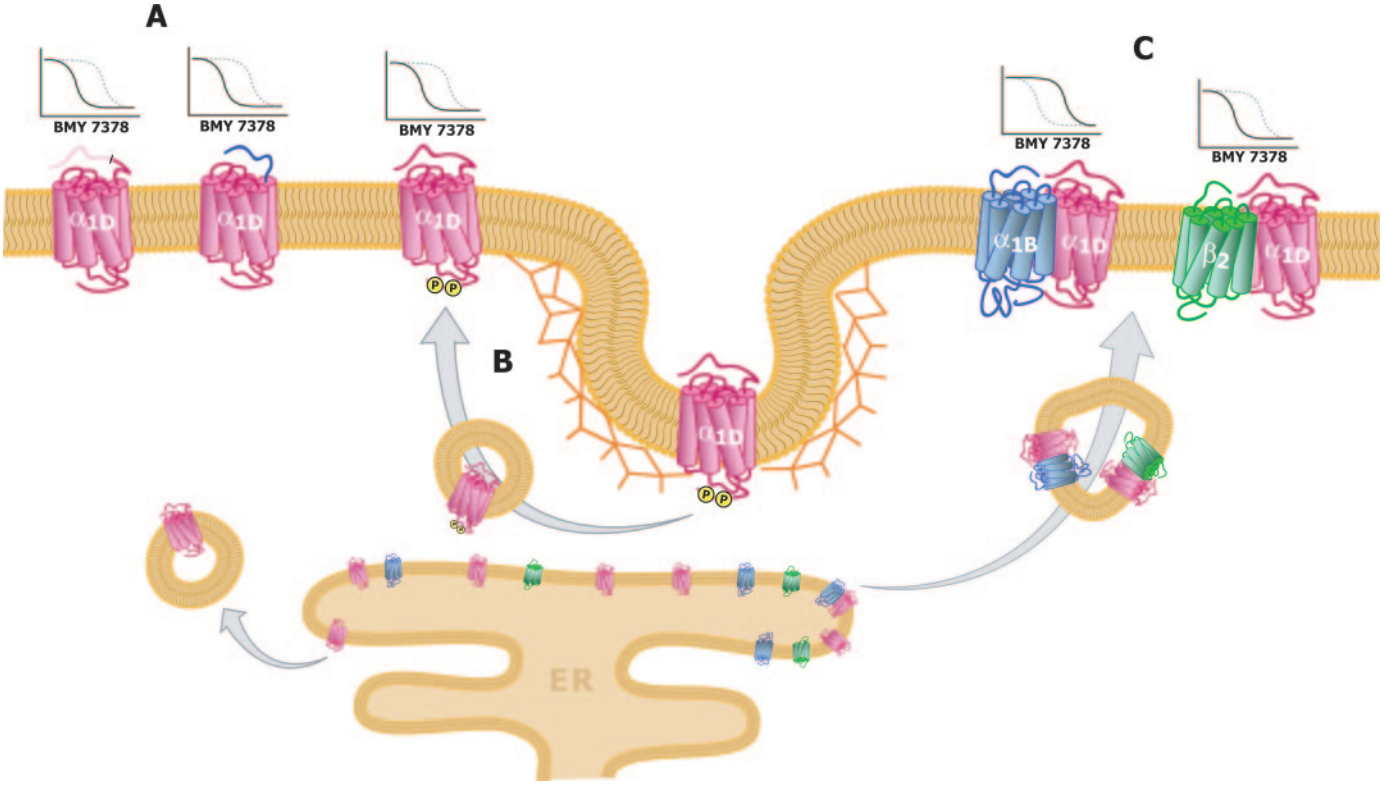

Fig. 1. Going to the ball: expression of the α_{1D}-AR on the cell membrane. The α_{1D}-AR is poorly expressed on the cell surface. It has been proposed that this is due to the length of the α_{1D}-AR's N terminus, in that truncation of the N terminus or substitution of it with the N terminus of the α_{1B}-AR leads to increased cell surface expression (A), a result of constitutive activity of the receptor leading to internalization of the receptor (B), or the need for a chaperone in the form of either the α_{1B} or β_2-AR to facilitate cell surface expression (C). Dimerization of the α_{1D}-AR with the α_{1B}-AR results in a single pharmacological entity that has low affinity for the α_{1D}-AR selective antagonist BMY-7378; in contrast, dimerization with the β_2-AR or monomeric expression of the α_{1D}-AR leads to a receptor with high affinity for BMY-7378.

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, Rho-conotoxin TIA, recently isolated from the venom of the predatory marine snail *Conus tulipa* by Sharpe et al. (2001), that 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 previously shown to allow 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 coexpressed 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 Rho-conotoxin TIA. They then show that even when the α_{1B}-AR is coexpressed 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 (2006) provide evidence that coexpression of the two α_1-AR subtypes results in a unique complex that now signals with greater potency then either subtype alone and that this response is still seen even with coexpression of the α_{1D}-AR with a signaling incompetent α_{1B}-AR mutant.

Although 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 coexpressed, 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 cross-talk 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}-knockout 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-ARs 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 (Esbenshade et al., 1993; Bockman et al., 2004), 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, although at the heart of the ball, must surely await another evening.

Acknowledgments

We are most grateful to David Williamson for producing the figure.

References

Bockman CS, Bruchas MR, Zeng W, O'Connell KA, Abel PW, Scofield MA, and Dowd FJ (2004) Submandibular gland acinar cells express multiple α1-adrenoceptor subtypes. *J Pharmacol Exp Ther* **311:**364–372.

Chen Z, Rogge G, Hague C, Alewood D, Colless B, Lewis RJ, and Minneman KP (2004) Subtype-selective noncompetitive or competitive inhibition of human α1-adrenergic receptors by rho-TIA. *J Biol Chem* **279:**35326–33.

Daly CJ, Deighan C, McGee A, Mennie D, Ali Z, McBride M, and McGrath JC (2002) A knockout approach indicates a minor vasoconstrictor role for vascular alpha1B-adrenoceptors in mouse. *Physiol Genomics* **9:**85–91.

Deighan C, Methven L, Naghadeh MM, Wokoma A, Macmillan J, Daly CJ, Tanoue A, Tsujimoto G, and McGrath JC (2005) Insights into the functional roles of alpha(1)-adrenoceptor subtypes in mouse carotid arteries using knockout mice. *Br J Pharmacol* **144:**558–565.

Esbenshade TA, Han C, Murphy TJ, and Minneman KP (1993) Comparison of α_1-adrenergic receptor subtypes and signal transduction in SK-N-MC and NB41A3 neuronal cell lines. *Mol Pharmacol* **44:**76–86.

Foord SM and Marshall FH (1999) RAMPs: accessory proteins for seven transmembrane domain receptors. *Trends Pharmacol Sci* **20:**184–187.

Garcia-Sainz JA, Vazquez-Cuevas FG, and Romero-Avila MT (2001) Phosphorylation and desensitization of alpha(1d)-adrenergic receptors. *Biochem J* **353:**603–610.

Garcia-Sainz JA and Villalobos-Molina R (2004) The elusive alpha(1D)-adrenoceptor: molecular and cellular characteristics and integrative roles. *Eur J Pharmacol* **500:**113–120.

Graham RM, Perez DM, Hwa J, and Piascik MT (1996) alpha(1)-Adrenergic receptor subtypes—molecular structure, function and signaling. *Circ Res* **78:**737–749.

Hague C, Chen Z, Pupo AS, Schulte NA, Toews ML, and Minneman KP (2004a) The N terminus of the human α_{1D}-adrenergic receptor prevents cell surface expression. *J Pharmacol Exp Ther* **309:**388–397.

Hague C, Lee SE, Chen Z, Prinster SC, Hall RA, and Minneman KP (2006) Heterodimers of α_{1B}- and α_{1D}-adrenergic receptors form a single functional entity. *Mol Pharmacol* **69:**45–55.

Hague C, Uberti MA, Chen Z, Hall RA, and Minneman KP (2004b) Cell surface expression of alpha1D-adrenergic receptors is controlled by heterodimerization with α_{1B}-adrenergic receptors. *J Biol Chem* **279:**15541–9.

Hosoda C, Tanoue A, Shibano M, Tanaka Y, Hiroyama M, Koshimizu TA, Cotecchia S, Kitamura T, Tsujimoto G, and Koike K (2005) Correlation between vasoconstrictor roles and mRNA expression of α_1-adrenoceptor subtypes in blood vessels of genetically engineered mice. *Br J Pharmacol* **146:**456–466.

Khorana HG (1992) Rhodopsin, photoreceptor of the rod cell. An emerging pattern for structure and function. *J Biol Chem* **267:**1–4.

Lomasney JW, Cotecchia S, Lefkowitz RJ, and Caron MG (1991) Molecular-biology of alpha-adrenergic receptors—implications for receptor classification and for structure-function-relationships. *Biochim Biophys Acta* **1095:**127–139.

McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, and Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature (Lond)* **393:**333–339.

Noda K, Saad Y, Graham RM, and Karnik SS (1994) The high-affinity state of the β_2-adrenergic receptor requires unique interaction between conserved and non-conserved extracellular loop cysteines. *J Biol Chem* **269:**6743–6752.

Perez DM, Piascik MT, and Graham RM (1991) Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA. *Mol Pharmacol* **40:**876–883.

Pupo AS, Uberti MA, and Minneman KP (2003) N-terminal truncation of human alpha(1D)-adrenoceptors increases expression of binding sites but not protein. *Eur J Pharmacol* **462:**1–8.

Sharpe IA, Gehrmann J, Loughnan ML, Thomas L, Adams DA, Atkins A, Palant E, Craik DJ, Adams DJ, Alewood PF, et al. (2001) Two new classes of conopeptides inhibit the alpha1-adrenoceptor and noradrenaline transporter. *Nat Neurosci* **4:**902–907.

Sharpe IA, Thomas L, Loughnan M, Motin L, Palant E, Croker DE, Alewood D, Chen S, Graham RM, Alewood PF, et al. (2003) Allosteric α_1-adrenoreceptor antagonism by the conopeptide rho-TIA. *J Biol Chem* **278:**34451–7.

Tanoue A, Nasa Y, Koshimizu T, Shinoura H, Oshikawa S, Kawai T, Sunada S, Takeo S, and Tsujimoto G (2002) The α(1D)-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J Clin Investig* **109:**765–775.

Uberti MA, Hague C, Oller H, Minneman KP, and Hall RA (2005) Heterodimerization with β_2-adrenergic receptors promotes surface expression and functional activity of α_{1D}-adrenergic receptors. *J Pharmacol Exp Ther* **313:**16–23.

Uberti MA, Hall RA, and Minneman KP (2003) Subtype-specific dimerization of α_1-adrenoceptors: effects on receptor expression and pharmacological properties. *Mol Pharmacol* **64:**1379–1390.

Yang M, Reese J, Cotecchia S, and Michel MC (1998) Murine α_1-adrenoceptor subtypes. I. Radioligand binding studies. *J Pharmacol Exp Ther* **286:**841–847.

Yang M, Verfurth F, Buscher R, and Michel MC (1997) Is alpha1D-adrenoceptor protein detectable in rat tissues? *Naunyn-Schmiedeberg's Arch Pharmacol* **355:** 438–446.

Address correspondence to: Dr. Robert M. Graham, Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst, 2010 NSW, Australia. E-mail: b.graham@victorchang.unsw.edu.au