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Constitutive activity and inverse agonism at the $\alpha_1$adrenoceptors

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ABSTRACT

Mutations of G protein-coupled receptors (GPCR) can increase their constitutive (agonist-independent) activity. Some of these mutations have been artificially introduced by site-directed mutagenesis, others occur spontaneously in human diseases. The α_{1B}adrenoceptor was the first GPCR in which point mutations were shown to trigger receptor activation. This article briefly summarizes some of the findings reported in the last several years on constitutive activity of the $\alpha_1$adrenoceptor subtypes, the location where mutations have been found in the receptors, the spontaneous activity of native receptors in recombinant as well as physiological systems. In addition, it will highlight how the analysis of the pharmacological and molecular properties of the constitutively active adrenoceptor mutants provided an important contribution to our understanding of the molecular mechanisms underlying the mechanism of receptor activation and inverse agonism.

1. The $\alpha_1$adrenoceptors: structure–functional relationships

The mammalian G protein-coupled receptors (GPCRs) can be divided in three main classes according to sequence homology: class I or rhodopsin-like (which is the largest subfamily), class II or secretin-like, and class III or glutamate-metabotropic-like [1]. Within class I, the adrenoceptors (AR) mediate the functional effects of epinephrine and norepinephrine. The AR family includes nine different gene products: three β (β_1, β_2, β_3), three α_2 (α_{2A}, α_{2B}, α_{2C}) and three α_1 (α_{1A}, α_{1B}, α_{1D}) receptor subtypes.

Within the subfamily of the α_1AR subtypes [2], extensive mutational analysis performed by various investigators helped to identify the structural determinants involved in each of the three main ''classical'' functional properties of GPCRs: (1) ligand-binding; (2) coupling to G protein-effector systems; (3) desensitization. Beyond these classical features, a number of additional functional paradigms of GPCRs have recently emerged including constitutive activity, oligomerization and their interaction with a variety of signaling proteins.

The molecular interactions of the endogenous catecholamines, epinephrine and norepinephrine, with different AR subtypes has been explored in different studies. Epinephrine and norepinephrine contain a protonated amino group separated from the aromatic catechol ring by a β-hydroxylethyl chain. Mutagenesis studies of the α_{1B}AR [3,4] suggested that the amino group of the catecholamines makes an electrostatic interaction with the carboxylate side chain of an aspartate on helix 3, $Asp^{125(3.32)}$, which is highly conserved in all GPCR binding biogenic amines (Fig. 1) (the amino acid numbering in parentheses, used only for the amino acids in the helical bundle, is that proposed in Ref. [5]). Similar findings were obtained mutating the homologous aspartate in the α_{1A}AR subtype (S.C., unpublished results). For both the α_{1A} and α_{1B}AR, the catechol *meta*- and *para*-hydroxyl groups of epinephrine and norepinephrine make weak hydrogen

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Abbreviations: GPCR, G protein-coupled receptor; AR, adrenoceptor; CAM, constitutively active mutant; MD, molecular dynamics

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bonding interactions with serine residues in helix 5 which are conserved in all catecholamine-binding GPCRs (Fig. 1) [3,4].

The roles of a cluster of aromatic amino acids in helices 6 and 7 in ligand binding of the α_{1B}AR were also investigated [6]. The results indicated that, among these aromatic residues, Phe$^{310(6.51)}$ is the only one involved in binding epinephrine probably interacting with the catechol ring of the ligand.

In contrast, very little is known so far about the amino acids of the α_{1B}AR which interact with different antagonists as well as about the structural basis underlying receptor selectivity for different ligands. Mutation of Asp$^{125(3.32)}$ (Fig. 1) to alanine profoundly impaired the ability of the hamster α_{1B}AR to bind antagonists [3]. Experimental mutagenesis studies suggested that a conserved tyrosine in the extracellular half of helix 7 should interact with prazosin [3].

Activation of the α_1AR subtypes causes polyphosphoinositide hydrolysis catalyzed by phospholipase C *via* pertussis toxin-insensitive G proteins of the $G_{q/11}$ family in almost all tissues where this effect has been examined. Polyphosphoinositide hydrolysis results in the increase of intracellular inositol phosphate production. Several lines of evidence demonstrated that the i3 loop contains the main structural determinants involved in α_{1B}AR coupling to G proteins of the $G_{q/11}$ family. A detailed analysis of the molecular basis of the receptor–G_q coupling was carried on by combining computational modeling and experimental mutagenesis of α_{1B}AR [7]. The functional analysis of a large number of receptor mutants in conjunction with the predictions of molecular modelling support the hypothesis that Arg^{254} and Lys^{258} in the i3 loop as well as Leu^{151} in the i2 loop (Fig. 1) are directly involved in receptor-G protein interaction and/or receptor mediated activation of the G protein [8]. It is important to highlight that mutations of the homologous leucine or hydrophobic residue in the i2 loop resulted in receptor–G protein uncoupling for other GPCRs as well [1].

The α_{1B}AR expressed in various cell types can undergo phosphorylation and desensitization upon exposure to agonists as well as to the protein kinase-C (PKC) activator phorbol-myristate-acetate [9,10]. In particular, we demonstrated that a stretch of serines in the C-tail of the receptor represents the main phosphorylation sites (Fig. 1) [11]. Three of them (Ser^{404}, Ser^{408} and Ser^{410}) are involved in agonist-induced phosphorylation whereas two others (Ser^{394} and Ser^{400}) represent the sites for PKC-mediated phosphorylation of the α_{1B}AR. Agonist-induced regulation of the receptor seems to be mainly mediated by members of the G protein-coupled receptor kinase (GRK) family. In fact, GRK_2-mediated phosphorylation of Ser^{404}, Ser^{408} and Ser^{410} is crucially involved in the desensitization of the α_{1B}AR.

2. The discovery of constitutively active receptor mutants and its implications

The α_{1B}AR was the first GPCR in which point mutations were shown to trigger receptor activation [12]. A conservative substitution (Ala^{293}Leu) in the cytosolic extension of helix 6 (Fig. 1) of the α_{1B}AR resulted in its constitutive (agonist-independent) activity. In the absence of agonist, cells expressing the mutated receptor exhibited higher basal levels of inositol phosphates as compared to cells expressing the wild type α_{1B}AR. To further assess the role of this amino acid, Ala$^{293(6.34)}$ was systematically mutated by substituting each of the other 19 amino acids [13]. Remarkably, all possible amino acid substitutions of Ala^{293} in the α_{1B}AR induced variable levels of constitutive activity which was the highest for the Ala^{293}Glu mutant.

To extend the generality of this finding within the AR family, similar mutations were performed in the β_2 and α_{2A}AR which are coupled to G_s-mediated stimulation or G_i-mediated inhibition of adenylyl cyclase, respectively [14,15]. Both β_2 and α_{2A}AR mutants exhibited increased constitutive activity leading to increased or decreased agonist-independent adenylyl cyclase activity, respectively.

The discovery of the constitutively active mutants (CAMs) in the AR family catalyzed the interest of a large number of groups towards the elucidation of two main aspects of GPCR function and drug action: (a) the activation process of GPCRs and (b) the identification of ligands with negative efficacy. In addition, the discovery of the CAM GPCRs encouraged the search for spontaneously occurring activating mutations of different receptors which are responsible for a number of human diseases [16].

This article will briefly review some of the findings obtained in the last several years on constitutive activity of the $\alpha_1$adrenoceptor subtypes and its implications on our understanding of inverse agonism.

3. Constitutively activating mutations in the $\alpha_1$adrenoceptor subtypes

Among the three α_1AR subtypes, the largest number of activating mutations have been described in the α_{1B} (Fig. 1), few in the α_{1A} and none in the α_{1D}AR subtype (Table 1). For all the CAMs their constitutive activity was mainly assessed measuring inositol phosphate accumulation in whole cells whereas for few of them other biochemical pathways, like phospholipase A_2 or D, have also been explored.

As mentioned above, in the α_{1B}AR all possible amino acid substitutions of Ala$^{293(6.34)}$ in the cytosolic extension of helix 6 induced variable levels of constitutive activity [13]. No quantitative relationship was found between the physicochemical properties of the substituting amino acids and the levels of agonist-independent activity of the CAMs. The greatest increase in constitutive activity was observed for the mutation of Ala$^{293(6.34)}$ into glutamate. All CAMs displayed increased affinity for the full agonist epinephrine which was, at least to some extent, correlated with their degree of constitutive activity.

Studies from our laboratory combining site-directed mutagenesis of the α_{1B}AR and molecular dynamics (MD) simulations on computational models of the receptor highlighted the potential role played in receptor activation by the Glu/AspArgTyr (E/DRY) motif at the cytosolic end of helix 3 (Fig. 1), which is highly conserved in GPCRs of the rhodpsin-like class. Mutations of the aspartate (Asp$^{142(3.49)}$) of the E/DRY motif resulted in high constitutive activity (Fig. 2). Similarly to the Ala$^{293(6.34)}$ replacements, all the 19 possible natural amino acid substitutions of the aspartate resulted in variable levels of