

Invited review

Recent progress in α_1 -adrenergic receptor researchZhong-jian CHEN, Kenneth P MINNEMAN¹*Department of Pharmacology, School of Medicine, Emory University, Atlanta, GA 30322, USA***Key words**

alpha1-adrenergic receptor; noncompetitive drug; protein-protein interaction; dimerization; cell surface expression

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Abstract

α_1 -Adrenergic receptors (AR) play an important role in the regulation of physiological responses mediated by norepinephrine and epinephrine, particularly in the cardiovascular system. The three cloned α_1 -AR subtypes (α_{1A} , α_{1B} , and α_{1D}) are G protein-coupled receptors that signal through the $G_{q/11}$ signaling pathway, each showing distinct pharmacological properties and tissue distributions. However, due to the lack of highly subtype-selective drugs, the functional roles of individual subtypes are still not clear. Development of new subtype-specific drugs will greatly facilitate the identification of the functions of each subtype. Conopeptide ρ -TIA has been found to be a new α_{1B} -AR selective antagonist with different modes of inhibition at α_1 -AR subtypes. In addition, recent studies using genetically engineered mice have shed some light on α_1 -AR functions *in vivo*, especially in the cardiovascular system and brain. Several proteins have been shown to interact directly with particular α_1 -AR, and may be important in regulating receptor function. Receptor heterodimerization has been shown to be important for cell surface expression, signaling and internalization. These new observations are likely to help elucidate the functional roles of individual α_1 -AR subtypes.

Introduction

α_1 -Adrenergic receptors (AR) are members of the G protein-coupled receptor (GPCR) superfamily that mediate physiological responses to norepinephrine (NE) and epinephrine (EPI). Pharmacological analysis and molecular cloning have shown that this receptor family has three subtypes (α_{1A} , α_{1B} , α_{1D}), which have different pharmacological properties and amino acid sequences^[1]. Four C-terminal splice variants of the α_{1A} -AR have been found^[2,3]. Stimulation of all three subtypes results in activation of the $G_{q/11}$ signaling pathway, involving activation of phospholipase C, generation of the second messengers inositol (1,4,5) triphosphate and diacylglycerol, and mobilization of intracellular calcium. Because of their clinical importance, research on α_1 -AR has been very active for many years. Several recent reviews have summarized this research from different perspectives^[4–9]. In this review, we mainly focus on recent developments in subtype-selective drugs, genetically engineered mouse models, interacting proteins, receptor dimerization, and factors controlling receptor cell surface expression.

 α_1 -AR subtype selective drugs

Although all three α_1 -AR subtypes activate the same $G_{q/11}$ protein signaling pathway, their different tissue distributions suggest that they play distinct functional roles. For example, the α_{1A} -AR subtype has been considered to be the dominant receptor subtype controlling benign prostatic hypertrophy, and is an important therapeutic target for treatment of this disease^[10]. Therefore, identifying the specific functions of individual α_1 -AR subtypes is of considerable therapeutic interest. Unfortunately, this has proved difficult because of difficulties in identifying highly subtype-selective drugs. Despite extensive efforts to address this difficulty, only a few subtype-selective compounds have been characterized^[11,12]. WB 4101^[13], (+)-niguldipine^[14], and 5-methylurapidil^[15] have been used extensively as α_{1A} -AR selective antagonists, and BMY7378^[16] is widely used to characterize α_{1D} -AR. However, there has been little progress in identifying α_{1B} -AR selective antagonists. Chlorethyclonidine was originally characterized as an α_{1B} -AR selective site-directed alkylating agent^[17]; however, it has limited selectivity in alkylating the cloned

subtypes, and is no longer widely used. Recently, a conopeptide isolated from a sea snail, known as ρ -TIA, was identified as a non-competitive α_{1B} -AR antagonist^[18,19] that may serve as an allosteric modulator, but acts as a competitive inhibitor at the other two subtypes^[20,21]. This suggests that ρ -TIA may interact with a novel antagonist binding site specific to the α_{1B} -AR and serve as a template for development of highly selective α_{1B} -AR drugs.

Genetically engineered mouse models

Since attempts to elucidate the function of individual receptor subtypes has been limited by a lack of agonists, antagonists, and antibodies with adequate subtype selectivity, recent studies using genetically engineered mice have shed some light on the contributions of each subtype to physiological responses to NE and EPI. In general, α_1 -AR knockout mice do not show overt physical abnormalities (Table 1). Studies in knockout mice lacking a single α_1 -AR subtype have shown that all three subtypes seem to be involved in the regulation of blood pressure. Consistent with previous pharmacological characterization *in vitro*, studies in α_{1D} -AR knockout mice have shown that the α_{1D} -AR subtype plays a dominant role in aortic contraction^[22]. Not only do genetically engineered mouse models help clear up some of the confusing cardiovascular effects mediated by individual subtypes, they also provide clues as to the functional roles of each subtype in the central nervous system, where these receptors are expressed in high concentrations, but their functions have been difficult to determine. The involvement of α_{1B} -AR in the regulation of locomotion has been suggested by pharmacological manipulation^[23]. Interestingly, knockout mice lacking α_{1B} -AR also showed decreased locomotor hyperactivity in response to psychostimulants and opiates, suggesting that targeting the α_{1B} -AR might be a useful therapeutic strategy in the treatment of drug abuse^[24]. In addition to the altered phenotypes being examined in the knockout or transgenic models, the underlying molecular mechanisms have been investigated using oligonucleotide microarrays. Alteration in the expression of NMDA receptors, GABA_A receptors, and apoptotic and calcium regulatory genes shown in transgenic murine brains may provide a potential molecular basis for neurodegeneration induced by overexpressed constitutively active α_{1B} -AR^[25]. Despite the new insights provided by the genetically engineered studies, some discrepancies have been noticed from different studies, such as the contribution of α_{1B} -AR in the aortic contractile response^[26,27]. In addition, some of the altered phenotypes observed in the α_{1B} -AR knockout model may result from compensatory effects of other receptor subtypes^[28]. Further

investigation using classic pharmacological approaches with highly subtype-selective drugs would be useful to facilitate future interpretations of those data.

Receptor interacting proteins

Recent studies have revealed that GPCR can interact with various cellular proteins in addition to the cognate G proteins, thereby expanding the receptor signaling network and establishing the distinct functional roles of closely-related receptor subtypes within the same family^[29,30]. Those interacting proteins include cytoplasmic and membrane proteins that may play regulatory roles in receptor pharmacology, trafficking and signaling^[31]. Although all three α_1 -AR subtypes couple to G_{q/11} signaling pathways, previous studies have shown that the three subtypes can activate distinct downstream signaling components in the G_{q/11} signaling pathway or couple to different signaling pathways^[1]. Because of their relatively long C-termini, which have the least sequence homology among the three subtypes, most attention has been focused on finding binding partners interacting with this region. In addition, the sequence diversity in the third intracellular loop (I3 loop) is also attractive due to its importance in coupling to G_{q/11}. In a similar manner, it has been shown that the three β -AR subtypes differentially associate with a variety of proteins other than G proteins^[32]. However, only a handful of interacting proteins have been identified for α_1 -AR subtypes (Table 2). A few of these interactions have been shown to have functional consequences, but most of them require further evaluation. For example, gC1qR and the mu2 subunit of the AP2 clathrin adaptor complex are involved in α_{1B} -AR trafficking or internalization. Although transglutaminase II (Gh) is the first non-G_{q/11} binding partner found to specifically associate with the α_{1B} - and α_{1D} -AR^[33,34], the α_1 -AR signaling pathways seem to remain intact in Gh knockout mice^[35]. Nevertheless, the search for novel binding partners should be considered as an alternative approach to study the molecular differences among the three α_1 -AR subtypes.

Receptor dimerization

Unlike the conventional view that GPCR are monomers, a growing body of evidence indicates that GPCR are able to form dimers or oligomers that are required for their pharmacology, function and/or cell surface expression. This concept has gained great appreciation for the class III GPCR subfamily, including the GABA_B receptors^[36,37] and taste receptors^[38]. However, the significance of class I GPCR dimerization has been under debate, due to difficulties in identify-

Table 1. Characteristics of α_1 -AR knockout or overexpression models.

	No alteration	Altered phenotype	Reference
Knockout			
α_{1A}	Cardiac output and renal function	Decreased basal blood pressure; reduced blood pressure response to α_1 agonist (PE)	[55]
α_{1B}	Basal blood pressure	Reduced blood pressure and aorta contractile responses to α_1 agonists (NE, PE)	[26]
	General physical mobility (motor activity)	Impaired modulation of memory consolidation and fear-motivated exploratory activity	[56]
		Increased exploratory response to novelty	[57]
		Attenuated hypertrophic growth after vascular injury	[58]
	Blood glucose, insulin and free fatty acid levels in the fed state	Elevated leptin concentration in the fed state; impaired glucose homeostasis and free fatty acid levels in the fasted state; increased parasympathetic activity	[59]
	Basal dopaminergic activity and basal locomotor behavior	Reduced locomotor responses induced by psychostimulants and opiates	[24]
α_{1D}	Cardiac function	Decreased basal blood pressure; decreased aorta contractile response to α_1 agonists (NE, PE)	[22]
	Renal function	Attenuated increase in blood pressure in salt-induced hypertension	[60]
	Mechanical and chemical nociception	Impaired thermal nociception	[61]
	Basal locomotor activity	Better motor coordination; impaired working memory or attention	[62]
	Hypertrophic growth after vascular injury		[58]
α_{1A}/α_{1B}	Heart sizes for female mice; basal blood pressure	Smaller heart sizes for male mice; decreased heart rate	[63]
		Increased myocardial contraction; decreased responsiveness to β -AR stimulation	[64]
Overexpression			
α_{1A}	Basal blood pressure	Enhanced cardiac contractility	[65]
α_{1B}	Basal blood pressure	Cardiac hypertrophy	[66]
		Granulovascular neurodegeneration; locomotor impairment and seizure	[67]
		Myocardial hypertrophy and hypertension	[68]
	Basal cardiac parameters (heart rate, blood pressure)	Decreased inotropic response to PE	[69]

NE, norepinephrine; PE, phenylephrine.

ing unique functional responses or pharmacological properties. Our group has shown that the three α_1 -AR subtypes can form homodimers and subtype-specific heterodimers with other AR^[39,40], which has been confirmed by data from other groups^[41,42]. Because truncation of either the amino or the carboxyl terminus of the receptor does not affect receptor dimerization^[40], the transmembrane domains or associated loops have been proposed to be involved in this interaction. Because the pharmacological analyses of the three cloned α_1 -AR subtypes in heterologous expression systems have not yet recapitulated all the receptor sub-

types previously defined by pharmacological criteria in tissues, such as the α_{1L} -AR^[43], the newly found receptor dimers have been hypothesized to perform atypical α_1 -AR pharmacology, which has been seen in the opioid receptor family^[44]. Although the homo- or heterodimers formed by α_{1A} -AR C-terminal splice variants have failed to show any novel pharmacology when studied with existing selective drugs^[45], it is still hard to conclude that the dimerization does not have effects on receptor pharmacology, because the available drugs are limited and the particular receptor dimers may yet be identified. On the other hand, the α_1 -AR sub-

Table 2. Receptor interacting proteins other than G_{q/11} with potential functional roles.

Subtypes	Interacting proteins	Interacting domains	Roles	Reference
α_{1A}	Regulator of G protein signaling 2 (RGS2)	I3 loop	Signaling	[70]
	Bone morphogenetic protein-1 (BMP-1)	C-terminus	Unknown	[71]
	nNOS	Unknown	Unknown	[72]
	Active Bcr-related protein			[73]
	Filamin C	C-terminus	Unknown	[73]
α_{1B}	Tissue transglutaminase (Gh)	I3 loop	Signaling	[33,34]
	Spinophilin	I3 loop	Signaling	[74]
	gC1qR	C-terminus	Cellular localization	[75]
	AP2 clathrin adaptor complex, μ_2 subunit	C-terminus	Internalization	[76]
	nNOS	Unknown	Unknown	[72]
α_{1D}	Filamin C	C-terminus	Unknown	[73]
	Tissue transglutaminase (Gh)	I3 loop	Signaling	[34]
	gC1qR	C-terminus	Unknown	[77]
	nNOS	Unknown	Unknown	[72]
	Filamin C	C-terminus	Unknown	[73]

type-specific dimerization has been found to be important for receptor trafficking and signaling (summarized in the next section).

α_{1D} -AR cell surface expression

Theoretically, all three α_1 -AR subtypes should be present at the cell surface to be recognized by their highly hydrophilic natural ligands that are unlikely to cross cell membranes. However, when expressed in recombinant systems, the α_{1D} -AR subtype has been noted to show almost exclusively intracellular expression^[46,47], which makes them difficult to characterize. We recently reported that cell surface expression of the α_{1D} -AR could be specifically rescued by coexpression with the α_{1B} -AR but not the α_{1A} -AR^[48]. The coexpressed receptors seem to form a new receptor entity, and then modulate signaling and internalization of each receptor subtype in the complex. Recently, the β_2 -AR was also reported to be able to translocate α_{1D} -AR^[49]. Besides receptor dimerization, removal of the long amino-terminus of the α_{1D} -AR has also been shown to facilitate translocation of intracellular receptors to the cell surface^[50]. Subsequent studies using receptor N-terminal chimeras showed that this N-terminal domain might convey a retention signal to prevent receptor cell surface expression^[51]. Moreover, the density of α_{1D} -AR cell surface expression was shown to increase upon sequential truncation^[52].

Future directions

In the past few years, our knowledge of the functional

roles of the α_1 -AR family has been dramatically expanded using many different approaches. These findings generate several interesting directions that may be worth pursuing.

1 Further development of highly subtype-selective drugs, especially non-competitive antagonists: most of the specific α_1 -AR drugs available are competitive antagonists with moderate subtype-selectivity, which also target other cell surface proteins. Since the three α_1 -AR subtypes have relatively high homologies among the transmembrane domains that are believed to form the ligand binding pocket, designing new subtype-selective drugs that compete for this site has not been easy. However, development of noncompetitive drugs may be a good strategy because those drugs normally recognize a different site with less homology. This strategy has been successfully applied in designing highly subtype-selective drugs for other GPCR^[53].

2 Investigate the physiological relevance of receptor oligomers: the success of this direction requires the advance of two other research fields, characterization of subtype-specific antibodies and development of new α_1 -AR drugs. Although receptor dimerization and protein-protein interactions have been identified in recombinant systems, and their importance in advancing our knowledge of the α_1 -AR family has been recognized, their physiological relevance *in vivo* cannot be confirmed and exploited without subtype-specific antibodies. On the other hand, new subtype-selective drugs may recognize the discrete pharmacology of receptor dimers, therefore expanding the existing cloned α_1 -AR family and providing new therapeutic targets.

3 Characterize subtype-specific interacting proteins: the

growing list of GPCR interacting proteins has elucidated the molecular mechanisms of the differences among subtypes, which could lead to the development of drugs specifically targeting to such interactions. Because previous evidence suggests that the three α_1 -AR subtypes might couple to different signaling pathways, it is likely that more interacting proteins would be identified through the approaches that have been successfully used, such as yeast two-hybrid screenings and pull-down assays with fusion proteins.

4 Study the functional role of α_1 -AR in the central nervous system^[5]. In fact, almost all typical and atypical antipsychotics are α_1 -AR antagonists, although they show little, if any, subtype selectivity^[54]. In addition, tricyclic antidepressants are also α_1 -AR antagonists, and it is possible that this property may contribute to their therapeutic efficacy. We believe that studies focusing on those directions would further improve our understanding of the functional roles of each α_1 -AR subtype.

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