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α_1-ADRENERGIC RECEPTOR SUBTYPES

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INTRODUCTION

Norepinephrine (NE) and epinephrine (EPI) are important neurotransmitters and hormones in the periphery and in the central nervous system (CNS). NE is released from postganglionic sympathetic nerves, and both NE and EPI are secreted from the adrenal medulla. NE and EPI play key roles in controlling cardiovascular function, airway reactivity, energy metabolism, and other processes. The adrenergic receptors (ARs) through which these compounds act are targets for many therapeutically important drugs, including those for cardiovascular diseases, asthma, prostatic hypertrophy, and nasal congestion.

In 1948, pharmacological studies in isolated tissues led Ahlquist to propose distinct α- and β-AR subtypes (1). The existence of β_1- and β_2-AR subtypes was first recognized in 1967 (2), and two different α-AR subtypes (α_1 and α_2) were discovered in the 1970s (3). In the late 1980s, the development of more selective drugs and the use of molecular cloning technology resulted in the surprising realization that ARs had substantially more subtypes than previously suspected (4–8). Nine different subtypes have now been cloned and pharmacologically characterized, and additional subtypes may still be identified.

Three main AR families (α_1, α_2, and β) each contain at least three distinct but closely related subtypes. These families are subdivided according to sequence homology, drug specificity, and mechanism of signal transduction. Subtypes within a family have about 70–75% sequence homology, and the

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sequence homology between families is about 40% (4, 8). Each family has a characteristic pharmacological profile, although the drug specificities for subtypes within each family also differ. All members within a particular family appear to activate the same, or similar, signal transduction mechanisms. Each subtype is a product of a separate gene and has a unique drug specificity and tissue distribution. The existence of so many subtypes suggests that additional highly selective drugs with possible therapeutic advantages could be developed.

This review focuses on α_1-AR subtypes and summarizes what is known about the number, properties, and signaling mechanisms of these receptors. α_1-ARs play a dominant role in control of smooth muscle contraction and are important in control of blood pressure, nasal congestion, prostate function, muscle growth, and other processes (5–7). As such, the number of α_1-AR subtypes and the drug specificities of each subtype are of particular therapeutic interest. However, the number of α_1-AR subtypes remains controversial. As discussed below, two subtypes can be clearly distinguished pharmacologically (7), while three cDNAs have been cloned (8). The relationship between the pharmacologically defined receptors and the cDNA clones is still confusing.

PHARMACOLOGICAL CHARACTERIZATION

In the mid 1980s, converging lines of evidence strongly supported the existence of two pharmacologically distinct α_1-AR subtypes (7). Morrow & Creese (9) were the first to observe pharmacological heterogeneity among α_1-AR binding sites in rat brain membranes. Analysis of inhibition curves for the competitive antagonists WB 4101 and phentolamine showed that the competition for ^{3}H-prazosin binding sites was unexpectedly complex. Other competitive antagonists did not exhibit such complexity, and this observation suggested the presence of two different affinity states that represented pharmacologically distinct α_1-AR subtypes. Morrow & Creese (9) suggested that these subtypes be called α_{1A}- and α_{1B}-ARs. Researchers working independently of Morrow & Creese found that the site-directed alkylating agent chloroethylclonidine (CEC) inactivated only about half of the α_1-AR binding sites in membranes from rat cerebral cortex (10, 11). This result contrasted with those for other site-directed alkylating agents such as phenoxybenzamine and benextramine, which inactivated all α_1-AR binding sites in a monophasic manner. An examination of different tissues revealed two populations of α_1-AR binding sites and responses. Some were potently and completely inactivated by CEC (CEC-sensitive), and others were completely resistant to CEC-pretreatment (CEC-insensitive) (11). Later studies showed that CEC and WB 4101 appeared to be distinguishing the same

subpopulations of binding sites (12). The site with a high affinity for the competitive antagonist WB 4101 and its congeners (α_{1A}) correlated with the CEC-insensitive sites, while the binding sites with a low affinity for WB 4101 (α_{1B}) correlated with the CEC-sensitive sites. Functional studies on contraction of isolated smooth muscles showed a similar correlation between a low affinity for WB 4101 and a sensitivity to CEC inactivation (11), thus providing evidence that these different binding sites were actually functional receptor subtypes.

The existence of two pharmacologically distinct α_1-AR subtypes is now widely accepted, and other more highly selective drugs have been identified that distinguish between these subtypes. Substantial evidence suggests that α_{1A}-ARs have a 20- to 100-fold higher affinity for the competitive antagonists 5-methylurapidil (13, 14), (+)-niguldipine (15), WB 4101 (9, 12), and benoxathian (16) than do α_{1B}-ARs. As yet, no competitive antagonists have been reported that have substantial selectivity for the α_{1B} subtype. However, α_{1B}-ARs are highly sensitive to inactivation by the site-directed alkylating agent CEC. CEC binds equally well to both α_{1A}- and α_{1B}-ARs, but it does not inactivate the α_{1A} subtype (10–12). Presumably the α_{1A} subtype lacks the appropriate reactive group for covalent attachment of CEC.

MOLECULAR CLONING

Three α_1-AR cDNA clones have been isolated, but their relationship to the pharmacologically defined α_{1A}- and α_{1B}-AR subtypes remains controversial.

α_{1B}-*Adrenergic Receptor*

The first α_1-AR cDNA clone was isolated from a hamster smooth muscle DDT_1-MF_2 cell library (17). This clone encoded a protein of 515 amino acid residues, which was predicted by hydropathy analysis to have the 7 membrane-spanning domains typical of a G protein–linked receptor. Expression of this clone in COS-7 cells resulted in a receptor with a drug specificity similar to that of the native α_{1B} subtype (17), which stimulated inositol phosphate formation when activated. Northern analysis showed the mRNA for this clone had a tissue distribution similar to that predicted for the α_{1B}-AR (17). Investigators now generally agree that this cDNA encodes the hamster α_{1B} subtype and rat (18, 19), dog (20), and human (21) homologs have been isolated.

α_{1C}-*Adrenergic Receptor*

An additional cDNA that encoded a 466-residue polypeptide was isolated from a bovine brain library (22). This clone was homologous to the hamster α_{1B} clone (72% in the membrane spanning regions) but was localized to a

different human chromosome. Expression of this cDNA resulted in a novel subtype with a unique drug specificity that had a relatively high affinity for α_{1A}-selective drugs but was sensitive to inactivation by CEC. Interestingly, mRNA for this clone showed an extremely restricted distribution and was found in detectable quantities in only human hippocampus and rabbit liver (22). Based on its unique pharmacology and very limited tissue distribution, researchers concluded that this clone encoded a novel subtype, which was designated α_{1C} (22).

$\alpha_{1A/D}$-*Adrenergic Receptor*

Lomasney et al (19) cloned a third α_1-AR by using homology screening from a rat cerebral cortex library. This clone, which encoded a protein of 560 amino acids (and was 73% identical to the hamster α_{1B} clone in membrane-spanning domains), was assumed to encode the α_{1A} subtype. This conclusion was based mainly on the distribution of mRNA for this clone in rat tissues, although the relatively high potency of WB 4101 at the expressed receptor supported this classification as well. However, oxymetazoline, which is known to be α_{1A} selective, had a low affinity for the receptor encoded by this clone (19), and (in contrast to the natively expressed α_{1A}) the expressed receptor was partially CEC-sensitive. Perez et al (23) isolated an essentially identical clone from a rat hippocampal library that differed in only

al (19). They performed a more extensive pharmacological analysis of the properties of the expressed receptor and found that the affinities of (+)niguldipine and 5-methylurapidil for the receptor expressed by this clone were more than 100-fold lower than expected for the native α_{1A} subtype, which meant they were more similar to those expected for the native α_{1B} subtype (23). In addition, mRNA for this subtype is found in rat spleen, which is known from pharmacological experiments to express only α_{1B}-like receptors. Perez et al (23) concluded that this was another novel clone (with mainly α_{1B}-like pharmacology) and called it the α_{1D} (23). Schwinn & Lomasney (24) confirmed the same drug specificity for their clone, and both groups agree that they appear to have the same clone. The two different codons most likely result from sequencing errors or minor individual variations. Most investigators now agree that this clone does not code for the α_{1A} subtype (Table 1) but encodes a fourth subtype, the α_{1D}.

A sequence for a human homologue of this cDNA (known as α_{1A} but probably α_{1D}) has been reported (25), although this cDNA has never been expressed to show that it encodes a functional receptor. In addition, the 5′ ends of rat and human α_{1A}/α_{1D} cDNAs differ substantially. Although the 5′ end of the rat and human α_{1B}-AR clones are the same length and essentially identical for the two species, the open reading frame of the

Table 1 Comparison of the K_I (nM) values of native α_{1A}- and α_{1B}-ARs from rat tissues with those of various cDNA clones[a]

	Membranes		Clones		
Antagonist	Rat α_{1A}	Rat α_{1B}	Rat $\alpha_{1A/D}$	Rat/Hamster α_{1B}	Bovine α_{1C}
WB 4101	0.3[b] (.08–1.0)	34.0 (4.7–251)	2.0 (1.9–2.1)	30.0 (29–31)	0.5 (.5–.6)
(+) Niguldipine	0.4 (.05–2.4)	165 (32–847)	225 (46–1100)	854 (8–1700)	80 —
5-Methyl-urapidil	1.0 (.6–1.5)	101 (28–363)	70 (15–330)	190 (41–340)	7 —
Oxymetazoline	3.1 (3–3.2)	193 (178–209)	2100 —	560 —	42 (27–70)

[a] This table compares the pharmacological properties of α_{1A}- and α_{1B}-ARs natively expressed in membranes from various rat tissues with those of various cDNA clones expressed in COS, CHO, or HeLa cells.

[b] Mean values for the K_I for each compound are compiled from literature reports referenced in the text. Underneath in parentheses are the ranges of reported values for each compound and each receptor subtype.

human α_{1A}/α_{1D} clone is about 100 bp shorter than the rat clone, which accounts for a predicted 30-amino acid difference in size. Also, the sequence identity between these two clones is relatively low for the next 180 bp, after which it becomes very high. The relationship between the human and rat α_{1A}/α_{1D} clones remains to be clarified.

Where Is the α_{1A} *Clone?*

Although three clones have been isolated and expressed, none of them can yet account for the pharmacologically defined α_{1A} subtype. Most investigators now agree that the α_{1A} subtype has probably not yet been cloned (23, 24), despite extensive searches in tissues enriched in this subtype. The reason why is not clear. For example, screening of a cDNA library from rat hippocampus, which should be enriched in the α_{1A} subtype, by using degenerate polymerase chain reaction (PCR) primers resulted in identification of primarily (95%) α_{1B} clones, although a single novel clone, the α_{1D} (23) was also found. Thus, all cDNAs found in a tissue enriched in the α_{1A} subtype coded for other subtypes. Because other investigators have had similar experiences, it is possible the α_{1A} may not bear much sequence homology to known subtypes. This would explain why homology-based screening approaches have not succeeded in picking up this clone.

However, the possibility that the α_{1D} clone is related to the α_{1A} subtype remains viable, as suggested by several lines of evidence. First, mRNA for

the α_{1D} subtype is generally localized to rat tissues known to be enriched in the α_{1A} subtype (19, 23). The major exception to this observation is rat spleen, where α_{1D} mRNA is found, but where the α_1-ARs are all of the pharmacologically defined α_{1B} subtype (7, 23). Second, no α_{1A} cDNA has been isolated, despite extensive searches in tissues known to express this subtype. Finally, intriguing reports have emerged from the Garcia-Sainz laboratory (26–28) on the α_1-AR subtype in guinea pig liver. Guinea pig liver expresses mRNA only for the α_{1D} subtype, which is referred to as α_{1A} in the papers, and not for the α_{1B} or α_{1C} subtypes (26–28). However, the pharmacology of the natively expressed receptors in guinea pig liver resembles the rat and human α_{1A}, with a high affinity for 5-methylurapidil (0.75–3.0 nM) and a complete lack of sensitivity to CEC (26–28). Species differences in the guinea pig α_{1D} homologue might result in a receptor with an α_{1A}-type pharmacology, but because the rat α_{1D} clone does not express a rat α_{1A}-type pharmacology, this seems less likely. Although the α_{1D} and α_{1A} might coexist in guinea pig liver, the pharmacological observations in this system suggest the presence of a homogeneous α_{1A} population (26–28).

These observations raise the possibility that the α_{1D} clone is somehow related to the pharmacologically defined α_{1A} subtype. Most studies of other G protein–linked receptors suggest that the pharmacological properties of the expressed clones agree well with those of the natively expressed subtypes (see below). However, we cannot rule out the possibility that alternate splice variants, tissue-specific processing or environment, or unexpected interactions between coexisting subtypes might produce an α_{1A}-type pharmacology out of the known clones.

SPECIES VARIANTS, ALTERNATIVE SPLICING, OR TISSUE-SPECIFIC PROCESSING?

Because currently available clones are derived from several different species, small structural differences between proteins could complicate comparison of the drug specificities of subtypes derived from different species. Such a situation has been observed with the mouse and human homologues of the α_{2A}-AR, in which a single amino acid difference causes interspecies variations in affinity for the antagonist yohimbine (29). However, interspecies variations cannot explain the problem with the α_{1A} subtype, because most pharmacological data are from rat tissues (7) and both α_{1A}/α_{1D} clones that have been identified and characterized are derived from rat libraries (19, 23). In addition, expression of hamster (17), rat (19), and human (21) α_{1B} clones results in receptors with essentially identical drug specificities, which suggests little interspecies variations in the ligand binding properties of this subtype. However, the possibility that structural differences may alter ligand

recognition properties must always be considered when comparing clones from different species.

The possibility that the α_{1A} subtype is the result of a splice variant, or some cell-specific posttranslational modification, of one the cDNAs already isolated must also be considered. α_1-AR genes are unusual among G protein–linked receptors in that they contain introns in their protein-coding regions. Introns have been reported in the genes for the hamster (8) and human (21) α_{1B} and bovine α_{1C} subtypes (22). In most cases, complete genomic sequences for these receptors are not yet available. Alternative splicing of multiple exons, resulting in multiple splice variants, could conceivably contribute to the current confusion. In this case, of course, it is still surprising that a full-length cDNA for the α_{1A} subtype has not been identified despite searches in tissues enriched in this subtype. Another possibility is that clones derived at least in part from genomic DNA (19, 21) lacked an exon that might contribute to altered recognition properties of the expressed receptor.

For example, the human and rat β_3-ARs were first thought to be encoded by single exons (30, 31); however, an additional exon coding for receptor sequence of both species was reported recently (32). The additional amino acids reside at the carboxy-terminal tail and not in the transmembrane domains, but they may still be related to the puzzling pharmacological differences between native and recombinant human β_3-ARs (33). However, long and short alternatively spliced dopamine D_2 receptor variants, which differ in the length of the third intracellular loop, appear to have no pharmacological or functional differences (34).

Cell-specific modifications might also alter the binding specificity of a receptor. Contractile studies of different arteries have suggested that agonist affinity for α_1-ARs might be influenced by the tissue environment in which the receptor is found (35, 36). Bevan et al (35) reported a more than 200-fold difference in agonist affinity for NE in different rabbit arteries but found few or no differences in antagonist affinity (36). Such studies suggest that cell-specific modifications or microenvironments might play a role in determining the ligand-binding specificity of a receptor protein. In this case, expression of a cDNA in a foreign cell would not necessarily result in a receptor with pharmacological properties identical to the natively expressed subtype. However, there is generally little biochemical or molecular precedent for such a result. Much evidence suggests that the binding properties of a receptor are a product of its primary amino acid sequence (37), and the pharmacological properties of specific receptor cDNAs expressed in a wide variety of cell types have varied remarkably little. In fact, both β_1 and β_2-ARs retain their normal drug specificities even when expressed in *Escherichia coli* (38). However, the possibility of altered ligand binding

properties caused by cell-specific environment or processing cannot be eliminated until it is subjected to direct experimental test.

Clearly, the lack of success in obtaining an α_{1A} clone does not result from pharmacological variations in species homologues. The possibility that alternative splice variants, cell-specific processing, or environment could be contributing to the current confusion has not been conclusively eliminated, but this hypothesis is supported by little direct precedent. Most likely, the α_{1A}-AR clone has yet to be isolated.

ADDITIONAL SUBTYPES?

Evidence from several pharmacological studies appears to suggest the existence of additional α_1-AR subtypes. These include receptors with very low affinities for specific competitive antagonists and receptors with intermediate and partial sensitivities to CEC.

Functional studies have suggested that some α_1-AR subtypes may have different affinities for the prototype α_1-AR antagonist, prazosin (39–41). In rat thoracic aorta, for example, the pA_2 for prazosin is substantially higher than in other rat tissues (40), which suggests that different receptors have different affinities for prazosin. Studies on α_1-AR–mediated contractile responses in several isolated blood vessels led Muramatsu and coworkers (41) to develop a classification scheme for α_1-AR subtypes based primarily on differential affinities for prazosin. However, such studies have largely not been confirmed with direct binding assays (5, 9, 42); thus whether they result from pharmacologically distinct α_1-AR subtypes is uncertain.

The highly α_{1A}-selective antagonist (+)niguldipine has also provided evidence for additional α_1-AR subtypes. This compound shows a remarkable selectivity in blocking two different biochemical responses to α_1-AR activation in slices of rat cerebral cortex. (+)Niguldipine blocks the increase in inositol phosphates caused by α_1-AR activation with a relatively low affinity, characteristic of the α_{1B} subtype (43). However, it is almost completely ineffective in blocking the potentiation of cAMP accumulation caused by α_1-AR activation in the presence of activators of other G protein–linked receptors (e.g. isoproterenol, adenosine) (44, 45). This observation raises the possibility of an additional α_1-AR subtype in rat brain with a very low affinity for (+)niguldipine. Additional data for a subtype with a low affinity for (+)niguldipine have been obtained recently by using radioligand binding studies on rat tissues (46).

In fact, careful pharmacological analysis of second-messenger responses in rat brain preparations shows that the α_{1A}/α_{1B} classification scheme is inadequate to completely explain any of the second messenger responses studied (45). Although the inositol phosphate response to NE in brain slices

appears to be primarily mediated by the α_{1B} subtype (43), it shows a stereoselectivity for niguldipine enantiomers not seen in radioligand binding studies of the α_{1B} subtype in the same tissue preparation (44). In addition, the α_{1A}-selective antagonist 5-methylurapidil potently blocks this response (45), and pretreatment with the α_{1B}-selective alkylating agent CEC does not block this response (10, 45).

Some of these complications will probably be explained by interactions between coexisting α_1-AR subtypes linked to the same functional response. In addition, three closely related (α_{1B}-like) subtypes are known from molecular cloning studies, and slight differences between these subtypes may complicate pharmacological analysis. Overall, it seems likely that there are additional α_1-AR subtypes, but their properties are not yet clear.

SUBTYPE-SELECTIVE DRUGS

To date, only a few drugs have been found to have substantially different affinities for the pharmacologically defined α_{1A}- and α_{1B}-AR subtypes, and even fewer drugs differ substantially in affinity for the cloned subtypes. Most of these drugs have no potential for therapeutic utility because they also interact potently with other biological molecules. A major effort in drug design and screening should be directed at obtaining selective compounds that will be useful in analyzing the interactions and functional roles of these subtypes and that might have potential therapeutic applications.

The two most selective agents currently available are 5-methylurapidil (13, 14) and (+)niguldipine (15), both of which are at least 50- to 100-fold selective for the α_{1A} over the α_{1B} subtypes. However, 5-methylurapidil is also a potent serotonin receptor agonist (13, 14) and (+)niguldipine is a potent dihydropyridine-type calcium channel blocker (15). Although these actions do not complicate radioligand binding studies, they greatly confound functional differentiation of these subtypes. This is particularly true in smooth muscle, in which both serotonin receptors and dihydropyridine sensitive Ca^{2+} channels play major functional roles, and where defining the functional roles of α_1-AR subtypes is of major interest.

Other α_{1A}-selective antagonists that show less selectivity (10- to 20-fold) include WB 4101, benoxathian, and phentolamine (5, 9, 16). The selectivity shown by these drugs is sufficient for distinguishing between subtypes only under the simplest and most well-controlled conditions, such as radioligand binding assays in membrane preparations. They have only limited use in more complex situations in which responses are more complicated, drug concentration is less easily controlled, or coexisting subtypes interact with each other in complex ways. Oxymetazoline shows about a 50- to 100-fold higher affinity for the α_{1A} subtype than the α_{1B} subtype (11); however, the

partial agonist properties of this compound (which also activates serotonin and α_2-AR receptors) also restrict its use primarily to radioligand binding assays.

Currently no drugs are available that show any large degree of selectivity for the α_{1B} subtype. Spiperone showed a slight (13-fold) selectivity for the α_1-ARs in rat liver membranes (α_{1B}) over those in rat salivary gland membranes (α_{1A}) (47), but this compound has not proven particularly useful in subsequent studies. No other α_{1B}-selective competitive antagonists have been identified. As discussed above, the site-directed alkylating agent CEC is selective for the α_{1B} subtype. This compound does not have different binding affinities for α_{1A}- and α_{1B}-ARs, and the selectivity appears to reside in the lack of an appropriate reactive group in the α_{1A} subtype to which CEC can covalently attach (11, 12).

Few available drugs show substantial selectivity between the three cloned subtypes (α_{1B}, α_{1C}, α_{1D}) (Table 1). WB 4101 has a higher affinity for the receptors expressed by the $\alpha_{1A/D}$ and α_{1C} clones than for the receptor expressed by the α_{1B} clone. 5-Methylurapidil is more potent at the expressed α_{1C} clone than at either of the other two. The receptors expressed by the three clones show some differences in sensitivity to CEC inactivation ($\alpha_{1B} > \alpha_{1C} > \alpha_{1A/D}$), but this selectivity is rather small. Otherwise, the receptors expressed by these three clones do not differ pharmacologically.

Some evidence also suggests differential alkylation of α_{1A}- and α_{1B}-ARs by the alkylating prazosin analog SZL 49, or prazobind (48, 49). Piascik et al (48, 49) reported that injection of SZL 49 into rats in vivo causes a partial inactivation of α_1-ARs and presented some evidence that this inactivation resulted from selective alkylation of the α_{1A} subtype. However, the selectivity of this compound seems to be related to its route of administration rather than true subtype selectivity because it inactivates both α_{1A}- and α_{1B}-ARs equally well in membrane preparations (50).

Very little is known about the subtype selectivity of α_1-AR agonists. Both phenylephrine and methoxamine, although generally considered full agonists at α_1-ARs in smooth muscle and other isolated tissue preparations, show only weak intrinsic activity relative to NE in activating second messenger responses in tissues or cell lines (7). These compounds are probably partial agonists with only an intermediate efficacy, and their effects in intact tissues are likely magnified by large receptor reserves. Tsujimoto et al (51) reported that methoxamine is selective for the α_{1A} subtype in both affinity and efficacy. Because of the complexity of evaluating these parameters and the still uncertain state of α_1-AR subclassification, drawing definite conclusions can be difficult. One must remember these considerations when using these compounds.

Garcia-Sainz et al (26) used hepatocytes from guinea pigs, rats, and

rabbits, which express $\alpha_{1A/D}$-, α_{1B}-, and α_{1C}-ARs respectively, to examine the agonist pharmacology of these subtypes. Looking at labelling of phosphatidylinositol, they found that methoxamine had a high intrinsic activity in guinea pig liver ($\alpha_{1A/D}$) but not in rat or rabbit liver, while oxymetazoline had a high intrinsic activity in rabbit liver (α_{1C}) but not in guinea pig or rat liver (26). These observations indicate that methoxamine may be an $\alpha_{1A/D}$-selective agonist, whereas oxymetazoline may be an α_{1C}-selective agonist. One must view these results with caution, however, because the receptor reserves of the different subtypes in the different liver preparations are unknown, and efficacy cannot be obtained directly from intrinsic activity. In fact, an examination of other responses in the same hepatocyte preparations produced different relative intrinsic activities for these agonists (26). Nevertheless, these results do suggest that commonly used agonists may show different efficacies at different α_1-AR subtypes.

SIGNAL-TRANSDUCTION MECHANISMS

In most cells, the primary functional consequence of α_1-AR activation is an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) (7). This increase appears to result from the release of Ca^{2+} from internal stores and/or the influx of extracellular Ca^{2+} into the cell. The specific signaling mechanisms involved are only partly understood. Activation of α_1-ARs is coupled to the enzyme phospholipase C via a G-protein (Gp or Gq), which when activated can metabolize phosphatidylinositol(4,5)bisphosphate and produce inositol(1,4,5)trisphosphate [Ins(1,4,5)P_3] and diacylglycerol (DAG) (52). Ins(1,4,5)P_3 can interact with specific receptors on intracellular organelles to release stored Ca^{2+}. DAG can activate protein kinase C, which then phosphorylates many cellular proteins (including Ca^{2+} channels) that may regulate $[Ca^{2+}]_i$ (53). Evidence also indicates that α_1-AR activation can increase influx of extracellular Ca^{2+} via voltage-dependent (54) as well as non-voltage-dependent Ca^{2+} channels (55). Most of the data that support the existence of an α_1-AR coupled to the activation of voltage-dependent Ca^{2+} channels comes from studies using isolated vascular smooth muscle preparations in which dihydropyridine Ca^{2+} channel antagonists can inhibit contractions elicited by α_1-AR agonists (7, 56, 57).

In addition to the role each of these mechanisms plays separately in the elevation of $[Ca^{2+}]_i$, these signaling pathways might interact with each other to regulate increases in $[Ca^{2+}]_i$. For example, when α_1-AR activation depletes intracellular pools of Ca^{2+}, a sustained influx of extracellular Ca^{2+} into the cell through dihydropyridine-insensitive Ca^{2+} channels often occurs (58). Conversely, the opening of voltage-dependent Ca^{2+} channels may allow the initial influx of Ca^{2+} to activate release of Ca^{2+} from intracellular

stores (59). Additionally, other signaling pathways known to be mediated by α_1-ARs such as activation of phospholipases A_2 (60) and D (61) and potentiation of adenylate cyclase activity (44, 45) may also be involved in the regulation of $[Ca^{2+}]_i$ and must be considered as potential mechanisms by which α_1-ARs control $[Ca^{2+}]_i$.

It was suggested early on that α_{1A}- and α_{1B}-ARs might increase intracellular Ca^{2+} levels by different mechanisms (5). Studies in isolated smooth muscles showed that dihydropyridine Ca^{2+} channel blockers or removal of extracellular Ca^{2+} blocked contractions caused by α_{1A} activation, while contractions elicited by α_{1B} activation were insensitive to these treatments (5, 14, 16, 51, 56, 57). Han et al (16) suggested that α_{1A}-ARs might gate Ca^{2+} influx through voltage-gated channels, while α_{1B}-ARs mobilized intracellular Ca^{2+}.

Investigators now generally agree that activation of α_{1B}-ARs increases formation of DAG and Ins(1,4,5)P_3, and that Ins(1,4,5)P_3 mobilizes Ca^{2+} from intracellular stores. The signaling mechanism activated by the α_{1A} subtype, however, is less clear. Despite the evidence for influx of extracellular Ca^{2+} through voltage-gated channels (5, 14, 16, 51, 56, 57), α_{1A}-like receptors also increase inositol phosphate formation (62, 63). In addition, α_{1B}-ARs can activate Ca^{2+} influx (64), although not usually through voltage-gated channels. α_1-ARs have also been linked to activation of both pertussis toxin–sensitive (60, 65) and –insensitive (66, 67) G proteins, to arachidonic acid release (60), cAMP formation (44, 45), and phospholipase D (61). Although clues and correlations have been noted, the relationship between specific subtypes and signaling mechanisms remains a mystery.

Studies of cloned α_1-AR subtypes have suggested that, like other G protein–linked receptors, these receptors can activate multiple signaling mechanisms in transfected cells. All known clones can activate inositol phosphate formation in transfected cells (8, 17, 19, 22–24, 68), but interpretation of signaling by transfected subtypes is complicated. Receptor theory predicts that the density of a particular receptor subtype on a cell should be related in a predictable manner to the maximum response to receptor activation and the potency of agonists in activating that response. As receptor density increases, maximum response should increase to a ceiling level at which other components of the response (G protein, effector, substrates, etc) become limiting. At this point, further increases in receptor density are predicted to increase the potency of the agonist in activating the response without affecting the maximum achievable response, referred to as spare receptors or a receptor reserve. Presumably, by progressively increasing receptor density by transfection of receptor cDNAs, one could progressively increase maximum response and subsequently agonist potency. Under conditions of extremely high receptor expression, receptors might couple to

signaling mechanisms that they would not activate under more normal expression levels.

This observation is particularly important in studying signaling by G protein–linked receptors, because these receptors often couple to multiple signaling mechanisms when transfected into mammalian cells. Since transfections are usually done using vectors with strong constitutively active promoters, transfection usually results in extremely high levels of receptor expression. The observation that such transfected receptors can couple to several different second messenger systems is often difficult to interpret, because we cannot say whether such coupling would occur at more normal levels of receptor expression. Given the homologies between G protein–linked receptors of different classes, high-level expression of a particular receptor subtype would probably result in coupling to a particular signaling mechanism that might not normally be activated by that receptor subtype.

This conundrum is particularly confusing in studies of the signaling mechanisms associated with α_1-AR subtypes, as both native and cloned subtypes have been suggested to couple to various signaling mechanisms (5), as discussed above. The α_{1B} subtype expressed in MDCK cells increases inositol phosphate formation, mobilizes intracellular Ca^{2+}, and promotes arachidonic acid release in addition to promoting Ca^{2+} influx (64). Expression of both the α_{1B} and α_{1C} cDNA clones results in the expected coupling to inositol phosphate formation and Ca^{2+} mobilization, but also to Ca^{2+} influx and increases in cAMP (68). Although phospholipase C responses to α_{1B}-AR activation are usually insensitive to pertussis toxin (66), expression of this subtype in *Xenopus laevis* oocytes results in activation of phospholipase C through a pertussis toxin-sensitive G_o pathway (69). Whether there is any specificity of coupling, or whether specific subtypes preferentially couple to different signaling mechanisms is not yet known. Each subtype can probably couple to different signalling mechanisms, but this might occur at different receptor densities. These relationships remain to be worked out in detail.

CONCLUSIONS

Investigators have not yet reached a consensus on the number and signaling mechanisms of α_1-adrenergic receptor (AR) subtypes. Two native subtypes (α_{1A} and α_{1B}) can be distinguished pharmacologically, and three subtypes (α_{1B}, α_{1C}, and α_{1D}) have been cloned. One of the cloned subtypes (α_{1D}) was originally thought to encode the pharmacologically defined α_{1A} subtype. However, recent data suggest otherwise, and many investigators now agree that the α_{1A} subtype has probably not yet been cloned. The relationship between the cloned receptors and the native subtypes must be understood,

and any additional cDNA clones obtained, before the drug specificities and second messenger pathways of α_1-AR subtypes can be clearly defined.

Little is yet known about the cellular and tissue distribution of these subtypes, their developmental profiles, or their functional importance. Molecular cloning of complementary DNA sequences for the remaining subtypes will help to clarify the number and properties of these subtypes. Identification of drugs that can selectively target particular subtypes is an important goal that may result in therapeutic advances in numerous disease states, including benign prostatic hyperplasia. The newly recognized complexity of the adrenergic receptors presents us with both important challenges and new therapeutic targets. The potential impact of this field on medical therapeutics remains to be clearly defined.

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