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Structure and Function of α-Adrenoceptors

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I. Introduction 475
II. α-Adrenoceptor classification 476
A. α_1/α_2-Adrenoceptor classification 476
1. Anatomical classification 476
2. Functional classification 477
3. Pharmacological classification 477
B. α_1-Adrenoceptor heterogeneity 477
C. α_2-Adrenoceptor heterogeneity 480
III. α-Adrenoceptor signal transduction mechanisms 483
A. Second messenger systems 483
1. Calcium translocation 483
2. Membrane phospholipid metabolism 484
3. Inhibition of adenylyl cyclase 486
4. Sodium/hydrogen exchange 487
B. Correlation between α-adrenoceptor occupancy and function 488
1. Occupancy-response relationships 488
2. Relationship between α-adrenoceptor occupancy and activation of signal transduction mechanisms 489
3. Guanine nucleotide regulatory proteins and α-adrenoceptor function 490
IV. Molecular biology of the α-adrenoceptors 493
A. Cloning of α_1-adrenoceptor DNAs 493
B. Functional expression of α_1-adrenoceptor complementary DNAs 495
C. Cloning of α_2-adrenoceptor DNAs 496
D. Expression of α_2-adrenoceptor DNAs 498
V. Summary, conclusions, and future directions 499
A. α-Adrenoceptor subclassification 499
B. α-Adrenoceptor signal transduction 500
C. Molecular biology of the α-adrenoceptors 500
VI. References 501

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I. Introduction

THE endogenous catecholamines, epinephrine and norepinephrine, exert their physiological effects on target tissues through direct binding interactions with cell surface receptors. Pharmacological studies of metabolic effects provoked by adrenoceptor agents, both in vivo and in vitro, provided indirect evidence for the existence of specific receptor moieties. Following an evaluation of these data, Ahlquist (1948) proposed a division of adrenoceptor responses into two general classes, termed α and β, based on pharmacological criteria. The concept of two discrete types of adrenoceptor responses was further supported by the development of potent antagonists that are highly selective for the adrenoceptor subtypes, making them particularly useful in the classification and in the biochemical characterization of α- and β-adrenoceptors.

Since the original definition of α- and β-adrenoceptor responses, extensive studies of adrenoceptor pharmacology have pointed to further division of each receptor family into types. The β-adrenoceptor responses were divided into two classes, termed β_1 and β_2, based on the relative potencies of isoproterenol, epinephrine, and norepinephrine (Lands et al., 1967). β_1-Adrenoceptors demonstrate approximately equal affinity for epinephrine and norepinephrine, whereas β_2-adrenoceptors recognize epinephrine with higher affinity than norepinephrine. α-

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Adrenoceptor-mediated responses have also been subdivided into two types, α_1 and α_2, which are differentiated primarily by the relative potency of selective agonists and antagonists (Berthelsen and Pettinger, 1977; Langer, 1974). For example, α_1-adrenoceptors are stimulated by phenylephrine and blocked by prazosin, whereas α_2-adrenoceptors are stimulated by clonidine and blocked by yohimbine. Recently, data have been reported that support the notion of heterogeneity within each of the α-adrenoceptor types (Minneman, 1988; Bylund, 1985, 1988).

It is also possible to distinguish between α- and β-adrenoceptor responses based on mechanisms of signal transduction. The link between the activation of α_1- and α_2-adrenoceptors and the final functional response is a complex series of events that is only now starting to be understood in molecular terms. Activation of α_1- and α_2-adrenoceptors can initiate a variety of changes in membrane function and intracellular biochemical events including regulation of plasma membrane ion channels, activation of plasma membrane ion exchange mechanisms, and stimulation and inhibition of plasma membrane-associated enzyme systems leading to changes in the intracellular concentration of a wide variety of mediators, each of which is capable of producing many different changes in cell function. It is now believed that guanine nucleotide-binding regulatory proteins (G proteins) play a central role in the transduction of receptor activation to initiation of the primary step in the transduction pathway. One of the problems facing us now is the determination of which of these many possible events are involved in the signal transduction process and which occur as a by-product of the signal transduction process but play no role in the process itself. The process of signal amplification as it occurs at the different stages of α-adrenoceptor signal transduction is now being understood and can be useful in interpreting data from functional experiments.

Adrenoceptors, both α and β, appear to belong to a larger superfamily of membrane receptors that transmit information into the interior of cells through coupling to G proteins (Dohlman et al., 1987; Strader et al., 1989; Lefkowitz and Caron, 1988; Gilman, 1987). However, the signaling mechanisms of the α- and β-adrenoceptor classes are distinct. β-Adrenoceptors are linked to the activation of adenylyl cyclase resulting in the generation of the second messenger cAMP.* This is true for all β-adrenoceptor types (Lefkowitz et al., 1983; Emorine et al., 1989). Agonist binding to α_1-adrenoceptors leads to the activation of phospholipase C and the alteration in the intracellular concentration of Ca^{2+} (Minneman, 1988). α_2-Adrenoceptors appear to be linked to adenylyl cyclase but in an inhibitory fashion (Jakobs et al., 1976).

The isolation of pure α-adrenoceptor proteins, and the subsequent cloning and expression of several distinct α-adrenoceptors, has provided confirmation of the presence of multiple subtypes of both α_1- and α_2-adrenoceptors and has provided important clues regarding the actual molecular conformation of the receptor. Like many other neurotransmitter receptors, the primary structure of the α-adrenoceptors is consistent with seven membrane-spanning regions, which are highly conserved between different subtypes. Specific probes now allow the determination of α-adrenoceptor subtype distribution in an individual tissue. Much of this information has only been recently obtained, and many details remain unknown, such as the precise nature of the agonist interaction with the α-adrenoceptor or, indeed, the actual site at which the agonist or second messenger binds to effect a response. Although both functional and molecular studies show the presence of multiple α-adrenoceptor subtypes, an exact correspondence between the functional receptors and the expressed proteins has not yet been established.

II. α-Adrenoceptor Classification

A. α_1/α_2-Classification

1. Anatomical classification. It has been known for many years that α-adrenoceptor antagonists increase the overflow of norepinephrine that is produced by sympathetic nerve stimulation. Brown and Gillespie (1957) suggested that this effect was due to blockade of postjunctional α-adrenoceptors, thus preventing the combination of released norepinephrine with α-adrenoceptors on effector cells, thereby leading to an increase in norepinephrine overflow. In contrast, Thoenen et al. (1964) proposed that the increased overflow of norepinephrine was due to inhibition of neuronal uptake of released norepinephrine unrelated to α-adrenoceptor blockade, whereas Langer (1970) proposed that blockade of extraneuronal uptake may be responsible. Subsequently, Starke et al. (1971a,b) and Langer et al. (1971) postulated that this effect of α-adrenoceptor antagonists on norepinephrine release was a prejunctional phenomenon mediated by α-adrenoceptor blockade. Moreover, it was demonstrated that α-adrenoceptor agonists could inhibit the neurogenic release of norepinephrine by a mechanism involving prejunctional α-adrenoceptors and that norepinephrine could inhibit its own release by this negative feedback mechanism (Starke, 1972a,b). Further studies demonstrated that certain agonists, such as clonidine, and certain antagonists, such as phenoxybenzamine, could discriminate between pre- and postjunctional α-adrenoceptors (Starke et al., 1974, 1975; Dubocovich and Langer, 1974), thus providing evidence that these recep-

*Abbreviations: cAMP, cyclic adenosine 5′-monophosphate; CEC, chlorethylclonidine; SK&F 104078, 9-[(3-methyl-2-butenyl)oxy]-6-chloro-3-methyl-2,3,4,5-tetrahydro-3-benzazepine; PtdIns, phosphatidylinositol; PtdIns(4,5)P2, phosphatidylinositol-4,5-biphosphate; DAG, 1,2-diacylglycerol; INS(1,4,5)P3, inositol-1,4,5-trisphosphate; PA, phosphatidic acid; mRNA, messenger ribonucleic acid; cDNA, complementary deoxyribonucleic acid; PCR, polymerase chain reaction.

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tors, which were obviously in anatomically distinct locations, were also pharmacologically distinct entities. As a result of this differentiation between pre- and postjunctional α-adrenoceptors in vitro, Langer (1974) proposed that the postjunctional α-adrenoceptor that mediates effector organ response be termed α_1 and the prejunctional α-adrenoceptor that regulates neurotransmitter release be called α_2. Support for the differentiation of pre- and postjunctional α-adrenoceptors in vivo came from Drew (1976), who provided evidence that pre- and postjunctional α-adrenoceptors in the pithed rat could be distinguished pharmacologically from one another.

2. Functional classification. Frequently, the anatomical classification of prejunctional α_2- and postjunctional α_1-adrenoceptors holds true. However, it became clear that anatomical location alone cannot be used to classify α-adrenoceptors because the inhibition of α-melanocyte-stimulating hormone-induced melanin granule dispersion in frog skin (Pettinger, 1977) and the inhibition of isoproterenol-induced glycolysis and lipolysis in hamster isolated epididymal adipocytes (Schimmel, 1976), both being postjunctional events, are produced by agonists that are highly selective for α_2-adrenoceptors. This led Berthelsen and Pettinger (1977) to suggest a functional classification of α-adrenoceptors based not on receptor location but, rather, on the type of function mediated by the receptor subtype. Thus, α_2-adrenoceptors were proposed to be those that mediated inhibitory responses, whereas α_1-adrenoceptors were thought to mediate responses that were excitatory in nature.

However, the functional classification based on inhibitory α_2- and excitatory α_1-adrenoceptors was later shown not to apply in all instances. Following the development of the highly selective α_1-adrenoceptor antagonist, prazosin, Drew and Whiting (1979) were able to demonstrate that the vasoconstrictor response to norepinephrine in the rat and the cat was inhibited not only by prazosin but also by the selective α_2-adrenoceptor antagonist, yohimbine, thereby demonstrating that this postjunctional excitatory response could be mediated by α_2-adrenoceptors, as well as by α_1-adrenoceptors. Therefore, neither the anatomical location nor functional activity can be used reliably to classify α-adrenoceptors. Thus, a new method for α-adrenoceptor classification needed to be developed which is the now universally accepted pharmacological method of receptor subclassification based solely upon the relative affinities of highly selective antagonists and, to some extent, agonists.

3. Pharmacological classification. During the past 15 years, a pharmacological classification of α-adrenoceptors has evolved and is based on the relative potency of a series of agonists and antagonists. For example, an α-adrenoceptor that is activated by either methoxamine, cirazoline, or phenylephrine and is blocked in a competitive manner by low concentrations of prazosin, WB-4101, or corynanthine is classified as an α_1-adrenoceptor. Conversely, responses to either α-methylnorepinephrine, UK-14,304, B-HT 920, or B-HT 933 which are competitively antagonized by low concentrations of either yohimbine, rauwolscine, or idazoxan are classified as being mediated via α_2-adrenoceptors. The natural sympathetic neurotransmitter, norepinephrine, is a relatively nonselective adrenoceptor agonist, and the adrenal hormone, epinephrine, shows only a slight selectivity for α_2-adrenoceptors. The receptor subtype selectivity of several α-adrenoceptor agonists and antagonists is presented in table 1.

Several of these agonists and antagonists have now been radiolabeled and have proven to be useful ligands with which to characterize and classify α-adrenoceptors in radioligand-binding studies. Another use of such radiolabeled compounds has been to study the localization and distribution of α-adrenoceptors in various tissues using autoradiographic techniques. The use of these compounds has led to the development of several further subclassifications of both α_1- and α_2-adrenoceptors. However, not all of these further subclassifications are mutually compatible, and there is as yet no universally accepted subclassification beyond that of α_1- and α_2-adrenoceptors.

B. α_1-Adrenoceptor Heterogeneity

Findings from several laboratories suggest that the α_1-adrenoceptor can be pharmacologically subdivided further into distinct subtypes. This subdivision is based on several criteria, such as differential sensitivity of α_1-adrenoceptor-mediated responses to blockade by prazosin and other α_1-adrenoceptor antagonists and different requirements of α_1-adrenoceptor-induced responses for extracellular calcium. In addition, species differences in the affinity of certain α_1-adrenoceptor agonists (Ruffolo and Waddell, 1982; Ruffolo et al., 1984a; Ruffolo, 1985) and the demonstration of postjunctional α-adrenoceptors having characteristics of both α_1- and α_2-adrenoceptors (Hieble and Woodward, 1984, Skarby et al., 1983) would suggest that the α_1-adrenoceptor population may not represent one homogeneous class.

In general, comparison of antagonist affinities in different α_1-adrenoceptor assays shows excellent correla-

TABLE 1
Adrenoceptor agonists and antagonists commonly used in the subclassification of α_1- and α_2-adrenoceptors

	α_1-Adrenoceptor selective	Nonselective	α_2-Adrenoceptor selective
Agonists			
	Phenylephrine	Norepinephrine	Clonidine
	Methoxamine	Epinephrine	α-Methylnorepinephrine
	Cirazoline		B-HT 933
	Amidephrine		B-HT 920
	Sgd 101/75		UK-14,304
Antagonists			
	Prazosin	Phentolamine	Rauwolscine
	WB-4101	Tolazoline	Yohimbine
	Corynanthine		Idazoxan

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tion (Hieble et al., 1982; Timmermans et al., 1981). However, there are some reported data that suggest possible differences between α_1-adrenoceptors in different tissues. Prazosin is generally accepted as a potent and highly selective antagonist of α_1-adrenoceptors. If the receptor dissociation constants for prazosin against a variety of α_1-adrenoceptor-mediated responses are examined, there is nearly a 100-fold range in antagonist affinity observed (Agrawal et al., 1984; Medgett and Langer, 1984; Drew, 1985; Flavahan and Vanhoutte, 1986a). Medgett and Langer (1984) suggested that the wide range in prazosin affinity is a result of its ability to differentiate between two subtypes of α_1-adrenoceptors. They proposed a directly innervated α_1-adrenoceptor subtype with a high affinity for prazosin ($K_B < 0.4$ nM) and that the remaining α_1-adrenoceptors are located farther away from the nerve terminals and have a lower sensitivity to prazosin ($K_B > 1.6$ nM). Based on their observation of a high affinity (K_B 0.2 nM) for prazosin using methoxamine as the agonist in the isolated rat caudal artery, as compared to when norepinephrine is used as the agonist (K_B 1.3 nM), Medgett and Langer (1984) suggested that methoxamine may selectively stimulate the α_1-adrenoceptor subtype having high affinity for prazosin. Corynanthine, another selective α_1-adrenoceptor antagonist, does not appear to discriminate between these two subtypes, as evidenced by equal K_B values against methoxamine and norepinephrine in the rat caudal artery.

In vitro studies in the rabbit pulmonary artery also support a subdivision of the α_1-adrenoceptor into subtypes having high and low affinity for prazosin (Holck et al., 1983). Similarly, Flavahan and Vanhoutte (1986a) used differences in prazosin affinity observed in a variety of tissues to support the subdivision of α_1-adrenoceptors. However, in the rabbit pulmonary artery, in contrast to the rat caudal artery, methoxamine appears to activate the α_1-adrenoceptor subtype having low affinity for prazosin, based on a prazosin dissociation constant of 4.3 nM (Holck et al., 1983). In the rabbit pulmonary artery, the contractile response to clonidine has been shown to be mediated via the prazosin high-affinity site.

A widely studied subdivision of α_1-adrenoceptors has been proposed based on the ability of several α_1-adrenoceptor antagonists, most notably chlorethylclonidine, WB-4101, (+)-niguldipine, and 5-methylurapidil, to show biphasic displacement of [^{3}H]prazosin binding in membranes from a variety of central and peripheral tissues and the ability of these compounds to produce a differential antagonism of functional responses in intact tissues. Morrow et al. (1985) first demonstrated that, whereas indoramin and dihydroergocryptine produced monophasic displacement of specific [^{3}H]prazosin binding in rat cerebral cortical membranes, phentolamine and WB-4101 exhibited biphasic displacement that could be resolved into two components that each represented approximately 50% of the total binding. Thus, it was suggested that these α-adrenoceptor antagonists could discriminate between two α_1-adrenoceptor subtypes in the rat cerebral cortex. Further studies by Morrow and Creese (1986) confirmed and extended these findings and designated the α_1-adrenoceptor with subnanomolar affinity for WB-4101 as α_{1A} and the α_1-adrenoceptor with lower affinity as α_{1B}. More recent studies have shown that 5-methylurapidil has a greater selectivity (40- to 80-fold) than WB-4101 (10- to 30-fold) for the α_{1A}- vis-à-vis the α_{1B}-adrenoceptor (Gross et al., 1988) and that these α_1-adrenoceptor subtypes also exist in the human cerebral cortex obtained either at autopsy or during surgery (Hanft et al., 1989).

Minneman and coworkers (1988) proposed the existence of two α_1-adrenoceptor subtypes based upon functional and radioligand-binding studies which support the proposed subdivision into α_{1A}- and α_{1B}-adrenoceptors. These functional studies have shown that the two α_1-adrenoceptors appear to be linked to two different intracellular processes. Contraction of smooth muscle mediated by α_1-adrenoceptor activation is mediated through an increase in the intracellular concentration of ionized calcium. This increase may result from either the release of calcium from various intracellular stores or via the translocation of extracellular calcium through calcium channels located in the cell membrane (Nichols and Ruffolo, 1988). Within a tissue, there are differences among α_1-adrenoceptor agonists in their ability to utilize extracellular and intracellular calcium stores to produce smooth muscle contraction, and between tissues there are marked differences in the relative roles of intracellular and extracellular calcium in the response to a single agonist. Johnson and Minneman (1987) demonstrated that the alkylating analog of clonidine, CEC, could inactivate only approximately 50% of the α_1-adrenoceptors in the rat cerebral cortex and could not inactivate any α_1-adrenoceptors in rat hippocampus despite the fact that all of the α_1-adrenoceptors in both brain regions were sensitive to alkylation by phenoxybenzamine. Thus, it appeared that CEC could discriminate between α_1-adrenoceptor subtypes and that these subtypes had a differential distribution within different regions of the brain. Further studies showed that CEC could inactivate nearly all of the α_1-adrenoceptors in rat liver and spleen but very few of the α_1-adrenoceptors in the rat vas deferens (Han et al., 1987a). Thus, two tissues in which smooth muscle contractile responses can also be measured had been identified in which CEC could discriminate between apparent α_1-adrenoceptor subtypes. It was then shown that CEC selectively antagonized the contractile response to norepinephrine in the spleen without having any significant effect in the vas deferens (Han et al., 1987a). These α_1-adrenoceptor subtypes were designated α_{1a} for the CEC-insensitive receptor and α_{1b} for the CEC-sensitive receptor. Further studies showed that

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WB-4101 had a higher affinity for the CEC-insensitive site than the CEC-sensitive site, which suggested that CEC was selective for the α_1-adrenoceptor subtype previously designated by Morrow and coworkers as α_{1B} (Han et al., 1987b).

Moreover, the CEC-insensitive response to norepinephrine in the rat vas deferens was sensitive to inhibition by calcium channel blockers or chelation of extracellular calcium with ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,n′-tetraacetic acid, whereas the CEC-sensitive response in the rat spleen was insensitive to calcium channel blockers or chelation of extracellular calcium, suggesting that these two receptor subtypes are linked to different signal transduction processes. It was proposed, therefore, that α_{1a}-adrenoceptors were linked to the influx of extracellular calcium and α_{1b}-adrenoceptors were linked to the release of intracellular calcium (Han et al., 1987b). More recent studies have strengthened the argument that the CEC-sensitive α_1-adrenoceptor (α_{1b}) is the same as the WB-4101-insensitive α_1-adrenoceptor (α_{1B}) and that this receptor is linked predominantly or exclusively to the generation of intracellular inositol phosphates and the subsequent release of intracellular calcium (Minneman et al., 1988; Michel et al., 1990b). However, it may be prudent to retain the α_{1A}/α_{1B} terminology for binding studies and the α_{1a}/α_{1b} terminology for functional studies until a more definitive confirmation of identity is obtained.

The subdivision of α_1-adrenoceptors into those that are sensitive to inhibition by calcium channel blockers but insensitive to alkylating agents, and those with the inverse properties, was previously proposed by Timmermans and coworkers. For example, Timmermans et al. (1983a) suggested that two different α_1-adrenoceptor subtypes differentially produce either the release of intracellular calcium or the influx of extracellular calcium to account for the ability of calcium channel blockers to inhibit pressor responses in pithed rats produced by some α_1-adrenoceptor agonists, such as Sgd 101/75, but not others, such as cirazoline. Similarly, McGrath (1983) proposed that two α_1-adrenoceptor subtypes may be linked to the two different modes of calcium translocation in the rat anococcygeus muscle based on differing agonist potency series for contractile responses that are produced in the presence of extracellular calcium and those that are produced in calcium-free medium. Later, Timmermans et al. (1983a, 1985) and Ruffolo et al. (1984b) showed that the α_1-adrenoceptor-mediated pressor responses in pithed rats that are resistant to inhibition by calcium channel blockers are highly sensitive to inhibition by phenoxybenzamine and that elimination of the phenoxybenzamine- sensitive component renders the residual response highly sensitive to inhibition by calcium channel blockers. Thus, if two α_1-adrenoceptor subtypes are indeed responsible for these phenomena, then the α_1-adrenoceptor subtype that is sensitive to calcium channel blockers would be equivalent to the α_{1a}-adrenoceptor and the phenoxybenzamine-sensitive α_1-adrenoceptor subtype would be equivalent to the α_{1b}-adrenoceptor. However, the studies of Han et al. (1987a) in which CEC could discriminate between two α_1-adrenoceptor subtypes demonstrated that phenoxybenzamine could not discriminate between the two α_1-adrenoceptor subtypes. Thus, under some conditions, phenoxybenzamine appears to be relatively selective for α_{1b}-adrenoceptors, whereas under other conditions, it shows no α_1-adrenoceptor subtype selectivity.

Interestingly, the subdivision of α_1-adrenoceptors into the α_{1A}- and α_{1B}-subtypes was, and still is, based primarily on the inability of [^{3}H]prazosin to discriminate between the postulated α_{1A}- and α_{1B}-adrenoceptor subtypes, a phenomenon that is inconsistent with the previously described subdivision of α_1-adrenoceptors based upon a differential potency of prazosin against different agonists and in different tissues. However, it is possible that in those studies in which the ability of antagonists to displace [^{3}H]prazosin from a single site have been investigated have only studied the high-affinity [^{3}H] prazosin-binding site and that there exists, in addition to the α_{1a}- and α_{1b}-adrenoceptors, an additional α_1-adrenoceptor subtype(s) that is characterized by a relatively lower affinity for prazosin.

Recent studies have suggested that there may indeed be an α_1-adrenoceptor subtype that cannot be classified as either α_{1a} or α_{1b}. Thus, in the rat aorta, it appears that only one α_1-adrenoceptor subtype mediates the response to different agonists, despite the fact that these agonists have a differential reliance upon intracellular and extracellular calcium. It has been shown that, in the rat aorta, the partial α_1-adrenoceptor agonist, (−)-dobutamine, is highly sensitive to inhibition by nifedipine (Oriowo et al., 1990) and is only a weak partial agonist at inducing inositol phospholipid metabolism (Motley et al., 1990), suggesting a relatively selective interaction with α_{1a}-adrenoceptors. In contrast, the response to (−)-norepinephrine, which is proposed to interact with both of the α_1-adrenoceptor subtypes (Minneman, 1988), is relatively insensitive to inhibition by nifedipine (Oriowo et al., 1990) and produces a large increase in inositol phospholipid metabolism (Motley et al., 1990). Phenoxybenzamine (30 nM) abolishes the contractile response to (−)-norepinephrine, and pretreatment of the tissues with norepinephrine significantly protects against receptor alkylation by phenoxybenzamine. Moreover, (−)-dobutamine also protects the α_1-adrenoceptors in rat aorta from inactivation by phenoxybenzamine and to the same extent as (−)-norepinephrine. Thus, the equal abilities of (−)-norepinephrine (nonselective) and (−)-dobutamine (α_{1a}-selective) to protect against α_1-adrenoceptor alkylation by phenoxybenzamine, which is proposed to interact selectively with α_{1b}-adrenoceptors, are consistent with the concept of both agonists interacting with a

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single α_1-adrenoceptor that can activate different signal transduction processes. Additional support for the existence of an α_1-adrenoceptor that cannot be classified as being of either the α_{1a}- or α_{1b}-subtype comes from studies of the α-adrenoceptor that mediates the vasoconstrictor response to sympathetic nerve stimulation in the perfused rat caudal artery. Sulpizio and Hieble (1991) showed that the initial phasic constrictor response is exclusively dependent upon the release of intracellular calcium and the secondary tonic response is exclusively dependent upon the influx of extracellular calcium. According to the hypothesis of Minneman (1988) the phasic response would be mediated by α_{1b}-adrenoceptors and selectively antagonized by CEC, whereas the tonic response would be mediated via α_{1a}-adrenoceptors and be selectively antagonized by WB-4101, (+)-niguldipine and 5-methylurapidil. However, both phases of contraction can be abolished by low concentrations of WB-4101, (+)-niguldipine, and 5-methylurapidil which are selective for α_{1a}-adrenoceptors and by CEC up to 100 μM which selectively inactivates α_{1b}-adrenoceptors. Thus, it would appear that in this tissue exogenous norepinephrine activates a single receptor that is sensitive to both α_{1a}- and α_{1b}-adrenoceptor antagonists.

Although the above data provide evidence to support the concept of α_1-adrenoceptor heterogeneity, at the present time, there is no consistent mechanism by which to subdivide the α_1-adrenoceptor. However, receptor-cloning and expression studies have provided data that support the existence of α_1-adrenoceptor subtypes (see section IV). One must acknowledge that differences in α_1-adrenoceptor characteristics do exist between tissues and/or species, but in contrast to the clear distinction between α_1- and α_2-adrenoceptors, the heterogeneity that exists for α_1-adrenoceptors does not yet fit a consistent pattern. Thus, a subdivision of α_1-adrenoceptors that satisfies all of the experimental data is not yet at hand. However, it is likely that there are indeed additional subtypes of the α_1-adrenoceptor in view of the data from cloning experiments and that there may exist α_1-adrenoceptor subtypes that interact exclusively with one signal transduction process or G protein and other α_1-adrenoceptor subtypes that interact nonexclusively with multiple intracellular signal transduction processes.

C. α_2-Adrenoceptor Heterogeneity

There is now convincing evidence to suggest that α_2-adrenoceptors do not represent one homogeneous population of receptors. A comprehensive review of the literature reporting pharmacologically determined affinities of the α_2-adrenoceptor antagonist, yohimbine, led Drew (1985) to conclude that there may exist two different populations of α_2-adrenoceptors unrelated to their synaptic location. The major division appeared to be based on species variation, such as rodent versus non-rodent, although there are examples of intertissue variation within the same species. Consistent with this interspecies heterogeneity of α_2-adrenoceptors, Waterfall et al. (1985) reported that the affinities of rauwolscine, yohimbine, and a series of substituted benzoquinoxolazines were approximately equivalent at prejunctional α_2-adrenoceptors in the rat vas deferens, whereas the affinities of yohimbine and rauwolscine were approximately 100-fold greater than those of the benzoquinoxolazines for the prejunctional α_2-adrenoceptor in the rabbit vas deferens.

Radioligand-binding studies also suggest the existence of species differences in α_2-adrenoceptors. Summers et al. (1983) demonstrated that α_2-adrenoceptors in human cerebral cortex are similar or identical with those present on human platelets in which yohimbine has a high affinity and prazosin has a low affinity (Cheung et al., 1982). However, although the α_2-adrenoceptors in human cerebral cortex and human platelets appear to be identical with each other, both appear to be different from those in rat cerebral cortex, where yohimbine and rauwolscine have significantly lower affinities and prazosin has a relatively higher affinity (Cheung et al., 1982). Moreover, in the neonatal rat lung, the α_2-adrenoceptor is characterized by a relatively low affinity for yohimbine and a relatively high affinity for prazosin (Latifpour et al., 1982).

In addition to those differences between antagonist affinities, species variation in guanine nucleotide regulation of agonist binding are apparent. *p*-Aminoclonidine binding to porcine submandibular gland and lung is reduced in a dose-dependent manner by guanosine 5′-triphosphate as a result of a decrease in agonist affinity, whereas in the rat submandibular gland, guanosine 5′-triphosphate increases agonist binding by elevating the number of specific binding sites (Feller and Bylund, 1984). These species-dependent differences in α_2-adrenoceptor-binding characteristics have been confirmed in studies using soluble α_2-adrenoceptors from various tissues and species (Cheung et al., 1986; McKernan et al., 1986), thus eliminating potential artifacts that may arise when studying cell membranes from different species.

True receptor heterogeneity should be demonstrated in tissues from a single species rather than in tissues from different species. Work from Bylund and associates has been instrumental in establishing such a heterogeneity for α_2-adrenoceptors. For example, careful analysis of the ability of prazosin to inhibit [^{3}H]yohimbine binding to various regions of both rat (Bylund, 1985) and human (Petrash and Bylund, 1986) brain revealed discrete regions of heterogeneity. In some regions, the inhibition is characterized by prazosin competing with low affinity for only one site, and in others it is characterized by prazosin competing with two sites, one with a low affinity identical with that seen in the human platelet and the other with a high affinity identical with that seen in the neonatal rat lung. Interestingly, these regional differences in the ability of prazosin to identify two α_2-adrenoceptor-binding sites were not the same in

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the rat and the human brain, with the rat cortex demonstrating two sites with approximately equal densities but the human cortex demonstrating only one site. Two α_2-adrenoceptor-binding sites were identified within the human caudate in approximately equal proportions. Bylund (1985) proposed a subclassification of α_2-adrenoceptors based on the relative potency of prazosin, with the receptor having a low affinity for prazosin, typified by the human platelet α_2-adrenoceptor, being termed α_{2A}, and the receptor having a relatively higher affinity for prazosin, typified by the neonatal rat lung α_2-adrenoceptor, being termed α_{2B}. More recent studies have confirmed this subclassification of α_2-adrenoceptors and have identified other tissues and cell lines that possess only one of these α_2-adrenoceptor subtypes. Thus, the rabbit spleen (Michel et al., 1990a) and the human colonic adenocarcinoma cell line, HT29 (Bylund et al., 1988), contain only the α_{2A}-adrenoceptor subtype, whereas the rat kidney (Michel et al., 1990a) and neuroblastoma × glioma hybrid cell line , NG108–15 (Bylund et al., 1988), contain only the α_{2B}-adrenoceptor. Moreover, several ligands have been shown to have a good degree of selectivity for one or the other of these α_2-adrenoceptor subtypes. Thus, benoxathian, oxymetazoline, and WB-4101 are relatively selective for α_{2A}-adrenoceptors, whereas chlorpromazine, imiloxan, and prazosin are relatively selective for α_{2B}-adrenoceptors (Michel et al., 1990a). Importantly, functional studies of the inhibition of α_2-adrenoceptor-mediated attenuation of cAMP production with subtype-selective antagonists in HT29 and NG108–15 cells have confirmed the existence and definition of the α_{2A}-and α_{2B}-adrenoceptor subtypes (Bylund and Ray-Prenger, 1989).

More recently, a third α_2-adrenoceptor subtype, the α_{2C}-adrenoceptor, was proposed (Bylund, 1988). This receptor, which was identified on OK cells, an opossum kidney-derived cell line (Murphy and Bylund, 1988), has characteristics that are similar to those of the α_{2B}-adrenoceptor (e.g., a relatively high affinity for prazosin), but the ratio of the affinities of prazosin and yohimbine is intermediate between that of the α_{2A}- and the α_{2B}-adrenoceptors (Murphy and Bylund, 1988). As yet this receptor has not been identified in human tissues. Thus, the possibility still exists that an α_{2C}-adrenoceptor subtype exists only in the opossum kidney.

The evidence is now strongly in favor of a species-dependent heterogeneity of α_2-adrenoceptors, but more important, there is good evidence of tissue-dependent heterogeneity that is independent of species. Earlier studies of the effect of rauwolscine and prazosin on the contractile response to norepinephrine in isolated blood vessels suggested that there may exist an α-adrenoceptor that has properties that are intermediate between those of α_1- and α_2-adrenoceptors. For example, Skarby et al. (1983) found that contraction of cat mesenteric arteries produced by norepinephrine was competitively antagonized by both prazosin and rauwolscine. In this preparation, prazosin is only 20-fold more potent than rauwolscine, in contrast to the much higher ratio of nearly 1000-fold found in tissues with classical α_1-adrenoceptors. Similarly, in canine splenic artery, prazosin is only 10-fold more potent than rauwolscine in antagonizing the responses to norepinephrine, and in canine splenic vein, the ratio is only 3 (Hieble and Woodward, 1984).

Despite earlier unsuccessful attempts to differentiate between pre- and postjunctional α_2-adrenoceptors, it has been demonstrated that there exists a population of prejunctional α_2-adrenoceptors that are pharmacologically distinct from a population of postjunctional α_2-adrenoceptors. An excellent correlation exists between the affinities of a series of antagonists at prejunctional α_2-adrenoceptors in guinea pig atrium and postjunctional α_2-adrenoceptors in canine saphenous vein, suggesting that there is no pre/postjunctional α_2-adrenoceptor heterogeneity. This correlation holds true for members of several chemical classes, including yohimbine alkaloids (yohimbine and rauwolscine), imidazolines (phentolamine), tetralones (BE-2254), 3-benzazepines (SK&F 86466), benzodioxanes (piperoxan), and tetrahydroisoquinolines (SK&F 72223) (Hieble et al., 1986). Similarly, initial studies in vivo provided no convincing evidence for a significant differential antagonism of either pre- or postjunctional α_2-adrenoceptors by any of the currently available antagonists (Paciorek et al., 1984; Docherty and Hyland, 1985).

However, using a pharmacological approach, several groups have proposed that pre- and postjunctional α_2-adrenoceptors can be differentiated. de Jonge et al. (1981) found that several 2,5-disubstituted imidazolines were selective agonists at prejunctional α_2-adrenoceptors. However, the interpretation of these data is difficult because studies with agonists are complicated by potential differences in agonist intrinsic efficacy and tissue receptor reserve (i.e., differences in pre- and postjunctional α_2-adrenoceptor density).

Studies with a series of α_2-adrenoceptor antagonists of the 3-benzazepine class have provided convincing evidence for a population of prejunctional α_2-adrenoceptors that are pharmacologically distinct from those found postjunctionally in vascular smooth muscle. SK&F 104078 has been shown to be an antagonist at postjunctional vascular α_1- and α_2-adrenoceptors at concentrations that do not block prejunctional α_2-adrenoceptors in several models. The ability of SK&F 104078 to block postjunctional α_2-adrenoceptors in vitro has been shown many times (Hieble et al., 1988; Connaughton and Docherty, 1988; Kelly et al., 1989, Akers et al., 1989), and this compound has little or no antagonist activity at prejunctional α_2-adrenoceptors in atria from several species (Hieble et al., 1988) and in the guinea pig ileum (Hieble et al., 1988; Akers et al., 1989). Moreover, in contrast to rauwolscine and yohimbine, SK&F 104078 has little ef-

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fect on norepinephrine overflow from rabbit aorta, guinea pig atrium, guinea pig vas deferens, and human saphenous vein. Species differences cannot account for these observations because it has been shown that SK&F 104078 has antagonist activity at postjunctional α_2-adrenoceptors in saphenous vein from both dog and rabbit without having any significant effect on prejunctional α_2-adrenoceptors in atria from these species (Hieble et al., 1988). In addition, the ability of SK&F 104078 to discriminate between pre- and postjunctional α_2-adrenoceptors has even been demonstrated in a single tissue. Thus, in human saphenous vein, SK&F 104078 inhibits postjunctional α_2-adrenoceptors at concentrations that do not block prejunctional α_2-adrenoceptors (Hieble et al., 1991). However, data obtained from in vivo studies suggest that, similar to the original classification of α-adrenoceptors into α_1- and α_2-adrenoceptor subtypes, anatomical location cannot be used as a means of subclassification for the α_2-adrenoceptor subtypes. For example, although SK&F 104078 can antagonize the postjunctional α_2-adrenoceptor-mediated pressor response to selective α_2-adrenoceptor agonists in the pithed rat, the degree of antagonism, in contrast to that obtained with yohimbine and rauwolscine, is limited to a maximal 3- to 4-fold shift in the agonist dose-response curve. Thus, there would appear to be an SK&F 104078-resistant component to the postjunctional vascular α_2-adrenoceptor-mediated vasoconstrictor response in the pithed rat. Moreover, Akers et al. (1989) showed that a dose of SK&F 104078 that produces a significant inhibition of postjunctional vascular α_2-adrenoceptor-mediated pressor responses in pithed rats has no effect on prejunctional α_2-adrenoceptor-mediated inhibition of neurogenic tachycardia produced by B-HT 933, similar to that originally demonstrated by Hieble et al. (1988). However, this dose of SK&F 104078 did inhibit xylazine-induced inhibition of neurogenic tachycardia. Thus, it is possible that multiple prejunctional α_2-adrenoceptors exist, only one of which is insensitive to inhibition by SK&F 104078 and the other(s) which is sensitive. Thus, SK&F 104078 can distinguish between α_2-adrenoceptor subtypes that show a preferential, although not absolute, differential distribution between prejunctional and postjunctional locations.

Further support for the hypothesis that multiple prejunctional α_2-adrenoceptors exist, only one of which is insensitive to SK&F 104078, comes from the work of Ruffolo et al. (1991a). These authors showed that in the rat vas deferens rauwolscine is equipotent at inhibiting the prejunctional α_2-adrenoceptor-mediated inhibition of neurotransmitter release whether UK-14,304, xylazine, B-HT 933, B-HT 920, clonidine, or M-7 was used as the agonist (K_B 3.5 nM). In contrast, the prejunctional α_2-adrenoceptor agonist activity of only clonidine and M-7 was inhibited by SK&F 104078 with a K_B of 400 to 500 nM, whereas UK-14,304, xylazine, B-HT 933, and B-HT 920 were insensitive to SK&F 104078 at concentrations up to 10 μM. Comparative studies showed that SK&F 104078 could not antagonize the prejunctional α_2-adrenoceptor activity of any of the agonists in the guinea pig atrium, whereas it was capable of inhibiting the postjunctional α_2-adrenoceptor agonist activity of all of the agonists in the canine saphenous vein with a K_B of 400 nM (Ruffolo et al., 1991a). Thus, it would appear that the sympathetic neurons in the rat vas deferens, but not in the guinea pig atrium, possess a mixed population of prejunctional α_2-adrenoceptors that show a differential sensitivity to inhibition by SK&F 104078 and that those prejunctional α_2-adrenoceptors that are sensitive to SK&F 104078 are similar, or identical with, those found postjunctionally in the vasculature.

A recently identified α-adrenoceptor antagonist, SK&F 104856, has a similar profile to SK&F 104078, blocking α_1-adrenoceptors and postjunctional α_2-adrenoceptors, with no effect at prejunctional α_2-adrenoceptors in atrial preparations (Hieble, et al., 1991). Like SK&F 104078, SK&F 104856 produces weak blockade of the prejunctional α_2-adrenoceptor-mediated neuroinhibitory action of UK 14,304 in the rat vas deferens (DeMarinis et al., 1991). Whether the antagonist potency of SK&F 104856 in the rat vas deferens is dependent on the α_2-adrenoceptor agonist used has not yet been determined.

One of the problems of studying prejunctional α_2-adrenoceptors has been the inability to perform radioligand-binding studies on these receptors. The reason for this is not certain but is most likely due to the relatively low density of α_2-adrenoceptors on sympathetic neurons within a tissue, and the nerve terminals themselves are in a relatively low density compared with other tissue elements. However, recent studies by Bylund and coworkers suggest that the bovine pineal gland may represent a preparation in which receptors that resemble prejunctional α_2-adrenoceptors exist. The α_2-adrenoceptors in the bovine pineal gland can be distinguished from all other α_2-adrenoceptors through the demonstration of a relatively low affinity for SK&F 104078 compared with the other proposed α_2-adrenoceptor subtypes (Bylund and Iversen, 1990). Thus, these binding experiments support the hypothesis that SK&F 104078 can distinguish between α_2-adrenoceptor subtypes and that the SK&F 104078-insensitive receptor is a novel α_2-adrenoceptor. In the α_2-adrenoceptor subclassification scheme devised by Bylund and Iversen (1990), this SK&F 104078-insensitive receptor has been designated the α_{2D}-adrenoceptor.

Molecular biology has added credence to the concept of α_2-adrenoceptor subtypes. Although confusion remains regarding the correlation between the α_2-adrenoceptors studied by molecular biological techniques and those studied using the more traditional pharmacological techniques, pharmacologically distinct α_2-adrenoceptors

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have been cloned and several have been expressed (see section IV). Interestingly, none of the cloned α_2-adrenoceptors has a low affinity for SK&F 104078, suggesting that there is indeed a distinct SK&F 104078-insensitive α_2-adrenoceptor, termed the α_{2D}-adrenoceptor by Bylund and Iversen (1990), and that this receptor has yet to be cloned.

Radiolabeled derivatives of several imidazoline-containing molecules can bind to both the α_2-adrenoceptor and to additional, pharmacologically distinct, sites. Such sites can be labeled by either idazoxan, which acts functionally as a selective α_2-adrenoceptor antagonist, or by clonidine and *p*-aminoclonidine, which act as preferential agonists at the α_2-adrenoceptor, and have been detected both in the central nervous system (Boyajian et al., 1987; Boyajian and Leslie, 1987; Bricca et al., 1989; Ernsberger et al., 1990) and in several peripheral tissues (Langin et al., 1990; Michel et al., 1989; Senard et al., 1990). Clonidine and *p*-aminoclonidine do not label the same site as idazoxan (Brown et al., 1990). Because radioligand binding to this novel site cannot be displaced by the physiological α-adrenoceptor ligands, norepinephrine and epinephrine, it is more properly considered a non-adrenergic-binding site rather than a subtype of the α_2-adrenoceptor, although increasing evidence suggests that such a site may serve as a pharmacological receptor mediating, at least in part, the antihypertensive action of clonidine and other imidazolines (Ernsberger et al., 1990; Tibirica et al., 1991).

III. α-Adrenoceptor Signal Transduction Mechanisms

A. Second Messengers

1. Calcium translocation. Before the demonstration of the coexistence of postjunctional vascular α_1- and α_2-adrenoceptors, it was already known that calcium ions are involved in α-adrenoceptor-mediated vasoconstriction (Casteels et al., 1978; van Breemen et al., 1978). This phenomenon has been studied in great detail in vascular tissue in vitro and in vivo. van Meel et al. (1981) first suggested that an influx of extracellular calcium ions was necessary for arteriolar vasoconstriction mediated via α_2-adrenoceptors in vivo based on the sensitivity of α_2-adrenoceptor-mediated pressor responses to inhibition by a variety of organic and inorganic calcium channel antagonists. The role of an influx of extracellular calcium ions in α_2-adrenoceptor-mediated vasoconstriction was confirmed in one of the few in vitro arterial preparations in which α_2-adrenoceptor-mediated vasoconstriction has been demonstrated, namely, the rat isolated tail artery (Medgett and Rajanayagam, 1984). In vitro studies in isolated veins, particularly the canine saphenous vein, provided convincing evidence for a similar role for calcium ions in α_2-adrenoceptor-mediated vasoconstriction in the venous circulation. Such responses are reduced by organic and inorganic calcium channel antagonists, and are virtually abolished in calcium-free medium containing the calcium chelator, ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (Matthews et al., 1984a; Cooke et al. 1985). Furthermore, the α_2-adrenoceptor agonist, B-HT 920, produces an influx of $^{45}Ca^{2+}$ which is inhibited by the calcium channel antagonists, verapamil and nifedipine (Matthews et al., 1984a), but does not induce intracellular calcium release (Jim and Matthews, 1985). The influx of calcium produced by α_2-adrenoceptor agonists in the canine saphenous vein is not likely to be due to activation of voltage-dependent calcium channels in the smooth muscle plasma membrane because there is little change in membrane potential during smooth muscle contraction (Matthews et al., 1984b). In addition, much higher concentrations of the organic calcium channel antagonist, nimodipine, than those required to abolish depolarization-induced contraction of the canine saphenous vein are required to inhibit α_2-adrenoceptor-mediated contraction (Cooke et al., 1985). In contrast to the dog, α_2-adrenoceptor-mediated contraction of the rat saphenous vein is accompanied by membrane depolarization (Cheung, 1985a) and results from extracellular calcium ion influx initiated by opening of voltage-sensitive calcium channels (Cheung, 1985b). Thus, there is no uniform mechanism by which α_2-adrenoceptors induce calcium ion influx in vascular smooth muscle. At present, the relationship between the proposed inhibition of adenylyl cyclase in vascular smooth muscle produced by α_2-adrenoceptor agonists and the influx of extracellular calcium ion is unknown.

The relative roles of extracellular calcium influx and intracellular calcium release in the coupling of α_1-adrenoceptor activation to response has been the subject of much controversy in recent years. Most studies investigating calcium utilization in α_1-adrenoceptor-mediated responses have used vascular smooth muscle of both arterial and venous origin. Pressor responses in vivo mediated by postjunctional vascular α_1-adrenoceptors were found to be resistant to inhibition by calcium channel antagonists (van Meel et al., 1981; Cavero et al., 1983), suggesting that, unlike α_2-adrenoceptors, α_1-adrenoceptors may not rely heavily upon extracellular calcium to produce vasoconstriction. However, not all selective α_1-adrenoceptor agonists produce vasoconstriction in vivo that is resistant to inhibition by calcium channel blockers. For example, St 587, Sgd 101/75, and (−)-dobutamine all produce α_1-adrenoceptor-mediated pressor responses in pithed rats which are inhibited by calcium channel blockers (Ruffolo et al., 1984b). Moreover, in isolated arterial tissues in vitro, vasoconstriction elicited by α_1-adrenoceptor activation may or may not be sensitive to inhibition by calcium channel blockers. Thus, it would appear that some α_1-adrenoceptor agonists produce vasoconstriction via an influx of extracellular calcium, whereas others do not.

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Studies of $^{45}Ca^{2+}$ flux in rabbit aorta showed that α_1-adrenoceptor activation does indeed produce an influx of extracellular calcium (Deth and Lynch, 1981; Cauvin et al., 1982; Awad et al., 1983). Moreover, even α_1-adrenoceptor agonists that are relatively insensitive to inhibition by calcium channel antagonists (e.g., cirazoline) produce a significant influx of extracellular calcium of a magnitude that is greater than that produced by agonists that show a high degree of sensitivity to inhibition by calcium channel antagonists (e.g., St 587) (Chiu et al., 1986). Studies of the efflux of $^{45}Ca^{2+}$ have shown that α_1-adrenoceptor agonists also induce a release of calcium from intracellular stores (Deth and Lynch, 1981; Cauvin et al., 1982; Awad et al., 1983). However, it is only those agonists that have a high intrinsic efficacy that produce a significant release of intracellular Ca^{2+} (Chiu et al., 1986). Such $^{45}Ca^{2+}$ flux studies have revealed that the initial phasic component of α_1-adrenoceptor-mediated contraction of rabbit aorta and the secondary tonic component use different calcium pools. Leijten et al. (1985) proposed the following model of calcium mobilization produced by α_1-adrenoceptor activation in rabbit aorta and mesenteric artery. During α_1-adrenoceptor activation, calcium is released from a small labile intracellular store on the inner surface of the plasma membrane. This calcium does not activate smooth muscle contraction directly but, rather, triggers the release of calcium from the sarcoplasmic reticulum. Simultaneously, calcium bound on the outer surface of the plasma membrane is released and enters the cell through receptor-operated calcium channels. These two processes, which are responsible for the early phasic component of contraction, are followed by an influx of calcium from the free extracellular calcium pool which produces the secondary tonic contraction. However, the relative contribution of intracellular and extracellular calcium in α_1-adrenoceptor-mediated vasoconstriction varies with species and with anatomical location (Cauvin and Malik, 1984; Cauvin et al., 1984).

Thus, the data taken together suggest that both α_1- and α_2-adrenoceptors mediate an influx of extracellular calcium in both arterial and venous tissues and that α_1-adrenoceptors may additionally induce the release of intracellular calcium. A detailed analysis of the occupancy-response relationships for the influx of extracellular calcium and the release of intracellular calcium has helped us to understand the apparent discrepancy between the magnitude of the influx of extracellular calcium and the sensitivity to inhibition by calcium channel antagonists (see below).

An increase in the levels of free intracellular Ca^{2+} cannot be responsible for the prejunctional α_2-adrenoceptor-mediated inhibition of neurotransmitter release as it is for the postjunctional α_2-adrenoceptor-mediated vasoconstrictor response. Indeed, because an increase in intracellular calcium at the site of neurotransmitter release is believed to be intimately involved in the release process, it might be expected that α_2-adrenoceptor-mediated inhibition of neurotransmitter release is associated with a reduction in intracellular Ca^{2+}. This phenomenon has been observed in rat cortical synaptosomes in which α_2-adrenoceptor activation has been shown to reduce intracellular Ca^{2+} (Adamson et al., 1987). Thus, in contrast to postjunctional vascular α_2-adrenoceptors, activation of presynaptic α_2-adrenoceptors reduces intracellular Ca^{2+}. The mechanism by which this occurs, however, is unknown.

2. Membrane phospholipid metabolism. Following delineation of the role of the various calcium pools involved in responses mediated by α_1- and α_2-adrenoceptors, the biochemical events occurring after α-adrenoceptor activation and before the mobilization of calcium and the subsequent initiation of a functional response have subsequently been studied. In most systems, calcium translocation produced by agonist interaction with α_1-adrenoceptors is believed to be secondary to enhanced PtdIns turnover, due to activation of phospholipase C, in the so-called "PI response" (Michell, 1979). This pathway is shown diagrammatically in fig. 1. However, it appears that α_2-adrenoceptor-mediated responses are not the result of an increase in PtdIns turnover (Reese and Mathews, 1986) but, rather, may, in some cases, be associated with the inhibition of adenylyl cyclase. However, in hamster fibroblasts that have been transfected with clones expressing either the human kidney or platelet α_2-adrenoceptor, epinephrine has been shown to produce a small, but significant, increase in water-soluble inositol phosphates, suggesting that α_2-adrenoceptor activation can, under certain circumstances, stimulate phospholi-

FIG. 1. Schematic diagram of the cyclic nature of PtdIns turnover showing the potential role of various metabolites in signal transduction pathways. PIP, phosphatidylinositol-4-phosphate; IP_2, inositol-1,4-bisphosphate; IP, inositol-1-phosphate; CDP.DG, cytidine diphosphate diacylglycerol; PKC, protein kinase C; PLC, phospholipase C; ATP, adenosine triphosphate; CTP, cytidine triphosphate.

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pase C activity (Cotecchia et al., 1990). α_1-Adrenoceptor-mediated contraction of rabbit aorta correlates well with increases in PtdIns turnover (Villalobos et al., 1982), thereby suggesting a causative link between α_1-adrenoceptor-mediated activation of phospholipase C leading to hydrolysis of PtdIns and subsequent smooth muscle contraction. It was later observed that α_1-adrenoceptor stimulation of phospholipid turnover was the result not of hydrolysis of PtdIns but, rather, of the hydrolysis of polyphosphoinositides, particularly PtdIns(4,5)P2 (Berridge, 1983). The products of this hydrolysis, DAG and INS(1,4,5)P3, have been proposed to be the second messengers responsible for transduction of the α_1-adrenoceptor signal (Berridge, 1984). INS(1,4,5)P3 has been shown to release calcium ions from the endoplasmic reticulum in liver (Burgess et al., 1984; Joseph et al., 1984) and skinned porcine coronary artery smooth muscle cells (Suematsu et al., 1985), whereas DAG activates cytosolic protein kinase C (Kishimoto et al., 1980) which, under certain conditions, may induce slowly developing vascular smooth muscle contraction (Danthuluri and Deth, 1984; Rasmussen et al., 1984) possibly via an interaction with myosin light chain kinase (Nishikawa et al., 1984). In addition, PA, formed primarily via the phosphorylation of DAG by DAG kinase, is a calcium ionophore (Tyson et al., 1976) and causes calcium-dependent smooth muscle contraction (Salmon and Honeyman, 1980). It is possible, therefore, that PA may also be involved in the transduction of α_1-adrenoceptor activation into the final biological response.

Studies by Campbell et al. (1985) correlated phosphoinositide hydrolysis with calcium fluxes and smooth muscle contraction in rabbit aorta. They found that PtdIns(4,5)P2 hydrolysis, which yields INS(1,4,5)P3, is temporally associated with the release of intracellular calcium and the early phasic contraction, whereas PA production is temporally correlated with an influx of extracellular calcium and the maintenance of the tonic contraction. The precise role, if any, of DAG in the contractile process is unknown. It is possible that DAG, and not PA, may produce the influx of extracellular calcium, because PA production also reflects DAG production, and activation of protein kinase C produces tonic vascular contraction via an influx of extracellular calcium (Forder et al., 1985). In addition, DAG may moderately amplify the contractile response to the increase in intracellular calcium by a protein kinase C-mediated increase in calcium sensitivity. If this series of biochemical events is compared with the model of calcium fluxes described by Leijten et al. (1985), it must be assumed that the INS(1,4,5)P3-induced release of calcium from the sarcoplasmic reticulum is restricted to that area located on the inner surface of the plasma membrane, because the activator calcium is released by this small initial calcium release. Because this INS(1,4,5)P3-induced calcium release occurs at the same site as the production of INS(1,4,5)P3 (i.e., at the plasma membrane), the time lapse between PtdIns(4,5)P2 hydrolysis and the primary calcium signal and the amount of PtdIns(4,5)P2 required to produce a sufficient concentration of INS(1,4,5)P3, are minimized.

A hypothesis that links extracellular calcium influx and intracellular calcium release into a single complex process has been developed. There are obvious links between extracellular and intracellular calcium in that extracellular calcium is required for the maintenance of the intracellular calcium pool in the endoplasmic reticulum and that refilling of the intracellular stores from the extracellular space can occur without a significant increase in the level of free intracellular calcium (Poggioli and Putney, 1982; Aub et al., 1982). Because it is believed that INS(1,4,5)P3 is produced, and the initial intracellular calcium release occurs, at the interface between the plasma membrane and the endoplasmic reticulum (see above), it has been proposed that the influx of extracellular calcium that is measured is merely a reflection of the refilling of the internal calcium stores that are released as a result of an INS(1,4,5)P3-triggered event (Putney, 1987). Thus, in this scheme PtdIns metabolism and the generation of INS(1,4,5)P3 are the second messenger pathways for the release of intracellular calcium and the influx of extracellular calcium. There are several problems with this hypothesis, however, which lead to the conclusion that for α_1-adrenoceptor-mediated calcium mobilization, at least in the vasculature, such a scheme cannot explain all the available data. For example, this hypothesis would argue against the existence of agonists that could only produce an influx of extracellular calcium without a prior release of intracellular calcium. This clearly is not the case because it has been shown that certain α_1-adrenoceptor agonists, e.g., Sgd 101/75, will only produce an influx of extracellular calcium with no release of intracellular calcium (Chiu et al., 1986). Moreover, activation of α_1-adrenoceptors in the perfused rat caudal artery produces a biphasic response consisting of an initial rapid phasic component followed by a slower tonic component of vasoconstriction. It is possible to abolish the initial phasic component by inhibiting the release of intracellular calcium with ryanodine without affecting the subsequent tonic response. In addition, the secondary tonic response can be inhibited by blocking extracellular calcium influx with nifedipine without affecting the initial phasic response (Sulpizio and Hieble, 1991). These data demonstrate that, even if the primary role of the influx of extracellular calcium is to replenish the intracellular calcium pool, an excess of calcium enters such that the intracellular calcium concentration increases to a level that can support a significant level of smooth muscle contraction. More important, however, these data demonstrate that the two calcium mobilization processes that are activated by α_1-adrenoceptors can operate independently of each other

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and that intracellular calcium release is not required for the influx of extracellular calcium. Thus, it is clear that INS(1,4,5)P3 is not necessarily part of the transduction process for the α_1-adrenoceptor-mediated influx of extracellular calcium in vascular smooth muscle.

α_1-Adrenoceptor activation has been shown to stimulate phospholipase C and increase the production of INS(1,4,5)P3 in a variety of organs and cells. For example, α_1-adrenoceptor activation in the myocardium is associated with a rapid increase in INS(1,4,5)P3 production (Scholz et al., 1988) and a sustained increase in DAG production (Okumura et al., 1988). It has been shown that α_1-adrenoceptor activation in rat left ventricular papillary muscle produces an initial rapid increase in contractility which slowly declines and is temporally associated with the rapid but transient INS(1,4,5)P3 production followed by a secondary prolonged increase in contractility that is associated with the slower more prolonged production of DAG (Otani et al., 1988). Thus, in the myocardium membrane, phospholipid hydrolysis appears to play a crucial and complex role in the transduction of the α_1-adrenoceptor-mediated positive inotropic response.

Membrane phospholipid hydrolysis is also believed to underlie the development of desensitization of α_1-adrenoceptor responses in vascular smooth muscle. Desensitization of α_1-adrenoceptor-mediated contraction of rabbit aorta by exposure to epinephrine for 7 hours is not associated with a loss of postjunctional vascular α_1-adrenoceptors, or a change in agonist affinity, but rather is a consequence of a marked blunting of the epinephrine-induced increase in PtdIns turnover (Lurie et al., 1985). Desensitization of α_1-adrenoceptors by longer-term exposure (24 hours) to an α_1-adrenoceptor agonist is characterized by a decrease in receptor number with no change in affinity and a reduction in PtdIns turnover that is 50% greater than the reduction in receptor number (Bobik et al., 1984). It appears, therefore, that desensitization of vascular α_1-adrenoceptors results, at least in part, from an uncoupling between α_1-adrenoceptors and membrane phospholipid hydrolysis. The mechanism of this uncoupling may involve activation of protein kinase C by DAG. Activation of protein kinase C by phorbol esters markedly reduces the turnover of inositol phospholipids produced by α_1-adrenoceptor activation in cultured cells from rabbit aorta (Cotecchia et al., 1985) and hamster vas deferens (Leeb-Lundberg et al., 1985). This uncoupling is associated with phosphorylation of α_1-adrenoceptors in cultured cells from hamster vas deferens (Leeb-Lundberg et al., 1985). Thus, it appears that the complex metabolism of membrane inositol phospholipids is responsible, in part, for the transduction of α_1-adrenoceptor activation into the final biological response and is also a mediator of desensitization produced by α_1-adrenoceptor activation.

3. Inhibition of adenylyl cyclase. The inhibition of adenylyl cyclase has been postulated to be a component of the transduction mechanism between receptor activation and response in most, if not all, cells that possess functional α_2-adrenoceptors (Fain and Garcia-Sainz, 1980). Such an inhibition has been directly observed to occur in human platelets (Mills, 1975), neuroblastoma × glioma hybrid cells (Sabol and Nirenberg, 1979), rat renal cortex (Woodcock and Johnston, 1982), porcine thyroid (Muraki et al., 1984), and rat pancreatic islets (Yamazaki et al., 1982) and has been postulated to occur as a result of prejunctional neuronal α_2-adrenoceptor activation in rat neocortex (Schoffelmeer and Mulder, 1983) and after postjunctional vascular α_2-adrenoceptor activation in pithed rats (Boyer et al., 1983). However, the actual importance of this effect in the transduction of α_2-adrenoceptor activation into effector response in all systems is open to some doubt. Although (−)-epinephrine induces human platelet aggregation and inhibits adenylate cyclase, it has been observed that some α_2-adrenoceptor agonists induce platelet aggregation without producing inhibition of adenylate cyclase (Clare et al., 1984). In addition, other agonists that induce platelet aggregation are, in fact, antagonists of (−)-epinephrine-induced inhibition of adenylyl cyclase activity. Further evidence against inhibition of adenylyl cyclase as being a necessary intermediate in α_2-adrenoceptor-mediated platelet aggregation is provided by the work of Haslam (1975) in which it was shown that the aggregatory response produced by (−)-epinephrine is not accompanied by a decrease in intracellular levels of cAMP in resting platelets and that a reduction in intracellular cAMP is observed only after prior elevation by prostaglandin E_1.

α_2-Adrenoceptor-mediated inhibition of adenylyl cyclase is regulated by a guanine nucleotide-binding regulatory protein (G protein) that acts to couple α_2-adrenoceptor activation to a reduction in the catalytic activity of adenylyl cyclase in many cells. The precise mechanism of the interaction is at present unknown. However, it has been shown that pertussis toxin inactivates this G protein, termed G_i, via adenosine 5′-diphosphate ribosylation of the 41,000 Da α-subunit of the G_i protein (Murayama and Ui, 1983). Inhibition of α-adrenoceptor-mediated responses by pertussis toxin has been used as evidence for the critical role of an inhibition of adenylyl cyclase in the transduction of those responses. For example, α_2-adrenoceptor-mediated lipolysis in hamster adipocytes (Garcia-Sainz, 1981), insulin release from pancreatic islets (Katada and Ui, 1980), and vasoconstriction in pithed rats (Boyer et al., 1983; Nichols et al., 1988, 1989) are all inhibited by pertussis toxin. Boyer et al. (1983) interpreted their data as showing that α_2-adrenoceptor activation in vascular smooth muscle produces activation of G_i to reduce the activity of adenylyl cyclase. However, Nichols et al. (1988, 1989) interpreted these data, in conjunction with other data (see below), as showing that pertussis toxin is acting to inhibit the

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influx of extracellular calcium by inactivating a G protein that regulates the function of receptor-operated calcium channels in vascular smooth muscle. It was argued by Nichols et al. (1988, 1989) that, for an inhibition of adenylyl cyclase to be responsible for the transduction of an α-adrenoceptor-mediated response, there must, under normal conditions, be a level of adenylyl cyclase activity sufficient to keep intracellular cAMP levels at a concentration that is sufficient to produce an effect, i.e., vasorelaxation. Thus, if inhibition of adenylyl cyclase was truly responsible for α_2-adrenoceptor-mediated vasopressor responses, there must be a sufficient activation of adenylyl cyclase to keep levels of cAMP high enough to reduce blood pressure. In the pithed rat, this level of activation would need to be extremely high because resting blood pressure is very low and marked pressor responses (>80 mm Hg) can be attained. Because there is little likelihood of any circulating agents that can produce such a stimulatory effect on adenylyl cyclase in the pithed rat, it is unlikely that inhibition of adenylyl cyclase plays any significant role in the transduction of α_2-adrenoceptor-mediated vasoconstriction, even though these pressor responses are very sensitive to inhibition by pertussis toxin. However, this is not to say that, if adenylyl cyclase is activated and cAMP levels are elevated in vascular smooth muscle, α_2-adrenoceptor activation will not reduce levels of intracellular cAMP (Stubbs et al., 1988).

It has also been proposed that a pertussis toxin-sensitive inhibition of adenylyl cyclase is involved in the transduction of prejunctional α_2-adrenoceptor-mediated inhibition of neurotransmitter release in rabbit hippocampal slices (Allgaier et al., 1985) and rat vas deferens. However, in both cases, the degree of inhibition of the prejunctional α_2-adrenoceptor-mediated response by pertussis toxin was small. In contrast, Musgrave et al. (1988) showed that in vivo treatment of mice with pertussis toxin does not produce an inhibition of prejunctional α_2-adrenoceptor-mediated inhibition of [^{3}H]norepinephrine release from atria isolated from the treated animals. In addition, Nichols et al. (1988) showed that treatment of rats with pertussis toxin can abolish the postjunctional α_2-adrenoceptor-mediated pressor response without any significant effect on the prejunctional α_2-adrenoceptor-mediated cardioinhibitory response to B-HT 933 in pithed animals. Taken together, these data suggest that a pertussis toxin-sensitive G protein is not involved in prejunctional α_2-adrenoceptor-mediated inhibition of norepinephrine release or at most would appear to mediate a small and insignificant effect compared with the postjunctional α_2-adrenoceptor-mediated vasoconstriction.

In contrast to platelets and vascular smooth muscle, α_2-adrenoceptor-mediated inhibition of adenylyl cyclase through activation of a pertussis toxin-sensitive G_i protein probably does underlie the transduction of α_2-adrenoceptor activation to changes in water permeability in rat papillary collecting ducts. Thus, Gellai and Ruffolo (1987) showed that α_2-adrenoceptor agonists produce a positive free water clearance in the rat, most likely via an effect to inhibit the action of vasopressin on the collecting tubule (Stanton et al., 1987). This effect of renal tubular α_2-adrenoceptor activation was directly shown to be associated with a pertussis toxin-sensitive inhibition of vasopressin-stimulated adenylyl cyclase activity in the distal collecting duct (Edwards and Gellai, 1987).

It would appear, therefore, that in some systems, inhibition of adenylyl cyclase leading to a reduction in intracellular cAMP is an important intermediate step between α_2-adrenoceptor activation and effector organ response. However, there are some α_2-adrenoceptor-mediated responses that do not appear to use this second messenger system, despite being sensitive to inhibition by pertussis toxin.

4. Sodium/hydrogen exchange. Changes in calcium mobilization, inhibition of adenylyl cyclase, and stimulation of phospholipase C cannot account for all α_1- and α_2-adrenoceptor signal transduction processes. Thus, other intracellular second messenger systems must be involved in those systems in which the previously described pathways do not play a significant role. Such a pathway of intermediates between α_2-adrenoceptor activation and the second wave of aggregation in human platelets has been proposed by Limbird and coworkers (Limbird and Speck, 1983; Limbird, 1984; Sweatt et al., 1985). In this complex scheme, shown diagrammatically in fig. 2, it is postulated that α_2-adrenoceptor activation stimulates a plasma membrane-bound Na^+/H^+ exchange (antiporter) system which leads to an increase in intracellular pH, resulting from the enhanced extrusion of intracellular H^+, and a concomitant release of plasma membrane-bound calcium into the cell. Elevation of both intracellular pH and calcium ion concentration, acting together, increases the activity of phospholipase A_2 sufficiently to release small amounts of arachidonic acid from the phospholipids in the cell membrane. This small release of arachidonic acid leads to the production of thromboxane A_2 and the endoperoxide intermediates, prostaglandin G_2 and prostaglandin H_2, which subsequently produce potent activation of membrane-bound phospholipase C. Activation of phospholipase C increases the conversion of PtdIns(4,5)P2 into INS(1,4,5)P3 and DAG. DAG is hydrolyzed sequentially by a di- and monoacylglycerol lipase or can be phosphorylated to PA acid which can serve as a substrate for phospholipase A_2 to release large amounts of arachidonic acid. This arachidonic acid is converted into the potent aggregatory prostanoids, prostaglandin G_2, prostaglandin H_2, and thromboxane A_2.

Studies have been performed to try to determine whether a similar pathway to that proposed by Limbird and coworkers exists for either postjunctional α_1- or α_2-

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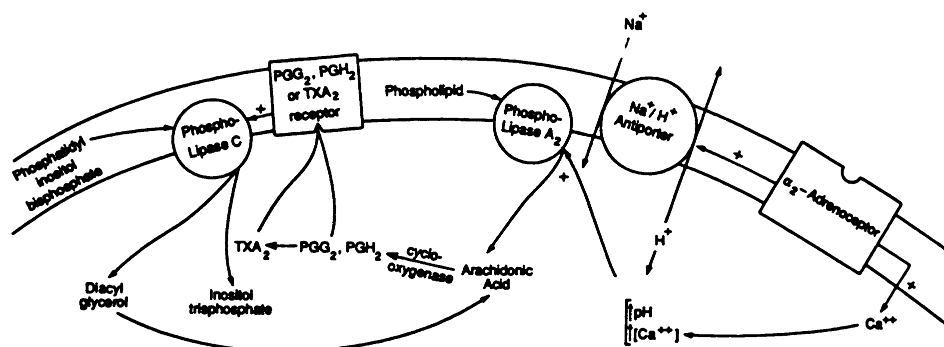

FIG. 2. Diagrammatic representation of the role of Na^+/H^+ exchange in the transduction of α_2-adrenoceptor-mediated secondary aggregation and secretion in human platelets. TXA_2, thromboxane A_2; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2.

adrenoceptor-mediated vasoconstriction or prejunctional α_2-adrenoceptor-mediated inhibition of norepinephrine release in pithed rats. It was shown that inhibition of thromboxane A_2 production by indomethacin, thromboxane A_2 receptor blockade by sulotroban, and inhibition of Na^+/H^+ exchange by amiloride had no effect on α_2-adrenoceptor-mediated vasoconstriction or inhibition of neurotransmitter release in vivo (Motley et al., 1988). Thus, it is unlikely that activation of Na^+/H^+ exchange or any signal transduction pathway involving arachidonic acid metabolism via the cyclooxygenase pathway is involved in postjunctional α_1- or α_2-adrenoceptor-mediated vasoconstriction or prejunctional α_2-adrenoceptor-mediated inhibition of norepinephrine release.

B. Correlation between α-Adrenoceptor Occupancy and Function

1. Occupancy-response relationships. The relationship between α_1-adrenoceptor occupancy by agonists and end-organ response has been studied extensively in cardiovascular tissues in vitro. Full α_1-adrenoceptor agonists commonly have a nonlinear occupancy-response relationship in most arterial vessels, and most full agonists (e.g., norepinephrine, epinephrine, phenylephrine, and cirazoline) produce half-maximal responses at approximately 5 to 10% α_1-adrenoceptor occupancy in large blood vessels such as rat aorta (Ruffolo et al., 1979), guinea pig aorta (Ruffolo and Waddell, 1982b), canine aorta (Sastre et al., 1984), rabbit aorta (Besse and Furchgott, 1976; Purdy and Stupecky, 1984), and rabbit ear artery (Purdy and Stupecky, 1984). Because of the difficulty in obtaining α_2-adrenoceptor-mediated contractile responses in arterial vessels in vitro, no direct comparison of the α_1- and α_2-adrenoceptor occupancy-response relationships has been made in arterial blood vessels.

In contrast to studies in vitro, α_2-adrenoceptor-mediated arterial vasoconstriction may be readily demonstrated in vivo where comparisons between the apparent occupancy-response relationships for α_1- and α_2-adrenoceptor-mediated vasopressor effects can be made. Irreversible alkylation of postsynaptic vascular α_1-adrenoceptors by phenoxybenzamine in conscious rabbits and pithed rats produces marked rightward shifts in the pressor dose-response curves to α_1-adrenoceptor agonists before depressions of the maximal response are observed. In contrast, alkylation of α_2-adrenoceptors by phenoxybenzamine is associated with depressed maximal vasoconstrictor responses with only small rightward shifts in the dose-response curves to α_2-adrenoceptor agonists (Hamilton et al., 1983; Reid et al., 1983; Ruffolo and Yaden, 1984). These results are highly suggestive of a more favorable occupancy-response relationship for α_1-adrenoceptors than for α_2-adrenoceptors in the arterial circulation. This differential pattern of α_1- and α_2-adrenoceptor responses following alkylation of vascular α-adrenoceptors was analyzed further in the pithed rat using cirazoline as an α_1-adrenoceptor agonist and B-HT 933 as an α_2-adrenoceptor agonist (Ruffolo and Yaden, 1984). Cirazoline possesses a hyperbolic relationship between the maximal pressor effect that can be obtained and the fraction of α_1-adrenoceptors remaining available for interaction with cirazoline after phenoxybenzamine treatment, suggesting the existence of a large α_1-adrenoceptor reserve. In contrast, for the α_2-adrenoceptor agonist, B-HT 933, a linear relationship was found to exist between the maximal pressor response obtainable and the proportion of intact α_2-adrenoceptors available for interaction with the agonist (Ruffolo and Yaden, 1984), a situation highly characteristic of a system with no receptor reserve (Ruffolo, 1982). The α_1-adrenoceptor-mediated vasopressor effect of cirazoline had a 5-fold more favorable occupancy-response relationship than was found for the α_2-adrenoceptor-mediated vasopressor effect of B-HT 933, which is consistent with the hypothesis that there may be spare α_1-adrenoceptors, but not α_2-adrenoceptors, in the arterial circulation for these agonists in vivo. This apparent large difference between the α_1- and α_2-adrenoceptor occupancy-response relationships in the arterial circulation may partly explain the difficulty of studying postjunctional vascular α_2-adrenoceptors in arteries in vitro compared to the ease with which α_1-adrenoceptors can be studied (Ruffolo, 1986).

α-Adrenoceptor occupancy-response relationships have been studied in preparations of the femoral and

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saphenous veins maintained in vitro. Similar to canine aorta (Sastre et al., 1984), canine saphenous vein possesses a large α_1-adrenoceptor reserve for full agonists, such as cirazoline (Ruffolo and Zeid, 1985; Flavahan and Vanhoutte, 1986b) and phenylephrine (Flavahan and Vanhoutte, 1986b). Thus, the nonlinear relationship between α_1-adrenoceptor occupancy and vascular contractile response is similar in canine aorta and saphenous vein. In contrast to the saphenous vein, the femoral vein of the dog possesses a very small or no α_1-adrenoceptor reserve for phenylephrine (Flavahan and Vanhoutte, 1986b). Thus, it is apparent that the degree of coupling between α_1-adrenoceptor occupancy and response can vary between vessels of different anatomical location and serving different functions. Flavahan and Vanhoutte (1986c) suggested that this difference in α_1-adrenoceptor density between femoral and saphenous veins may result from the different thermoregulatory role of these vessels. Cooling, which acts as a functional antagonist, reduces α_1-adrenoceptor-mediated vasoconstriction in the femoral vein because there is little α_1-adrenoceptor reserve. However, the α_1-adrenoceptor-mediated responses of the saphenous vein are buffered from the effect of cooling because of the large α_1-adrenoceptor reserve, and thus, canine saphenous vein can still constrict in response to α_1-adrenoceptor agonists when cooled. This leads to maintained constriction of the saphenous vein but markedly reduced constriction of the femoral vein which, in vivo, will divert blood from the superficial areas to the deeper layers of the leg and thus retard heat loss.

In contrast to the arterial circulation in vivo, it has been reported that the canine saphenous vein in vitro possesses an α_2-adrenoceptor reserve for B-HT 933, such that a hyperbolic occupancy-response relationship is observed for this agonist (Ruffolo and Zeid, 1985). However, in these experiments there was also found to be a 4-fold larger α_1-adrenoceptor reserve for cirazoline than α_2-adrenoceptor reserve for B-HT 933 (Ruffolo and Zeid, 1985), which is similar to the 5-fold difference in the occupancy-response relationships observed for these agonists in the arterial circulation of the pithed rat (Ruffolo and Yaden, 1984). In contrast to the findings of Ruffolo and Zeid (1985), Flavahan and Vanhoutte (1986b) found no α_2-adrenoceptor reserve in canine saphenous vein for UK-14,304, a potent, full α_2-adrenoceptor agonist. At present, it is not understood why the studies of Ruffolo and Zeid (1985) and Flavahan and Vanhoutte (1986b) have yielded different estimates of α_2-adrenoceptor reserve for full α_2-adrenoceptor agonists in the canine saphenous vein. Nevertheless, it is obvious that α_1-adrenoceptors predominate over α_2-adrenoceptors, and are associated with a larger receptor reserve, in both venous and arterial vascular smooth muscle.

The relationship between α-adrenoceptor occupancy and response has also been studied in non-cardiovascular tissues. It would appear that there is also a reserve for presynaptic α_2-adrenoceptors present on the postganglionic cholinergic neurons in the guinea pig ileum. Increasing concentrations of the irreversible α-adrenoceptor antagonist, benextramine, initially produce rightward shifts in the dose-response curve for the α_2-adrenoceptor agonist, α-methylnorepinephrine, with no depression of the maximal response, after which higher concentrations of benextramine produce a reduction of the maximal response (Mottram and Thakar, 1984). Analysis of these data by the method of Furchgott (1966) to yield a dissociation constant for α-methylnorepinephrine allows for the construction of an occupancy-response relationship that clearly demonstrates a receptor reserve for α-methylnorepinephrine at prejunctional α_2-adrenoceptors on postganglionic neurons in the guinea pig ileum, such that only 7% of the α_2-adrenoceptors need be occupied to produce a half-maximal response (Ruffolo et al., 1988).

2. Relationship between α-adrenoceptor occupancy and activation of signal transduction mechanisms. Although studies of the relationship between receptor occupancy by an agonist and the final functional response can yield important information, more information can be obtained by studying occupancy-response relationships for the various components of the signal transduction process. However, in many cases there is very little or no data to determine accurate relationships for these intracellular processes. Only now are data becoming available to allow us to get a clear picture of the full pathway of events with their respective quantitative interrelationships for the activation of vascular smooth muscle by α_1-adrenoceptors. The relationships that exist between α_1-adrenoceptor activation and intra- and extracellular Ca^{2+} fluxes must be considered when approaching this problem. In vitro studies of rat aorta have shown that there is a large α_1-adrenoceptor reserve for the overall contractile response to norepinephrine but that there appears to be little or no α_1-adrenoceptor reserve for that component of the response that is dependent upon the release of intracellular Ca^{2+} (Heaslip and Rahwan, 1983). Furthermore, norepinephrine is less potent at inducing intracellular Ca^{2+} release than at producing vasoconstriction, and Sgd 101/75, which is a partial α_1-adrenoceptor agonist with respect to the contractile response, has very little effect on intracellular Ca^{2+} release (Chiu et al., 1986). However, release of intracellular Ca^{2+} has a relatively high capacity because the response to the full agonist, norepinephrine, is reduced by only approximately 30% when extracellular Ca^{2+} influx is totally inhibited by nifedipine (Chiu et al., 1986).

In contrast, there must exist an α_1-adrenoceptor reserve for that component of the response to full agonists that is dependent upon the influx of extracellular Ca^{2+}, and this process must have a relatively low capacity. This is supported by the finding that the partial agonist, Sgd 101/75, which is dependent almost exclusively upon the influx of extracellular calcium for its contractile re-

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sponse, is a full agonist relative to norepinephrine for the influx of extracellular Ca^{2+} but produces a maximal contractile response of only approximately 40% of the norepinephrine maximal contractile response (Chiu et al., 1986).

Thus, it appears that the component of the α_1-adrenoceptor-mediated response in vascular smooth muscle that is produced by the influx of extracellular Ca^{2+} has an extremely high efficiency of coupling (albeit relatively low capacity), giving rise to an α_1-adrenoceptor reserve for this component of Ca^{2+} utilization, whereas the intracellular component has a low efficiency of coupling (but with high capacity), leading to a low α_1-adrenoceptor reserve for the mobilization of intracellular calcium. This information regarding the differences in the occupancy-response curves for the influx of extracellular Ca^{2+} and the release of intracellular Ca^{2+} was used to propose a mathematical model of the two transduction processes activated by a single α_1-adrenoceptor converging on one final response, namely, vascular smooth muscle contraction (Nichols and Ruffolo, 1988). This model (fig. 3) can explain why full α_1-adrenoceptor agonists produce maximal influxes of extracellular calcium but are relatively insensitive to inhibition by calcium channel antagonists, whereas partial α_1-adrenoceptor agonists may produce the same degree of Ca^{2+} influx but are highly sensitive to inhibition by calcium channel antagonists. Moreover, this model can explain why full α_1-adrenoceptor agonists can become sensitive to inhibition by calcium channel antagonists following removal of a portion of the α_1-adrenoceptor reserve by irreversible antagonists. For a detailed description of this mathematical model, see the papers by Nichols and Ruffolo (1988) and Ruffolo and Nichols (1988).

An α_2-adrenoceptor reserve has been proposed to exist in the human platelet for one of the postulated intermediate responses between receptor activation and platelet aggregation, namely, the inhibition of adenylyl cyclase. Lenox et al. (1985) found that the agonist dissociation constant for (−)-epinephrine (5.2 μM) was 20-fold greater than the concentration required to produce 50% inhibition of adenylyl cyclase activity (240 nM). From their radioligand-binding and functional studies, Lenox et al. (1985) calculated that only approximately 10% of the α_2-adrenoceptors needed be occupied to produce a half-maximal response. However, the value obtained for the dissociation constant of (−)-epinephrine (5.2 μM) is somewhat higher than that reported previously for α_2-adrenoceptors on human platelets [100 nM by Swart et al. (1984); 156 nM by Kawahara and Bylund (1985)] and human cerebral cortex [69 nM by Summers et al. (1983)], values that are, in fact, lower than the EC_{50} for inhibition of adenylyl cyclase. Thus, the existence of an α_2-adrenoceptor reserve for (−)-epinephrine in this system is open to some doubt. Furthermore, the relationship between (−)-epinephrine binding to α_2-adrenoceptors in human platelets and induction of platelet aggregation was studied by Swart et al. (1984) who found that the agonist dissociation constant was 5-fold lower than the EC_{50} for aggregation. Similarly, the EC_{50} values for the inhibition of adenylyl cyclase and induction of platelet aggregation are equivalent (Clare et al., 1984). It would appear, therefore, that there is no α_2-adrenoceptor reserve in human platelets.

3. Guanine nucleotide regulatory proteins and α-adrenoceptor function. The majority of neurotransmitters bind to and activate membrane receptors that are coupled to "transducer molecules" associated with the plasma membrane. These transducer molecules form a family of G proteins which have a heterotrimeric structure consisting of α-, β-, and γ-subunits (Gilman, 1987). After they are activated by an agonist-occupied receptor, G proteins modify the activity of the transduction systems that generate intracellular signals leading ultimately to physiological or pharmacological responses. The involvement of G proteins in a variety of intracellular signal transduction systems has been established, as has the fact that G proteins do not represent one homogeneous population of proteins but, rather, are a family of structurally related proteins serving to couple receptors to intracellular signal transduction systems (Gilman, 1987). It is likely that, similar to activation of adenylyl cyclase by G_s, it is the free guanosine 5′-triphosphate-activated α-subunit that is responsible for the transduction of the signal from receptor to effector (Birnbaumer, 1990). However, in some cases the $\beta\gamma$-subunits may be the transducer (see below).

As discussed previously, α_2-adrenoceptor activation can produce an inhibition of adenylyl cyclase. This inhibition of adenylyl cyclase can be abolished by pertussis toxin which inactivates G_i by adenosine diphosphate ribosylation of the 41,000-Da α-subunit (Murayama and Ui, 1983). The mechanism by which α_2-adrenoceptor-mediated activation of G_i inhibits adenylyl cyclase is at present unclear. However, several possible mechanisms have been proposed (fig. 4), all of which may occur under differing conditions (Birnbaumer, 1990). It has been proposed that activation of G_i results in the liberation of the $\beta\gamma$-subunits which can combine with free α_s, the active component of G_s that activates adenylyl cyclase, to yield the inactive α_s-$\beta\gamma$ complex, thus indirectly inhibiting adenylyl cyclase (Gilman, 1987). However, because adenylyl cyclase can be inhibited in cell systems that do not contain G_s, e.g. S49 cyc^- cells, this cannot be the only mechanism by which G_i inhibits adenylyl cyclase. Two other potential mechanisms of G_i-mediated inhibition of adenylyl cyclase have been proposed: (*a*) α_i directly inhibits the activity of the catalytic subunit and (*b*) α_i competes with α_s for binding to the catalytic subunit (Gierschik and Jakobs, 1988).

It has recently been shown that G proteins also partic-

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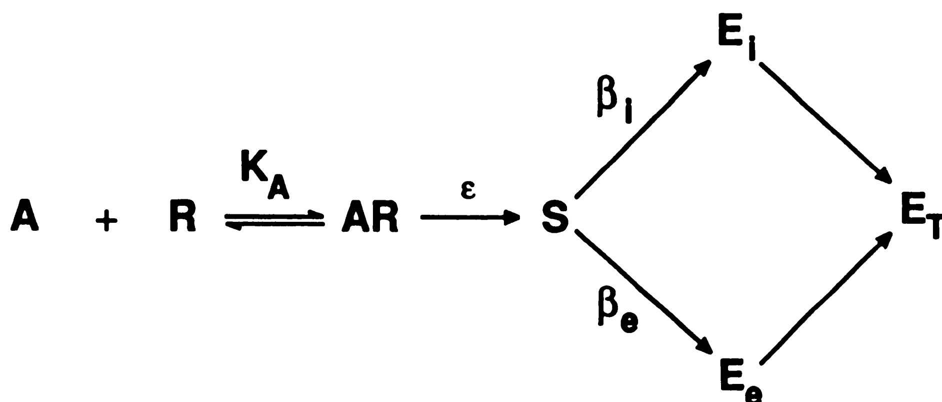

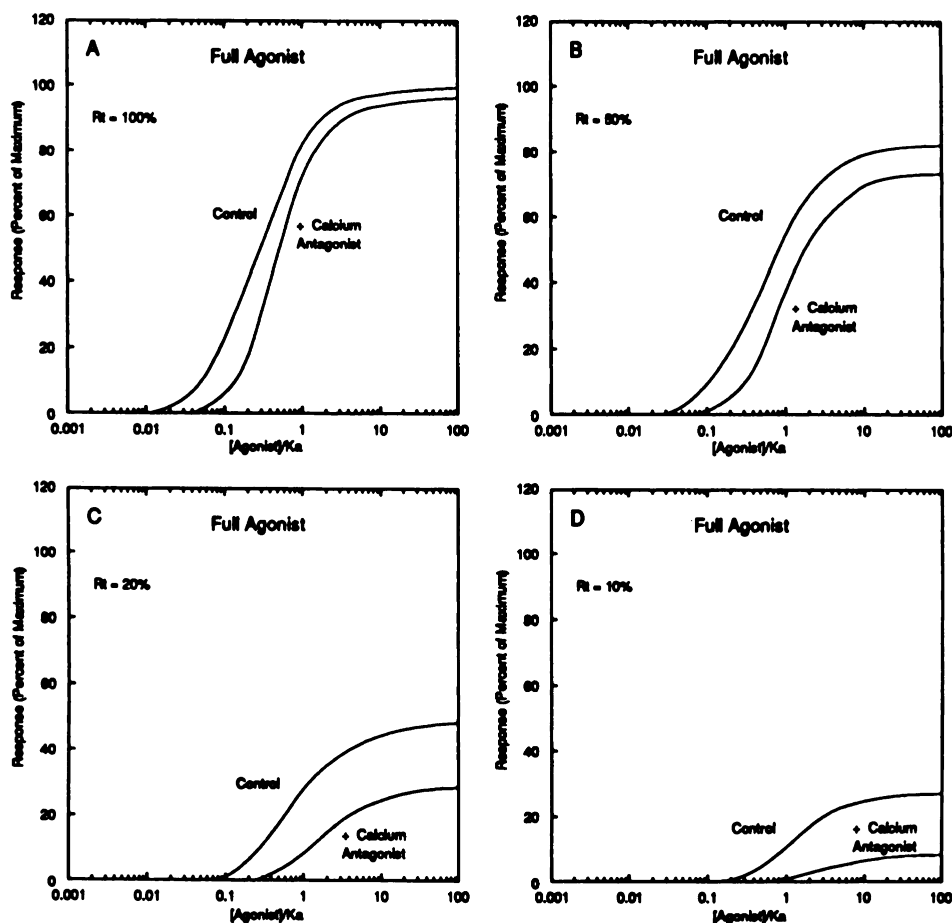

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FIG. 3. Mathematical model of two signal transduction processes activated by a single α_1-adrenoceptor converging on one final response. An agonist, A, interacts with a receptor, R, with a dissociation constant, K_A, and intrinsic efficacy, ϵ, to produce an initial stimulus, S (change in receptor conformation?). This initial stimulus is manifest as the ability to interact with G proteins. If the receptor can interact with multiple G proteins (as in this model), multiple signal transduction processes will be activated. The stimulus, S, is transduced into intracellular calcium release, E_i, and extracellular calcium influx, E_e, with efficiencies of coupling (amplification), β_i and β_e, respectively. The relative maxima of E_i and E_e and magnitudes of β_i and β_e (which can vary from tissue to tissue) will determine the relative magnitudes of E_i and E_e for a given stimulus, S. In this model, we have assumed a linear combination of E_i and E_e to give the final contractile response, E_T, based on the relatively linear relationship between intracellular calcium concentration and α_1-adrenoceptor-mediated contractile response in vascular smooth muscle. In vascular smooth muscle (as in many other tissues), the efficiency of coupling between an activated receptor and the release of intracellular calcium is low, but the amount of calcium that can be released is high. In contrast, the efficiency of coupling between the activated receptor and the influx of extracellular calcium is high, but the amount of calcium that can enter the cell this way is relatively low. Thus, an agonist that produces a large stimulus because of a large receptor number and high intrinsic efficacy will produce a proportionally greater release of intracellular calcium than influx of extracellular calcium. In contrast, an agonist that produces a small stimulus because of a small receptor number, a low intrinsic efficacy, or both will produce a proportionally greater influx of extracellular calcium than release of intracellular calcium.

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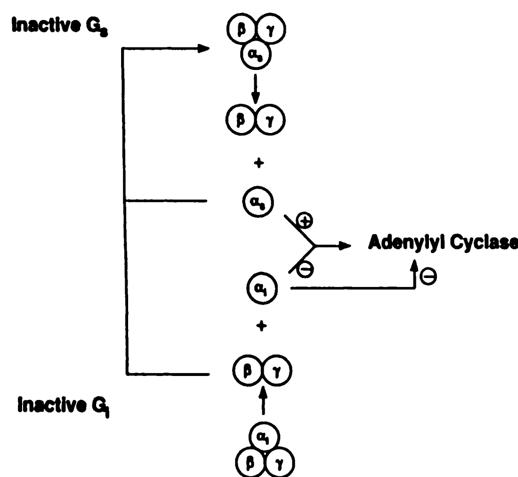

FIG. 4. Postulated mechanisms of α_2-adrenoceptor-mediated inhibition of adenylyl cyclase by G_i. Activation of G_i and G_s results in the dissociation of the α-subunits from the β-γ complex. The β-γ complex of G_i is very similar or identical with that of G_s, and thus β-γ complexes released from G_i could deactivate the active α_s-subunit by forming the inactive $\alpha_s\beta$-γ complex. In addition, α_i may inhibit the activity of adenylyl cyclase by either directly inhibiting the catalytic subunit or by competing with active α_s for binding sites on the catalytic subunit.

ipate in the regulation of transmembrane calcium fluxes mediated by receptors (Rosenthal et al., 1988), including postjunctional vascular α_1- and α_2-adrenoceptors (Nichols et al., 1988, 1989). Postjunctional α_2-adrenoceptor-mediated vasoconstriction is completely inhibited in rats pretreated with pertussis toxin (Nichols et al., 1988, 1989). These results indicate that α_2-adrenoceptor-mediated vasoconstriction, which is exclusively dependent upon the translocation of extracellular calcium through membrane calcium channels, involves a pertussis toxin-sensitive G protein. Interestingly, prejunctional α_2-adrenoceptor-mediated inhibition of neurotransmitter release is not affected by pretreatment with pertussis toxin (Nichols et al., 1988; Musgrave et al., 1988; Docherty, 1988), suggesting that, if a G protein is involved with prejunctional α_2-adrenoceptors, it must be different from that associated with postjunctional α_2-adrenoceptors in the vasculature (Nichols et al., 1988). We recently showed that pre- and postjunctional α_2-adrenoceptors are also pharmacologically distinct (Ruffolo et al., 1987; Hieble et al., 1988), and this may be, in part, responsible for these receptors utilizing different G proteins.

Because pertussis toxin-sensitive G proteins are known to link α_2-adrenoceptors to the inhibition of adenylyl cyclase, it is tempting to speculate that vasoconstriction produced by α_2-adrenoceptor agonists results from the inhibition of adenylyl cyclase through a process that involves a pertussis toxin-sensitive G protein, possibly G_i. However, as indicated above, although α_2-adrenoceptors do inhibit adenylyl cyclase in vascular smooth muscle, this process appears not to be relevant to the production of vasoconstriction (Nichols et al., 1989). Furthermore, the activation of a Na^+/H^+ antiporter in vascular smooth muscle by α_2-adrenoceptors likewise does not appear to contribute to the process of vasoconstriction (Motley et al., 1988), nor is the activation of phospholipase C leading to the hydrolysis of inositol phospholipids (Reese and Mathews, 1986). Because none of the known processes for signal transduction associated with α_2-adrenoceptors appears to be involved in the production of α_2-adrenoceptor-mediated vasoconstriction, it has been proposed that the α_2-adrenoceptor may be coupled to calcium channels in vascular smooth muscle either directly through a pertussis toxin-sensitive G protein or indirectly through the action of a currently unidentified signal transduction process which involves a pertussis toxin-sensitive G protein (Nichols et al., 1989). A direct interaction between G proteins and calcium channels was previously proposed (Rosenthal, 1988).

The involvement of pertussis toxin-sensitive G proteins in α_1-adrenoceptor-mediated responses is, like calcium utilization, more complex than that for α_2-adrenoceptors. Vasoconstriction produced by an α_1-adrenoceptor full agonist, which relies on both the translocation of extracellular calcium as well as on the mobilization of intracellular stores of calcium (Chiu et al., 1986), and predominantly the latter (Nichols and Ruffolo, 1988), is only slightly inhibited by pertussis toxin. The degree of inhibition by pertussis toxin of α_1-adrenoceptor-mediated vasoconstriction by a full agonist is comparable to the inhibition observed with a calcium channel blocker, suggesting that only that component of α_1-adrenoceptor-mediated vasoconstriction that is dependent upon the translocation of extracellular calcium involves a pertussis toxin-sensitive G protein (Nichols et al., 1989). By inference, the other component of α_1-adrenoceptor-mediated vasoconstriction by a full agonist that is dependent upon the mobilization of intracellular stores of calcium and that involves the activation of phospholipase C would likely involve a G protein that is insensitive to pertussis toxin, possibly G_p (Nichols et al., 1989).

α_1-Adrenoceptor-mediated vasoconstriction to partial agonists with low intrinsic efficacy, which involves nearly exclusively the translocation of extracellular calcium (Chiu et al., 1986; Nichols and Ruffolo, 1989), is highly sensitive to inhibition by pertussis toxin (Ruffolo et al., 1991b), again indicating that a pertussis toxin-sensitive G protein is involved in that component of α_1-adrenoceptor-mediated vasoconstriction that is dependent upon the translocation of extracellular calcium (Ruffolo et al., 1991b). Consistent with this notion is the observation that the degree of inhibition of α_1-adrenoceptor-mediated vasoconstriction to a partial agonist by pertussis toxin is qualitatively and quantitatively similar to the degree of inhibition produced by calcium channel blockers (Ruffolo et al., 1991b).

Nichols and Ruffolo (1988) proposed a model of the interaction among α_1- and α_2-adrenoceptors, G proteins, and calcium mobilization in the vasculature (fig. 5). In

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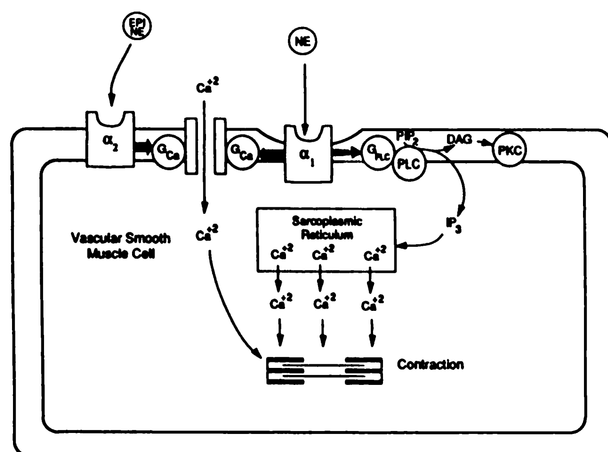

FIG. 5. Schematic representation of the proposed interaction among α_1- and α_2-adrenoceptors, G proteins, and calcium mobilization in the vasculature. A single α_1-adrenoceptor is coupled to both phospholipase C (PLC) and membrane calcium channels by two distinct G proteins. G_{PLC} refers to a pertussis toxin-insensitive G protein that couples the α_1-adrenoceptor to phospholipase C and G_{C_a} refers to a pertussis toxin-sensitive G protein that couples both α_1- and α_2-adrenoceptors to calcium channels. EPI, epinephrine; NE, norepinephrine; PKC, protein kinase C; IP_3, inositol-1,4,5-trisphosphate; PIP_2, phosphatidylinositol-4-bisphosphate.

this model, both α_1- and α_2-adrenoceptors are linked to membrane calcium channels by pertussis toxin-sensitive G proteins in vascular smooth muscle. α_2-Adrenoceptor agonists evoke the translocation of extracellular calcium through calcium channels that are coupled to the receptor through a pertussis toxin-sensitive G protein. Likewise, α_1-adrenoceptor-mediated vasoconstriction to both full agonists and partial agonists is also linked to calcium channels through a pertussis toxin-sensitive G protein. In addition, however, those α_1-adrenoceptor full agonists with high intrinsic efficacy are also coupled to phospholipase C and the ultimate mobilization of intracellular calcium (Legan et al., 1985; Chiu et al., 1986), presumably by a different G protein which, in the rat vasculature, is not sensitive to inhibition by pertussis toxin. As indicated above, a pertussis toxin-insensitive G protein linked to phospholipase C, termed G_p, is known (Harden et al., 1986), as is its involvement with α_1-adrenoceptor-mediated responses (Putney, 1987). A single α_1-adrenoceptor linked to two different G proteins may represent the biochemical basis for our proposal that α_1-adrenoceptor agonists with different intrinsic efficacies can utilize calcium from different functional pools by inducing either extracellular calcium mobilization or by mobilizing both intracellular and extracellular pools of calcium (Nichols et al., 1989; Nichols and Ruffolo, 1988). We conclude that the ultimate determinant of whether one or both of these G proteins is recruited by the activated α_1-adrenoceptor is the degree of stimulation and/or conformational change induced in the α_1-adrenoceptor by the interaction between the agonist and the receptor, which in turn is dependent upon agonist intrinsic efficacy.

This model has been proposed to explain the data obtained from experiments in which α_1- and α_2-adrenoceptor-mediated vasoconstriction were studied. This does not necessarily hold true for all α-adrenoceptor-mediated responses. However, this model may be a specialized form of a more general model in which receptors can interact with multiple G proteins to activate multiple signal transduction pathways with different efficiencies and in which multiple receptors may interact with the same G proteins (Ross, 1988).

IV. Molecular Biology of the α-Adrenoceptors

The characterization of adrenoceptors has been expedited by two technological advances. The development of radioligands that discriminate among the adrenoceptor types permitted their direct identification and characterization in broken cell preparations, and the development of highly potent and selective affinity ligands for labeling receptors in situ or for purification by biospecific affinity chromatography have made it possible to obtain sufficient quantities of adrenoceptors to define their biochemical properties. Both techniques have substantiated the pharmacological subclassification of adrenoceptors and have begun to define adrenoceptor subtypes in molecular terms.

The purification and characterization of adrenoceptors was a prerequisite to exploring their molecular biology. These approaches have shed new light on several important questions. First, what is the molecular basis for adrenoceptor subtypes? Do receptor subtypes result from posttranslational modification of a single gene product, do they arise from alternative splicing of mRNA transcribed from a single gene, or is each receptor subtype a product of a distinct gene? Second, what genetic elements regulate the expression of adrenoceptors in specific tissues, and can these genetic elements be controlled for therapeutic gain? Third, what is the molecular basis for signal transduction? Each receptor, by definition, serves two functions. One is to recognize agonist hormones and neurotransmitters through high-affinity binding interactions and the second is to initiate transmission of information into the target cell. Molecular biology, in conjunction with pharmacology and biochemistry, can help to identify the domains of the receptor molecules that are involved in ligand recognition and signal transduction. It should be pointed out that the application of molecular biology to the study of α-adrenoceptors is only very recent and, therefore, the studies discussed below raise as many questions as they answer.

A. Cloning of α_1-Adrenoceptor DNAs

Based on photoaffinity-labeling experiments (Leeb-Lundberg et al., 1984; Shreeve, 1988) and characterization of purified protein (Lomasney et al., 1986), the α_1-adrenoceptor has been identified as a glycoprotein with a molecular weight of 80,000 Da. This molecular weight differs considerably from that reported for the α_2-adrenoceptor purified from human platelets (67,000 Da) (Re-

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gan et al., 1984, 1986). This and the fact that peptide mapping of the two purified receptors is different suggested that they may be separate gene products. To gain a better understanding of the α_1-adrenoceptor, the isolation of its cDNA was undertaken. The cloning strategy required peptide sequences derived from the purified receptor protein. Purification of the α_1-adrenoceptor was a considerable undertaking, because 1 nmol of receptor was eventually stockpiled from the processing of 1600 liters of cultured DDT$_1$MF-2 cells, a hamster smooth muscle cell line (Cotecchia et al., 1988). The α_1-adrenoceptor was purified by detergent extraction from cell membranes and subsequent chromatographic procedures, including affinity chromatography using an immobilized prazosin analog, wheat germ lectin chromatography, and size-exclusion high-performance liquid chromatography. The purified receptor was then cleaved at methionine residues by cyanogen bromide, and three peptides were recovered from reverse phase high-performance liquid chromatography. An oligonucleotide probe was constructed based on the amino acid sequence of one of the peptides. The radiolabeled probe was then used to screen a hamster genomic library. A genomic library was screened because previous work had shown that the β_2-adrenoceptor gene (Kobilka et al., 1987a) and the α_2-adrenoceptor gene (see below) were without introns, thus increasing the probability that a full-length clone could be isolated from such a library. It seemed likely that the α_1-adrenoceptor gene might also be intronless. Indeed, a single clone that hybridized with the probe under high-stringency conditions was identified. The clone contained a 1.6-kilobase restriction fragment with an open reading frame, but the sequence proved to be incomplete. The sequence of only two of the three peptides derived from the purified receptor could be identified. The genomic fragment was then used to probe a cDNA library constructed from DDT$_1$MF-2 cell mRNA. A single 2-kilobase clone encoding the entire α_1-adrenoceptor sequence, including all three peptides, was obtained. The deduced amino acid sequence encoded for a protein of 515 amino acids with a calculated molecular weight of 56,000 Da. These results suggest that 30% of the molecular weight of the purified α_1-adrenoceptor is carbohydrate. In this regard, four potential glycosylation sites were identified near the NH_2 terminus of the protein primary sequence.

Hydrophobicity analysis of the α_1-adrenoceptor primary sequence revealed seven separate stretches of 20 to 25 hydrophobic residues that are connected by hydrophilic sequences. These hydrophobic regions have been proposed to represent putative transmembrane-spanning domains that are connected by hydrophilic loops that extend alternately into the extracellular and intracellular spaces from the plasma membrane (Dohlman et al., 1987; Strader et al., 1989; Lefkowitz and Caron, 1988). This protein pattern has been proposed as the common trademark of a large multigene family of receptors that couple to guanine nucleotide regulatory proteins during signal transduction (fig. 6).

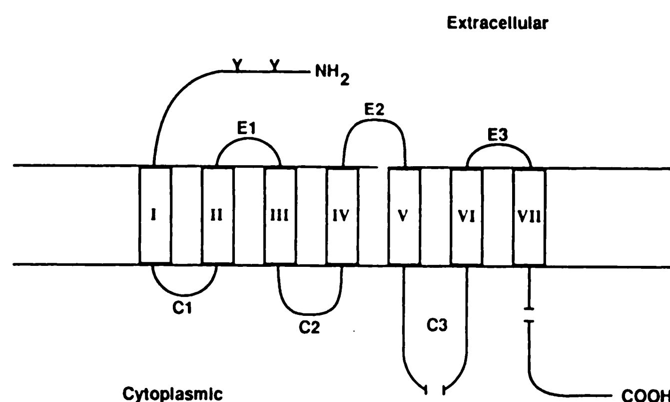

FIG. 6. Schematic model of G protein-coupled receptor proteins. Domains I to VII are proposed to span the membrane and are connected by hydrophilic sequences that extend into the extracellular (E) and cytoplasmic (C) space. Site(s) for N-linked glycosylation (Y) have been identified near the amino terminus. Among G protein-coupled receptors a high degree of variability has been observed in the third cytoplasmic loop (C3) and the carboxy tail.

An oligonucleotide probe of 66 bases encoding about half of the seventh membrane-spanning domain and carboxy terminus of the hamster α_1-adrenoceptor was used to screen a human genomic library in hopes of identifying the human receptor homolog (Schwinn et al., 1990). A single clone was identified under high-stringency conditions with a truncated open reading frame. The protein encoded by the human genomic fragment showed high homology to the transmembrane sequence of the hamster probe (67%), but the remaining sequence was quite divergent from the hamster α_1-adrenoceptor COOH-terminal tail. This clone probably represented a fragment of a human α_1-adrenoceptor isoform. A restriction fragment of the human genomic DNA encoding for most of its open reading frame was subsequently used to probe a bovine brain cDNA library. A single clone with a 3.1-kilobase insert was isolated and characterized. The cDNA encoded a protein of 466 amino acids giving a molecular weight of 51,000 Da. Hydrophobicity analysis indicated a similar alternating pattern of hydrophobic and hydrophilic sequences as was observed for the hamster α_1-adrenoceptor.

An additional α_1-adrenoceptor subtype was recently identified from a rat cerebral cortex cDNA library (Lomasney et al., 1991). An oligonucleotide probe derived from the cDNA of the hamster α_1-adrenoceptor under low-stringency conditions was used to identify two clones. Sequence analysis of the first clone proved it to be the rat homolog of the hamster α_1-adrenoceptor subtype. The open reading frame of the second clone was incomplete but provided suitable primer sequences to identify a full-length clone from rat genomic DNA using PCR. Sequence analysis of the coding region of the genomic clone revealed a protein of 560 amino acids.

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Comparison of the predicted primary sequences of the hamster, rat, and bovine α_1-adrenoceptors demonstrated that the proteins were significantly related. The regions of highest homology occurred in the seven putative membrane-spanning domains (65–73%). This degree of homology suggests that the proteins represent α_1-adrenoceptor subtypes and is consistent with relatedness among β-adrenoceptor subtypes (75%) and α_2-adrenoceptor subtypes (75%) when hydrophobic sequences are compared. The interspecies differences in primary sequence for a single adrenoceptor subtype is <15%. The hydrophobic regions of the α_1-adrenoceptors are also related (40 to 45% homologous) to other adrenoceptor types. This might be expected, because the membrane-spanning domains are thought to be involved in ligand binding and all the adrenoceptors share the common property of recognizing epinephrine and norepinephrine. The hydrophilic sequences in the hamster, rat, and bovine α_1-adrenoceptors are more divergent, particularly in the NH_2-terminal and COOH-terminal domains as well as the third intracellular loop. These regions may participate in defining the unique functional properties of these α_1-adrenoceptors.

B. Functional Expression of α_1-Adrenoceptor Complementary DNAs

Confirmation that a cloned DNA sequence encodes the protein of interest requires an assessment of function following expression in an appropriate cell system. The cDNAs encoding the hamster and bovine α_1-adrenoceptors were ligated into mammalian expression vectors under the control of the Rous sarcoma virus promoter (Schwinn et al., 1990). These vectors were transfected into monkey kidney COS-7 cells for transient expression. Crude membranes prepared from cell lysates were characterized for α_1-adrenoceptor expression using the radiolabeled α_1-adrenoceptor antagonist, 2-[β-(4-hydroxy-3-[^{125}I]iodophenyl)ethylaminomethyl]-tetralone. Saturation binding revealed efficient expression of both the hamster and bovine α_1-adrenoceptors, with a B_{max} of 15 pmol/mg protein and a K_D for the radioligand between 50 and 100 pM. Competition binding experiments were conducted using a variety of adrenoceptor agonists and antagonists (table 2). The data revealed that both the hamster and bovine clones display the appropriate pharmacology for α_1-adrenoceptors. Thus, prazosin, WB-4101, and phentolamine were more potent than yohimbine and rauwolscine for inhibiting the binding of 2-[β-(4-hydroxy-3-[^{125}I]iodophenyl)ethylaminomethyl]-tetralone, and oxymetazoline and phenylephrine were more potent than dopamine or isoproterenol. However, under closer inspection, differences between the two α_1-adrenoceptor clones were readily identified. Oxymetazoline and methoxamine were approximately 10-fold more potent in membranes expressing the bovine α_1-adrenoceptor compared to the hamster α_1-adrenoceptor. Moreover, the antagonists, WB-4101 and phentolamine, were more

TABLE 2

*Competition by agonists and antagonists for the binding of 2-[β-(4-hydroxy-3-[^{125}I]iodophenyl)ethylaminomethyl]-tetralone to membranes prepared from transfected COS-7 cells expressing either bovine or hamster α_1-adrenoceptors**

	K_i (nM)		Hamster α_1/ bovine α_1
	Bovine α_1	Hamster α_1	
Agonists			
Oxymetazoline	0.027	0.29	11
p-Aminoclonidine	0.59	0.68	1
Epinephrine	6.55	4.82	0.7
Phenylephrine	15.0	15.8	1
Norepinephrine	16.5	9.6	0.6
Methoxamine	75	1200	16
Dopamine	230	400	2
Serotonin	230	330	1
Antagonists			
Prazosin	0.27	0.26	1
WB 4101	0.55	8.5	16
Phentolamine	4.8	155	32
Indoramin	6	84	14
Corynanthine	78	640	8
Yohimbine	320	1300	4
Idazoxan	1500	1100	0.7

* Data from Schwinn *et al.* (1990).

than one order of magnitude more potent at the bovine α_1-adrenoceptor than at the hamster receptor. This pattern of differential affinities has been proposed as evidence for the existence of α_1-adrenoceptor subtypes (Minneman, 1988). According to the current nomenclature, the bovine α_1-adrenoceptor would be termed α_{1A} and the hamster receptor α_{1B}.

However, additional data raises questions as to the pharmacological classification of the bovine α_1-adrenoceptor. First, the susceptibility of α_1-adrenoceptors to inactivation by the alkylating agent, CEC, has been used as a criterion for distinguishing between α_{1A}- and α_{1B}-adrenoceptor subtypes (Minneman, 1988). The α_{1A}-adrenoceptor subtype is reported to be insensitive to inactivation by CEC, whereas the α_{1B}-adrenoceptor can be completely inhibited. Following expression of the bovine α_1-adrenoceptors in COS cells, it was shown that the receptors were partially inhibited (68%) by CEC, whereas the hamster α_1-adrenoceptors were totally inactivated under the same conditions (Schwinn et al., 1990). Second, the tissue distribution of α_1-adrenoceptor mRNA, as determined by Northern blot analysis, did not support a clear classification for the bovine α_1-adrenoceptor. Although mRNA for the α_{1B}-adrenoceptor, as detected with the hamster cDNA as probe, was abundant in liver, heart, and cerebral cortex, cDNA for the bovine α_1-adrenoceptor was unable to detect mRNA in any bovine or rat tissues (Schwinn et al., 1990). These results suggest that the bovine α_1-adrenoceptor subtype is expressed in only very select tissues or that mRNA levels are below detectability. The data suggest that the bovine α_1-adrenoceptor subtype is probably not the α_{1A} subtype but a close and novel relative in the α_1-adrenoceptor family.

A rat α_1-adrenoceptor gene distinct from the bovine

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and hamster genes has recently been isolated (Lomasney et al., 1991). The tissue distribution of the mRNA from this gene matches that of the α_{1A}-adrenoceptor as assessed by binding of WB-4101 and CEC and thus may represent the gene for the α_{1A}-adrenoceptor. However, a full pharmacological characterization of this receptor is required before a definitive assignment can be made. Interestingly, this α_1-adrenoceptor, but not the hamster α_{1B}-adrenoceptor or the bovine α_1-adrenoceptor, has been found in the rat aorta, a tissue in which pharmacological studies cannot classify the α_1-adrenoceptor as being of either the α_{1A}- or α_{1B}-subtype (Oriowo et al., 1990; Mir and Fozard, 1990).

Further characterization of the functional expression of the hamster α_{1B}-adrenoceptor has been reported (Cotecchia et al., 1990). Exposure of COS-7 cells expressing the hamster α_1-adrenoceptor to 100 μM epinephrine or norepinephrine resulted in a stimulation of PtdIns turnover that was completely blocked by antagonists with the appropriate specificity. Stimulation of total inositol phosphates was approximately 3-fold over basal levels with an EC_{50} for epinephrine of 200 to 300 nM. High-performance liquid chromatography analysis was used to show that INS(1,4,5)P3, thought to be the active second messenger for Ca^{2+} mobilization, was rapidly (<30 s) induced by epinephrine in the transfected cells.

Interestingly, in COS-7 cells expressing α_1-adrenoceptors, norepinephrine or epinephrine stimulated a 4-fold increase in cellular cAMP accumulation (Cotecchia et al., 1990). High levels of the β-adrenoceptor antagonist, propranolol, were included in these experiments to eliminate a β-adrenoceptor-dependent response. This increased cAMP accumulation could be completely blocked by prazosin and was not observed in nontransfected COS-7 cells. This effect on cAMP metabolism, mediated by α_1-adrenoceptors, does not appear to be the result of a direct effect on the adenylyl cyclase because no stimulation is observed in broken cell preparations. An α_1-adrenoceptor-dependent cAMP accumulation has been reported in liver (Morgan et al., 1983) and brain (Johnson and Minneman, 1986). The mechanism is not known but may involve the regulation of adenylyl cyclase by protein kinase C.

C. Cloning of α_2-Adrenoceptor DNAs

The strategy for cloning DNA encoding the α_2-adrenoceptor(s) was similar to that described above for the α_1-adrenoceptor. The first step entailed large-scale purification of the human platelet α_2-adrenoceptor to obtain the amino acid sequence. Approximately 1 nmol of α_2-adrenoceptor was purified from 1400 units of human platelets (Kobilka et al., 1987b). Following detergent solubilization, the receptor was purified to homogeneity by a combination of affinity chromatography, wheat germ lectin chromatography, and heparin-Sepharose chromatography. Peptides were generated by incubating the purified receptor with cyanogen bromide either alone or in combination with protease. Four peptides were isolated by reverse phase high-performance liquid chromatography and their amino acid sequences determined. Two overlapping 39-base oligonucleotide probes were constructed based on the amino acid sequence of one of the peptides. The probes were radiolabeled and used to screen a human genomic library. Three clones were identified under high-stringency conditions and were found to have identical inserts by restriction enzyme mapping. A 5.5-kilobase fragment of the genomic DNA that hybridized to both probes was isolated and characterized. The sequence of the open reading frame encoded for a protein of 450 amino acids, and the sequences of all four of the peptides derived from the purified α_2-adrenoceptor were identified within the sequence. Because the open reading frame encoding the human platelet α_2-adrenoceptor was continuous, the gene for this receptor was uninterrupted by introns. Hydrophobicity analysis of the translated primary protein sequence revealed a common motif, with seven distinct hydrophobic domains of 20 to 25 amino acids connected by hydrophilic loops composed primarily of polar and charged residues (fig. 7). The porcine homolog of the human platelet α_2-adrenoceptor was recently reported (Guyer et al., 1990).

Southern blot analysis of a *Pst*I restriction digest of human genomic DNA, using the *Pst*I restriction frag-

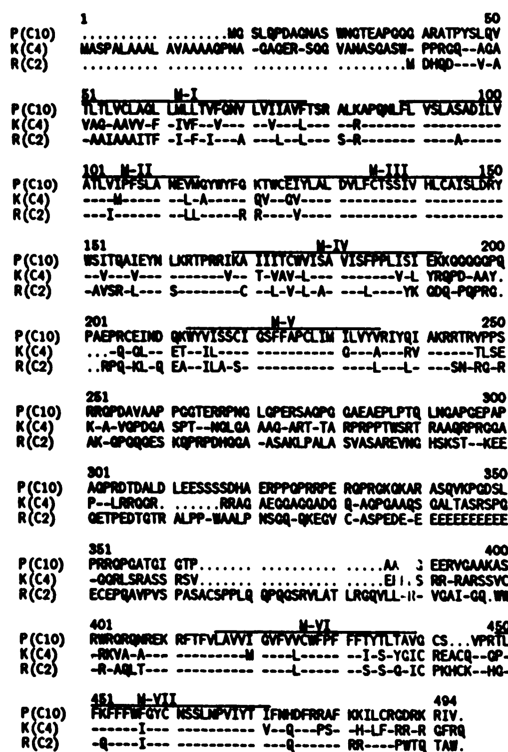

FIG. 7. Alignment of the amino acid sequences for three human α_2-adrenoceptors. The three human α_2-adrenoceptors are designated platelet [P(C10)], kidney [K(C4)], and PCR-isolated [R(C2)]. Hyphens, identity with the sequence for the platelet α_2-adrenoceptor; periods, gaps necessary for optimal alignment. The single letter amino acid code is used. Horizontal bars above the sequences, seven membrane-spanning domains.

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ment of the α_2-adrenoceptor gene, identified 3 distinct hybridizing species at low stringency (Kobilka et al., 1987b). The sizes of the bands were 0.95, 1.8, and 5.9 kilobases. The 0.95-kilobase band was identical with the probe itself, but observation of two additional bands raised the possibility of three closely related genes. The three distinct genes could be localized to different human chromosomes (C2, C4, and C10) by somatic cell hybridization, further supporting the notion of distinct α_2-adrenoceptor genes.

The *Pst*I fragment from the human platelet α_2-adrenoceptor was used to probe a human kidney cDNA library (Regan et al., 1988). Two clones were identified. Neither insert alone proved to be full length, but restriction analysis indicated complementary sequences. From the two fragments, a full-length clone could be constructed by ligation and then the entire coding sequence determined and analyzed (fig. 7). Somatic cell hybridization showed that the gene for the kidney α_2-adrenoceptor was located on chromosome 4, and the gene for the platelet α_2-adrenoceptor was localized to chromosome 10.

A gene for a third member of the α_2-adrenoceptor family has been cloned using PCR technology (Lomasney et al., 1990). Primers were prepared using conserved sequences in the third transmembrane-spanning domains and the third intracellular loops of the human platelet and kidney α_2-adrenoceptors. A 900-base pair fragment was generated by PCR from a sheared human genomic DNA library. A clone was identified, at high stringency, with a full-length open reading frame that contained no introns and encoded for a protein of 450 amino acids (fig. 7). The PCR fragment hybridized specifically to a 1.6-kilobase fragment on Southern blot analysis of *Pst*I-digested human genomic DNA. This 1.6-kilobase fragment had previously been localized to human chromosome 2 (Kobilka et al., 1987b), indicating that the three proposed genes for α_2-adrenoceptors have now been identified. The cDNA for the apparent rat homolog of this third α_2-adrenoceptor subtype (85% sequence homology) has also recently been cloned (Zeng et al., 1990).

Analysis of the translated primary sequence of the α_2-adrenoceptor clones revealed some striking similarities and differences. The unmistakable characteristics ascribed to G protein-coupled receptors were evident (fig. 8). Hydrophilic sequences of varying lengths connect seven hydrophobic regions. The hydrophobic sequences are of sufficient length to form transmembrane-spanning helices and, therefore, the hydrophilic sequences would be predicted to extend out from the plasma membrane, alternating between the extracellular space and cytoplasm. One striking characteristic of the α_2-adrenoceptor sequences is the length of the putative third cytoplasmic loop which contains approximately 150 amino acids. This is of interest because this third cytoplasmic loop has

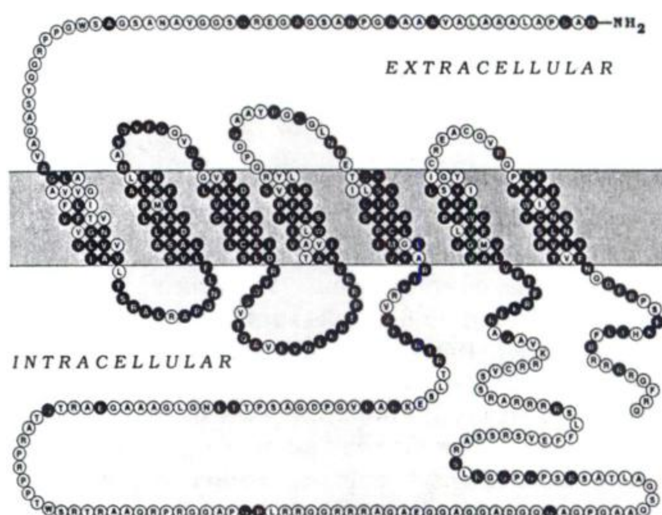

FIG. 8. Primary structure of the human kidney α_2-adrenoceptor. The amino acid sequence is represented by the one letter code. The arrangement of the receptor structure within the membrane is based on a model for rhodopsin and is thought to be general for other G protein-coupled receptors. The darkened residues represent amino acid identities between the human kidney and platelet α_2-adrenoceptors. From Regan et al., 1988.

been implicated in receptor-G protein coupling (Dixon et al., 1987; O'Dowd et al., 1988). This loop in the α_2-adrenoceptors is approximately 2 to 3 times the length of that found in the α_1- and β-adrenoceptor subtypes. However, the length is comparable to that found in the muscarinic cholinergic receptor subtypes (Peralta et al., 1987). In this regard, it was of interest to compare the sequence of the cardiac M_2-cholinergic receptor to the α_2-adrenoceptor, because stimulation of these receptor subtypes is associated with the inhibition of adenylyl cyclase activity. This comparison proved futile, however, because very little sequence homology for the third intracellular loops could be found among the α_2-adrenoceptor subtypes compared to that for the muscarinic receptor. These results suggest that other primary sequences, or possibly secondary or tertiary structural determinants, contribute to receptor-G protein coupling.

The NH_2-terminal sequences of the three α_2-adrenoceptor subtypes are also divergent (fig. 7). This is of interest because this segment of the receptors is the proposed site of asparagine-linked glycosylation. Indeed, the human platelet and kidney α_2-adrenoceptor sequences each contain two consensus sites for covalent attachment of carbohydrates within this domain. In contrast, the NH_2-terminal sequence of the human α_2C2-adrenoceptor identified by PCR or the rat α_2-adrenoceptor homolog is relatively short and does not appear to be glycosylated. Photoaffinity labeling of α_2-adrenoceptors partially purified from neonatal rat lung revealed a receptor protein of 44,000 Da that also appeared to be deficient in posttranslational glycosylation (Lanier et al., 1988). Thus, the lack of an NH_2-terminal glycosylation site is consistent with the α_2-adrenoceptor clone identified by PCR being a candidate for the α_{2B}-subtype.

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When the primary sequences of the α_2-adrenoceptor are compared with those of the hamster α_1-, human β_1-, or human β_2-adrenoceptors, the highest degree of homology is found in the putative membrane-spanning domains. These homologies break down as follows: β_1-adrenoceptor (45%), β_2-adrenoceptor (39%), and α_1-adrenoceptor (44%). Examination of the three α_2-adrenoceptor sequences again showed the highest degree of homology in the hydrophobic regions (75%) which are thought to form the ligand-binding pocket of the receptors.

Lanier et al. (1991) recently reported evidence for a fourth distinct α_2-adrenoceptor subtype. By screening rat genomic library at low stringency with an oligonucleotide probe based on the third putative membrane-spanning domain of the human α_2C4-adrenoceptor, these investigators isolated two clones. The first proved to encode for the rat homolog of the α_2C4-adrenoceptor subtype, whereas the sequence of the second clone was 89% homologous to the human α_2C10-adrenoceptor. Initial characterization of the expressed receptor suggests that this rat clone is not simply the homolog of the α_2C10-adrenoceptor subtype (see below).

D. Expression of α_2-Adrenoceptor DNAs

The ligand-binding properties of three α_2-adrenoceptor subtype clones were examined after the DNAs were expressed in *Xenopus* oocytes (Kobilka et al., 1987b), COS cells (Regan et al., 1988; Lomasney et al., 1990; Zeng et al., 1990), and Chinese hamster cells (Fraser et al., 1989). In all three expression systems, the α_2-adrenoceptor specificity was documented using competition binding of both agonists and antagonists for selective radiolabeled α_2-adrenoceptor antagonists, such as [^{3}H]rauwolscine or [^{3}H]yohimbine. A direct comparison of the ligand-binding properties of the human platelet α_2C10-adrenoceptor and kidney α_2C4-adrenoceptor was accomplished after transient expression in COS-7 cells (Regan et al., 1988). Saturation binding of [^{3}H]rauwolscine to crude membrane preparations showed efficient receptor expression, 6.9 and 25 pmol/protein for the kidney and platelet α_2-adrenoceptors, respectively. The binding affinity for [^{3}H]rauwolscine was higher for the kidney α_2-adrenoceptor, K_D 0.43 nM, than for the platelet receptor, K_D 2.1 nM.

A comparison of the relative potencies of a variety of agonists and antagonists to compete for [^{3}H]yohimbine binding revealed a number of pharmacological differences among the three human α_2-adrenoceptor clones following expression in COS cells (table 3). The platelet α_2C10-adrenoceptor showed relatively low affinity for prazosin and high affinity for oxymetazoline. In contrast, oxymetazoline bound to the α_2C2-adrenoceptor isolated by PCR with low affinity, and prazosin bound with relatively high affinity. Finally, the kidney α_2C4-adrenoceptor clone demonstrated an intermediate affinity for oxymetazoline and a high affinity for prazosin.

The pharmacological differences among the three human α_2-adrenoceptor clones expressed in a single cell type suggest that they represent distinct subtypes. Bylund (1985, 1988) previously suggested a subclassification scheme for α_2-adrenoceptors. According to his nomenclature, the platelet α_2C10-adrenoceptor would be classified as the α_{2A}-type. Based on existing data, the human kidney (α_2C4) and PCR-isolated (α_2C2) clones are candidates for either α_{2B} or α_{2C} subtypes. They clearly encode for distinct α_2-adrenoceptor subtypes based on the 10-fold differences in affinity for oxymetazoline and WB-4101 (Table 3). Biochemical data showing that α_{2B}-adrenoceptors in neonatal rat lung are not glycosylated suggest that the α_2C2 clone could be a peripheral α_{2B}-subtype. However, when used as a probe, this α_2-adrenoceptor DNA did not hybridize to mRNA prepared from neonatal rat lung (Lomasney et al., 1990). In contrast, Zeng et al. (1990) reported identification of a mRNA from neonatal rat lung using the apparent rat homolog of the PCR-isolated human receptor as a probe, lending support to the notion that α_2C2 encodes the α_{2B}-adrenoceptor subtype. Recent experiments, using the DNAs for the three human α_2-adrenoceptors as probes to localize receptor expression by Northern blot analysis of mRNA prepared from a variety of rat tissues, did not definitively resolve the α_2-adrenoceptor subclassification beyond confirming that the platelet α_2C10-adrenoceptor cDNA represents the α_{2A} subtype (Lorenz et al., 1990).

Additional experiments are clearly necessary to definitively assign α_2-adrenoceptor subtypes to the cloned DNAs. The studies to date demonstrate that the molecular basis for distinct pharmacological properties of α_2-adrenoceptor subtypes is separate genes encoding individual proteins. It is noteworthy that an additional compound, SK&F 104078, did not discriminate among the three α_2-adrenoceptors in competition binding assays (Lomasney et al., 1990). This antagonist had been reported to differentiate between pre- and postjunctional α_2-adrenoceptors in pharmacological experiments in the cardiovascular system (Hieble et al., 1988). These obser-

TABLE 3
*Competition by agonists and antagonists for the binding of [^{3}H] yohimbine to membranes prepared from transfected COS-7 cells expressing human α_2-adrenoceptors**

	K_i (nM)		
	Kidney α_2	Platelet α_2	PCR-isolated α_2
Antagonists			
Rauwolscine	2.1	7.1	11
WB 4101	13	47	132
SK&F 104078	41	97	105
Phentolamine	14.4	6.2	9.2
Prazosin	67.7	2237	293
Corynanthine	182	1188	1002
Agonists			
Oxymetazoline	125	13.2	1506
p-Aminoclonidine	97	31	120
Epinephrine	318	1671	1851
Norepinephrine	606	3677	1250

* Data from Lomasney *et al.* (1990).

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vations raise the possibility that genes for additional α_2-adrenoceptor subtypes are yet to be identified.

Along these lines, an apparently distinct α_2-adrenoceptor subtype cloned from a rat genomic library has been characterized following expression of NIH 3T3 fibroblasts (Lanier et al., 1991). Radioligand-binding studies revealed some unique pharmacological properties. The expressed receptor demonstrated relatively low affinity for rauwolscine and yohimbine (K_i 15 to 60 nM) and a distinctly lower affinity for SK&F 104078 (K_i ~500 nM) compared with the α_2C10-adrenoceptor (K_i ~100 nM). These distinguishing characteristics make this rat genomic clone a candidate for the α_{2D}-adrenoceptor subtype, but more extensive characterization, including tissue distribution of mRNA, will be necessary for definitive classification.

The signal transduction pathways activated by agonist occupancy of α_2-adrenoceptors is currently an active research area (Limbird, 1989). The platelet α_2C10-adrenoceptor is known to be coupled to the inhibition of adenylyl cyclase and has served as the primary model system in which to study this pathway. The signaling pathway for the kidney α_2C4-adrenoceptor is not well established, although inhibition of adenylyl cyclase activity is again thought to contribute (Edwards and Gellai, 1987). The DNA clones for the two forms of the α_2-adrenoceptors were expressed in Chinese hamster lung fibroblasts to examine signaling mechanisms (Cotecchia et al., 1990). These fibroblasts were shown to lack endogenous α_2-adrenoceptors. The distinct pharmacological properties of the α_2-adrenoceptor subtypes, as defined by radioligand-binding experiments, were retained following stable expression in the hamster cells. The level of receptor expression for both the kidney α_2C4- and platelet α_2C10-adrenoceptors were comparable. Following stimulation of adenylyl cyclase activity by forskolin, epinephrine inhibited cAMP accumulations in a dose-dependent manner for cells expressing either of the α_2-adrenoceptor subtypes. Both the extent of inhibition (55 to 65%) and the EC_{50} values (approximately 300 nM) were similar for the two clones. Epinephrine also inhibited prostaglandin E_1-stimulated adenylyl cyclase activity to the same extent in the two cell clones. When adenylyl cyclase activity was measured in membranes prepared from clones expressing the platelet α_2C10-adrenoceptor, maximal inhibition of basal adenylyl cyclase activity by epinephrine approached 70%, whereas in membranes prepared from cells expressing the kidney α_2C4-adrenoceptor, the maximal inhibition by saturating concentrations of the same agonist was only 27% (Cotecchia et al., 1990). The elucidation of the molecular basis for this discrepancy will require further experimentation.

In Chinese hamster ovary cells expressing the platelet α_2C10-adrenoceptor, epinephrine at concentrations <100 nM inhibited adenylyl cyclase activity, whereas at concentrations >100 nM this agonist potentiated the forskolin effect on the cyclase (Fraser et al., 1989). Both the inhibitory and potentiating effects of epinephrine on forskolin-stimulated adenylyl cyclase activity were shown to be directly dependent on the expression level of the receptor. Pertussis toxin, which uncouples receptor-G protein communication, completely inhibited the ability of epinephrine to attenuate forskolin-stimulated adenylyl cyclase activity but at the same time enhanced potentiation of cAMP synthesis. These results are consistent with previously reported pharmacological studies, which demonstrated that stimulation of α_2-adrenoceptors resulted in both inhibition and potentiation of forskolin-stimulated adenylyl cyclase activity in brain (Duman et al., 1986) and islet cells (Ullrich and Wollheim, 1984). The pertussis toxin appears to discriminate between the two mechanisms regulating adenylyl cyclase activity.

In hamster fibroblast clones expressing either the human kidney α_2C4- or platelet α_2C10-adrenoceptors, epinephrine promoted a small but significant increase in soluble inositol phosphates, suggesting that α_2-adrenoceptors can stimulate phospholipase C activity (Cotecchia et al., 1990). In these experiments, the transfected cells were preincubated with [^{3}H]myoinositol to label membrane phospholipids. Challenging these cells with 100 μM epinephrine resulted in a 20 to 30% increase in inositol phosphate metabolites, including the rapid production of INS(1,4,5)P3. Increased inositol phosphate production in response to an agonist could be observed for either α_2-adrenoceptor subtype. This effect of epinephrine was not observed in untransfected cells and could be specifically blocked by rauwolscine. The EC_{50} for epinephrine-induced PtdIns turnover was 5 μM, nearly an order of magnitude greater than the EC_{50} for adenylyl cyclase inhibition in the same cells. Preincubation of the transfected COS cells with pertussis toxin abolished the stimulation of inositol phosphates as well as adenylyl cyclase inhibition mediated through either α_2-adrenoceptor subtype. Although pertussis toxin pretreatment of transfected cells inhibited α_2-adrenoceptor coupling to both adenylyl cyclase and phospholipase C, additional experiments will be required to determine whether a single or distinct G protein mediates α_2-adrenoceptor coupling to different signaling pathways.

V. Summary, Conclusions, and Future Directions

A. α-Adrenoceptor Subclassification

The classification and subclassification of α-adrenoceptors has advanced from the definition of the α-adrenoceptor proposed by Ahlquist in which α-adrenoceptors were classified as those adrenoceptors with the agonist potency series epinephrine > norepinephrine > isoproterenol to the present time when there may be at least seven subtypes of α-adrenoceptors. Pharmacological techniques have led to the division of α-adrenoceptors

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into two main classes, α_1 and α_2. Further subdivisions based on pharmacological criteria, and now being supported by molecular biological criteria, have been proposed during the last decade such that α_1-adrenoceptors may have three subtypes and α_2-adrenoceptors may have four subtypes. However, in most cases there are very few compounds with a high degree of selectivity for any particular subtype and some of the subtypes have only been found to exist in particular tissues or species and may, therefore, be peculiar to that tissue in that species. Further studies will be required to demonstrate whether all of these subtypes exist in man, and, if so, what their localization and functions are.

B. α-Adrenoceptor Signal Transduction

The signal transduction systems involved in coupling α-adrenoceptor activation to its effector response have been studied extensively. Many α_1-adrenoceptor-mediated responses involve INS(1,4,5)P3 as the second messenger. Actions of the other product of PtdIns hydrolysis, DAG, on protein kinase C may also contribute to signal transduction or desensitization of the α_1-adrenoceptor. α_2-Adrenoceptor agonists can inhibit adenylyl cyclase, acting through G_i, although some α_2-adrenoceptor-mediated responses cannot be explained by an action on adenylate cyclase alone, and other signal transduction mechanisms, such as Na^+/H^+ exchange, or yet unknown systems, may be involved.

The vasoconstriction induced by activation of either α_1-adrenoceptors or α_2-adrenoceptors is dependent on an elevation of intracellular calcium. The source of this calcium in the case of α_2-adrenoceptor-mediated vasoconstriction is extracellular, because calcium channel blockade or removal of calcium ion from the bathing solution will markedly attenuate the response. α_1-Adrenoceptor agonists can also induce the translocation of extracellular calcium but in addition can release intracellular calcium stores, at least in the case of full agonists. A model to explain α_1-adrenoceptor agonist-induced vasoconstriction has been proposed, in which the α_1-adrenoceptor is coupled to two second messengers, a pertussis toxin-sensitive G protein-mediated translocation of extracellular calcium and a phospholipase C-mediated release of intracellular calcium stores. Full agonists at the α_1-adrenoceptor can activate both second messenger systems, whereas partial agonists can act only through the pertussis toxin-sensitive G protein system.

C. Molecular Biology of the α-Adrenoceptors

Although the application of molecular genetics to α-adrenoceptors is only relatively recent, a number of conclusions can be drawn from these studies. The investigations clearly confirm and extend the existing pharmacological data suggesting the subdivision of both α_1- and α_2-adrenoceptors. As had been found for β-adrenoceptors and muscarinic acetylcholine receptors previously, α-adrenoceptor subtypes arise from distinct gene products. Also consistent with previous studies of β-adrenoceptors and muscarinic receptors, molecular biology is likely to identify new α-adrenoceptor subtypes more quickly than will pharmacological studies.

An interesting parallel can be drawn between studies of signal transduction by α-adrenoceptor subtypes and muscarinic receptor subtypes. Following expression of receptors in transfected cells, it was possible to show that individual receptor subtypes can couple to more than one signal transduction pathway, albeit at different agonist concentrations (Cotecchia et al., 1990; Ashkenazi et al., 1987, 1989). Thus, α_2-adrenoceptor subtypes and M_2 and M_3 muscarinic receptors all inhibit adenylyl cyclase activity and at high agonist concentrations also stimulate PtdIns turnover. These studies also show that multiple pathways exist for different receptors to stimulate a single effector (e.g., phospholipase C). Previous reconstitution studies have shown selective, but not exclusive, coupling of adrenoceptors to G proteins (Cerione et al., 1985, 1986; Ansano et al., 1984). In the cellular expression systems for the α-adrenoceptor and muscarinic receptor DNAs, the G proteins mediating coupling to the two different signal transduction pathways remain to be identified. These studies are consistent with the notion that specificity of a cellular response to a hormone or neurotransmitter depends not only on the repertoire of receptors on the cell surface but also on the variety and stoichiometry of the G proteins available for coupling (Ross, 1988).

Future application of molecular biology to investigate α-adrenoceptors will provide new insights to a number of areas. The domains of the receptor protein involved in ligand binding and G protein coupling need to be explored in detail. Preliminary results point to the hydrophobic core, composed of seven membrane-spanning helices as contributing to ligand binding (Kobilka et al., 1988; Matsui et al., 1989), and the unusually large third intracellular loop suggests specificity of function in signal transduction. Mutagenesis experiments will begin to define the α-adrenoceptor structures.

As stated above, the family of α-adrenoceptor subtypes needs to be defined. Molecular biology will contribute to our understanding of α-adrenoceptor subtype function through expression experiments and by providing the tools to explore receptor localization and distribution throughout the body. Finally, as the gene sequences become available, an understanding of genetic regulation of α-adrenoceptor expression becomes possible. To date only very basic genetic structures in the nucleotide sequences flanking the structural genes for α-adrenoceptors have been reported (Fraser et al., 1989).

Now that the DNAs for a variety of adrenoceptor subtypes have been cloned, rapid progress in understanding their mechanisms of action is anticipated. It will be of interest to compare and contrast studies of α- and β-adrenoceptors in an attempt to arrive at general princi-

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ples governing the structure, function, and regulation of G protein-coupled receptors.

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