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PHARMACOLOGIC AND THERAPEUTIC APPLICATIONS OF α_2-ADRENOCEPTOR SUBTYPES

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INTRODUCTION

There has been a rapid accumulation of new experimental data relating to the pharmacology of α_2-adrenoceptors, including the identification of multiple receptor subtypes and new therapeutic applications for α_2-adrenoceptor agonists and antagonists. In view of the diverse pharmacology of receptors or binding sites defined as "α_2", one must redefine exactly what this designation now includes. For this review, an α_2-adrenoceptor is defined as one that is sensitive to both the physiological catecholamine agonists, norepinephrine and epinephrine, as well as selective agonists, such as B-HT 933 and UK-14,304, and is antagonized by agents such as rauwolscine, yohimbine, and idazoxan. Some α_2-adrenoceptor subtypes also have a high affinity for prazosin, previously thought to interact only with α_1-adrenoceptors, and others have a relatively low affinity for yohimbine and rauwolscine, compared to other α_2-adrenoceptor antagonists.

The three α_2-adrenoceptor subclassification schemes based on: (*a*) receptor cloning and expression, (*b*) correlation of radioligand binding affinity, and (*c*) antagonist potency in functional assays are described, as well as discrepancies between the subtypes identified using these schemes. In addition to their long-known efficacy in hypertension, α_2-adrenoceptor agonists are now being utilized clinically for several new indications. The many functions

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mediated by the α_2-adrenoceptor suggest that even more may be possible. While the clinical application of α_2-adrenoceptor blockade has been limited, new studies suggest several interesting potential applications.

α_2-ADRENOCEPTOR SUBCLASSIFICATION

Molecular Biology of α_2-Adrenoceptor Subtypes

The application of molecular cloning techniques has had a dramatic impact on the investigation of adrenoceptors. The cloning of the DNAs encoding rhodopsin, α- and β-adrenoceptors, as well as muscarinic cholinergic receptors, has defined a superfamily of G protein-coupled receptor genes (1, 2) and provided new approaches and insights into the structure, function, and regulation of the receptor family. As exemplified by the identification of several genes encoding α_2-adrenoceptors, recombinant DNA technology has helped to clarify the molecular basis for receptor subtypes. However, questions still remain as to the pharmacological definition and physiological significance of these newly identified adrenoceptor structures.

The initial strategy for cloning DNA encoding α_2-adrenoceptor(s) entailed large scale purification of α_2-adrenoceptor protein from an appropriate tissue source to obtain an amino acid sequence. Approximately 1 nmol of α_2-adrenoceptor was purified from 1400 units of human platelets (3). Following detergent solubilization, the receptor was purified to homogeneity, and 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 HPLC and their amino acid sequences determined. Two overlapping 39-base long 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 by restriction enzyme mapping to have identical inserts. A 5.5-kb 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. The same clone for a human α_2-adrenoceptor was subsequently described by Fraser et al (4). Since the open reading frame encoding the human platelet α_2-adrenoceptor was continuous, the gene for this receptor was uninterrupted by introns.

Southern blot analysis of a Pst1 restriction digest of human genomic DNA, using the Pst1 restriction fragment of the α_2-adrenoceptor gene, identified three distinct hybridizing species at low stringency (3). The sizes of the bands were 0.95 kb, 1.8 kb and 5.9 kb, respectively. The 0.95-kb band was identical

to 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.

The Pst1 fragment from the human platelet α_2-adrenoceptor was used to probe a human kidney cDNA library (5). 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. Somatic cell hybridization showed that the gene for the kidney α_2-adrenoceptor was located on chromosome 4, while the gene for the platelet α_2-adrenoceptor localized to chromosome 10.

A gene for a third member of the α_2-adrenoceptor family has been cloned using oligonucleotide screening (6) or polymerase chain reaction (PCR) technology (7). Primers for PCR 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-bp 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. The PCR fragment hybridized specifically to a 1.6-kb fragment on Southern blot analysis of Pst1-digested human genomic DNA. This 1.6 kb-fragment had previously been localized to human chromosome 2 (3), indicating that the three proposed genes for α_2-adrenoceptors have now been identified.

The apparent rat homologs for the three human α_2-adrenoceptor subtypes have also been identified by hybridization of oligonucleotide sequences derived from the human genes and applied to rat cDNA or genomic libraries at reduced stringencies (8–10). The porcine homolog of the human platelet α_2C10-adrenoceptor, i.e. α_2-adrenoceptor on chromosome 10, has also been reported (11).

Analysis of the translated primary sequence of the α_2-adrenoceptor clones revealed some striking similarities and differences. Hydrophobicity analysis of the translated primary protein sequences of all the α_2-adrenoceptors revealed seven distinct hydrophobic domains of 20–25 amino acids connected by hydrophilic loops composed primarily of polar and charged residues. The hydrophobic regions have been proposed to represent putative transmembrane-spanning domains connected by hydrophilic loops that extend alternately into the extra- and intracellular space from the plasma membrane (Figure 1; 1, 2). This protein pattern has emerged as the signature motif of the large multigene family of receptors that couple to guanine nucleotide regulatory proteins (G proteins) during signal transduction. One striking characteristic of the α_2-adrenoceptor sequences is the length of the putative third cytoplasmic loop,

which contains approximately 150 amino acids (Figure 1). This is of interest because the third cytoplasmic loop has been implicated in receptor-G protein coupling (12, 13). This loop in the α_2-adrenoceptors is approximately 2–3 times longer than that found in the α_1- and β-adrenoceptor subtypes. However, the length is comparable to that found in the muscarinic cholinergic receptor subtypes (14), and both receptor types can couple to the inhibition of adenylyl cyclase activity. There exists little sequence homology in the third intracellular loops of the α_2-adrenoceptor subtypes as compared to the muscarinic receptors. These results suggest that other primary sequences, or possibly secondary or tertiary structural determinants, contribute to receptor-G protein coupling.

The N-terminal sequences of the three α_2-adrenoceptor subtypes are also divergent. 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 N-terminal sequence of the α_2C2-adrenoceptor identified by PCR, or its rat

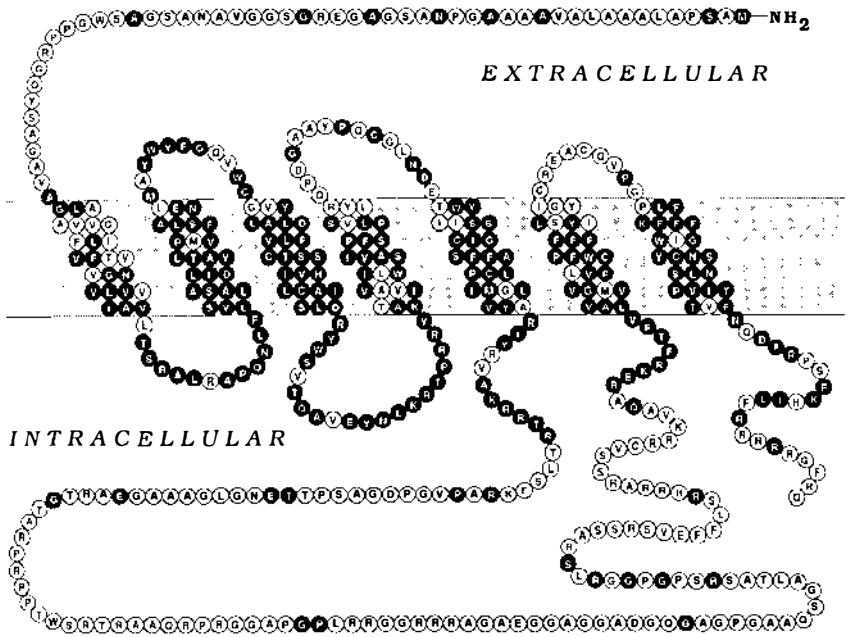

Figure 1 Primary structure of the human kidney α_2-adrenoceptor. The amino acid sequence is represented by the one letter code. The arrangement of the receptor structure within the membrane is based on a model of rhodopsin and is thought to represent a general model for other G protein-coupled receptors. The darkened residues represent amino acid identities between the human kidney and platelet α_2-adrenoceptors (From (5)).

a $_2$-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 daltons that also appeared to be deficient in posttranslational glycosylation sites (15). Thus, the lack of an N-terminal glycosylation site is consistent with the α_2C2-adrenoceptor clone being the same subtype as the α_2-adrenoceptor purified from neonatal rat lung.

When comparing the primary sequences of the α_2-adrenoceptor to 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%). Certainly some degree of homology in these domains is expected, since all of the adrenoceptors recognize and bind endogenous catecholamines, and mounting evidence points to the transmembrane-spanning domains as forming the ligand binding site.

The pharmacological classification of receptor subtypes is based on the relative potency of selective agonists and antagonists. The ligand-binding properties of the three α_2-adrenoceptor subtype clones were examined after the DNAs were expressed in *Xenopus* oocytes (3), COS cells (5, 7–10) and mouse fibroblasts (6). 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 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-adrenoceptors subtypes following expression in COS cells. The 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 (16, 17) 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 existent data, the human kidney α_2C4 and PCR-isolated α_2C2 clones are candidates for either the α_2B or α_2C subtypes. They clearly encode for distinct α_2-adrenoceptor subtypes based on a tenfold difference in affinity for oxymetazoline and WB 4101. Biochemical data showing that α_2B-adrenoceptors in neonatal rat

lung are not glycosylated suggests that the PCR-isolated clone (i.e. α_2C2) is 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 (7). In contrast, Zeng et al (8) reported identification of a messenger RNA from neonatal rat lung using the apparent rat homolog of the PCR-iso- lated human receptor as a probe, lending support to the notion that α_2C2 encodes the α_{2B}-adrenoceptor subtype. It has been suggested that α_2C4 may represent a central, and α_2C2 a peripheral, α_{2B}-adrenoceptor subtype (18) consistent with tissue-distribution data determined by northern blot analysis (19).

The pharmacological definition of the three rat α_2-adrenoceptor clones is somewhat more controversial. Although the pharmacology of human and rat homologs for α_2C2 and α_2C4 are consistent across species (10, 18, 20, 21), the subtype definition of the rat α_2C10-like clone has not yet been settled. The rat clone identified by Lanier et al (10) as RG20 shows 89% protein sequence homology to the the human platelet α_2C10-adrenoceptor, providing strong evidence that the two receptors are homologs. However, following expression of RG20 DNA in COS cells, the receptor demonstrated an unusually low affinity for [^{3}H]-rauwolscine or [^{3}H]-yohimbine (K_d = 30–60 nM). Based on the relative potency of rauwolscine/yohimbine compared to other adrenergic agonists and antagonists, Lanier et al (10) proposed that rat clone RG20 encoded a novel α_2-adrenoceptor subtype. A correlation could be shown between the pharmacology of the expressed RG20 receptor and data derived from binding studies using rat submaxillary gland as the α_2-adrenoceptor source (22). The α_2-adrenoceptor from this tissue has been cited as an example of the α_2D subtype (22, 23). However, other investigators (20) have designated RG20 and independent clones with the identical sequence as true α_2C10 homologs, while acknowledging certain pharmacological discrepancies that may be species-dependent.

The current data demonstrate that the molecular basis for distinct pharmacological properties of α_2-adrenoceptor subtypes is separate genes encoding individual proteins. Identification of an additional DNA encoding a novel α_2-adrenoceptor from either a rat or human source would provide proof of four subtypes. Although there is no current precedent, posttranslational processing of a receptor must still be considered as an alternative mechanism for alterations in pharmacological selectivity. It is noteworthy that an additional compound, SKF 104078, did not discriminate among the three α_2-adrenoceptors in competition binding assays (7, 20, 21). However, this antagonist does differentiate between pre- and postjunctional α_2-adrenoceptors in pharmacological experiments in the cardiovascular system (24, 25). Existent pharmacological data spurs on the search for additional α_2-adrenoceptor subtype genes.

Radioligand Binding Studies

Most of the evidence for multiple α_2-adrenoceptor subtypes has come from radioligand binding assays. Bylund (16) observed that [^{3}H] rauwolscine binding sites can be differentiated into two groups, those insensitive to prazosin, (K_i >1 μM, designated as α_2A) and those in which prazosin has high affinity (K_i >100 nM, designated as α_2B). Tissue sources containing essentially pure populations of α_2A (HT29 cells, human platelet, rabbit spleen) and α_2B (NG108-15 cells, neonatal rat lung, rat kidney) have been identified (17, 22, 23), and analysis of binding displacement curves has been used to demonstrate the presence of both α_2-adrenoceptor subtypes in rat and human brain (26, 27).

This subclassification scheme has been supported by the cloning and expression of α_2-adrenoceptor proteins having either high or low affinity for prazosin (see above), as well as functional studies measuring the ability of prazosin to inhibit UK-14,304-induced stimulation of adenylate cyclase in cell lines containing homogenous populations of α_2A and α_2B adrenoceptor binding sites (see below). Several agonists and antagonists capable of differentiating α_2A and α_2B adrenoceptors have also been identified (Table 1). With the exception of imiloxan, all of the selective α_2B antagonists have high affinity for the the α_1-adrenoceptor. Interestingly, recent studies show that the ability of several selective agents to discriminate between α_2A and α_2B adrenoceptors is markedly reduced when agonist radioligands, such as [^{3}H] clonidine or [^{3}H] UK14,304, are used to label the α_2-adrenoceptor population (28).

When the ability of a diverse series of antagonists to inhibit [^{3}H] rauwolscine

Table 1 α_2-Adrenoceptor subtype selectivity of agonists and antagonists

Compound	K_i (nM)[a]			Ratio
	α_1	α_{2A}	α_{2B}	$[K_i(\alpha_{2A})/K_i(\alpha_{2B})]$
Imiloxan	6760	3020	55	55
ARC-239	11.5	466	9.6	49
Prazosin	0.05	779	22.5	35
SK&F 104856	85	257	7.8	33
Chlorpromazine	3.5	870	52	17
Rauwolscine	235	2.0	1.5	1.3
UK 14,304	1800	0.2	1.1	0.18
Benoxathian	3.2	22	26	0.05
BRL 44408	180	5.4	204	0.02
Oxymetazoline	208	2.1	225	0.009

[a] K_i values for displacement of [^{3}H] prazosin (α_1) or [^{3}H] rauwolscine (α_{2A}, α_{2B}) binding.

binding was compared in a variety of tissues, it became apparent that there were more than two α_2-adrenoceptor binding sites. It is currently thought that at least four α_2-adrenoceptors can be identified by this technique (17, 29, 30). The two additional sites have been designated as α_2C and α_2D. Although the α_2C and α_2D sites resemble the α_2A and α_2B, respectively, antagonists capable of differentiating the four sites are available (29). It has been postulated that the α_2C and α_2B subtypes are species variants, since the α_2C subtype was initially observed only in opossum kidney or in a cell line derived from opossum kidney (31). However, a human-derived retinoblastoma cell line (Y79) has been found to have [^{3}H] rauwolscine binding sites typical of the α_2C subtype, suggesting that it does not merely represent a species variant of the α_2B adrenoceptor (32). The α_2C- and α_2D-adrenoceptors are also characterized by an unusually high or unusually low affinity for rauwolscine, respectively, compared to the α_2A and α_2B adrenoceptors. The α_2D adrenoceptors found on bovine pineal (30) and rat submaxillary or sublingual gland (23, 32) may correspond to the α_2-adrenoceptors, having an unusually low affinity for rauwolscine or yohimbine identified on adipocytes of rat (33), rabbit (34), and hamster (35), on rat jejunal enterocytes (36), and on a cell line derived from rat pancreatic islet cells (37). Calculation of affinity ratios for prazosin, oxymetazoline, and yohimbine in these tissues shows a close similarity (37).

A recent study suggests that additional α_2-adrenoceptor binding sites may exist. Analysis of saturation and displacement curves using another α_2-adrenoceptor antagonist radioligand, [^{3}H] RX 821002, suggests that the rat kidney may possess three distinct α_2-adrenoceptor sites, the α_2A plus two α_2B sites, which were designated as α_2B1 and α_2B2 (38). Since the only compound having substantial selectivity between the two α_2B sites, guanoxabenz, has not been evaluated in other tissue sources, it is not known which of these α_2B sites corresponds to the α_2B identified by other investigators, or whether two α_2B subtypes are found in other tissue sources.

Functional Subclassification of α_2*-Adrenoceptors*

While some evidence from functional studies supports the α_2A/α_2B subclassification scheme derived from radioligand binding studies, many functional studies suggest that α_2-adrenoceptors can be subdivided in yet a different manner.

UK-14,304 inhibits stimulated adenylate cyclase activity in both HT29 and NG108 cells, which possess essentially pure populations of α_2A- and α_2B-adrenoceptors, respectively (39). Interestingly, UK-14,304 was substantially more potent as an inhibitor of adenylate cyclase activity
cells; this observation, in conjunction with a tenfold higher affinity of [^{3}H]

UK-14,304 for α_2-adrenoceptor binding sites in HT29 vis-à-vis NG108 cells (28), suggests that UK-14,304 may have some selectivity for the α_2A-adrenoceptor subtype.

Yohimbine is equipotent as an antagonist of the response to UK-14,304 in the two cell lines (K_B=5.6 nM in HT29 and 3.4 nM in NG108); however, the α_2B selective antagonists, prazosin and ARC-239, are 50–100-fold more potent in NG108 cells, providing a functional correlate to the binding results.

Although α_2B binding sites appear to be present in a variety of tissues, it has been difficult to demonstrate functional responses attributable to activation of this receptor subtype. Most of the classical "α_2-adrenoceptor"-mediated responses have characteristics of the α_2A-adrenoceptor. Indeed, many of the responses were defined as α_2- by their high sensitivity to yohimbine and rauwolscine and resistance to prazosin.

There is some evidence for an α_2B-adrenoceptor contribution to prejunctional control of neurotransmitter release in certain tissue preparations. Moderate concentrations of prazosin antagonize norepinephrine-induced inhibition of K^+-induced increases in [^{3}H] overflow from rat cortical slices (40), and potentiate stimulation-induced release of endogenous norepinephrine from rat submandibular gland (41). However, studies measuring the effects of prazosin on stimulation-induced transmitter overflow are difficult to interpret due to the ability of prazosin to enhance basal transmitter release. Studies correlating the ability of a series of antagonists to interact with prejunctional α_2-adrenoceptors in rat atrium and rat vas deferens suggest these receptors to have α_2B and α_2A characteristics, respectively, based on differential potencies of ARC-239 and prazosin (42, 43). Further studies of antagonist potency correlations are consistent with the postjunctional α_2-adrenoceptor of human saphenous vein having α_2B characteristics, and with the prejunctional α_2-adrenoceptor of the rat submandibular gland being α_2A (43).

No conclusive functional evidence supports the existence of discrete α_2C- and α_2D-adrenoceptors. The stereoselectivity of mianserin enantiomers is greater at α_2C-adrenoceptors, compared to α_2A- and α_2B-adrenoceptors (29). Hence, the ability of the active enantiomer of mianserin to block the effect of exogenous norepinephrine on K^+-induced stimulation of norepinephrine efflux from rat cortical synaptosomes (44) has been used as evidence in support of a functional α_2C-adrenoceptor in the rat central nervous system (29).

Several antagonists have been identified that can clearly differentiate between pre- and postjunctional α_2-adrenoceptor subtypes on a functional basis. SK&F 104078 produces competitive blockade of postjunctional α_2-adrenoceptors, while not affecting the prejunctional α_2-adrenoceptors at several

sites, including atria of several species (24, 25, 45), guinea pig ileum (45, 46) and human saphenous vein (47). Controversy exists regarding the ability of SK&F 104078 to block prejunctional α_2-adrenoceptors in rat vas deferens (47). However, data in this tissue suggest the presence of multiple prejunctional α_2-adrenoceptor subtypes (48).

SK&F 104856, a derivative of SK&F 104078, also has the capacity to block postjunctional α_2-adrenoceptors, but with somewhat greater potency than SK&F 104078 (47). The selectivity of SK&F 104856 for postjunctional vs prejunctional α_2-adrenoceptors has been confirmed in vivo by experiments showing a lack of activity on prejunctional α_2-adrenoceptor-mediated increases in plasma catecholamines in anesthetized rats (49) and myocardial contractility in anesthetized instrumented dogs (50).

Other antagonists can also selectively antagonize postjunctional α_2-adrenoceptors. As is the case with SK&F 104078 and SK&F 104856, naftopidil antagonizes postjunctional α_2-adrenoceptors but not prejunctional α_2-adrenoceptors (51). Abbott-65265 is at least tenfold more potent at postjunctional than prejunctional α_2-adrenoceptors.

Currently available data show no apparent correlation between the functional α_2-adrenoceptor subclassification based on selectivity of SK&F 104078 and SK&F 104856 and the subclassification based on radioligand binding affinity described above. Although SK&F 104078 appears to be tenfold weaker in the α_2D test systems (bovine pineal, rat submaxillary gland) compared to α_2A, α_2B and α_2C adrenoceptor models (30), this profile is not observed with SK&F 104856, which appears to be selective for the α_2B subtype, with no selectivity for α_2D vs α_2A or α_2C adrenoceptors (30, 32). SK&F 104078 has equivalent affinity for the three expressed human α_2-adrenoceptor clones (31). However, one group finds a relatively low affinity (K_i= 531 nM) in one of the rat clones (RG20) (10). This low affinity for SK&F 104078 was part of the evidence used by these investigators to suggest that the RG20 clone represented a receptor discrete from the α_2A, α_2B, or α_2C subtypes. However, another group found the K_i value for SK&F 104078 in cells expressing the RG20 clone to be 162 nM, not substantially different from its affinity for another expressed rat α_2-adrenoceptor clone (20).

In guinea pig atrium or guinea pig ileum, the ability of SK&F 104078 to block α_2-adrenoceptor-mediated inhibition of neurotransmission is not dependent on the particular α_2-adrenoceptor agonist employed (46–48). However, in the rat vas deferens, the K_B values for SK&F 104078 differ substantially and depend upon the particular agonists used (46, 48, 52, 53). These data suggest that the rat vas deferens, but not the guinea pig atrium or guinea pig ileum, contains a heterogeneous population of prejunctional α_2-adrenoceptors, with some being sensitive and others insensitive to SK&F 104078 (48).

FUNCTIONS MEDIATED BY α_2-ADRENOCEPTORS

α_2-Adrenoceptors mediate a multitude of functions in both peripheral organs and within the central nervous system. At present, the nature of the particular α_2-adrenoceptor subtype (i.e. α_2A, α_2B, etc) is not known in most cases.

Central α_2-Adrenoceptor Function

CARDIOVASCULAR REGULATION Stimulation of central α_2-adrenoceptors in the ventrolateral medulla induces a reduction in sympathetic outflow, and an augmentation in parasympathetic outflow, to the periphery, manifested as a reduction in arterial blood pressure accompanied by bradycardia. Quantitative structure-activity studies have shown excellent correlation between the α_2-adrenoceptor agonist potency of a series of clonidine analogs and blood pressure reduction, provided a lipophilicity term is included to correct for penetration through the blood-brain barrier that is required to gain access to the site of action within the central nervous system (54–56).

The characteristic response to intravenous administration of an α_2-adrenoceptor agonist is an immediate pressor response, due to stimulation of peripheral arterial postjunctional α_1- and α_2-adrenoceptors (56). This pressor response is relatively short-lived, and is followed by a slow decline in arterial blood pressure to levels lower than those observed prior to drug administration. This long-lasting depressor/antihypertensive response is a result of central α_2-adrenoceptor stimulation. Heart rate declines immediately following administration, and continues to be reduced for the duration of drug action. If the α_2-adrenoceptor agonist is administered directly into the central nervous system, or via the vertebral artery that allows for easy access to the central nervous system, the initial pressor response is not observed (54–56).

The antihypertensive action of α_2-adrenoceptor agonists is likely to result from stimulation of postsynaptic α_2-adrenoceptors in the brainstem. Animal experiments have shown that catecholamine depletion with reserpine, or destruction of sympathetic neurons by treatment with 6-hydroxydopamine, does not generally attenuate the ability of α_2-adrenoceptor stimulation to decrease sympathetic outflow (57, 58), although Dollery & Reid (59) showed a slight attenuation by 6-hydroxydopamine. This would indicate that the central α_2-adrenoceptor involved in this response is not located prejunctionally on a catecholaminergic neuron.

A brainstem site of action is indicated, based on the inability of transection at the intercollicular level or at the pontomedullary junction to attenuate the antihypertensive activity of clonidine (60). Although the nucleus tractus solitarius has often been considered as the principal site of action of central α_2-adrenoceptor agonists (61), studies using microinjections of clonidine

suggest the lateral reticular nucleus in the ventrolateral medulla as a more likely candidate (62) for the reduction in sympathetic outflow. However, since α_2-adrenoceptors have also been identified in the dorsal vagal nucleus (63), it is possible that α_2-adrenoceptor agonists produce a direct action at this site to enhance vagal outflow.

REGULATION OF AFFECT, AROUSAL, AND PAIN Catecholamines, and particularly norepinephrine, play a role in control of affective state. The locus coeruleus, which has a relatively low resting level of activity, is stimulated by α_2-adrenoceptor blockade, presumably by blocking an ongoing α_2-adrenoceptor-mediated inhibition of firing, possibly by the action of norepinephrine released from the recurrent axon collaterals. Inhibition of locus coeruleus activity by α_2-adrenoceptor activation reduces the noradrenergic input to the hippocampus which, in turn, produces behavioral depression. However, in view of the low level of normal spontaneous activity in the locus coeruleus, this behavioral depression may be only minor. Conversely, blockade of α_2-adrenoceptors in the locus coeruleus, which is under a tonic α_2-adrenoceptor-mediated inhibition, produces an increase in firing rate (64). Moreover, the release of norepinephrine from the locus coeruleus axon terminals in the hippocampus is regulated by prejunctional α_2-adrenoceptors. Activation of these α_2-adrenoceptors reduces norepinephrine release and inhibition of the receptors enhances norepinephrine release. Thus, α_2-adrenoceptor antagonists increase the activity of the locus coeruleus and enhance the release of norepinephrine from the axon terminals in the hippocampus and produce a reversal of depression. Indeed, this is the basis for the proposed use of α_2-adrenoceptor antagonists in the treatment of depression.

Sedation is observed as a common side effect with clonidine (65, 66). Sedative effects of clonidine and other α_2-adrenoceptor agonists have also been observed in a variety of animal models (67). In addition to decreases in motor activity and potentiation of anesthesia, clonidine and other α_2-adrenoceptor agonists can also induce sleep (68).

Penetration of α_2-adrenoceptor agonists into the central nervous system is required to produce sedation, since peripherally acting α_2-adrenoceptor agonists are ineffective in producing sedation or sleep (67). Two possible sites at which α_2-adrenoceptors produce sedation have been proposed. As described above, α_2-adrenoceptor-mediated inhibition of locus coeruleus firing reduces the amount of norepinephrine released from axon terminals in the thalamus and cerebral cortex, and thereby reduces sensory information reaching the cortex and making the cortex less receptive to sensory information. In addition, activation of prejunctional α_2-adrenoceptors reduces the amount of norepinephrine released from the axon terminals in the thalamus and the cortex, thus producing the same effect as a reduction in locus coeruleus

activity. It appears that the latter effect is the prominent mechanism of α_2-adrenoceptor-mediated sedation.

Clonidine produces analgesia in the rat (69). If administered centrally, other α_2-adrenoceptor agonists, such as St-91, that do not cross the blood-brain barrier also produce analgesia (69). This effect is not mediated by endogenous opioid peptides, since it can be blocked by yohimbine, but not by naloxone (70). Most evidence supports a presynaptically mediated α_2-adrenoceptor mechanism for this effect (71). It is possible that α_2-adrenoceptors may participate in the physiologic analgesia produced by stress (71).

MODULATION OF PITUITARY HORMONE RELEASE Growth hormone (GH) secretion is pulsatile under basal conditions but can be enhanced by a variety of stimuli, such as sleep, exercise, stress, and insulin-induced hypoglycemia. The secretion of GH from the adenohypophysis is under the control of two hypothalamic peptides, growth hormone releasing hormone (GHRH), which is released from neurons in the ventromedial nucleus and arcuate nucleus region and stimulates the release of GH, and somatostatin (SRIF), which is released from neurons in the preoptic area and inhibits GH release. The basal release of GH is regulated by both GHRH and SRIF (72). There is evidence that α_2-adrenoceptors are involved in the regulation of GH release via mechanisms that involve changes in the secretion of both GHRH and SRIF. The importance of α_2-adrenoceptors in the regulation of GH release is exemplified by the fact that GH release in humans induced by a variety of stimuli, including insulin-induced hypoglycemia, stress, and exercise, is inhibited by the α-adrenoceptor antagonist, phentolamine. Activation of α_2-adrenoceptors stimulates GH release (72). The α_2-adrenoceptor-mediated enhancement in the release of GH is blocked by anti-GHRH antibodies and potentiated by anti-SRIF antibodies, suggesting that α_2-adrenoceptor activation increases the release of GHRH (72). Yohimbine inhibits pulsatile GH release (72, 73) and abolishes the increased secretion induced by inactivation of SRIF by anti-SRIF antibodies (72, 73), suggesting that endogenous activation of α_2-adrenoceptors enhances GHRH release. Moreover, yohimbine inhibits the release of GH produced by exogenous GHRH via a mechanism that is abolished by anti-SRIF antibodies, suggesting that the effect of yohimbine to abolish GHRH-induced GH release is due to enhanced release of SRIF.

Peripheral α_2-Adrenoceptor Function

INHIBITION OF NEUROTRANSMITTER RELEASE The prejunctional α_2-adrenoceptor serves as a key element in a local feedback system modulating neurotransmitter release. Activation of these prejunctional α_2-adrenoceptors

by either norepinephrine or epinephrine, the natural physiologic ligands, or by synthetic molecules having α_2-adrenoceptor agonist activity, such as B-HT 920, B-HT 933, UK-14,304 and clonidine, will inhibit stimulation-evoked neurotransmitter release from nerve terminals (74). Conversely, α_2-adrenoceptor antagonists, such as idazoxan, yohimbine, rauwolscine, and SK&F 86466, potentiate stimulation-evoked norepinephrine release (74). This potentiation shows that the prejunctional α_2-adrenoceptor is normally under active tone as a result of endogenously released norepinephrine. The results of activation or blockade of prejunctional α_2-adrenoceptors can be demonstrated in vivo in both animals (75) and in humans (76), as well as in isolated tissues. Since prejunctional α_2-adrenoceptors have been found in all sympathetically innervated tissues thus far examined, this neuromodulatory system appears to play an important role in the control of sympathetic tone.

The magnitude of prejunctional α_2-adrenoceptor-mediated effects are dependent upon the pattern of neuronal activity. As the amount of norepinephrine in the synaptic cleft is increased by a higher frequency and/or duration of nerve stimulation, the prejunctional α_2-adrenoceptor is activated to a greater degree by the neuronally released norepinephrine. Hence, the ability of an exogenously administered α_2-adrenoceptor agonist to inhibit sympathetic neurotransmission will decrease as stimulation frequency or duration is increased, since the prejunctional α_2-adrenoceptor-mediated autoinhibition system will already be maximally activated. Conversely, potentiation of neurotransmitter release by an α_2-adrenoceptor antagonist will be enhanced as stimulation parameters are increased to yield a more intense stimulation of prejunctional α_2-adrenoceptors (74, 77). In this regard, an α_2-adrenoceptor antagonist would not be expected to potentiate the release of neurotransmitter induced by a single pulse of nerve stimulation, since there would be no tone at the prejunctional α_2-adrenoceptor under such conditions.

VASCULAR SMOOTH MUSCLE CONTRACTION It is now widely accepted that arterial vasoconstriction may be mediated by a mixed population of postjunctional vascular α_1- and α_2-adrenoceptors. By using a variety of α_1-selective, α_2-selective and nonselective α-adrenoceptor antagonists, Yamaguchi & Kopin (78) observed that the pressor responses to exogenously administered catecholamines were selectively antagonized by α_2-adrenoceptor blockers. Conversely, the pressor response evoked by sympathetic nerve stimulation was selectively antagonized by α_1-adrenoceptor blockers. It was postulated, therefore, that postjunctional vascular α-adrenoceptors located at the neuroeffector junction (i.e. junctional receptors) were of the α_1-subtype, while those located away from the neuroeffector junction (i.e. extrajunctional receptors) were of the α_2-subtype. This observation relating to the junctional location of α_1-adrenoceptors and the extrajunctional location of α_2-ad-

renoceptors in arterial smooth muscle has been confirmed independently by a number of laboratories (79–81).

The physiologic role of the extrajunctional α_2-adrenoceptors is not fully understood. It has been suggested that the extrajunctional α_2-adrenoceptors would not normally interact with neuronally liberated norepinephrine since they are located at some distance away from the adrenergic nerve terminal, and the highly efficient neuronal uptake pump keeps synaptic levels of norepinephrine sufficiently low and thereby prevents diffusion of the neurotransmitter to the extrajunctional sites (79). It has been proposed that the extrajunctional α_2-adrenoceptors may respond to circulating epinephrine acting as a blood-borne hormone (80). Although circulating catecholamines are normally well below the levels required to exert a physiologic effect, it has been suggested that in times of stress, these levels may be sufficiently elevated such that postjunctional vascular α_2-adrenoceptors are activated (82). It has also been suggested that the contribution made by arterial extrajunctional α_2-adrenoceptors to total peripheral vascular resistance may be greater in hypertensive patients than in normotensive patients (83), implying that postsynaptic vascular α_2-adrenoceptors may play an important role in pathophysiological states such as hypertension and possibly congestive heart failure, where circulating catecholamine levels are high (84).

It is now clear that postjunctional α_2-adrenoceptors play an important role in the regulation of venous function. Activation of both α_1- and α_2-adrenoceptors produces a reduction in venous capacitance (85, 86), with a greater response being seen with α_2-adrenoceptor activation (85). Furthermore, the release of norepinephrine from sympathetic neurons by tyramine decreases total venous capacitance (i.e. causes venoconstriction) by activation of both α_1- and α_2-adrenoceptors (87), thus indicating the importance of both α-adrenoceptor subtypes in the sympathetic control of total systemic venous capacitance.

RENAL EXCRETORY FUNCTION Radioligand binding studies in rat kidney reveal that the major concentration of phentolamine displaceable [^{3}H]-rauwolscine binding sites is found in the renal cortex, with a particularly high density associated with the proximal tubules. Much lower densities of binding sites are found in the distal tubules, blood vessels, and glomeruli (88). In contrast to this predominant proximal tubular location of α_2-adrenoceptors as assessed by radioligand binding methodology, physiologic studies suggest a more important functional role for α_2-adrenoceptors in the distal tubule. α_2-Adrenoceptor activation weakly attenuates parathyroid hormone-induced activation of adenylyl cyclase in rat isolated proximal convoluted tubule, but more potently inhibits vasopressin-evoked stimulation of adenylyl cyclase in the medullary and cortical collecting tubules, with no effect in the medullary

and cortical thick ascending limb (89). In addition, α_2-adrenoceptor stimulation antagonizes the vasopressin-induced reduction in sodium and water excretion in isolated rat perfused kidney (90) and water reabsorption in isolated rabbit cortical collecting tubules (91).

Studies on the role of α_2-adrenoceptors in renal excretory function in vivo demonstrate that α_2-adrenoceptor activation in the conscious rat increases sodium and water excretion by a mechanism consistent with antagonism of vasopressin action on the cortical collecting tubule/duct (92). However, the relevance of this response observed in rats to other species is currently in doubt (93).

PLATELET AGGREGATION Epinephrine induces aggregation of human platelets and potentiates aggregation induced by other agents, such as ADP and thrombin (94). The response is normally biphasic, consisting of a reversible, partial aggregation followed by a rapid, irreversible aggregation. Selective α_2-adrenoceptor antagonists, such as yohimbine, block the effect of epinephrine (95), suggesting an α_2-adrenoceptor-mediated mechanism. However, many synthetic α_2-adrenoceptor agonists, such as clonidine, do not usually induce human platelet aggregation. The failure of most α_2-adrenoceptor agonists to induce platelet aggregation is likely a result of their low efficacy combined with low α_2-adrenoceptor density in platelets. In most studies, clonidine blocks epinephrine-induced platelet aggregation, a typical finding for a partial agonist. UK-14,304, a highly potent and selective α_2-adrenoceptor agonist, produces an effect comparable to that produced by epinephrine (96). As an explanation for this observation, UK-14,304 has a higher efficacy than clonidine and most other imidazolines at the α_2-adrenoceptor (97).

The functional role of α_2-adrenoceptor-mediated platelet aggregation is not clearly understood. Many substances normally present in blood can induce aggregation, and the in vitro concentrations of epinephrine required to induce platelet aggregation are generally higher than those found in vivo. The most likely situation is that the physiologic control of platelet aggregation involves the action of multiple aggregatory hormones, each present at levels below those necessary for induction of platelet aggregation individually. It is possible that elevated levels of catecholamines in the systemic circulation during stress, and in the coronary circulation during myocardial ischemia, may produce platelet aggregation and/or platelet hyperaggregability. This is supported by observations in a canine model of platelet-dependent coronary artery thrombosis in which stenosis of an endothelial-damaged coronary artery produces platelet-dependent cyclic flow reductions. In this model, the α_2-adrenoceptor antagonist, yohimbine, but not the α_1-adrenoceptor antagonist, prazosin, reduces the incidence of cyclic flow reductions (98). Thus, it would appear that epinephrine can act to potentiate the actions of aggregating agents in vivo

such that blockade of this effect produces an inhibition, albeit incomplete, of platelet aggregation.

GASTROINTESTINAL TRACT MOTILITY AND SECRETORY FUNCTION α_2-Adrenoceptors mediate several responses at different levels of the gastrointestinal tract, such as regulation of gastric and intestinal motility and secretions. Adrenergic regulation of gastric motility via an indirect mechanism was demonstrated by Jannson & Martinson (99) who showed that sympathetic nerve stimulation inhibits excitatory gastric smooth muscle responses produced by vagal stimulation, but not those produced by exogenously administered acetylcholine in the cat stomach in situ. α-Adrenoceptor-mediated inhibition of neurogenic contraction of isolated gastric fundus from dog (100) and rat (101) has been shown to be mediated via α_2-adrenoceptors located on the postganglionic parasympathetic neurons in the stomach, leading to a decrease in acetylcholine release. α_2-Adrenoceptors also cause an inhibition of vagally mediated gastric acid secretion from parietal cells in the pylorus-ligated rat (102), as well as that resulting from electrical nerve stimulation (103), via a prejunctional inhibition of acetylcholine release.

These peripheral gastric inhibitory α_2-adrenoceptors may be located on the intramural parasympathetic ganglia (104), in addition to being present on the prejunctional terminals of the postganglionic cholinergic neurons (105). Furthermore, it has been suggested that α_2-adrenoceptors located in the central nervous system may be partly responsible for the inhibition of gastric acid secretion in pylorus-ligated rats (102). Thus, vagally mediated increases in motility and secretion may be inhibited by activation of α_2-adrenoceptors in several locations involving the periphery and central nervous system.

α_2-Adrenoceptors also mediate effects on motility and electrolyte and fluid transport in the small and large intestine. As in the stomach, stimulation of sympathetic nerves supplying the ileum inhibits the contractile response to parasympathetic nerve stimulation by an α_2-adrenoceptor-mediated mechanism (106). The α_2-adrenoceptor-mediated inhibition of small intestinal contraction that is induced by cholinergic nerve stimulation is accompanied by a reduction of acetylcholine release (104), thus indicating a prejunctional location of the α_2-adrenoceptors.

Stimulation of α-adrenoceptors in rabbit ileal mucosa promotes net Na^+ and Cl^- absorption (107). In addition, net HCO_3^- secretion is abolished, and the membrane potential across the mucosal cell is decreased (107). The α-adrenoceptor involved in this response has been examined in detail and has been shown to be of the α_2-subtype (108, 109). α_2-Adrenoceptors also mediate an inhibition of intestinal fluid secretion induced by PGE_1, VIP, dibutyryl cAMP, and cholera toxin in isolated rat jejunum (110, 111), presumably by increasing net mucosal ion absorption. Further support for the presence of

a $_2$-adrenoceptors on intestinal mucosal cells has come from radioligand binding studies (112). The effects of α_2-adrenoceptor activation on intestinal ion and water transport are not species-dependent, and occur both in the ileum and colon (113). In humans, it has been demonstrated that clonidine produces an inhibition of watery diarrhea (114).

PANCREATIC ISLET CELL FUNCTION The endocrine pancreas of most mammalian species is functionally innervated by the sympathetic nervous system (115). Activation of this sympathetic innervation by stimulation of either the splanchnic nerves (116) or the ventromedial hypothalamus (117) produces a reduction in insulin secretion in a manner similar to that produced by epinephrine infusion in vivo (116). Similarly, epinephrine inhibits glucose-stimulated insulin release from isolated rat pancreatic islet cells in vitro (118). This adrenergic inhibition of insulin release from the pancreas is blocked by phentolamine and by dihydroergocryptine, and is unaffected by propranolol, thus demonstrating that it is mediated via α-adrenoceptors on the islet-cells (115). These α-adrenoceptors have subsequently been characterized as being of the α_2-subtype in both radioligand binding (119) and functional (118) studies.

The physiologic importance of adrenergic regulation of insulin release mediated via α_2-adrenoceptors in vivo has been studied by investigating the responses elicited by selective α_2-adrenoceptor antagonists. Nakadate et al (120) found that phentolamine, dihydroergocryptine, and yohimbine markedly increased plasma immunoreactive insulin in mice, whereas phenoxybenzamine or prazosin had no effect. In accord with this, yohimbine increases plasma insulin concentration in the rat (121). These results suggest that insulin secretion in vivo is under tonic inhibition by activation of pancreatic α_2-adrenoceptors. There is evidence from studies in the in situ saline-perfused canine pancreas that the α_2-adrenoceptors on islet α-cells are directly innervated. Thus, it is possible that both circulating epinephrine released from the adrenal medulla, as well as norepinephrine released from sympathetic neurons, may both contribute to the α_2-adrenoceptor-mediated tonic suppression of insulin release in vivo.

UROGENITAL SYSTEM The urinary bladder is innervated by the hypogastric nerve, stimulation of which produces a biphasic response consisting of an initial contraction followed by a secondary relaxation (122). The excitatory response of the urinary bladder is mediated via α-adrenoceptors in experimental animals (122) and in humans (123). Studies on various regions of the rabbit urinary bladder indicate that the α-adrenoceptors are most prominent in the bladder base, with fewer α-adrenoceptors being present in the dome (124). Functional studies suggest that only α_1-adrenoceptors mediate the

contractile response of the bladder base of rats (125), rabbits (126), and humans (127), with little or no α_2-adrenoceptor component. However, radioligand binding studies have shown the additional presence of α_2-adrenoceptors in the bladder base but not in the dome (126). The functional role of these α_2-adrenoceptors, if any, is at present unknown.

Similar to the bladder, the urethra is also innervated by the sympathetic nervous system, and α-adrenoceptors mediate a contractile response to sympathetic nerve stimulation (128). It is believed that this α-adrenoceptor-mediated contractile response contributes to the maintenance of urethral smooth muscle tone and intraurethral pressure, and thus, to urinary continence (128). Radioligand binding studies have demonstrated the presence of both α_1- and α_2-adrenoceptors in the rabbit urethra, with the α_2-adrenoceptor density in the female being six times greater than that in the male (129). Thus, it is possible that α_2-adrenoceptors are regulated by female sex hormones, possibly estrogens, similar to the regulation observed in the uterus (see below).

Radioligand binding and functional studies suggest that α_2-adrenoceptor density and functional importance increase from the proximal to distal regions of the urethra (130). Recent studies have shown that activation of sympathetic neurons that innervate the urethra produces constriction via norepinephrine acting on junctional α_1-adrenoceptors with a concomitant effect of the released norepinephrine acting on prejunctional α_2-adrenoceptors to reduce the release of norepinephrine (131). In contrast, epinephrine released from the adrenal medulla can activate extrajunctional α_2-adrenoceptors to produce urethral constriction (131).

The prostate gland has received much attention in recent years, with particular interest directed at the role played by α-adrenoceptors in the maintenance of prostatic tone in benign prostatic hypertrophy. An analysis of receptor dissociation constants of selective α-adrenoceptor antagonists has shown that the response to norepinephrine in the human prostate is mediated primarily by activation of α_1-adrenoceptors, with only the possibility of a small α_2-adrenoceptor component that varies in magnitude between subjects (132). Radioligand binding studies have confirmed the presence of both α_1- and α_2-adrenoceptors in human prostate (133). In vivo studies in the dog have demonstrated that α_2-adrenoceptors do indeed play a role in the contractile response of the prostate (134). However, as yet there is no clinical evidence for a role of α_2-adrenoceptors in mediating contraction of the prostate in humans.

The uterus contains functional α-adrenoceptors, activation of which by either exogenous catecholamines or sympathetic nerve stimulation produces uterine contraction. Using radioligand binding studies, Hoffman et al (135) demonstrated the presence of both α_1- and α_2-adrenoceptors in the rabbit uterus. Subsequent studies revealed that, despite a preponderance of α_2- over

a $_1$-adrenoceptors in rabbit uterus, the contractile response to exogenous norepinephrine was mediated solely via the α_1-subtype (136).

The increase in total α-adrenoceptor number produced by estrogen treatment (137, 138) is due to a selective increase in α_2-adrenoceptor density (136). A similar increase in α_2-adrenoceptor number is observed with elevated estrogen levels in human myometrium (139). Uterine α_2-adrenoceptors also appear to be under the control of progesterone since elevated plasma levels of progesterone reduce human myometrial α_2-adrenoceptor density in both normal and estrogen-primed uteri (139).

Despite this regulation of uterine α_2-adrenoceptor number by gonadal steroids, no functional role has yet been demonstrated for these receptors in this tissue. It is possible that the α_2-adrenoceptors regulate some aspect of cellular metabolism important for uterine function, and that the steroid-induced changes in α_2-adrenoceptor number mediate changes in the metabolic activity of the uterus during the menstrual cycle and pregnancy. It does appear that the large population of uterine α_2-adrenoceptors does not reside prejunctionally on sympathetic nerve terminals since their number is not reduced by surgical or chemical denervation (136).

ENDOTHELIAL FUNCTION Vascular endothelial cells mediate relaxation of arterial smooth muscle in response to certain vasodilators, such as acetylcholine, bradykinin, and substance P, by the release of the so-called endothelium-derived relaxing factor (EDRF) (140). It has been proposed that activation of α_2-adrenoceptors on endothelial cells stimulates the release of EDRF (141, 142), an action that would tend to antagonize vasoconstriction produced by activation of postjunctional vascular α-adrenoceptors. Cocks & Angus (142) demonstrated that removal of endothelium enhanced the contractile response produced by norepinephrine in canine and porcine circumflex coronary artery, and that after blockade of α_1-adrenoceptors, norepinephrine could produce yohimbine- and idazoxan-sensitive relaxation of precontracted arteries only in the presence of an intact endothelium. Additional studies have shown that α_2-adrenoceptors mediate release of EDRF from carotid, mesenteric, renal, and femoral arteries from dogs and pigs, although there are anatomical and species differences in the magnitude of this response (143). Furthermore, it has been suggested that endothelial α_2-adrenoceptors mediate release of EDRF in coronary microvessels (143). Thus, α_2-adrenoceptor agonists do indeed appear to have the capability of modulating vascular responsiveness via stimulation of the release of EDRF in both large arteries and in the microcirculation.

LIPOPROTEIN METABOLISM A reciprocal adrenoceptor-mediated regulation of lipolysis in the adipocyte is well established, with β- and α_2-adrenoceptor

stimulation resulting in stimulation and inhibition of lipolysis, respectively. Epinephrine, which can activate both adrenoceptors, appears to be the primary hormone involved in the control of the lipolytic process. This modulation is mediated through adenylyl cyclase, via phosphorylation of hormone-sensitive lipase, the enzyme catalyzing the hydrolysis of adipocyte triglycerides to di- and monoglycerides (144). α_2-Adrenoceptor density can vary between species, and between different sites within the same species, and can be influenced by physiological parameters such as age, degree of obesity, and environmental temperature.

Inhibition of lipolysis by selective α_2-adrenoceptor agonists can be readily demonstrated in vitro. The basal lipolytic activity is inhibited, provided that adenosine deaminase is present to block the inhibitory effect of the high concentrations of adenosine attained under these conditions (145). Selective α_2-adrenoceptor agonists can also inhibit the elevation in lipolytic activity induced by β-adrenoceptor agonists, forskolin, or theophylline (146, 147). The effect of epinephrine on lipolysis in isolated adipocytes is dependent on the species and site from which the cells were isolated, and is proportional to the α_2/β-adrenoceptor density ratio. For example, human omental adipocytes, with a relatively low α_2-adrenoceptor density, show a concentration-related lipolytic response to epinephrine, whereas lipolytic activity is inhibited by epinephrine in femoral subcutaneous adipocytes, which have a high density of α_2-adrenoceptors (148). As expected, in the presence of α_2-adrenoceptor blockade, epinephrine induces a lipolytic response of similar magnitude to that produced by the selective β-adrenoceptor agonist, isoproterenol (149).

THERAPEUTIC APPLICATIONS

α2-Adrenoceptor Agonists

HYPERTENSION Central α_2-adrenoceptor stimulation has been utilized clinically for many years in the treatment of hypertension. The most commonly used drugs are clonidine, a moderately selective α_2-adrenoceptor agonist that can readily cross the blood-brain barrier, and α-methyldopa, which is actively transported into the brain, then metabolized to α-methyl-norepinephrine, which preferentially activates the α_2-adrenoceptor (150). The therapeutic and side-effect profiles of these two agents are quite similar (150).

SUPPRESSION OF OPIATE WITHDRAWAL α_2-Adrenoceptors and opiate receptors both mediate inhibition of the firing rate of the locus coeruleus, which sends projections throughout the central nervous system. The α_2-adrenoceptors and opiate receptors located on the noradrenergic cell bodies

within this nucleus may be linked through a common second-messenger system to a potassium channel, activation of which results in hyperpolarization (151). Many symptoms of opiate withdrawal can be attributed to elevated activity of the autonomic nervous system. The elevated autonomic activity may be caused both by increasing activity of the locus coeruleus and by increased activity of spinal centers controlling sympathetic outflow (152). The ability of clonidine to attenuate this elevated autonomic activity has led to a useful clinical approach for the treatment of opiate withdrawal.

Supported by data in experimental animals (153), clinical trials of clonidine in methadone-maintained opiate addicts showed significant reductions in both physiological and psychological signs and symptoms of withdrawal (154), although in both animals and in humans the efficacy of clonidine may be limited primarily to symptoms associated with elevated autonomic activity. The utility of clonidine in the treatment of opiate withdrawal has been confirmed in many clinical trials, both in in-patient (154) and out-patient (155) settings. As a result, clonidine has become the accepted alternative to methadone in the treatment of opiate withdrawal (156). Combined therapy with clonidine and naltrexone, a long-acting opiate antagonist, has been used as a rapid and effective mode of detoxification of addicted individuals (157).

Other α_2-adrenoceptor agonists have been evaluated as adjuncts to opiate withdrawal. These include guanabenz (158) and guanfacine (159), both, like clonidine, approved for the treatment of hypertension. A controlled trial suggests that guanfacine may sometimes show greater efficacy and reduced side-effects compared to clonidine (159).

OTHER CENTRAL NERVOUS SYSTEM INDICATIONS Clonidine has been shown to be beneficial in the treatment of attention-deficit hyperactivity disorder in adolescents and children. Placebo-controlled studies in this condition have shown clonidine to the superior to placebo (160) and generally equivalent in efficacy to the psychostimulant, methylphenidate (161). Since clonidine and methylphcnidate have a different pattern of optimal efficacy against the multiple symptoms associated with this disorder, patient populations more like to respond to each drug can be identified, and combination therapy with clonidine and methylphenidate may be effective in resistant cases (162).

Tizanidine, an α_2-adrenoceptor agonist structurally analogous to clonidine, is an effective muscle relaxant, useful in the treatment of spasticity resulting from stroke, cerebral trauma, or multiple sclerosis (163–165). Tizanidine is thought to act via an α_2-adrenoceptor-mediated modulation of the release of excitatory amino acid neurotransmitters from spinal interneurons (166).

Although no clinical studies have been performed, pretreatment with clonidine and dexmedetomidine, an α_2-adrenoceptor agonist having greater

efficacy and selectivity than clonidine, has been shown to improve neurologic outcome in rats subjected to incomplete cerebral ischemia via carotid ligation in conjunction with systemic hypotension (167). The therapeutic response to dexmedetomidine, which was more consistent than that to clonidine, could be reversed by atipamezole, a centrally acting α_2-adrenoceptor antagonist (168). These animal studies suggest that an α_2-adrenoceptor agonist may attenuate some of the sequelae of stroke.

α_2-ADRENOCEPTOR AGONISTS AS ANALGESICS Many studies, both in experimental animals and in human subjects, have shown α_2-adrenoceptor agonists to be potent analgesic agents. α_2-Adrenoceptor agonists are clinically effective either as monotherapy or in combination with opiates, where a synergistic effect is produced (169). Combinations of α_2-adrenoceptor agonists, opiates, and local anesthetics can be used for peri-operative analgesia, to inhibit pain by three different mechanisms, decreasing the doses required for each class of analgesic (170). Most studies have been performed with clonidine, which is effective via systemic, epidural, intrathecal, or intracerebroventricular administration (171). There is experimental support for the involvement of spinal α_2-adrenoceptors in the analgesic action of clonidine (171), and inhibition of the release of substance P may be involved.

Epidural administration of clonidine is effective as a postoperative analgesic following a variety of surgical procedures (172) and in the relief of severe neurogenic pain (173). However, clonidine when administered by this route was ineffective against the severe postoperative pain associated with thoracotomy (174). Synergistic effects between epidural clonidine and opiates have been observed (175). Intrathecal clonidine is an effective analgesic in cancer patients tolerant to opiates (176) and will prolong the effect of tetracaine (177).

α_2-ADRENOCEPTOR AGONISTS AS ADJUNCTS TO GENERAL ANESTHESIA Sedation is a commonly observed side effect when α_2-adrenoceptor agonists are administered to humans. The sedative activity makes this class of drug useful as preanesthetic medication, especially since several other pharmacologic effects of α_2-adrenoceptor agonists (e.g. analgesia, decreased salivary secretion, reduction in fear and anxiety, inhibition of sympatho-adrenal stress responses) are also considered to be beneficial for this indication. Intravenous administration of medetomidine induces sleep in normal human volunteers at a dose reducing blood pressure by only 10–15 mmHg (178). In dogs, this agonist reduces the minimum alveolar concentration of halothane required to maintain anesthesia by 95% (179). Similar effects have been observed with other highly selective α_2-adrenoceptor agonists, such as B-HT 920 and B-HT

933 (180). Studies with selective antagonists have confirmed the involvement of central α_2-adrenoceptors in this effect (181), and suggest that the lesser magnitude of anesthetic potentiation observed with clonidine may relate to its lower efficacy and/or selectivity at the α_2-adrenoceptor. Nevertheless, oral pretreatment with clonidine reduced isoflurane requirements by 40% in patients undergoing elective surgery (182). Premedication with dexmedetomidine or clonidine will also reduce the dose of short-acting barbiturate required to induce sleep (183).

α_2-Adrenoceptor agonists, such as xylazine, medetomidine, and detomidine, are commonly used in veterinary practice to produce sedation and as adjuncts to other anesthetic agents (184). An advantage of the α_2-adrenoceptor agonists in large animal surgery is that their anesthetic action can be quickly reversed by the administration of an α_2-adrenoceptor antagonist.

GLAUCOMA A selective α_2-adrenoceptor agonist, para-aminoclonidine (apraclonidine), is now in clinical use for the topical treatment of glaucoma (185). The increased hydrophilicity of apraclonidine vis-à-vis clonidine decreases access to the central nervous system and consequently reduces the incidence of systemic side effects following topical administration. Apraclonidine is now also widely used for the prevention of the acute rise in intraocular pressure observed following laser surgery in the anterior segment of the eye (186).

SHORT STATURE The ability of central α_2-adrenoceptor agonists to potentiate growth hormone secretion has suggested a potential utility of these agents in children with constitutional growth delay. Although the efficacy of clonidine for this indication has not always been demonstrable in controlled trials, several 6–12-month trials with clonidine in slowly growing children show a statistically significant improvement in growth rate, compared to placebo (187). When only those children responsive to clonidine (65% in one 12-month study of 112 subjects) are considered, the response is more impressive (188). It is suggested that some of the children with growth delay have a low secretion rate of endogenous growth hormone, and that clonidine can reverse this deficit via blockade of the inhibitory action of somatostatin on growth hormone secretion (189). Combined administration of clonidine and growth hormone releasing factor to normal human volunteers induced a more physiological episodic pattern of growth hormone release than when the releasing factor was administered alone, suggesting that therapy with these two agents together may be useful in children with short stature (190).

DIARRHEA Animal studies have shown that α_2-adrenoceptor agonists can both promote intestinal fluid absorption and inhibit intestinal motility (191),

and are effective in several animal models of experimental diarrhea (191). The proabsorptive effect appears to result from a peripheral action, since α2-adrenoceptor agonists incapable of entering the central nervous system also showed in vivo activity (191).

Studies in normal human volunteers showed clonidine to inhibit intestinal motility (192) and to promote absorption from the intestinal mucosa (192). Although clonidine produced a modest reduction in fecal electrolyte loss in cholera patients, no clinically significant benefit in fecal fluid loss was observed (193). However, several anecdotal reports show clonidine to be of significant benefit in diarrhea associated with diabetes (194). Clonidine was also effective in a patient with diarrhea induced by bronchogenic carcinoma (114), and diarrhea is one of the opiate- and alcohol-withdrawal symptoms most commonly relieved by clonidine (195).

α2-*Adrenoceptor Antagonists*

Despite the many functional effects mediated by the α2-adrenoceptors, there is currently little therapeutic application of α2-adrenoceptor blockade. Yohimbine, the only α2-adrenoceptor antagonist approved for use in human subjects, has a short plasma half-life and is also an antagonist at central serotonergic and dopaminergic receptors. Several potential therapeutic applications exist for a selective α2-adrenoceptor antagonist; novel drugs are currently being developed for some of these indications.

RAYNAUD'S PHENOMENON Data from experimental animals and humans indicates that the postjunctional α2-adrenoceptor plays an important role in the control of cutaneous blood flow (131, 196). The inverse relationship between ambient temperature and α2-adrenoceptor sensitivity (197) suggests that α2-adrenoceptors may play an even more important role under the cold conditions where Raynaud's attacks commonly occur. In contrast to the selective α1-adrenoceptor antagonists, which have little effect, yohimbine or rauwolscine will reverse cold-induced decreases in cutaneous flow induced by total body cooling (196) or localized skin cooling (198) in normal human volunteers. Since both the α1-adrenoceptor and α2-adrenoceptor play a role in the control of cutaneous blood flow, a nonselective α-adrenoceptor antagonist, or an antagonist blocking both α1-adrenoceptors and postjunctional α2-adrenoceptors may be the most effective approach to Raynaud's phenomenon, based on measurements of cutaneous blood flow in the rat (49).

IMPOTENCE Systemic administration of yohimbine has been evaluated in impotence, both of psychogenic and organic origin (199). In double-blind

trials, the yohimbine group consistently reported more benefit than the placebo group, although the differences did not consistently reach statistical significance. While a substantial percentage of patients (38–46%) report some subjective improvement, in one study only 5% were completely satisfied (200). The mechanism for the beneficial effect of yohimbine, and indeed the involvement of α_2-adrenoceptor blockade in its action, has not been established.

NONINSULIN-DEPENDENT DIABETES α_2-Adrenoceptor activation is known to inhibit the secretion of insulin from the β-cell of the pancreatic islet. α_2-Adrenoceptor antagonists, such as rauwolscine, idazoxan, and SK&F 86466, potentiate glucose-induced insulin secretion in the rat, and attenuate peak plasma glucose levels attained following an oral glucose challenge (201, 202). Studies in normal human subjects show phentolamine to potentiate the acute phase of insulin secretion (203). α_2-Adrenoceptor antagonists do not influence basal insulin levels (204). Hence, blockade of the α_2-adrenoceptor on the pancreatic islet cell may represent an approach to selectivity enhancing glucose stimulated insulin secretion in noninsulin-dependent diabetes (NIDDM).

Phentolamine enhances the insulin response to glucose challenge in NIDDM patients (205). Although a nonadrenergic component to the action of phentolamine on the islet cell has been proposed (206), a highly selective nonimidazoline α_2-adrenoceptor antagonist, MK-912, has been shown to produce a similar clinical effect (207).

SL 84,0418, a potent and selective α_2-adrenoceptor antagonist (208), is being developed specifically as an oral hypoglycemic agent for NIDDM. In a primate model in which idazoxan is ineffective, SL 84,0418 blunts the hyperglycemic response to oral glucose challenge and stimulates insulin secretion (209). In contrast to most α_2-adrenoceptor antagonists, SL 84,0418 can stimulate basal insulin release and produce symptomatic hypoglycemia in normal human volunteers (210), suggesting an action in addition to α_2-adrenoceptor blockade.

OBESITY Based on the ability of α_2-adrenoceptor stimulation to inhibit lipolysis in isolated human adipocytes, there is a rationale for the use of an α_2-adrenoceptor antagonist to promote the weight loss induced by caloric restriction (211). Since endogenous epinephrine can activate both the lipolytic β-adrenoceptor and the antilipolytic α_2-adrenoceptor, α_2-adrenoceptor blockade should promote lipolysis. This premise is supported by experimental observations showing that fat deposits known to be relatively unaffected by dietary restriction have a high density of α_2-adrenoceptors vis-à-vis β-ad-

renoceptors (212). Studies in dogs suggest that in addition to effects of lipolysis, yohimbine may exert a chronic effect to reduce food intake (213); however, the relationship between this effect and α_2-adrenoceptor blockade has not been established.

In normal volunteers, a single oral dose of yohimbine induces a fourfold increase in plasma glycerol and free fatty acids when administered following an overnight fast (214). The lipolytic effects of yohimbine are substantially attenuated by pretreatment of the subjects with propranolol, suggesting that most of the effect is indirectly mediated via an increase in plasma catecholamines. This is supported by studies in dogs showing that the ability of a series of α_2-adrenoceptor antagonists to increase plasma-free fatty acids correlates well with the degree of elevation of plasma norepinephrine (211). Nevertheless, in both human subjects and in dogs, a small, but statistically significant lipolytic effect remains after propranolol pretreatment, suggesting that direct blockade of adipocyte α_2-adrenoceptors may contribute to the lipolytic effect of the α_2-adrenoceptor antagonists. This is supported by experiments in conscious and anesthetized rats showing that SK&F 86466 will augment the lipolytic effect of exogenous epinephrine infusion (215).

DEPRESSION Depression may result from a depletion of norepinephrine and/or secretion at certain synapses within the central nervous system. The possibility exists that different classes of depression result from selective depletion of norepinephrine or serotonin, and that agents selectively elevating synaptic levels of these neurotransmitters have selective efficacy in alleviating the consequences of this depletion (216).

Classical antidepressants act via blocking the neuronal uptake of norepinephrine and/or serotonin. Blockade of α_2-adrenoceptors can accelerate the down-regulation of β-adrenoceptors induced by a neuronal uptake blocker (217), and may therefore be useful in the treatment of depression.

Although information on the clinical efficacy of selective α_2-adrenoceptor antagonists in depression is sparse, several such agents are currently being evaluated, including idazoxan. The data available suggest that these agents may be effective (218). In addition, several marketed antidepressants have α_2-adrenoceptor antagonist activity. While the relatively weak α_2-adrenoceptor antagonist activity of a potent neuronal uptake blocker, such as amitriptyline, is unlikely to contribute to its clinical profile, the situation may be different for some atypical antidepressants, such as mianserin and several of its structural analogs (219). There is interest in the development of molecules combining α_2-adrenoceptor antagonism with neuronal uptake blockade (220).

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