Biochemical Pharmacology, Vol. 41, No. 11, pp. 1541–1549, 1991
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

COMMENTARY

CLONIDINE-DISPLACING SUBSTANCE (CDS) AND ITS PUTATIVE IMIDAZOLINE RECEPTOR

NEW LEADS FOR FURTHER DIVERGENCE OF α_2-ADRENERGIC RECEPTOR ACTIVITY

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α_2-ADRENOCEPTORS—MULTIPLE EFFECTS

Alpha$_2$-adrenergic receptors (α_2AR*) are involved in the control of many physiologic functions in the central nervous system and in the periphery. Centrally mediated lowering of blood pressure is attributed mainly to stimulation of presynaptic α_2AR as autoreceptors located at specific nuclei in the midbrain. Activation of these receptors decreases sympathetic outflow and vasomotor tone which, in turn, decreases mean arterial pressure [for review see Refs. 1 and 2].

The most characteristic feature of α_2AR action is the lowering of cAMP levels which is often related to the various inhibitory responses mediated by α_2-adrenergic agonists in different cells. For example, in human platelets, α_2-adrenergic agonists aggregate platelets and inhibit adenylyl cyclase, similarly to fat cells where α_2-adrenergic agonists inhibit adenylyl cyclase and also mediate inhibition of lipolysis. Is lowering of cAMP levels sufficient to induce platelet aggregation or to inhibit lipolysis in adipocytes? This point is not yet fully understood.

Other responses to α_2-adrenergic agonists in the periphery are inhibition of acetylcholine release in the guinea pig ileum; inhibition of insulin secretion in pancreatic cells; and inhibition of the field-stimulated contraction of rat vas deferens, guinea pig vena cava and mesenteric arteries [for review see Ref. 1]. At the central nervous system, α_2-adrenergic agonists mediate numerous responses such as inhibition of neurotransmitter release, hypotension, sedation and induction of growth hormone release [1].

The importance of α_2-adrenergic agonists as hypotensive drugs gave birth to the production of a series of agonists, with an imidazoline moiety as the core structure such as clonidine, *p*-aminoclonidine and UK 14,304, as well as guanabenz and guanoxane, with a guanido moiety as the core structure. All the above-mentioned drugs induce a transient, believed to be peripherally mediated, increase of plasma adrenaline, followed by a centrally mediated decrease in blood pressure and, therefore, are used as antihypertensive drugs. In their tritiated form, α_2-agonists and antagonists are used to label both central and peripheral α_2AR.

Radioligand-binding studies provide evidence to sustain a multiplicity of α_2AR [2]. Re-subclassification of adrenergic receptors [3] reveals at least three subtypes of α_2AR (α_{2A}, α_{2B} and α_{2C}) where the main differences lie in the rank order potency of various selective α_2-adrenergic antagonists.

An extensive study in the pithed rat by Ruffolo and colleagues [4] shows that not all the effects mediated by imidazoline ligands, such as clonidine, can be attributed to an interaction at α_2AR only (see following sections). This was the first functional demonstration for an additional interaction site for clonidine-like drugs, most likely at sites different from α_2AR.

Multiple effects of clonidine analogs at the central nervous system which could not be mimicked by the "classical" endogenous α_2AR agonists, adrenaline and noradrenaline, suggested that α_2AR are a heterogenous population which can be further subdivided to novel subfamilies.

Bousquet *et al.* [5] pointed out functional differences between a series of α_2-agonists which possess an imidazoline structure (clonidine, ST 587, cirazoline) and phenylethylamine (α-methylnoradrenaline). Upon injection of imidazoline ligands at the rostral ventrolateral medulla, a marked decrease in mean arterial pressure was observed, whereas α-methylnoradrenaline, a potent phenylethylamine α_2-adrenergic agonists, was ineffective.

In 1987, Boyajian and Leslie [6] carried out a detailed mapping of α_2-adrenergic receptors in rat brain using tritiated yohimbine and tritiated idazoxan, both considered selective α_2-antagonists. The regional distribution as detected by the two radiolabeled antagonists reveals a unique population of idazoxan sites distinct from the population of sites labeled by yohimbine. These results show that idazoxan binds to an additional site which is not recognized by yohimbine and thus strengthens the hypothesis suggesting the existence of a novel class of α_2AR.

* Abbreviations: α_2AR, α_2-adrenergic receptors; cAMP, cyclic AMP; GTP, guanosine triphosphate; and CDS, clonidine-displacing substance.

Hence, further subclassification emerges which is based on interaction of the so-called selective α_2-adrenergic antagonist, also with a non-adrenergic site. By definition this site which does not recognize phenylethylamines could not be a subtype of α_2AR. The overwhelming cross-recognition of imidazolines and guanidine ligands at α_2AR and at novel target receptors, suggests similar structural features between the two sites.

PHYSIOLOGICAL STUDIES—EVIDENCE FOR IMIDAZOLINE NON-ADRENERGIC SITES

Mediation of inhibitory processes in various cell types is the main characteristic of α_2-adrenergic agonist action. However, the molecular mechanism which underlies α_2-adrenergic response has been demonstrated in only a few systems [7, 8].

Human platelets, fat cells and NG108-15 cells (a neuroblastoma × glioma hybrid cell) are among the few systems where coupling of α_2AR via a pertussis-toxin-sensitive GTP-binding protein (G_i) to adenylyl cyclase has been demonstrated. The coupling mediates inhibitory action of adenylyl cyclase to lower cAMP levels in the cells. However, the decrease in the cAMP level in these cells cannot account for the α_2-agonist-mediated responses, e.g. aggregation and lipolysis.

In pancreatic islets of Langerhans, α_2-adrenoceptor agonists inhibit the glucose-induced insulin release [reviewed in Refs 9 and 10]. The role played by cAMP in insulin release is equivocal. On the one hand, a few reports showed no involvement whatsoever for cAMP [11], while on the other hand dibutyryl cAMP has been shown to stimulate insulin release [12]. The ability of calcium ionophore to induce insulin release leaves part of the action to increase of intracellular calcium. Furthermore, inhibition of glucose-mediated insulin release by α_2-agonists occurs without a concomitant decrease in cAMP levels. Thus, a dissociation between the observed functional effect of α_2-agonists and adenylyl cyclase activity is evident. Similar results were obtained for platelet aggregation where no correlation between aggregation and inhibition of adenylyl cyclase was observed. Inhibition of intestinal secretion also could not be accounted for by a decrease in cAMP levels only. Taken together, these observations exclude adenylyl cyclase inhibition as the only pathway responsible for the α_2-mediated physiological effects [9]. α_2-Adrenergic agonists inhibit glucose-induced insulin release in pancreatic cells, similar to the inhibition of acetylcholine release in the gut, or inhibition of renin release in the kidney. Since all these exocytotic processes are Ca^{2+} dependent, it was suggested that the α_2-agonist may mediate its inhibitory effects via modulating calcium levels in the cells. However, this assumption has been ruled out in insulin-secreting RINm5F cells, whereby catecholamine inhibition of insulin release was shown not to involve a decrease in cytosolic free Ca^{2+} [13]. Recently, it was demonstrated that a voltage-dependent Ca^{2+} current recorded from NG108-15 cells was depressed by noradrenaline via α_2AR [14], an action which might lead to inhibition of release.

In a study by Holz *et al.* [15], norepinephrine induced inhibition of voltage-dependent Ca^{2+} channels in embryonic chick dorsal root ganglion cells. Since clonidine did not inhibit the voltage-dependent channels, the authors concluded that the effects are mediated by clonidine-insensitive, α_2-adrenergic-like receptors. Are there two different receptors or different binding domains for adrenaline and for imidazoline? Is it possible that certain α_2-adrenergic agonists may attenuate Ca^{2+} entry in neuronal cells via different receptors? Is it directly or indirectly via an increase in K^+ conductance?

Clonidine, an α_2-adrenergic agonist with an imidazoline structure, which acts in some cells as either a partial agonist or as an antagonist, is used frequently as a synthetic and, therefore, unmetabolized drug to study α_2AR in various systems. Indeed, most of the norepinephrine-mediated effect can be mimicked faithfully by clonidine. However, a few exceptions showed that clonidine had a wider range of action. One of the first leads for a possible unique clonidine site was the ability of cimetidine, an imidazole ligand, to antagonize the depressor effect of intraventricular administration of clonidine [16].

Configurational requirements at the α_2-adrenergic receptor were used as a tool to differentiate between imidazoline and phenylethylamine interactions. The Easson–Stedman hypothesis [17] established the first basis for possible interaction of phenylethylamines at their appropriate site of action. This hypothesis predicts a three-point attachment for binding of phenylethylamines evolving from the asymmetry at the agonist carbon atom, and was examined for imidazoline-like agonists [4].

Indeed, the Easson–Stedman hypothesis proved adequate, upon evaluation, with regard to the recent cloning and structure determination of adrenergic (α_2-, α_1- and β-adrenergic) receptors. Aspartic acid (113) residue in the third transmembranal helix is present in all known G-protein-coupled receptors, including α-adrenergic, β-adrenergic, muscarinic, 5-hydroxytryptamine (5-HT), and dopaminergic receptors, and therefore might serve as the negative charge which directs the positioning of the amine at the right binding conformation. Further possible interactions are provided by other amino acid side chains, e.g. the two hydroxyl groups of the catechol moiety hydrogen bond to serine 204 and serine 207, both at the 5th transmembranal helice [18]).

Ruffolo *et al.* [4] have tested the possible interaction points of a chimera between clonidine (imidazoline) and norepinephrine (phenylethylamine) at the α_2-adrenergic receptor, in comparison to interaction points of norepinephrine. The optically active catecholamidazoline and catecholamidine have been resolved for their optical enantiomers and tested for their pressor responses in the pithed rat [19]. These chimera analogs show a weak isometric activity difference in the pithed rat, unlike the highly restricted stereochemical constraints observed for the activity of phenylethylamine diastereoisomers. As a consequence, it would be difficult to assign a similar interaction for clonidine or its various analogues, and for norepinephrine, at the α_2AR. All these results strongly suggest an additional site

for clonidine which may be related but certainly is not identical to α_2AR. More data supporting the additional site came from direct injections of imidazoline agonists into the medulla oblongata, and monitoring changes in blood pressure [5].

CLONIDINE-DISPLACING SUBSTANCE (CDS)

In 1984, the isolation and partial purification of an endogenous clonidine-like substance from rat brain [20] and from bovine brain [21] were reported. The presence of an endogenous, non-catecholamine, low molecular weight brain substance, which competes with [^{3}H]clonidine in rat brain and [^{3}H]yohimbine in human platelets, suggested the existence of an additional α_2-adrenergic-like receptor.

The endogenous ligand, named clonidine-displacing substance, displaces [^{3}H]clonidine from bovine and rat brain membranes in a reversible and competitive manner and, hence, its given name. Further characterization of CDS established that the purified fraction, chromatographed by HPLC on a C_{18} reverse phase column, also competes with [^{3}H]yohimbine [21] and [^{3}H]rauwolscine [22] on α_2AR present in human platelets. It does not bind to α_1-adrenergic receptors since it does not displace [^{3}H]prazosin from brain membranes nor does it bind to β-adrenoceptors as no displacement of [^{125}I]cyanopindolol from turkey erythrocyte membranes was observed [21]. CDS is a low molecular weight substance of 587.8 ± 2 daltons, as determined by plasma desorption mass spectrometry (PDMS) [23], which is a good approximation of its molecular weight as determined by low molecular sieve chromatography. It is ninhydrin and fluorescamine negative, it is not a peptide, and it is heat and acid resistant [23]. Thus far its exact chemical structure has not been elucidated because of its low abundance. It is possible, however, to study its physiological and pharmacological properties because of its high potency.

Physiological properties of CDS

Rat vas deferens. Rat vas deferens can be stimulated electrically to produce a twitch response which is highly sensitive to an α_2-adrenergic agonist (e.g. clonidine [24]). CDS mimicks the clonidine-mediated inhibition of the twitch response, and its inhibitory action is partially antagonized by phentolamine (1 μM) and yohimbine (1 μM) [25].

A crude preparation of CDS, which was used prior to its separation on the HPLC column, was shown to induce contraction of the gastric fundus smooth muscle of the rat [26].

More recently, it was shown that CDS extracted from human plasma produced contractions of rat aortic rings, and these contractions were antagonized by rauwolscine but not by prazosin. The authors argue that since methoxamine-mediated contractions were relaxed by clonidine but not by CDS, and since clonidine-induced contractions were antagonized by both rauwolscine and prazosin, that CDS mediates its effect via receptors which are different from the clonidine sites, or that CDS is a more specific agonist than clonidine.

Intracerebral injection of CDS. The rostral ventrolateral medulla (RVL) is considered to be one of the nuclei regulating centrally mediated blood pressure. Clonidine and its analogs injected at this site produce a marked decrease in blood pressure; however, α-methylnoradrenaline injected at this site is ineffective [5].

Injection of CDS at the RVL of the cat (0.1 μL = 5 units) produced an increase in mean arterial pressure with a maximal rise at 2 min of 40 ± 0.8%, N = 9 [27]. Intracisternal administration of CDS in the anesthetized rat also caused a significant rise in mean arterial pressure which was maximal at 5 min (20 ± 4%; N = 5) [28]. Furthermore, preinjection of clonidine followed by CDS treatment resulted in a marked reduction in the hypotensive effect of clonidine (−15 ± 5 vs −30 ± 5%, $P < 0.001$) suggesting *in vivo* competition on a common site. Thus, it appears that imidazoline-type ligands and CDS share affinity for a common site, which is not an adrenergic receptor. Centrally activated α_2-adrenergic receptors also mediate growth hormone release which, to some extent, can be activated by idazoxan, an α_2-antagonist [29]. Although the mechanism of growth hormone release is not understood yet, these results, similar to CDS reversal of inhibition, can also be interpreted as alleviating the inhibitory action of an endogenous ligand agonist.

Based on these results it is tempting to speculate that the endogenous ligand (CDS) is an agonist for a new, non-adrenergic, imidazoline site, through which it mediates an increase in blood pressure. Following this scheme, clonidine is then an antagonist which relieves the hypertensive effects of the endogenous CDS via the imidazoline site.

Interestingly, CDS purified by a method similar to that of Atlas and Burstein [20, 21] competed with [^{3}H]*p*-aminoclonidine in brain membranes and produced a decrease in mean arterial pressure when injected into the ventro medullary C1 region of the rat [30]. Since the CDS preparation applied in the above study was a crude methanolic-chloroform extract it would be inappropriate to discuss the contradictory results. CDS extracted from bovine brain inhibited specific [^{3}H]*p*-aminoclonidine binding to a polyclonal antiserum against *p*-aminoclonidine [31]. The authors suggest structural similarity between CDS and clonidine. Further purification should be carried out in order to establish the structure of this form of CDS and determine its relationship to the original CDS.

Interaction of CDS with human platelets. The affinity of CDS for α_2AR, using [^{3}H]yohimbine, has been established in human platelets [20] and later was shown to be competitive with [^{3}H]rauwolscine with an apparent dissociation constant of 1.28 units [22, 23]. In human platelets, α_2AR are coupled to adenylyl cyclase via an inhibitory GTP-binding protein (G_i) where α_2-agonists induce inhibition of adenylyl cyclase activity [7]. CDS which binds to

* Synetos D, Manolopoulos VG, Atlas D and Pipili-Synetos E, An endogenous substance partially purified from plasma contracts the isolated rat aorta. Manuscript submitted for publication.

platelet membranes, was unable to inhibit adenylyl cyclase activity at concentrations up to 30-fold its affinity in binding nor was it able to reverse the epinephrine-induced-inhibition of the prostaglandin E_1 (PGE_1)-stimulated adenylyl cyclase [22]. In platelets, a characteristic physiologic response to α_2-adrenergic agonists is aggregation which bears no correlation to inhibition of adenylyl cyclase [reviewed in Ref. 9].

CDS was acting in a manner similar to clonidine and other α_2-adrenergic agonists [e.g. guanabenz, rilmenidine in human platelets (Diamant and Atlas, manuscript in preparation)]. It inhibited epinephrine-mediated aggregation, as well as potentiated ADP- and collagen-induced aggregation and, in turn, thromboxane B_2 content [22].

Finally, it appears that CDS plays a role similar to clonidine as a partial agonist: (a) it acts as an antagonist of α_2-mediated platelet aggregation (at concentrations 60-fold higher than its binding constant); (b) it potentiates ADP- and collagen-induced platelet aggregation (at concentrations 12-fold lower than its dissociation constant); and (c) it is poorly coupled to G_1 and, in turn, acts as a poor inhibitor of adenylyl cyclase, if at all.

Thus, in peripheral tissues (human platelets, smooth muscle, rat gastric fondus and vas deferens), CDS mimics the action of clonidine, unlike its action in the brain where, in contrast to clonidine, it acts to increase blood pressure.

CDS in human serum. The ability of CDS to interact with α_2AR in human platelets led us to explore for its presence in human serum. Methanolic extract from sera of patients with pregnancy-induced hypertension (PIH) showed increased levels of CDS (12.2 ± 1.5 units/mL serum), as compared to normal control pregnancy (4.2 ± 0.5 units/mL serum) or chronic hypertension (4.5 ± 0.3 units/mL serum) [32]. Further purification of the methanolic extract on a C_{18}-reverse phase column yielded a cleaner fraction of serum CDS, which contracted isolated rat aorta similar to the action of clonidine.*

The presence of CDS in human serum broadens the possible role played by CDS in both peripheral tissues and at the central nervous system.

IMIDAZOLINE RECEPTORS

The observations that clonidine and its analogs seem to mediate their effects not exclusively via α_2AR and that CDS, an endogenous non-catecholamine ligand, might account for some of these responses, suggest the existence of a putative imidazoline receptor. Binding studies carried out using [^{3}H]*p*-aminoclonidine, showed additional, non-adrenergic, [^{3}H]*p*-aminoclonidine-labeled sites (30% of total sites) in bovine ventrolateral medulla membranes [33]. This site was further characterized as a selective imidazole binding site which binds CDS with very high affinity [34]. It soon became apparent that rauwolscine (an isomer of yohimbine) labels a smaller population of sites in a variety of tissues, compared to a much larger population of sites labeled with idazoxan, both considered selective α_2-antagonists [reviewed in Ref. 35].

Using the imidazoline-structured α_2-antagonist [^{3}H]dazoxan (RX 81094), Coupry *et al.* [36] have characterized a peripheral imidazoline, non-catecholamine binding site in the rabbit renal proximal tubule. α_2AR, detected by [^{3}H]rauwolscine, and imidazoline receptors are both present in the rabbit kidney in a ratio of about 30 to 70% respectively. These results were further confirmed by others in rabbit kidney [37], in human kidney [38] and in pig kidney [39] where the existence of a non-adrenergic imidazoline site was monitored (Table 1). Recently we have shown in human platelets that idazoxan labels additional sites which are not recognized by rauwolscine or adrenaline ([41], Fig. 1). Similarly, in rat lung, using adrenaline for non-specific binding, α_2AR ($B_{max} = 175 \pm 20$ fmol/mg protein) represent about 30% of the total sites labeled by idazoxan ($B_{max} = 578 \pm 30$ fmol/mg protein) using cirazoline for non-specific binding ([41], Table 1). The most interesting results were observed in rat liver cells where noradrenaline and adrenaline display no affinity for sites labeled with idazoxan, indicating that the sites labeled by idazoxan are distinct from α_2AR ([41], Fig. 1). A significantly higher affinity of idazoxan for α_2AR (2–4 nM) was observed in most tissues studied, as compared to lower affinity (8–14 nM) displayed for the imidazoline site. Since both imidazoline sites and α_2AR bind [^{3}H]idazoxan, the affinity observed in tissues of mixed α_2 and imidazoline sites suggests an average affinity for both sites. These results further sustain the notion that idazoxan binds to an additional site which is not an α_2AR adrenergic receptor and displays lower affinity for this site compared to its affinity for conventional α_2AR.

IS THE IMIDAZOLINE RECEPTOR A SUBTYPE OF α_2AR?

Receptor density

As shown in Table 1, the number of idazoxan sites exceeds the number of α_2AR (determined by [^{3}H]yohimbine or [^{3}H]rauwolscine) in all tissues examined, with the exception of rat and rabbit cerebral cortex.

Specificity

As shown in Table 2, naphazoline, cirazoline and guanabenz display high affinity for liver imidazoline receptors ($K_i = 8.9 \pm 5.93$, 2.1 ± 0.4 and 33.8 ± 11.8 nM respectively), whereas norepinephrine, epinephrine and yohimbine are not recognized by these receptors ($K_i = 60{,}000$ nM, 60,000 nM, and >1,000,000 nM respectively). Thus, by definition imidazoline is not an adrenergic site.

Similarly, clonidine which competes with high affinity for the α_2AR in the brain ($K_d = 1.4$ nM) binds poorly (15 μM) to the liver imidazoline receptor (Table 2).

* Synetos D, Manolopoulos VG, Atlas D and Pipili-Synetos E, An endogenous substance partially purified from plasma contracts the isolated rat aorta. Manuscript submitted for publication.

Table 1. Distribution of [^{3}H]idazoxan sites (idazoxan) and [^{3}H]rauwolscine sites (adrenaline) in various cells

Tissue	Animal	B_{max} (fmol/mg protein) Idazoxan	Adrenaline	Reference
Liver	Human	600 ± 200	—	40
	Rabbit	600 ± 200	—	40
	Rat (memb.)	400 ± 37	0	41
	Rat (hepatocytes)	801 ± 23	0	41
Kidney	Human	413 ± 56	93	38
	Rabbit	566 ± 188	155 ± 28.5	36
	Rabbit	209 ± 64	58 ± 19	37
	Rat	385 ± 60	195	42
	Pig	160 ± 20	—	39
Fat cells	Rabbit	1370 ± 19	—	43
Cerebral cortex	Human	—	107	44
	Rabbit	70 ± 8.0	126 ± 55	37
	Rat	283 ± 7.0	132 ± 14	41
Lung	Rat	578 ± 30	175 ± 20	41
Platelets	Human	358 ± 102	212 ± 78	42
	Human	267 ± 11	160.4 ± 83	41
Myometrium	Human	127 ± 42	47 ± 13	42
HEL cells		355 ± 76	—	42

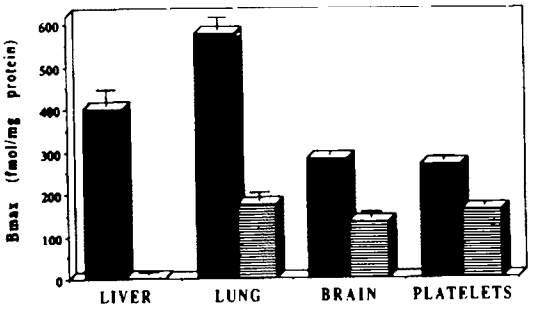

Fig. 1. Imidazoline and phenylethylamine sites in rat liver, lung and brain and in human platelets. Imidazoline sites were evaluated by direct binding using [^{3}H]idazoxan at increasing concentrations in the absence and in the presence of 20 μM cirazoline (shadowed columns). Specific binding sites for α_2-adrenergic receptors were evaluated by direct binding of [^{3}H]idazoxan at increasing concentrations in the absence and in the presence of 20 μM (−)noradrenaline (striped columns). B_{max} values were derived from Scatchard plots using the EnzFitter program. The "imidazoline" receptors represent both imidazoline specific and α_2-adrenergic receptors since cirazoline is also an α_2-adrenergic agonist.

Coupling to GTP-binding protein

Lack of effect of GppNHp or NaCl on the affinity of clonidine, UK 14,304 or guanabenz strongly indicates that they bind differently to imidazoline receptors than to α_2AR and, therefore, if they act as agonists, they are not coupled to G-proteins [41].

CDS affinity

CDS binds to imidazoline receptors in liver cells with an apparent K_i = 3.75 units which is about 10-fold less than its affinity for clonidine sites in the brain (Table 3). These results suggest divergence in affinity towards CDS which can be attributed to either heterogeneity of receptor subtypes or to tissue specificity.

Structural aspects

α_2AR belong to a receptor family characterized by 7 transmembrane domains, which are coupled to GTP-binding proteins. Since it is coded by an intronless gene, the diversity of the receptor molecule which can arise from alternative RNA splicing is excluded, unlike the dopamine receptor D_2 where alternative RNA splicing resulted in a 29 amino acid deletion in the third inner loop of the receptor leading to two different receptors [48, 49].

Thus, the multiplicity of α_2AR, as observed from binding studies, could stem from changes in the region coding for the binding domain, likely to emerge from different genes.

Specificity for cloned α_2AR and imidazoline receptors

α_2AR from human platelets and rat kidney were cloned, sequenced and expressed in COS 7 cells [45, 50]. The two receptors display similar although not identical pharmacological profiles, attributed to tissue specificity, and are present on two different chromosomes. Human platelet α_2AR are related to the α_{2A}-adrenergic subtype, and human kidney to the α_{2B} subtype, based on their relative affinities for

Table 2. Competition of various α_2-adrenergic ligands on liver membranes [41] and on cloned kidney and human platelet α_2-adrenergic receptors, expressed in COS cells [45]

α_2-Adrenergic ligands	K_i (nM) Imidazoline (liver)	K_i (nM) α_2-Adrenergic (kidney)	K_i (nM) α_2-Adrenergic (human platelets)
Agonists			
Epinephrine	60,000	170	1000
Norepinephrine	60,000	240	2400
Clonidine	15,067	—	—
p-NH_2-clonidine	42,000	81	74
Oxymethazoline	14,000	62	11
UK 14,304	42	210	72
Guanabenz	33	59	14
Naphazoline	9	—	—
Cirazoline	2.1	—	—
Antagonists			
Yohimbine	>1,000,000	0.93	1.6
Phentolamine	187,000	33	10.0
Piperoxan	68,000	—	—
Idazoxan	9.96	17	10

Table 3. Affinity of CDS in various cellular membranes

Animal tissue	Radiolabeled ligand	CDS (units)* IC_{50}	CDS (units)* K_i†	Reference
Rat brain	[^{3}H]Clonidine	1	0.5	21
Human platelets	[^{3}H]Rauwolscine	2	1.28	22
Rabbit kidney	[^{3}H]Idazoxan	2	1.00	47
Rat liver	[^{3}H]Idazoxan	6	3.75	41
Rat ventrolateral medulla	[^{3}H]p-Aminoclonidine	0.31	—	34

* One unit of activity is defined as the amount needed to displace 50% [^{3}H]clonidine (2 nM) specifically bound to rat brain membrane (250 μg protein) [20].

† K_i values were calculated according to Cheng and Prusoff [46] according to the equation $K_i = IC_{50}/1 + I/K_d$ where IC_{50} is the concentration needed to displace 50% of specifically bound radioligand; [I] is the concentration of the radioligand; and K_d its dissociation constant.

prazosin and oxymethazoline [51]. Thus, a valuable comparison of affinities between a pure α_2-adrenergic clone on the one hand and a unique imidazoline-receptor population on the other should underlie the main differences in their pharmacological selectivity. The rank order of agonist and antagonist potencies obtained from competition studies of α_2AR clone from rat kidney and human platelets expressed in COS cells, versus that of imidazoline receptors obtained from competition using [^{3}H]idazoxan in rat liver, is presented in Table 2.

Thus, the overwhelming affinity of imidazoline and guanidine α_2-adrenergic ligands, for the imidazoline receptors, the lack of affinity for adrenaline, noradrenaline or yohimbine, and the insensitivity for GTP analogs suggest that the imidazoline receptor is a unique receptor and not a subtype of α_2AR.

Furthermore, when the two types of α_2-adrenergic genes were used to transfect COS 7 cells, no idazoxan sites, in addition to α_2AR, were detected [38]. Therefore, it would be, most likely, an additional gene which would be coding for the imidazoline receptor. Some of the points discussed above are schematically presented in Fig. 2.

Subclassification of imidazoline receptors. A further point of divergence arises from the rather low affinity displayed by clonidine, p-aminoclonidine and CDS for imidazoline receptors, as clearly illustrated in liver cells (Tables 2 and 3). All three ligands mentioned above show high affinity for sites labeled by [^{3}H]clonidine [20], [^{3}H]p-aminoclonidine [34] and [^{3}H]rauwolscine [22] and low affinity for imidazoline receptors in rat liver and rabbit kidney [41, 47]. Furthermore, in membranes prepared from bovine ventrolateral medulla, phenylethylamine displaced 70% of the total [^{3}H]p-aminoclonidine-labeled sites and the remaining 30% were adrenaline-insensitive sites [33]. Since no physiological function has so far been related for imidazoline receptors, it

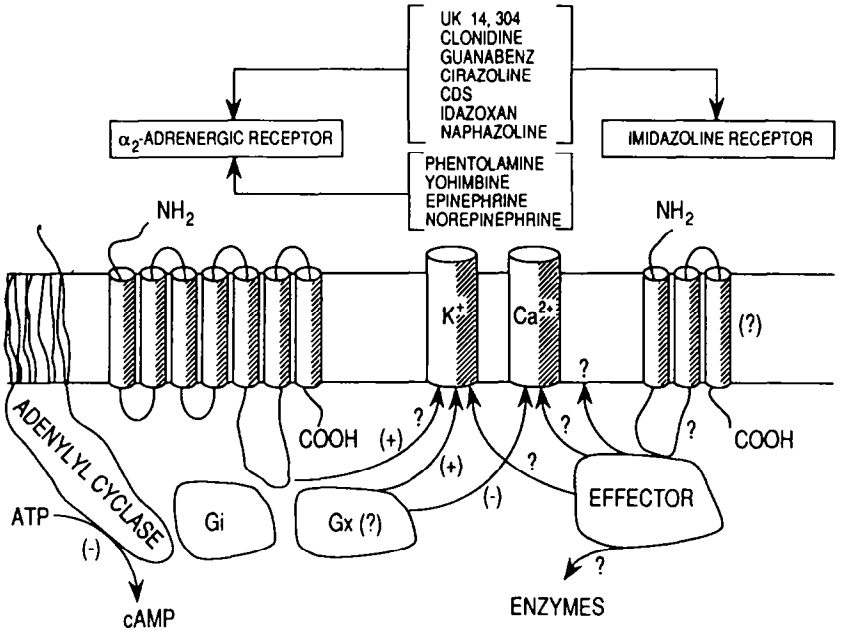

Fig. 2. Schematic representation of α_2-adrenergic receptors and an imaginary imidazoline site and their postulated sites of action in the cell.

would be difficult at this stage to refer to the lower affinity of these ligands at the imidazoline sites.

The non-adrenergic sites were further characterized for their specificity *vis-a-vis* guanidine- and imidazoline-structured ligands, as well as for their affinity for HPLC purified bovine CDS [47].

Although CDS competed with [^{3}H]idazoxan on imidazoline receptors in the purified basolateral kidney membranes with an apparent $K_d = 1$ unit (Table 3), it is still to be proven in a functional assay that CDS is the agonist of these sites. This would be shown biochemically once the physiological role of these sites in liver cells is determined.

Separation of α_2AR to two distinct proteins was reported recently [52]. The imidazoline/guanidinum binding site (IGRS) was separated from the α_2AR by lectin-affinity chromatography using solubilized rabbit kidney membranes. The solubilized α_2AR were retained by the resin and could be recovered afterward by high-salt elution. The IGRS-solubilized receptor, which was not detained on the column, expressed specificity and affinity for guanidine/imidazoline-type ligands, and for CDS, similar to native receptors in intact membranes [52].

Mechanistic aspects. There is ample evidence that inhibition of noradrenergic neurons at the locus coeruleus results from hyperpolarization due to the activation of outward potassium currents [53–55]. The coupling of α_2AR to K^+ channels was also suggested from intracellular recordings of the guinea pig submucus plexus neurons [56]. According to these studies, it was concluded that post-synaptic α_2AR mediate a K^+ conductance increase which underlies the inhibitory synaptic potentials. Further studies showed the involvement of a pertussis toxin sensitive GTP-binding protein in mediating the outward currents evoked by α_2-agonists in locus coeruleus neurons [57].

Although inhibition of neurotransmitter release and coupling to K^+-gating correlate with the pharmacology of α_2AR, many inconsistencies were reported which were attributed in part to the presence of presynaptic α_1AR or to different pathways by which α_2AR modify the function of noradrenergic terminal axons [reviewed in Refs 1 and 55].

Interestingly, binding of [^{3}H]idazoxan to imidazoline receptors in liver cells was sensitive to monovalent ions which interfere with K^+ channels, e.g. NH_4^+ and Cs^+, in addition to a significant inhibition of specific binding by 4-aminopyridine ($IC_{50} = 0.381 \pm 0.07$ mM [41]). In kidney membranes, K^+ was shown to increase the dissociation rate of idazoxan from 0.13 to 0.33 min^{-1}, suggesting binding of K^+ to an allosteric site of the imidazoline receptors [47].

Therefore, one may speculate that similarity between α_2AR and imidazoline receptors is carried further to a possible interaction with some sort of a K^+ channel. Additional studies on whole cells should shed more light on a possible function for imidazoline receptors.

In conclusion:

(a) separation of imidazoline receptors from α_2AR on a heparin-agarose column indicates that the two receptors are two distinct proteins.

(b) A strong similarity in the binding domains of α_2AR and imidazoline sites is suggested by the high affinity shared by imidazoline- and guanidine-type ligands for both sites.

(c) No binding of phenylethylamines at the imidazoline receptors is observed, thus indicating that,

by definition, the imidazoline receptor is not a subtype of α_2AR.

(d) Lack of GppNHp and Na^+ effect on the affinity of mutual α_2-adrenergic agonists at the imidazoline receptor strongly suggests a different mode of action of these ligands at the imidazoline receptor.

(e) Interaction of CDS with both α_2AR and imidazoline sites suggests its structural similarity to guanidine- and imidazoline-type ligands, as shown by their mutual affinity for the two receptors. The high affinity exhibited by CDS for antiserum (anti-*p*-aminoclonidine) binding to [^{3}H]*p*-aminoclonidine sites also indicates their structural similarity.

(f) Since CDS is an endogenous substance, it may represent the natural agonist to the new imidazoline receptor.

Hence, whole cell studies will become essential for establishing the functional role played by imidazoline receptors.

Perhaps via cloning and sequencing of imidazoline receptors on the one hand, and establishing the structure of CDS on the other hand, it would be possible to assign a functional role for this receptor and eventually for the endogenous CDS molecule.

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