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Heterogeneity of Alpha-2 Adrenergic Receptors

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BYLUND, D. B. *Heterogeneity of alpha-2 adrenergic receptors.* PHARMACOL BIOCHEM BEHAV 22(5) 835–843, 1985.—Alpha adrenergic receptors are subdivided into alpha-1 and alpha-2 subtypes on the basis of their pharmacologic properties. An evaluation of data from radioligand binding and functional experiments indicates that the receptors classified as alpha-2 are not a homogeneous group. The best example of this heterogeneity is the differences in the pharmacologic properties of alpha-2 receptors in rodent and non-rodent mammalian species. Prazosin generally has a high affinity for rodent alpha-2 receptors, but a low affinity for non-rodent alpha-2 receptors, while oxymetazoline is more potent at non-rodent alpha-2 receptors. A definition of alpha-2 adrenergic receptor subtypes is proposed with prazosin having a lower affinity (200–300 nM) at alpha-2A receptors and a higher affinity (5–10 nM) at alpha-2B receptors. Using this definition, the human platelet appears to have only the alpha-2A subtype, while all the receptors in the neonatal rat lung are of the alpha-2B subtype. The rat brain has roughly equal amounts of alpha-2A and-2B receptors while in the rat submandibular gland, about 85% of the receptors are alpha-2A. The ability to pharmacologically define putative alpha-2 adrenergic receptor subtypes should promote the development of additional subtype selective drugs which will increase our understanding of adrenergic pharmacology and may provide new therapeutic approaches.

Alpha-2 adrenergic receptors Receptor subtypes Prazosin Yohimbine Species differences

CLASSIFICATION OF ADRENERGIC RECEPTORS

Over a century ago, the receptor concept was put forth by Langley [31] in order to explain his observations concerning the effect of atropine and pilocarpine on salivary flow in the cat. Some years later Dale [17] extended this concept to include receptor subtypes in order to explain the differential effects of the ergot alkaloids on smooth muscle. He suggested that there might be subtypes of the adrenergic receptors at myoneural junctions, one mediating the excitatory effects and the other the inhibitory affects. Some 40 years later in a seminal paper, Ahlquist [1] suggested that it was more useful to define subtypes of adrenergic receptors on the basis of their pharmacology rather than on the basis of their function. In that study he compared the rank order of potency of five catecholamines using eight different physiological assay systems. He found that in five of his test systems, the catecholamines had the same relative affinities. The remaining three functions fell into a second group which had a markedly different order of potency as compared to the first group. He suggested that the receptor subtypes be defined on the basis of these two different orders of potencies, that is, on the basis of their pharmacology. He named the two receptor subtypes alpha and beta adrenergic receptors. This pharmacological classification scheme was subsequently validated by the use of antagonist drugs which selectively blocked either the alpha or the beta adrenergic response.

The beta adrenergic receptors were further subdivided into beta-1 and beta-2 receptor subtypes [30]. This subdivision also had a pharmacological basis since it resulted from comparing the rank order potency of 12 agonists in several isolated organ systems. This subclassification was subsequently validated by the development of subtype selective antagonists. It is important to emphasize that the beta-1 beta-2 subdivision is based solely on pharmacological data. The major biochemical mechanism of action for both beta-1 and beta-2 receptor subtypes appears to be the activation of adenylate cyclase. While beta-1 and beta-2 receptors coexist in most tissues, the pharmacological properties of each subtype is the same in all tissues. Furthermore is appears that the pharmacology of beta-1 and beta-2 receptors is identical in all mammalian species [39].

In contrast to the beta adrenergic system where the subclassification of the receptors using pharmacological criteria was relatively straight forward, the identification of alpha adrenergic receptor subtypes has been much more difficult. This has been due in part, to the more complex physiological responses elicited through alpha adrenergic receptors and to the slower development of subtype selective drugs. The initial subclassification was based on the presumed anatomic localization of the receptors with the suggestion that alpha adrenergic receptors could be subdivided into postsynaptic (alpha-1) and presynaptic (alpha-2) subtypes [48]. While it appears that all presynaptic adrenergic receptors are of the alpha-2 subtype, there are now many examples of receptors which are not presynaptic which have the same pharmacological properties. Thus this anatomic subdivision of alpha adrenergic receptors is not useful as a definition of alpha-1 and alpha-2 receptors. Berthelsen and Pettinger [4], realizing the limitations of the anatomic subdivision of alpha adrenergic receptors, suggested a functional basis for their

classification. According to their scheme alpha-1 and alpha-2 adrenergic receptors mediated excitatory and inhibitory responses respectively. However this classification scheme also lacks general applicability and thus can not be used as a definition of alpha-1 and alpha-2 adrenergic receptors. A third attempt to define alpha adrenergic receptor subtypes was based on a biochemical approach. According to this definition, alpha-1 receptors mediated effects secondary to an elevation of intracellular calcium while alpha-2 adrenergic receptors mediated effects secondary to the inhibition of adenylate cyclase [19,54]. While this definition of alpha adrenergic receptor subtypes may eventually be shown to be correct, it is not a generally applicable definition at the present time, because of our relative ignorance of the actual mechanisms for alpha adrenergic receptors in many tissues. Although the subclassification of adrenergic receptors will probably be based ultimately on their primary structure, it would appear prudent for the present to use a pharmacological subclassification scheme [14]. This is consistent with the primary division of adrenergic receptors into alpha and beta subtypes as well as the beta-1 versus beta-2 subclassification scheme. Presently the best definition of alpha-1 versus alpha-2 subtypes is based on the antagonists yohimbine (or its isomer rauwolscine) and prazosin. At alpha-1 receptors prazosin is more potent than yohimbine while at alpha-2 receptors yohimbine is more potent than prazosin [14].

Using this definition of alpha adrenergic receptor subtypes, one can then ask the question if alpha-1 and alpha-2 receptors have similar characteristics in all tissues and species as is the case with the beta-1 and beta-2 receptor subtypes. Over the past five years there has been increasing evidence that this is not the case, especially for the alpha-2 adrenergic receptors. It is the purpose of this review to present some of the mounting evidence for alpha-2 adrenergic receptor heterogeneity and approach the question of putative alpha-2 adrenergic receptor subtypes.

EVIDENCE FOR HETEROGENEITY IN ALPHA-2 ADRENERGIC RECEPTORS

With the introduction of the alpha-2 antagonist yohimbine as a [^{3}H]ligand in 1980 [40,56] (in addition to the partial agonist [^{3}H]clonidine which had been introduced several years earlier) many laboratories developed an interest in alpha-2 adrenergic receptor binding studies. The human platelet quickly became the "favored tissue" for these binding studies and has become the standard against which other tissues are compared. Using the platelet as a model system Hoffman and Lefkowitz [23] had developed a two state model for the alpha-2 adrenergic receptor. According to this model agonists bind with high affinity to the alpha-2(H) state of the receptor but with low affinity to the alpha-2(L) state. On the other hand, antagonists interact at both states with equal affinity.

Our interest in the alpha-2 adrenergic receptor was sparked by the remarkable increase in alpha-2 adrenergic receptor binding (using [^{3}H]clonidine) following denervation of the rat submandibular gland [10]. However in subsequent experiments we were unable to demonstrate any binding of [^{3}H]yohimbine to membranes prepared from denervated glands. Thus, in contrast to the platelet which had a significantly higher density of [^{3}H]yohimbine binding sites as apposed to [^{3}H]clonidine sites, the receptor in the submandibular gland bound the [^{3}H]agonist but not the [^{3}H]antagonist [11]. We were similarly surprised to find that in the one day old rat lung there was a considerable amount of [^{3}H]yohimbine binding but no [^{3}H]agonist binding [33]. It was possible, of course, that the effect in the submandibular gland was the result of the denervation and was not something that would happen in a "normal" tissue. In order to eliminate this possibility, we looked at the alpha-2 binding in the sublingual gland. We found that the gland from the adult animal had a high density of [^{3}H]clonidine binding, but no detectable [^{3}H]yohimbine binding.

These observations prompted us to compare the number of binding sites for [^{3}H]yohimbine and [^{3}H]clonidine in sev-

TABLE 1

RATIO OF [^{3}H]YOHIMBINE TO [^{3}H]CLONIDINE BINDING SITES*

Species Tissue	Ratio of Bmax values†
Rat Sublingual Gland	<0.02
Rat Cerebral Cortex	0.5
Guinea Pig Cerebellum	0.9
Rat Corpus Striatum	1.5
Cat Cerebellum	1.8
Human Cerebral Cortex	2.5
Human Platelet	2.5
Human Adipocyte	4.4
Rat Lung (Neonatal)	>65.0

*Data from [9].
†Bmax of [^{3}H]yohimbine/Bmax of [^{3}H]clonidine.

TABLE 2

COMPARISON OF [^{3}H]YOHIMBINE AND [^{3}H]CLONIDINE BINDING IN BRAIN REGIONS OF VARIOUS MAMMALS

Brain Region and Species	Bmax, fmol/mg [^{3}H]Yohimbine	Bmax, fmol/mg [^{3}H]Clonidine	Bmax Ratio	K_D, nM [^{3}H]Yohimbine	K_D, nM [^{3}H]Clonidine
Cerebral Cortex					
Rat	121 ± 6	235 ± 19	0.5	2.2 ± 0.3	1.8 ± 0.1
Guinea Pig	95 ± 9	170 ± 30	0.6	1.7 ± 0.3	0.7 ± 0.1
Cat	111	79	1.4	0.4	1.4
Human	201 ± 18	95 ± 12	2.1	0.46 ± 0.05	2.9 ± 0.5
Cerebellum					
Rat	37 ± 4	66 ± 7	0.6	1.1 ± 0.1	1.9 ± 0.2
Guinea Pig	23 ± 5	25 ± 3	0.9	0.8 ± 0.1	0.7 ± 0.1
Cat	258 ± 66	143 ± 49	1.8	0.6 ± 0.1	1.1 ± 0.2
Human	54 ± 5	29 ± 4	1.9	0.33 ± 0.03	2.9 ± 1.1
Hippocampus					
Rat	99 ± 13	156 ± 9	0.6	2.1 ± 0.3	2.3 ± 0.1
Guinea Pig	48	122	0.4	1.1	0.9
Corpus Striatum					
Rat	106 ± 6	72 ± 5	1.5	1.3 ± 0.1	1.6 ± 0.8
Guinea Pig	121 ± 27	88 ± 9	1.4	0.8 ± 0.1	1.2 ± 0.1
Human	71 ± 31	12 ± 6	5.9	1.4 ± 0.3	2.2 ± 0.4

TABLE 3

INHIBITION OF [^{3}H]YOHIMBINE AND [^{3}H]RAUWOLSCINE BINDING IN RAT BRAIN*

Drug	Cerebral Cortex		Hippocampus		Corpus Striatum	
	[^{3}H]Yoh†	[^{3}H]Rau	[^{3}H]Yoh	[^{3}H]Rau	[^{3}H]Yoh	[^{3}H]Rau
Yohimbine	5.4 ± 0.6	2.8 ± 0.6	3.3 ± 0.7	2.7 ± 0.4	1.8 ± 3.8	1.5 ± 0.4
Phentolamine	9.1 ± 2.2	6.1 ± 0.7	10.4 ± 3.4	8.5 ± 1.5	13.2 ± 1.7	10.8 ± 2.0
Prazosin	41 ± 6	37 ± 7	45 ± 7	49 ± 3	15 ± 2	12 ± 2
Norepinephrine	81 ± 15	—	138 ± 3		78 ± 11	
Epinephrine	15 ± 6	—	58 ± 12		22 ± 8	
Clonidine	8.1 ± 0.2	—	9.5 ± 0.5		14.4 ± 1.0	

*Values are K_i ± SEM in nM.
†Yoh, Yohimbine; Rau, Rauwolscine.

eral tissues and species [9]. Table 1 presents the results from some of these experiments as the Bmax value for [^{3}H]yohimbine divided by the Bmax value for [^{3}H]clonidine. Thus we are comparing [^{3}H]antagonist binding to [^{3}H]agonist binding. It is clear from this brief table that by choosing an appropriate tissue and species almost any ratio of these two binding sites can be obtained. This led us, in 1981, to suggest the possibility of alpha-2 adrenergic receptor subtypes [9].

Table 2 presents a comparison of [^{3}H]yohimbine and [^{3}H]clonidine binding in several brain regions of four different mammals. There is considerable variation in both the Bmax values for [^{3}H]yohimbine and [^{3}H]clonidine as well as in the ratio. The K_D values tend to be less variable. It is interesting to note that the Bmax ratio for the rodents (rat and guinea pig) is always lower than the ratio for the non-rodents (cat and human) in any given brain region.

If there are alpha-2 adrenergic receptor subtypes, then the relative proportions of those subtypes might vary among the brain regions in the rat. Furthermore, these putative receptor subtypes should have some differences in their pharmacological properties. We therefore compared the potencies of six drugs in three brain regions using both [^{3}H]antagonist and [^{3}H]agonist binding. Table 3 presents the data for the inhibition of [^{3}H]yohimbine and [^{3}H]rauwolscine by various drugs. None of the drugs seem to clearly differentiate between the brain regions with the possible exception of prazosin which was more potent against both [^{3}H]yohimbine and [^{3}H]rauwolscine binding in the corpus striatum as compared to the cerebral cortex and the hippocampus. Similarly as shown in Table 4 the three antagonists and the three agonists did not differ significantly in potency in inhibiting the binding of [^{3}H]p-aminoclonidine. From these studies which indicate similar pharmacology in the three brain regions, we concluded that alpha-2 adrenergic receptor subtypes, if indeed they exist, are present in roughly constant proportions in the various rat brain regions.

EVIDENCE FOR SPECIES DIFFERENCES IN ALPHA-2 ADRENERGIC RECEPTOR BINDING

Since the data in Tables 1 and 2 had indicated the possibility of species differences in the characteristics of alpha-2 adrenergic receptor binding, we decided to compare the properties of these binding sites in some detail. A careful evaluation of the data in the literature and from our laboratory, has uncovered three clear differences between rodent and non-rodent mammalian alpha-2 adrenergic receptors. First, the affinity of [^{3}H]yohimbine is five to ten times higher in non-rodent species as compared to rodents. Second, oxymetazoline is more potent in the non-rodent species. Third, prazosin is relatively more potent (as compared to yohimbine) at alpha-2 receptors in rodents as compared to non-rodents.

TABLE 4

INHIBITION OF [^{3}H]p-AMINOCLONIDINE BINDING IN RAT BRAIN*

Drug	Cortex	Hippocampus	Corpus Striatum
Yohimbine	37 ± 5	50 ± 4	23 ± 4
Phentolamine	2.2 ± 0.2	3.9 ± 1.5	3.1 ± 0.9
Prazosin	863 ± 123	606 ± 120	539 ± 34
Norepinephrine	3.5 ± 0.5	4.0 ± 0.1	3.3 ± 0.9
Epinephrine	1.4 ± 0.1	2.0 ± 0.3	1.5 ± 0.3
Clonidine	1.4 ± 0.3	1.2 ± 0.3	0.8 ± 0.1

*Values are K_i ± SEM in nM.

[^{3}H]Yohimbine is More Potent in Non-Rodent Species

Data from a large number of studies are summarized in Table 5. For the rodent species, including the NG108 mouse rat hybrid cell line, the K_D for [^{3}H]yohimbine binding to alpha-2 adrenergic receptors tends to be in the range of 5 to 10 nM. By contrast, the K_D for [^{3}H]yohimbine binding in non-rodent species such as the human, dog and pig as well as in the human adenocarcinoma cell line HT 29, is 2 nM or lower. We made the observation several years ago that use of a glycylglycine buffer increases the affinity of [^{3}H]yohimbine about three fold, and thus have used this buffer in all of our studies in preference to Tris, which is used by most other investigators. Thus these comparisons among species should be made in assays using the same buffer. For example, as noted in Table 5, we found a K_D value of 2 nM for rat cerebral cortex and 0.3 nM for human platelet, using

TABLE 5

COMPARISON OF [^{3}H]ANTAGONIST AFFINITY IN VARIOUS SPECIES

Tissue	K_D, nM	B_{max} fmol/mg prot	[^{3}H]Ligand*	Buffer*	Reference
Rodent					
Rat Cerebral Cortex	4	130	Rau	Tris	[36]
Rat Cerebral Cortex	2	108	Rau	Tris	[15]
Rat Cerebral Cortex	5	125	Yoh	Tris	[15]
Rat Cerebral Cortex	10	254	Yoh	Tris	[45]
Rat Cerebral Cortex	2	121	Yoh	Glygly	Table 2
Rat Intestine	6	37	Yoh	Tris	[41]
NG108 Neuroblastoma	9	258	Yoh	Tris	[28]
Rat Lung (neonatal)	2	302	Yoh	Glygly	[33]
Rat Submandibular Gland	6	205	Yoh	Glygly	[20]
Rat Renal Cortex	10	120	Yoh	Tris	[55]
Rat Renal Cortex	20	239	Yoh	Tris	[47]
Guinea Pig Cerebral Cortex	2	95	Yoh	Glygly	Table 2
Non-Rodent					
Human Platelet	0.5	144	Rau	Tris	[15]
Human Platelet	0.5	305	Rau	Glygly	[27]
Human Platelet	1.2	182	Yoh	Tris	[16]
Human Platelet	0.3	300	Yoh	Glygly	[27]
Human Adipocyte	0.4	585	Yoh	Glygly	[8]
Human Cerebral Cortex	2.1	135	Rau	Tris	[49]
Human Cerebral Cortex	0.5	201	Yoh	Glygly	Table 2
Dog Artery	1.1	50	Yoh	$NaKPO_4$	[5]
Dog Trachea	2.0	50	Yoh	Tris	[2]
HT 29 Cells	0.5	300	Yoh	Glygly	‡
Porcine Cerebral Cortex	0.3	170	Yoh	Glygly	[22]
Porcine Lung	0.2	129	Yoh	Glygly	[20]
Porcine Submandibular Gland	0.4	244	Yoh	Glygly	[20]
Bovine Cerebral Cortex	2	160	Rau	Tris	[42]

*Rau, Rauwolscine; Yoh, Yohimbine.

†The use of a TRIS or $NaKPO_4$ buffer instead of a glycylglycine (Glygly) buffer increases the K_D about 3-fold [27].

‡Turner, Ray-Prenger and Bylund, unpublished.

glycylglycine buffer, while for Tris buffer the affinity for the rat cerebral cortex is in the range of 5 to 10 nM and that for the platelet about 1 nM. The higher affinity of [^{3}H]yohimbine binding in the non-rodent species coupled with the previously mentioned generally higher Bmax value (as compared to [^{3}H]clonidine) result in significantly "better" [^{3}H]yohimbine binding in the non-rodent species.

Oxymetazoline is More Potent in Non-Rodent Species

The second pharmacological difference between rodent and non-rodent species is that oxymetazoline tends to be more potent in non-rodent species that it is in rodent species. Table 6 is a summary of K_i values for oxymetazoline in inhibiting [^{3}H]antagonist binding in both rodent and non-rodent species. As can be seen, in the rodent species the K_i values range from about 3 to 1,000, while in the non-rodent species the K_i value tends to be around 1 nM. As we have previously noted [13] the difference between the rodent and the non-rodent becomes even more dramatic when the ratio of the K_i values of prazosin to oxymetazoline are calculated. With the exception of the submandibular gland from the three-week old rat, this ratio is 10 or less for the rodent species while in the non-rodent species it is between 200 and 500.

Prazosin is Relatively More Potent in Rodent as Compared to Non-Rodent Species

The third difference which we have noted between rodent and non-rodent alpha-2 adrenergic receptors is that prazosin (when compared to yohimbine) is relatively more potent in the rodent species. This difference was actually noted very early in the studies using [^{3}H]yohimbine. For example, in a 1980 paper in which [^{3}H]yohimbine was used as a radioligand in rat kidney and brain, the authors' refrained from suggesting that yohimbine was binding to alpha-2 adrenergic receptors, presumably due to the high affinity of prazosin in inhibiting [^{3}H]yohimbine binding [56]. In a previous review [14] we initially used the affinity ratio of yohimbine to prazosin to differentiate between alpha-1 and alpha-2 receptor subtypes. However it was obvious from those data (see Table 3 of [14]) that this ratio also differentiated the rodent from non-rodent alpha-2 adrenergic receptors. Table

TABLE 6

COMPARISON OF THE AFFINITY OF OXYMETAZOLINE AND PRAZOSIN IN INHIBITING ^{3}H-ANTAGONIST BINDING IN RODENTS AND NON-RODENTS

Tissue	K_i, nM Oxymetazoline	Prazosin	Ratio	Reference
Rodent				
Rat Cerebral Cortex	15	138	9	[45]
Rat Cerebral Cortex	8	55	7	Table 8
Rat Submandibular Gland	3	169	56	[20]
Rat Lung (neonatal)	80	5	0.06	[33]
Rat Intestine	1000	900	0.9	[41]
NG108	270	42	0.16	[28]
Non-Rodent				
Bovine Cerebral CTX	2	1000	500	[42]
Human Platelet	0.7	240	350	[27]
Porcine Submandibular Gland	1	220	220	[20]
Porcine Lung	0.8	175	220	[20]
HT 29 Cell Line	1.1	320	290	*

*Turner, Ray-Prenger and Bylund, unpublished.

TABLE 7

COMPARISON OF RELATIVE AFFINITIES OF YOHIMBINE AND PRAZOSIN IN VARIOUS SPECIES

Tissue	K_i-Prazosin/K_i-Yohimbine [^{3}H]Antagonist Yoh	Rau	[^{3}H]Angonist Clonidine	PAC	Reference
Rodent					
Rat Cerebral Cortex	15	16	8, 30		[15, 26, 45, 51]
Rat Cerebral Cortex	8	13	23		Tables 3 and 4
Rat Submandibular Gland	13			53, 23	[18,20]
Rat Lung (Neonatal)	5				[33]
Rat Renal Cortex	14, 7		5		[37, 47, 55]
NG108 Neuroblastoma	5			58	[28]
Guinea Pig Kidney	27		6		[6,25]
Non-Rodent					
Human Adipocyte	42		4400		[3,50]
Human Platelet	450, 240	900	620		[15, 16, 27, 46]
Human Cerebral Cortex		260			[49]
Bovine Cerebral Cortex		200			[42]
Dog Trachea	470				[2]
Dog Artery	330				[5]
Porcine Submandibular Gland	500			2200	[20]
Porcine Lung	800			4500	[20]
Human Adenocarcinoma HT 29	640			1600	†

*Yoh, Yohimbine; Rau, Rauwolscine; PAC, p-aminoclonidine.
†Turner, Ray-Prenger and Bylund, unpublished.

7 presents a more extensive compilation of these data. For both [^{3}H]antagonist binding ([^{3}H]rauwolscine or [^{3}H]yohimbine) and [^{3}H]agonist binding ([^{3}H]clonidine or [^{3}H]p-aminoclonidine) the ratio of the K_i for prazosin to the K_i for yohimbine is markedly lower in the rodents as compared to the non-rodents. Against [^{3}H]antagonist binding for example, the values for rat cerebral cortex vary between 14 and 16 while for the human platelet they vary between 240 to more than 3,000. Against [^{3}H]agonist binding the ratios appear to be somewhat higher but the difference between the rodent and non-rodent is still about 100 fold.

In order to see if other adrenergic drugs might similarly differentiate between species, we compared the potencies of various drugs in inhibiting [^{3}H]yohimbine binding to human platelet and rat cerebral cortex (Table 8). The drugs in this table are arranged so that the drugs having the relatively higher potency in the human platelet are at the top while the drugs having relatively higher potency in the rat cortex are at the bottom. The calculated ratio of the K_i values is also given. This ratio as calculated from a similar study in another laboratory is also given [15]. For the 16 drugs there is a wide variation in the relative potencies. However, none appear to be significantly better than oxymetazoline and prazosin in differentiating between the two tissues.

FUNCTIONAL STUDIES

In the strictest sense of the word the term receptor implies a functional connection. Thus, before a binding site can be rigorously termed a receptor it must have a demonstrable function. We therefore asked the question if the pharmacological differences which we have observed in binding studies could also be observed in functional studies. In our laboratory we chose to look at this question by investigating the relative potencies of yohimbine and prazosin in potentiating norepinephrine release from rat submandibular gland slices. In this system endogenously released norepinephrine appears to inhibit norepinephrine release in a negative feedback loop mediated by alpha-2 adrenergic receptors. By blocking the alpha-2 adrenergic receptors this inhibition can be reversed and the release of norepinephrine increased. We found that yohimbine increased norepinephrine release by 52% with an ED_{50} of 140 nM. Prazosin had the same efficacy as yohimbine but was five fold less potent with an ED_{50} of 770 nM. Since we were unable to demonstrate an additive effect when the maximally affective concentrations of yohimbine and prazosin were used together, we concluded that both drugs were most likely acting through the alpha-2 receptor [52]. This potency difference is very similar to that

TABLE 8

INHIBITION OF [^{3}H]YOHIMBINE BINDING BY VARIOUS DRUGS IN RAT CORTEX AND HUMAN PLATELET

Drug	K_i, nM Human Platelet	K_i, nM Rat Cortex	Ratio Cortex/ Platelet	Ratio*
Oxymetazoline	0.94 ± 0.14	8.3 ± 1.0	8.8	—
Yohimbine	0.60 ± 0.20	3.9 ± 0.6	6.5	11
WB 4101	0.80 ± 0.10	4.3 ± 0.8	5.4	14
B-HT 958	18 ± 2	87 ± 20	4.8	—
UK 14,304	3.9 ± 0.6	15.5 ± 3.9	4.0	—
Guanabenz	7.8 ± 1.6	16.7 ± 3.5	2.1	—
Rauwolscine	0.28 ± 0.08	0.52 ± 0.10	1.9	6.0
ST 666	31 ± 4	60 ± 15	1.9	—
B-HT 933	202 ± 21	292 ± 72	1.4	—
Phentolamine	7.2 ± 1.0	9.1 ± 2.2	1.3	2.0
RX781094	0.67 ± 0.01	0.88 ± 0.35	1.3	—
Corynanthine	91 ± 11	108 ± 32	1.2	3.1
Norepinephrine	209 ± 34	81 ± 15	0.38	1.0
Clonidine	22 ± 9	8.1 ± 0.2	0.37	2.4
Prazosin	217 ± 53	55 ± 15	0.25	0.25
Epinephrine	95 ± 21	15 ± 6	0.16	2.1

*Values from [15].

TABLE 9

COMPARISON OF RELATIVE POTENCIES OF ANTAGONISTS OF ALPHA-2 RECEPTORS IN RODENTS AND NON-RODENTS

Tissue	Response	Prazosin IC_{50}/ Yohimbine IC_{50}	Reference
Rat Submandibular Gland	Norepinephrine Release	5	52
Rat Vas Deferens	Contraction	57	53
Hamster Adipocyte	cAMP Accumulation	25	21
Human Platelet	Adenylate Cyclase	~100	24
Human Platelet	Aggregation	>300	32
Human Adipocyte	Glycerol Release	>1000	7
Human Adipocyte	cAMP Accumulation	>1000	7

observed in rodent tissues with binding studies. Table 9 presents a summary of some studies in other laboratories also showing that prazosin is relatively more potent in rodent tissues as compared to non-rodent tissues. However, there are relatively few data available and much additional work needs to be done.

The second line of evidence suggesting pharmacological differences between rodent and non-rodent receptors is that oxymetazoline appears to be an agonist in rodent tissues but an antagonist in non-rodent tissues. In the human platelet oxymetazoline has an intrinsic activity of 0.09 (the intrinsic activity of a full agonist is 1.0 and that of an antagonist is 0) in inhibiting PGE_1 stimulated adenylate cyclase activity [24]. By contrast, oxymetazoline appears to be a full agonist in inhibiting intestinal secretions in the rat jejunum [41]. Additional studies are needed to determine if this is a general phenomenon. However the available data do illustrate pharmacological differences in alpha-2 adrenergic receptors using functional studies.

ALTERNATE HYPOTHESES

There are several alternate hypotheses which can also explain the different affinities of oxymetazoline and prazosin in rodent vs. non-rodent species. These include differential proteolysis of the receptors, degradation of the ligands and differences in membrane structure. We have conducted experiments using the rat cerebral cortex and human platelet as test systems to examine these hypotheses. The results of these experiments indicate that these hypotheses are false, thus supporting the original hypothesis that the differences represent actual pharmacological differences in the alpha-2 receptors. In order to test the first alternate hypothesis, supernatants from tissue homogenates from one species were incubated with membranes from the other species and subsequently [^{3}H]yohimbine inhibition experiments were conducted with the antagonist prazosin [29]. This manipulation did not alter the observed differences and thus soluble proteases can not account for those differences. Furthermore, inclusion of various proteolytic inhibitors did not alter the potency of the drugs in inhibiting binding in either tissue.

The second alternate hypothesis was eliminated by ligand rebinding experiments. In these experiments, incubation tubes were prepared in the normal manner to observe prazosin inhibition of [^{3}H]yohimbine binding, except that the incubation mixtures were centrifuged rather than filtered. The supernatant then was transferred to fresh membranes from the opposite species and the inhibition experiment conducted in the normal manner. In these experiments the potency of prazosin in inhibiting the binding was dependent upon the source of membranes in the second incubation, rather than the membrane source of the first incubation [29]. Thus metabolism of the ligands does not account for the observed pharmacologic differences. To test the third hypothesis, we solubilized the alpha-2 receptor from both the human platelet and rat cortex, and have shown that the pharmacological differences are retained [29].

PUTATIVE ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES

While receptor heterogeneity between species is important, the term subtype is usually reserved for heterogeneity which can be demonstrated within the same species. It has been previously suggested that the rat brain contains two subtypes of alpha-2 adrenergic receptors [44]. This conclusion was based on the observation that (−)-mianserin was an antagonist at the alpha-2 receptor which mediates the regulation of serotonin release but was without effect on the

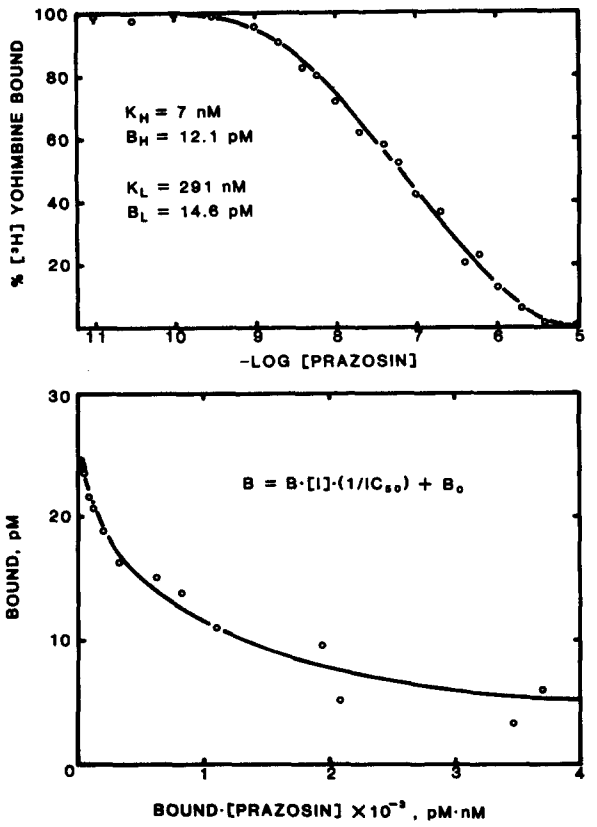

FIG. 1. Inhibition of [³H]yohimbine binding by prazosin in membranes from rat cerebral cortex. In the upper panel the data are plotted as percent of specific [³H]yohimbine bound as a function of the prazosin concentration on a logarithmic scale. Using non-linear curve fitting techniques a two site model gave a significantly better fit than a one site model. The parameters for the two site fit are given in the figure. In the lower panel, the dates are plotted as $B=B\cdot I(1/IC_{50}) + B_0$ where B is the concentration of [³H]yohimbine specifically bound, I is the concentration of prazosin and B_0 is the binding in the absence of prazosin. This method of plotting the data has significant advantages over the usual "Hofstee" plot of $P=(P/I)\cdot IC_{50} + 1$ where P is percent inhibition (Bylund, in preparation).

alpha-2 receptor mediating inhibition of norepinephrine release. By contrast (+)-mianserin was an effective antagonist at both receptor subtypes.

We observed that the pseudo Hill coefficient (n_H) for the inhibition of [³H]yohimbine binding by prazosin in the rat brain was low (0.61) and that this low value contrasted sharply with the other antagonists, phentolamine and yohimbine, which had Hill slopes not significantly different from 1. We chose to do more detailed inhibition curves using 22 concentrations of prazosin, and an example of such an experiment is given in Fig. 1. When these data are plotted as the amount of radioligand bound versus the bound radioligand times the concentration of prazosin, a curvilinear plot results which is consistent with the possibility of two non-interacting sites. Using standard computer techniques we were able to resolve the inhibition data into a high affinity site and a low affinity site. From the data in Fig. 1, 45% of the receptors had a 40 fold higher affinity for prazosin compared to the other 55% of the sites. Similar data were also obtained for the rat corpus striatum and these data are summarized in

TABLE 10

INHIBITION OF [³H]YOHIMBINE BINDING BY PRAZOSIN DEFINES PUTATIVE ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES*

Tissue	Subtype B		Subtype A	
	K_i, nM	% Sites	K_i, nM	% Sites
Rat Lung (neonatal)	5	100	—	0
Rat Cerebral Cortex	7	43	273	57
Rat Corpus Striatum	5	6	210	54
Rat Submandibular Gland	7	20	220	80
Human Platelet	—	0	240	100

*For each subtype, the K_i for the prazosin inhibition of [³H]yohimbine binding is given along with the percentage of sites which have that affinity.

TABLE 11

SELECTIVITY OF SUBTYPE SELECTIVE DRUGS FOR THE BETA-1 AND BETA-2 ADRENERGIC RECEPTORS*

Drug	K_i (nM) in Tissues Containing only one subtype		Fold Selectivity
	Beta-1	Beta-2	
Beta-1 Selective			
Practolol	2.4	58	24
Metoprolol	0.2	1.8	9
Atenolol	1.1	20	18
Beta-2 Selective			
Zinterol	1.0	0.04	25
Salmefamol	4.4	0.49	9
OPC 2009	5.5	0.21	26
IPS 339	0.014	0.0005	28
mean	—	—	20

*Data from [38].

Table 10. In the neonatal rat lung, prazosin has a high affinity in inhibiting [³H]yohimbine binding but has a Hill coefficient which is approximately 1.0 [33]. The human platelet also has a pseudo Hill coefficient which is near 1.0, but prazosin has a low affinity for this receptor. Thus the neonatal rat lung may have only the high affinity receptor, while the platelet may only have the low affinity subtype of receptor. The rat submandibular gland appears to contain mostly (85%) the receptor subtype with a low affinity for prazosin.

We have considered the possibility that the low Hill slope for the prazosin inhibition of yohimbine binding in the rat cortex was due to negative coopertivity. However experiments of [³H]yohimbine dissociation with and without prazosin did not support this hypothesis (Kawahara and Bylund, unpublished).

The data summarized in Table 10 provide the basis for our

current working hypothesis which suggests the existence of two subtypes of alpha-2 adrenergic receptors which we are tentatively calling type A and type B. The human platelet is the prototype tissue for the alpha-2A receptor. In non-rodent tissues this subtype appears to be the predominant one. The prototype tissue for alpha-2B receptors is the neonatal rat lung. The rat brain (both cerebral cortex and the corpus striatum) appear to contain approximately equal amounts of alpha-2A and alpha-2B subtypes. This hypothesis can explain the variation in the ratio of the affinities of prazosin and oxymetazoline noted in Table 6. The rat lung has a very low ratio indicative of the alpha-2B subtype. On the other hand the non-rodent tissues have high ratios indicative of the alpha-2A subtype. The rat cerebral cortex, which contains both subtypes, has an intermediate ratio. The rat submandibular gland, which is predominately, but not entirely, of the alpha-2A subtype, has a ratio higher than the rat cortex but lower than the non-rodent tissues.

It needs to be emphasized that these affinity differences are really quite large. Table 11 compares the K_i values for beta-1 and beta-2 selective drugs in tissues which contain only one of the two subtypes. The fold selectivity of that drug differentiating between the beta-1 and beta-2 receptor subtypes is also given. Thus for the beta system the drugs are only about 20 fold selective for the two subtypes. For the putative alpha-2 adrenergic receptor subtypes the differences are much larger. Assuming that the neonatal rat lung and the human platelet represent tissues which contain only one of the subtypes, the data in Table 6 indicate that the selectivity for prazosin is 48 fold (240/5) while for oxymetazoline the selectivity ratio is 110 (80/0.7). Thus the pharmacological differences in the putative alpha-2 adrenergic receptor subtypes are at least as great as those for the beta-1 and beta-2 receptor subtypes.

CONCLUSION

This paper has presented evidence suggesting that there is heterogeneity in alpha-2 adrenergic receptors. This heterogeneity is clearly demonstrated between rodent and non-rodent species and we have presented some preliminary results which suggest that it is useful to define alpha-2 adrenergic receptor subtypes.

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