0026-895X/91/030407-06$03.00/0

MOLECULAR PHARMACOLOGY, 40:407-412

Pharmacological Characterization of Rat α_2-Adrenergic Receptors

JEFFREY K. HARRISON, DREW D. D'ANGELO, DEWAN ZENG, and KEVIN R. LYNCH

Departments of Pharmacology (J.K.H., D.D.D., K.R.L.) and Biochemistry (D.Z., K.R.L.), University of Virginia School of Medicine, Charlottesville, Virginia 22908

Received March 1, 1991; Accepted June 3, 1991

SUMMARY

We described previously the molecular characterization of a rat α_{2B}-adrenergic receptor and have shown also that the rat genome contains three closely related α_2-adrenergic receptor genes. To characterize the ligand-binding properties of these receptor gene products, we expressed the DNAs encoding these receptors individually in COS-1 cells and studied their binding to a wide variety of typical and atypical adrenergic ligands. The receptors displayed high affinity binding to the radioligand [^{3}H]rauwolscine, with equilibrium dissociation constants ranging from 1.4 to 28 nM. Kinetic analysis of the binding of [^{3}H]rauwolscine to membranes from transfected cells was in very good agreement with data obtained from saturation analysis. We examined the ability of a number of agents to compete for the binding of [^{3}H]rauwolscine to the α_2-adrenergic receptor-transfected membranes. Whereas one of these receptors displayed a pharmacological profile typical of an α_{2A}-adrenergic receptor, the other two receptors showed similar pharmacological properties characteristic of an α_{2B}-adrenergic receptor. The two α_{2B}-like adrenergic receptors differed, however, in the ratios of K_i values for oxymetazoline and prazosin, as well as the K_i ratio of prazosin and yohimbine. In addition, the two α_{2B}-like adrenergic receptors had a 9-fold difference in affinity for chlorpromazine. The pharmacological characterization of the three rat α_2-adrenergic receptor gene products is consistent with the known pharmacology of α_2-adrenergic receptors, as documented using tissues and cell lines.

The existence of subtypes of α_2-ARs was first suggested by Bylund (1). These pharmacologically defined receptors display a unique rank order of potency for a variety of adrenergic drugs. Whereas all α_2-ARs display a high affinity for rauwolscine and its congener yohimbine, the subtypes of α_2-AR display widely differing relative affinities for the drugs prazosin and oxymetazoline. The α_2-AR found on human platelets, designated α_{2A}, has a high affinity for oxymetazoline and a low affinity for prazosin. The α_{2B} subtype, originally described in rat brain (2) and neonatal rat lung (3), displays the reverse order of potency, i.e., high affinity for prazosin and low affinity for oxymetazoline. A third subtype of α_2-AR was suggested from the work of Murphy and Bylund (4). They characterized a receptor (designated α_{2C}) on the opossum OK cell line that had similar, yet distinct, pharmacological features, compared with the α_{2B} receptor of neonatal rat lung. Information from both structural and functional studies also suggests that α_2-ARs are heterogeneous structures. For example, the α_{2B}-AR from neonatal rat lung is nonglycosylated (5).

This work was supported by a grant from the National Institutes of Health (PO1 HL19242). J.K.H. is the recipient of a National Research Service Award postdoctoral fellowship (F32 HL08223). K.R.L. is an Established Investigator of the American Heart Association, with partial funding provided by their Virginia affiliate.

The emergence of molecular cloning techniques in the study of receptor structure and function has allowed the unequivocal demonstration of multiple α_2-AR subtypes. To date, three closely related α_2-AR genes have been described in both human (6–9) and rat (10–14). Generic probes generated from a single α_2-AR gene hybridize to three distinct restriction enzyme-digested DNA fragments from either rat (15) or human (6), under conditions of reduced stringency. Each of these bands detected in Southern blot analysis can be accounted for by the three individual α_2-AR genes. In addition to the pharmacological differences in the α_2-AR proteins, the α_2-AR genes are expressed differentially in rat tissues (15, 16). Using subtype-specific hybridization probes in Northern analysis, our laboratory showed that two of the three α_2-AR genes are distributed widely and unevenly in the central nervous system, whereas very little, if any, of the third subtype is expressed there (15).

In this report, we describe the expression and pharmacological characterization of the three α_2-ARs found in the rat. A variety of typical and atypical adrenergic drugs were examined in competition for the binding of [^{3}H]rauwolscine to membranes prepared from COS cells transfected individually with the α_2-AR genes. Each of the α_2-ARs displays unique binding characteristics that account for their previously described pharmacology, as ascertained using animal tissues and cell lines.

ABBREVIATIONS: α_2-AR, α_2-adrenergic receptor; 8-OH-DPAT, 8-hydroxy-2-di-*N*-propylamino-tetralin.

Materials and Methods

Drugs. Sources of drugs were as follows: yohimbine, idazoxan, clonidine, prazosin, oxymetazoline, (−)-norepinephrine bitartrate, and corynanthine (Sigma Chemical Co., St. Louis, MO); atipamezole and dexmedetomidine (Farmos, Turku, Finland); SKF104,078 and chlorpromazine (SK&F Laboratories, Philadelphia, PA); BHT920 (Boehringer Ingelheim, Ridgefield, CT); phentolamine (CIBA-Geigy, Summit, NJ); 8-OH-DPAT (Research Biochemicals Inc., Natick, MA); and UK14,304 (bromoxidine) (Pfizer, Sandwich, Kent). [^{3}H]Rauwolscine (76.7 and 75.2 Ci/mmol) was from Dupont/New England Nuclear.

Expression of cloned α_2-AR genes and cDNAs. Previously we cloned and characterized a rat α_{2B}-AR cDNA (RNG) from rat kidney. The *Eco*RI fragment of this cDNA was filled in and blunt-end ligated into the filled-in *Hind*III site of the expression vector pRLDN-10B (gift from Dr. Mitch Reff, SK&F Laboratories). This vector contains an Rous Sarcoma Virus promoter, bovine growth hormone polyadenylation sequences, and an SV40 origin of replication. The genes for the rat analogs of the human α_2-AR genes (C-4 and C-10) were kindly provided by Dr. Steve Lanier (Massachusetts General Hospital, Boston, MA). These clones are designated as RG-10 and RG-20, respectively. The *Nco*I-*Xba*I fragment of RG-10 and the *Nco*I-*Sac*I fragment of RG-20 were subcloned also into the expression vector pRLDN-10B. This fragment of RG-20 was used as a hybridization probe in the screening of a rat genomic library (*Hae*III partial, λ charon4A; kindly provided by Dr. Tom Sargent, National Institutes of Health). The filled-in/exonuclease-treated *Nco*I/*Sac*I fragment of this independently derived clone was ligated into the filled-in *Hind*III site of pRLDN-10B. The three α_2-AR genes or cDNAs used in this study constitute the three α_2-AR genes of the rat. Each rat α_2-AR subtype shares 82–90% amino acid similarity with the corresponding human α_2-AR subtype.

COS-1 cells were transfected as described previously (10). After approximately 48 hr, membranes were prepared by washing the cell culture plates three times with ice-cold phosphate-buffered saline and then scraping the cells into ice-cold phosphate-buffered saline. The cell suspension was centrifuged at 7500 × *g* for 20 min. The pellets were homogenized in 20 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA (TME), and then centrifuged at 100,000 × *g* for 30 min. The final pellet was resuspended in TME at a protein concentration of 1–2 mg/ml, and the membrane suspension was frozen as aliquots in liquid nitrogen and stored at −70° until use. Protein concentrations were determined by the method of Bradford (17).

Binding studies. Saturation analysis with [^{3}H]rauwolscine was performed as previously described (10). Briefly, reactions contained 0.1–90 nM [^{3}H]rauwolscine and 10–20 μg of membrane protein in TME, in a final volume of 0.2 ml. Reactions were incubated for 60 min at 22°. At the end of this time, reactions were diluted with 4 ml of ice-cold 25 mM Tris·HCl, pH 7.5 (wash buffer), and filtered through Whatman GF/C filters. Filters were washed subsequently with 2 × 4 ml of ice-cold wash buffer. Filters were counted by liquid scintillation counting (Beckman Ready Safe, Beckman LS 3801 scintillation counter, 55% counting efficiency). Nonspecific binding was determined in the presence of either 10 μM yohimbine, 10 μM phentolamine, or 100 μM (−)-norepinephrine.

Experiments designed to ascertain the association and dissociation rate constants were conducted as follows. Reactions (4-ml final volume) were initiated by addition of membrane suspensions. At time points between 30 sec and 90 min, aliquots were withdrawn and binding was analyzed as described above. The nonspecific binding component was determined in the presence of 10 μM phentolamine. For dissociation experiments, reactions were allowed to reach steady state (90 min), at which time a small volume of phentolamine (10 μM final concentration) was added. At time points of 20 sec to 60 min, aliquots were withdrawn, and specific binding was determined as described above.

For competition experiments, 0.1 nM to 100 μM final concentrations of the indicated drugs were incubated with 0.9–2 nM [^{3}H]rauwolscine (RNG- and RG-10-transfected membranes) or 18–28 nM [^{3}H]rauwolscine (RG-20-transfected membranes), with 10–20 μg of membrane protein in TME, in a final volume of 0.2 ml. Binding reactions were processed and nonspecific binding was determined as described above.

Data were analyzed using the nonlinear regression analysis programs offered by GraphPAD INPLOT version 3.0 (GraphPAD Programs, San Diego, CA). IC_{50} values were converted to K_i values by the Cheng-Prussoff (18) equation: $K_i = IC_{50}\ [K_d/(L + K_d)]$, where L is the concentration of the radioligand and K_d is the equilibrium dissociation constant of the radioligand.

Results

We have described the molecular cloning of a rat α_{2B}-AR (RNGα_2) and recently obtained from S. M. Lanier two rat genomic clones (RG10α_2 and RG20α_2) encoding additional rat α_2-ARs. A comparison of the encoded amino acid sequences with those of the human α_2-AR clones reveals that the rat analog of C-10 is RG20α_2 (89% identical), the rat analog of C-4 is RG10α_2 (90%), and the rat analog of C-2 is RNGα_2 (82%). To characterize pharmacologically the proteins encoded by these DNAs, we have expressed them transiently in COS-1 cells. Fig. 1 shows the binding isotherms (steady state; see below) of [^{3}H]rauwolscine bound to membranes prepared from each of the α_2-AR DNA-transfected cells. Binding to the membranes was of high affinity, saturable, and specific and was modeled best to a single noninteracting population of sites. Equilibrium dissociation constants derived from analysis of several experiments revealed the following K_d values: RG-20α_2, 28.3 ± 2.1 (five experiments); RNGα_2, 2.4 ± 0.2 (four experiments); and RG-10α_2, 1.4 ± 0.1 (four experiments). Kinetic experiments were also conducted, to determine the binding constants of the three expressed α_2-ARs for [^{3}H]rauwolscine. Binding of [^{3}H]rauwolscine at 22° was rapid, reached steady state by 5 min (RG20), 15 min (RG10), or 30 min (RNG), and remained stable for at least 90 min (data not shown). Linear transformation of bound ligand at the early time points of association, as well as the binding during the dissociation phase of the experiment, is shown in Fig. 2. Assuming pseudo-first-order association and first-order dissociation kinetics, the linearity of the transformation reveals that the ligand is binding to a single site. The results of the calculations of forward and reverse rate constants and the subsequent calculations of the equilibrium dissociation constants are shown in Table 1. First, the kinetically derived equilibrium dissociation constants agree well with those derived from saturation analysis. Second, the relatively low affinity of the RG20α_2-AR for rauwolscine appears to be due to the rapid dissociation of this ligand from the receptor. We were concerned initially that this relatively high K_d for rauwolscine was a potential cloning artifact of the RG20α_2-AR gene, particularly in light of a report (11) on the cloning of a cDNA from brain and subsequent characterization of this gene as encoding an α_2-AR having a high affinity for yohimbine (K_d = 2.1 nM). Therefore, we isolated independently an additional genomic clone encoding the RG20α_2-AR. Comparison of the binding of rauwolscine at this receptor with the binding at the RG20α_2-AR revealed that the K_d values for rauwolscine at these two receptors were indistinguishable. Likewise, Lanier and colleagues have isolated additional cDNAs and also have found these expressed receptors to be indistinguishable from the original RG20α_2-AR.[1]

To characterize further the pharmacological profile of each expressed receptor, we examined the potency of a variety of

[1] S. M. Lanier, personal communication.

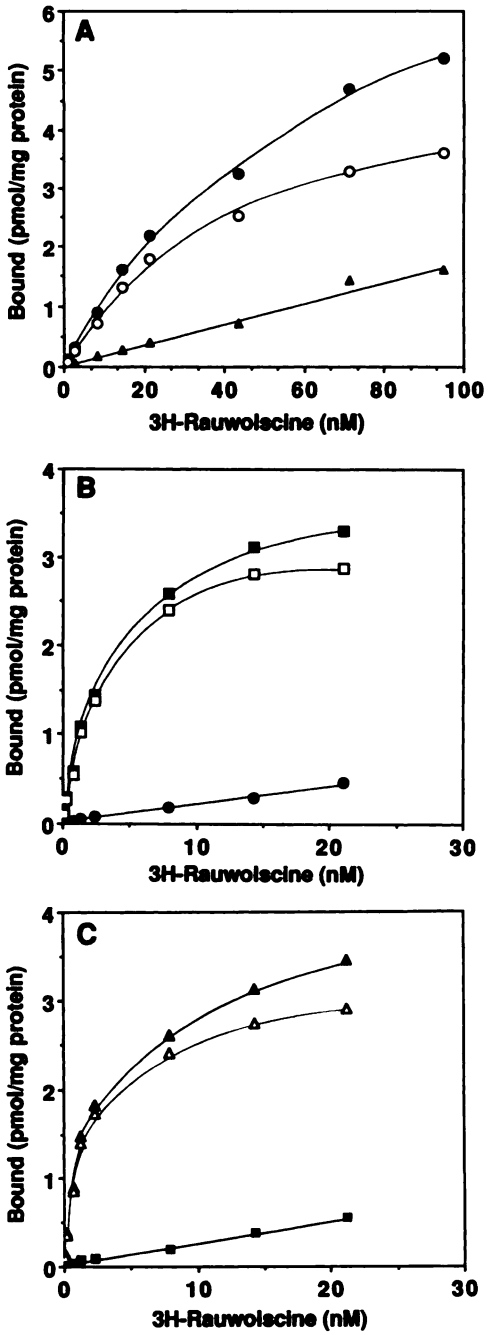

Fig. 1. Saturation binding of [^{3}H]rauwolscine. Equilibrium binding of [^{3}H] rauwolscine to COS-1 cell membranes transfected with the RG-20α_2-AR gene (A), RNGα_2-AR cDNA (B), and RG-10α_2-AR gene (C). Shown are representative experiments conducted as described in Materials and Methods. The total (A, ●; B, ■; C, ▲), specific (A, ○; B, □; C, △), and nonspecific (A, ▲; B, ●; C, ■) binding is indicated.

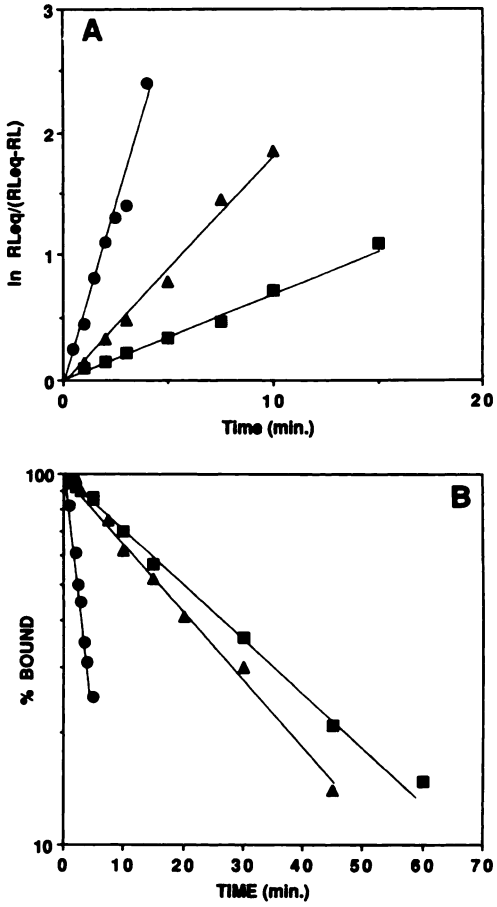

Fig. 2. Association and dissociation of [^{3}H]rauwolscine. Linear transformation of binding of [^{3}H]rauwolscine to RG-20- (●), RNG- (■), and RG-10- (▲) transfected COS cell membranes during the association (A) and dissociation (B) phases of the experiment. Results are representative of two experiments conducted as described in Materials and Methods. RL = number of receptor-ligand complexes, RLeq = number of receptor-ligand complexes at steady state.

TABLE 1

Summary of kinetic and saturation analyses

Binding of [^{3}H]rauwolscine to membranes of COS-1 cells transfected with the three rat α_2-ARs was measured. Kinetic and steady state experiments were conducted as described in Materials and Methods. Results for the equilibrium dissociation constants derived from saturation analysis are mean ± standard error.

	RG-20	RNG	RG-10
Kinetics			
k_{on} (M^{-1} min^{-1})	0.9×10^7	1.4×10^7	5.5×10^7
k_{off} (min^{-1})	0.28	0.034	0.041
K_d (nM)	31	2.3	0.8
n	2	2	2
Saturation			
K_d (nM)	28.3 ± 2.1	2.4 ± 0.2	1.4 ± 0.1
n	5	4	4

n = number of experiments.

typical and atypical adrenergic drugs in inhibiting the binding of [^{3}H]rauwolscine to the transfected cell membranes. Table 2 presents the inhibition constants (K_i values) for these drugs at each of the three expressed receptors. The affinity constants of these drugs for the receptor proteins spanned approximately 3 orders of magnitude. The slope factor (n_H) values for these ligands were near unity (0.7–1.0). As predicted from earlier studies with tissue or cell line homogenates, oxymetazoline had a relatively high affinity, and prazosin a relatively low affinity, for the RG20 receptor. In contrast, prazosin had a relatively high and oxymetazoline a relatively low affinity for the other two α_{2B}-AR-like receptors (RNGα_2-AR and RG10α_2-AR). Also consistent with previous data are the relative affinities of the three receptors for chlorpromazine. The RNGα_2- and RG10α_2-expressed receptors had a relatively high affinity for chlorpromazine, whereas the RG20α_2 protein had a low affinity for this drug. The RG20α_2-AR also displayed a relatively low affinity for yohimbine, a diastereomer of rauwolscine.

The K_i values of the 15 drugs, as well as the K_ds values for

TABLE 2

Drug affinities of the three rat α_2-ARs

Potencies of a variety of agents in competition for the binding of [^{3}H]rauwolscine to membranes of COS-1 cells transfected with the three rat α_2-ARs were measured. Results are mean ± standard error of three experiments conducted as described in Materials and Methods.

Drug	K_i		
	RG20	RNG	RG10
	nM		
Atipamezole	1.1 ± 0.3	1.0 ± 0.4	0.9 ± 0.2
Phentolamine	3.2 ± 1.0	12.4 ± 2.0	22.7 ± 6.1
Idazoxan	7.0 ± 1.6	24.3 ± 6.5	13.4 ± 2.0
Oxymetazoline	12.3 ± 2.5	430 ± 87	128 ± 35
Dexmedetomidine	14.8 ± 3.5	7.1 ± 1.3	7.9 ± 1.5
Clonidine	16.0 ± 4.0	53.2 ± 11.7	95 ± 16
Yohimbine	42.6 ± 7.2	6.9 ± 1.4	2.6 ± 0.6
UK 14,304	129 ± 20	125 ± 22	94.5 ± 13.8
SKF 104,078	162 ± 26	72 ± 26	87 ± 29
Norepinephrine	633 ± 122	209 ± 34	388 ± 22
Chlorpromazine	666 ± 126	5.3 ± 1.6	47.1 ± 4.7
BHT 920	983 ± 79	633 ± 150	328 ± 78
Corynanthine	1250 ± 290	273 ± 21	287 ± 41
8-OH DPAT	2170 ± 560	549 ± 167	711 ± 211
Prazosin	2530 ± 328	36.3 ± 8.3	80.0 ± 13.9

[^{3}H]rauwolscine, at each of the expressed receptors were compared. The affinity constants of the drugs binding to each receptor were compared with the affinity constants acting at each of the other receptors, and the correlations are shown in Fig. 3. These linear correlation techniques have been used to compare α_2-AR binding in tissues and cell lines (19). In Fig. 3, the thick line represents the line of identity, the thin lines are 1 order of magnitude away from identity, and the dotted lines are the correlation lines. Consistent with previous analyses of this type, a >10-fold difference in the affinities of a drug binding to two different receptors is criterion for demonstrating a subtype-selective agent. Fig. 3, A and B, demonstrates that oxymetazoline is selective for the RG20α_2-AR. Also, Fig. 3 shows that rauwolscine, chlorpromazine, and prazosin will differentiate either RNGα_2-AR or RG10α_2-AR from the RG20α_2-AR, whereas yohimbine differentiates the RG10α_2-AR from the RG20α_2-AR. Fig. 3C shows that the pharmacological profile of the RNGα_2-AR and the RG10α_2-AR are very similar. Of the drugs tested, chlorpromazine displayed the greatest relative difference in affinities for the RNGα_2-AR and the RG10α_2-AR (9-fold). The correlation coefficients of each of the sets of comparisons are as follows: RG20α_2-AR versus RNGα_2-AR, 0.580; RG20α_2-AR versus RG10α_2-AR, 0.708; and RNGα_2-AR versus RG10α_2-AR, 0.919.

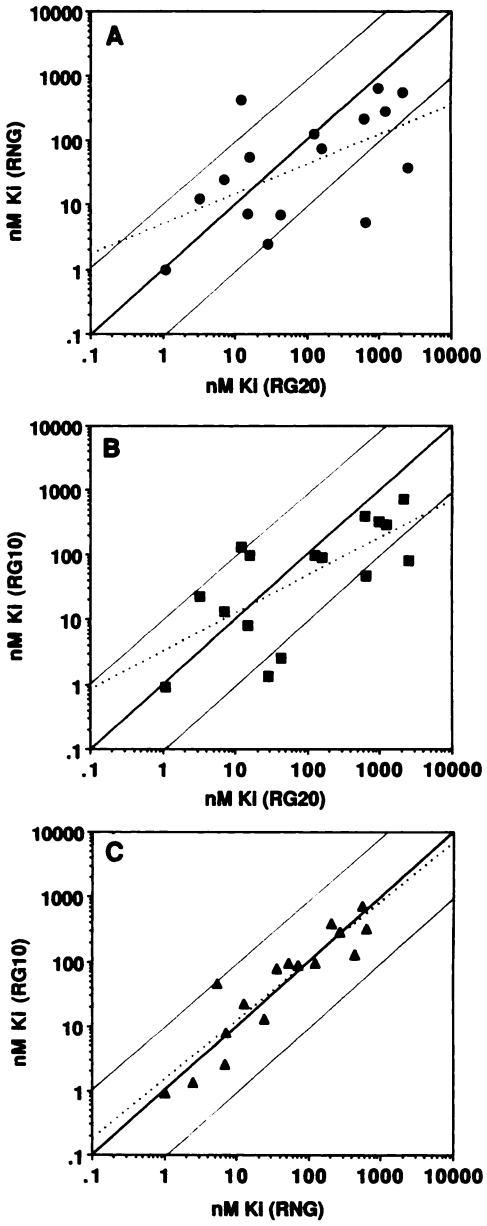

Fig. 3. Correlations between the logarithms of K_i values from COS-1 cell membranes expressing individually the three rat α_2-ARs. A, RNG and RG-20; B, RG-10 and RG-20; C, RNG and RG-10. *Thick lines*, line of identity; *thin lines*, 10-fold on either side of identity; *dotted lines*, correlation lines. The slopes of the correlation lines are: A, 0.477; B, 0.589; C, 0.929. The correlation coefficients are presented in the text.

Discussion

The focus of this study was the characterization of the ligand-binding properties of the three rat α_2-ARs. Like the human, the rat contains three closely related α_2-AR genes, as demonstrated by hybridization of restriction endonuclease-digested genomic DNA with probes derived from either human or rat α_2-AR DNAs (6, 15). To date, the cloning of all three α_2-AR genes or cDNAs from human (6–9) and rat (10–14) has been reported. Each of the rat α_2-AR genes is analogous to one of the human α_2-AR genes, as judged by cross-hybridization of subtype-specific regions (15), as well as by amino acid sequence comparison.

We expressed all three rat α_2-AR genes transiently in COS cells. Each of the expressed receptors displays high affinity, saturable binding of [^{3}H]rauwolscine. The affinities of the RNGα_2-AR and RG10α_2-AR for rauwolscine are similar to those of their human analogs. In contrast, the K_d of rauwolscine at the RG20α_2-AR is approximately 10-fold lower than the K_d for rauwolscine at its human counterpart (C-10). Despite the relatively low affinities of the RG20α_2-AR for rauwolscine and its congener yohimbine, this receptor displays the expected pharmacological profile of a human α_{2A}-AR. For example, the RG20α_2-AR has a relatively high affinity for the subtype-selective drug oxymetazoline and relatively low affinities for the subtype-selective drugs prazosin and chlorpromazine. A number of literature reports have suggested that the rat expresses a low affinity rauwolscine-binding α_2-AR. For example,

Boyajian *et al.* (20) have reported that [^{3}H]rauwolscine binding in rat brain fits better to a two-site model, with K_d values of 0.57 and 14.7 nM for the high and low affinity labeled sites, respectively. Data from this laboratory have demonstrated that RG20α_2-AR mRNA accumulates widely in rat brain (15). In addition, a low affinity rauwolscine-binding α_2-AR has been characterized in rat submaxillary gland (21). This binding site displays the expected pharmacological characteristics of an α_{2A}-AR subtype (i.e., high affinity for oxymetazoline and low affinity for prazosin and chlorpromazine). The similarity of the sequence of RG20α_2-AR to that of the human C-10 α_2-AR, as well as the pharmacological profile of the expressed receptor, suggests strongly that this protein is the rat α_{2A}-AR.

The assignment of the RNGα_2-AR and the RG10α_2-AR to the existing α_2-AR pharmacology is more complicated. Both of these proteins display somewhat similar pharmacological profiles. Whereas the RNGα_2-AR is the nonglycosylated α_2-AR and its mRNA accumulates in rat neonatal lung (the α_{2B}-AR prototypical tissue), the RG10α_2-AR mRNA accumulates in rat brain (15), which is another tissue reported to contain α_{2B}-AR subtypes (2). Murphy and Bylund (4) have characterized an α_2-AR in the opossum kidney cell line (OK) that has similar, yet distinct, pharmacological characteristics, compared with the α_2-AR found in neonatal rat lung. The differences in the pharmacology of the OK cell α_2-AR and the rat neonatal lung α_2-AR were revealed when a comparison of the ratio of K_i values for a pair of drugs was made. Table 3 shows a comparison of the K_i values of prazosin, oxymetazoline, and yohimbine and the prazosin to oxymetazoline, as well as the prazosin to yohimbine, K_i ratios of the three cloned rat α_2-ARs, the three human α_2-ARs, and the three prototypical tissues or cells containing the three α_2-AR subtypes. The α_{2A}-AR subtype displays a high ratio of K_i values of prazosin to oxymetazoline and a high ratio of prazosin to yohimbine. This is evident in the human platelet, the human C-10 α_2-AR, and the rat RG20α_2-AR, although the prazosin to yohimbine ratio of the RG20α_2-AR is significantly lower, due to the relatively higher K_i for yohimbine at the RG20α_2-AR. The K_i ratios of prazosin to oxymetazoline of the RNGα_2-AR and the human C-2 α_2-AR correlate better with the ratio observed in the neonatal rat lung, whereas the RG10α_2-AR and human C-4 α_2-AR ratios correlate better with the ratios seen with the OK cell α_2-AR. Likewise, the prazosin to yohimbine ratio of the RNGα_2-AR is closer to the neonatal lung α_2-AR ratio than is either the RG10α_2-AR or C-4 α_2-AR ratio. These latter receptors have prazosin to yohimbine K_i ratios nearer to the ratio determined in the OK cell. In addition, Lorenz *et al.* (16) have demonstrated that ^{32}P-labeled cDNA probes derived from the human C-4 α_2-AR gene hybridize to RNA extracted from OK cells. Therefore, we suggest that the α_2-AR from neonatal rat lung or the RNG/C-2 protein be designated as the α_{2B}-AR and the α_2-AR on the OK cell or the RG-10/C-4 product be designated as the α_{2C}-AR.

The list of drugs used in this study includes agonists and antagonists acting at functional α_2-ARs. Although agonists typically display broader competition curves in ligand-binding assays than do antagonists, all of the drugs tested competed for [^{3}H]rauwolscine binding with slope factors near unity (0.7–1). Pretreatment of the membranes or addition of guanyl nucleotides in the binding reaction had a negligible effect on the potency of norepinephrine in the competion experiments.[2] This is likely not surprising, due to the high level of expression of these receptors in COS cells and, therefore, a relatively high stoichiometric ratio of receptors to GTP-binding proteins. It is also possible that the appropriate GTP-binding proteins capable of interacting with α_2-ARs are not present at all in the COS cells.

In summary, we have expressed and pharmacologically characterized the three rat α_2-ARs in the context of a single cell type. The pharmacological profiles fit well into the classification scheme of α_2-AR subtypes. Although it is relatively easy to pharmacologically distinguish the RG20α_2-AR from either the RNGα_2-AR or the RG10α_2-AR, it is difficult to differentiate the latter two subtypes. The molecular cloning of the genes and cDNAs for the α_2-ARs has demonstrated unequivocally the existence of subtypes of α_2-ARs. It is on this, and perhaps future, information that the designation of the subtypes should be based. Characterization of their function, tissue-specific expression, and regulation will enhance our understanding of α_2-AR physiology. The advent of subtype-specific antisera, as well as the development of better subtype-specific drugs, should also prove useful.

[2] J. K. Harrison and K. R. Lynch, unpublished obeservations.

TABLE 3

Comparison of the affinities of α_2-ARs for oxymetazoline, prazosin, and yohimbine

The K_i values presented are from this study and binding studies reported elsewhere, as indicated.

Subtype	Clone/tissue	K_i			K_i ratio	
		Yohimbine	Prazosin	Oxymetazoline	Prazosin/oxymetazoline	Prazosin/yohimbine
			nM			
α_{2A}	Human platelet[a]	1.0	340	0.63	540	340
	C-10[b]	1.6	1800	11	164	1125
	RG-20	42.6	2530	12.3	206	59
α_{2B}	Neonatal rat lung[a]	1.0	5.4	52	0.10	5.4
	C-2[c]	9.5	293	1506	0.19	31
	RNG	6.9	36.3	430	0.08	5.3
α_{2C}	OK cell[d]	0.19	7.6	10	0.76	40
	C-4[b]	0.93	41	62	0.66	44
	RG-10	2.6	80	128	0.63	31

[a] From Ref. 19.
[b] From Ref. 7.
[c] From Ref. 9.
[d] From Ref. 4.

Acknowledgments

The authors wish to thank their colleagues at the University of Virginia for advice during the course of this study, particularly Drs. Patrice Guyenet and Amy Tucker. We also thank Ms. Cynthia Barber and Ms. Joan Gilrain for excellent technical assistance. We are particularly indebted to Dr. Stephen Lanier, Massachusetts General Hospital (Boston, MA), for his gifts of the rat genomic clones RG-20 and RG-10.

References

1. Bylund, D. B. Comparison of [^{3}H]-clonidine and [^{3}H]-yohimbine binding: possible subtypes of *alpha*-2 adrenergic receptors. *Pharmacologist* **23**:215 (1981).
2. Cheung, Y. D., D. B. Barnett, and S. R. Nahorski. [^{3}H]-Rauwolscine and [^{3}H]-yohimbine binding to rat cerebral and human platelet membranes: possible heterogeneity of α_2-adrenoceptors. *Eur. J. Pharmacol.* **84**:79–85 (1982).
3. Latifpour, J., S. B. Jones, and D. B. Bylund. Characterization of [^{3}H] yohimbine binding to putative *alpha*-2 adrenergic receptors in neonatal rat lung. *J. Pharmacol. Exp. Ther.* **223**:606–611 (1982).
4. Murphy, T. J., and D. B. Bylund. Characterization of α_2-adrenergic receptors in the OK cell, an opossum kidney cell line. *J. Pharmacol. Exp. Ther.* **244**:571–578 (1988).
5. Lanier, S. M., C. J. Homcy, C. Patenaude, and R. M. Graham. Identification of structurally distinct α_2-adrenergic receptors. *J. Biol. Chem.* **263**:14491–14496 (1988).
6. Kobilka, B. K., H. Matsui, T. S. Kobilka, T. L. Yang-Feng, U. Francke, M. G. Caron, R. J. Lefkowitz, and J. W. Regan. Cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptors. *Science (Washington D. C.)* **238**:650–656 (1987).
7. Regan, J. W., T. S. Kobilka, T. L. Yang-Feng, M. G. Caron, R. J. Lefkowitz, and B. K. Kobilka. Cloning and expression of a human kidney cDNA for an α_2-adrenergic receptor subtype. *Proc. Natl. Acad. Sci. USA* **85**:6301–6305 (1988).
8. Weinshank, R. L., J. M. Zgombick, M. Macchi, N. Adham, H. Lichtblau, T. A. Branchek, and P. R. Hartig. Cloning, expression and pharmacological characterization of a human α_{2B}-adrenergic receptor. *Mol. Pharmacol.* **38**:681–688 (1990).
9. Lomasney, J. W., W. Lorenz, L. F. Allen, K. King, J. W. Regan, T. L. Yang-Feng, M. G. Caron, and R. J. Lefkowitz. Expansion of the α_2-adrenergic receptor family: cloning and characterization of a human α_2-adrenergic receptor subtype, the gene for which is located on chromosome 2. *Proc. Natl. Acad. Sci. USA* **87**:5094–5098 (1990).
10. Zeng, D., J. K. Harrison, D. D. D'Angelo, C. M. Barber, A. L. Tucker, Z. Lu, and K. R. Lynch. Molecular characterization of a rat α_{2B}-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **87**:3102–3106 (1990).
11. Chalberg, S. C., T. Duda, J. A. Rhine, and R. K. Sharma. Molecular cloning, sequencing and expression of an *alpha*-2 adrenergic receptor complementary DNA from rat brain. *Mol. Cell. Biochem.* **97**:161–172 (1990).
12. Flordellis, C. S., D. E. Handy, M. R. Bresnahan, V. I. Zannis, and H. Gavras. Cloning and expression of a rat brain α_{2B}-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **88**:1019–1023 (1991).
13. Lanier, S. M., S. Downing, E. Duzic, and C. J. Homcy. Isolation of rat genomic clones encoding subtypes of the α_2-adrenergic receptor: identification of a unique receptor subtype. *J. Biol. Chem.*, in press.
14. Voigt, M. M., S. K. McCune, R. Y. Kanterman, and C. C. Felder. The rat α_2-C4 receptor gene encodes a novel pharmacological subtype. *FEBS Lett.* **278**:45–50 (1991).
15. Zeng, D., and K. R. Lynch. Distribution of α_2-adrenergic receptor mRNAs in the rat CNS. *Mol. Brain Res.*, in press.
16. Lorenz, W., J. W. Lomasney, S. Collins, J. W. Regan, M. G. Caron, and R. J. Lefkowitz. Expression of three α_2-adrenergic receptor subtypes in rat tissues: implications for α_2 receptor classification. *Mol. Pharmacol.* **38**:599–603 (1990).
17. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
18. Cheng, Y.-C., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
19. Bylund, D. B., C. Ray-Prenger, and T. J. Murphy. *Alpha*-2A and *alpha*-2B adrenergic receptor subtypes: antagonist binding in tissues and cell lines containing only one subtype. *J. Pharmacol. Exp. Ther.* **245**:600–607 (1988).
20. Boyajian, C. L., S. E. Loughlin, and F. M. Leslie. Anatomical evidence for *alpha*-2 adrenoceptor heterogeneity: differential autoradiographic distribution of [^{3}H]rauwolscine and [^{3}H]idazoxan in rat brain. *J. Pharmacol. Exp. Ther.* **241**:1079–1091 (1987).
21. Michel, A. D., D. N. Loury, and R. L. Whiting. Differences between the α_2-adrenoceptor in rat submaxillary gland and the α_{2A}- and α_{2B}-adrenoceptor subtypes. *Br. J. Pharmacol.* **98**:890–897 (1989).

Send reprint requests to: Kevin R. Lynch, Ph.D., Department of Pharmacology, Box 448, University of Virginia School of Medicine, 1300 Jefferson Park Ave., Charlottesville, VA 22908.