Characterization of α_2 Adrenergic Receptor Subtypes in Human Ocular Tissue Homogenates

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Purpose. To determine the predominant α_2 adrenergic receptor subtypes present in the human eye.

Methods. Saturation- and competition-receptor–binding experiments were performed with the radioligand [^{3}H]RX821002 in human ciliary body, retinal pigmented epithelium–choriocapillaris, iris, and neurosensory retina. The affinities of various adrenergic antagonists in these ocular tissues were compared with their affinities for the cloned α_{2A}, α_{2B}, and α_{2C} adrenergic receptor subtypes.

Results. The density of α_2 adrenergic receptors was highest in the iris (440 femtomoles/mg protein), lowest in the neurosensory retina (14 femtomoles/mg protein), and intermediate in the other two tissues (approximately 90 fmol/mg protein). The drug affinities in all four human ocular tissues were highly correlated (correlation coefficients between 0.94 and 0.97) with the affinities for the human α_{2A} adrenergic receptor subtype and poorly correlated (correlation coefficients between 0.15 and 0.66) with the α_{2B} and α_{2C} subtypes.

Conclusions. In agreement with previous studies in several animal species, the α_2 adrenergic receptors in the human ciliary body, retinal pigmented epithelium–choriocapillaris, iris, and neurosensory retina are predominately of the α_{2A} subtype. (*Invest Ophthalmol Vis Sci.* 1999;40: 2299–2306)

Glaucoma is characterized by a progressive loss of visual sensitivity resulting from optic nerve damage. Because high intraocular pressure is the most important risk factor for glaucoma, the treatment of glaucoma has emphasized the reduction of intraocular pressure.[1] Alpha-2 adrenergic agonists such as brimonidine and apraclonidine are effective ocular hypotensive agents,[2–4] although their mechanism of action is not clear.[5–7] The development of subtype-selective α_2 adrenergic agents for topical application is desirable to reduce both systemic and ocular side effects. An understanding of the distribution of α_2 receptor subtypes in the eye would be useful in designing new drugs with greater effectiveness and fewer adverse effects.

Based on both pharmacologic and molecular evidence, there are three major types of adrenergic receptors, α_1, α_2, and β, each of which is further divided into three or four subtypes.[8] The evidence for α_2 adrenergic receptor subtypes has come from binding and functional studies in various tissues and cell lines and more recently in cells transfected with the cDNA for the receptors.[9] On the basis of these studies, three α_2 adrenergic receptor subtypes have been defined. The α_{2A} adrenergic receptor subtype, for which prazosin has a relatively low affinity and oxymetazoline a relatively high affinity, is found in the human platelet and the HT29 cell.[8] The second subtype, the α_{2B}, was identified in neonatal rat lung and in the NG108 cell.[10] This subtype has a relatively high affinity for prazosin and a low affinity for oxymetazoline. A third subtype, the α_{2C}, has been identified in an opossum kidney cell line.[11] Although this subtype also has a relatively high affinity for prazosin and a low affinity for oxymetazoline, it is pharmacologically distinct from the α_{2B} subtype.[12] All three subtypes have been cloned from the human.[13–15] Using the homogenate radioligand-binding technique, α_2 adrenergic receptors have been identified in ocular tissues of several species, including the ciliary body, retinal pigmented epithelium (RPE)–choriocapillaris, iris, and neurosensory retina of both the cow[16,17] and the pig[18] and the ciliary body of the rabbit.[19] The results of these binding studies indicate that the α_{2A} subtype is the predominant, if not the only, α_2 adrenergic subtype in these ocular tissues, with the exception of the porcine neurosensory retina, which may contain a very low density (4 femtomoles/mg protein) of the α_{2C} subtype. Although similar binding studies have not yet been conducted with human ocular tissues, it appears likely that the predominant human ocular α_2 adrenergic subtype may also be the α_{2A}.

In contrast to these binding data, immunofluorescence labeling of the human ciliary body indicates the presence of α_{2B} and α_{2C} subtypes, but not the α_{2A} subtype.[20] Similarly, studies using polymerase chain reaction (PCR) suggest the presence of the α_{2B} and α_{2C} subtypes, but not the α_{2A} subtype, in a transformed cell line of human nonpigmented epithelium[20] and only the α_{2B} subtype in the human ciliary body.[21] To determine whether this apparent discrepancy is the result of species differences or of differences in techniques, we investigated the α_2 subtypes in human ocular tissues by the radioligand-binding technique. On the basis of receptor-binding experiments using the α_2 antagonist radioligand [^{3}H]RX821002, we conclude that the α_2 adrenergic receptors in the human ciliary body, RPE–choriocapillaris, iris, and neurosensory retina are predominately of the α_{2A} subtype.

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METHODS

Drugs and Chemicals

[^{3}H]RX821002 (specific activity 52–59 Ci/millimole) was obtained from Amersham International (London, UK); rauwolscine, WB 4101, and spiroxatrine from Research Biochemicals, (Natick, MA); and oxymetazoline from Sigma (St. Louis, MO). Prazosin and ARC-239 were generous gifts from Pfizer (Groton, CT) and Boehringer–Ingelheim (Ridgefield, CT), respectively. Drugs were prepared as 5- or 10-mM stock solutions and diluted in 5 mM HCl. The stock solution for prazosin was in methanol, for spiroxatrine in 80% dimethyl sulfoxide-20% 1 M HCl, and for all other drugs in 5 mM HCl.

Tissue

Human eyes were obtained frozen from the Missouri Lions Eye Bank and bisected at approximately 7 to 8 mm posterior to the limbus while bathed in 50 mM Tris buffer at 4°C. Under a dissecting microscope, the anterior hyaloid face and lens material were carefully dissected from the iris and ciliary body. The remnants of neurosensory retina, RPE, and choriocapillaris were removed from their attachments at the ora serrata in the anterior portion of the bisected globe. The iris was then removed by disinserting the iris root from the base of the ciliary body, thus releasing the base of the ciliary body then released from its attachment to the scleral spur anteriorly.

The various tissues were suspended in 25 ml ice-cold 50 mM Tris-HCl (pH 8 at 25°C) and homogenized (model TR-10 Tissumiser; Tekmar, Cincinnati, OH). The homogenate was filtered through a 53-μm nylon mesh, centrifuged at 1400 rpm for 10 minutes. The supernatant was transferred to another tube, recentrifuged at 20,000 rpm for 10 minutes and the pellet frozen at −80°C.

Radioligand-Binding Assays

Saturation- and competition-binding experiments were performed as described previously, using 25 mM sodium phosphate buffer at pH 7.4.[16,17,22] Briefly, saturation experiments were performed using two sets of duplicate tubes that contained 970 μl of membrane suspension and 20 μl [^{3}H]RX821002. The protein concentration was adjusted to ensure that the specifically bound radioligand was less than 10% of the total added radioligand. One set of tubes contained 10 μl (−)-norepinephrine (final concentration, 100 μM) to determine nonspecific binding. Specific binding was calculated as the difference between total and nonspecific binding. After a 40-minute incubation at room temperature, the suspensions were filtered through glass fiber filter strips (GF/B; Whatman, Clifton, NJ), which had been soaked overnight in 0.1% polyethylenimine, using a 48-sample manifold (Brandel Cell Harvester; Biomedical Research and Development, Gaithersburg, MD). The tubes and filters were washed twice with 5 ml ice-cold 50 mM Tris-HCl (pH 8.0), and the radioactivity on the filter was determined by liquid scintillation spectroscopy. The K_d and maximum binding (B_{max}) values were calculated from nonlinear regression of bound versus free ligand concentrations using a statistical software program (Prism; GraphPad, San Diego, CA). K_d values are geometric means and B_{max} values are arithmetic means. Protein concentrations were determined by the method of Bradford[23] with bovine serum albumin as the standard.

For inhibition experiments, 20 μl of a fixed concentration of radioligand [^{3}H]RX821002 (final concentration, 0.24 ± 0.04 nM, which is near the K_d concentration) and various concentrations of unlabeled drug (10 μl) were added to duplicate tubes containing 970 μl of the membrane suspension. Assays were then performed as described for saturation experiments. Competition binding data were analyzed (Prism; GraphPad) to determine the 50% inhibitory concentration (IC_{50}) assuming a one-site model. The pseudo Hill slope was determined by fitting the data to the four-parameter logistic equation. In some cases, the fit of the data to a one-site model was compared with the fit to a two-site model. IC_{50} values were converted to K_i values by the method of Cheng and Prusoff[24] and are presented as geometric means.

RESULTS

The selective α_2 adrenergic antagonist [^{3}H]RX821002 demonstrated saturable and high-affinity binding to membrane preparations from human ocular tissues (Fig. 1A). The density of receptor-binding sites (B_{max}) was highest in the iris, intermediate in the ciliary body and RPE–choriocapillaris, and lowest in the neurosensory retina (Table 1). The density was 30 times higher in the iris than in the neurosensory retina, and 5 times higher than in the ciliary body and RPE–choriocapillaris. The data were linear when plotted by the method of Rosenthal,[25] consistent with [^{3}H]RX821002 binding to a single class of sites (Fig. 1B). The affinity (K_d) of [^{3}H]RX821002 was essentially identical in the ciliary body, iris, and RPE–choriocapillaris but slightly lower (higher K_d) in the neurosensory retina (Table 1). The K_d values for these ocular tissues are similar to those obtained under identical binding conditions for the cloned α_{2A} subtype (0.25 nM), but lower than those for the α_{2B} and α_{2C} subtypes (0.89 and 0.58 nM, respectively),[22] indicating that the α_{2A} may be the major subtype in these tissues.

Inhibition radioligand-binding experiments were used to investigate further which α_2 adrenergic receptor subtypes are present in human ocular tissues. In the ciliary body, various adrenergic agents inhibited [^{3}H]RX821002 binding with the expected rank order for an α_{2A} receptor (Fig. 2). With the exception of the agonist norepinephrine, none of the slope factors (pseudo Hill coefficients) was significantly less than 1.0, indicating the presence of a single major receptor subtype. Oxymetazoline was approximately 300 times more potent than prazosin in inhibiting [^{3}H]RX821002 binding (Table 2), similar to the 100-fold difference found for the cloned α_{2A} subtype and much different from the prazosin and oxymetazoline ratios of 0.04 and 0.35 found for the cloned α_{2B} and α_{2C} subtypes, respectively.[26] The affinities of the antagonists used for the α_{2B} and α_{2C} subtypes relative to the α_{2A} subtype are shown in Table 3. The large range of relative affinities (230–0.02) indicates that this set of agents can easily differentiate the α_{2A} subtype from the α_{2B} and α_{2C} subtypes. The K_i values determined for the iris, RPE–choriocapillaris, and neurosensory retina were very similar to those of the ciliary body (Table 2), indicating that the α_{2A} subtype is also the predominant α_2 adrenergic receptor subtype in these tissues.

To compare the affinities in the four tissues more systematically, the logarithms of the K_i values (pK_i values) of the six antagonists listed in Table 2, as well as the K_d values of [^{3}H]RX821002 for the ciliary body, were plotted against those

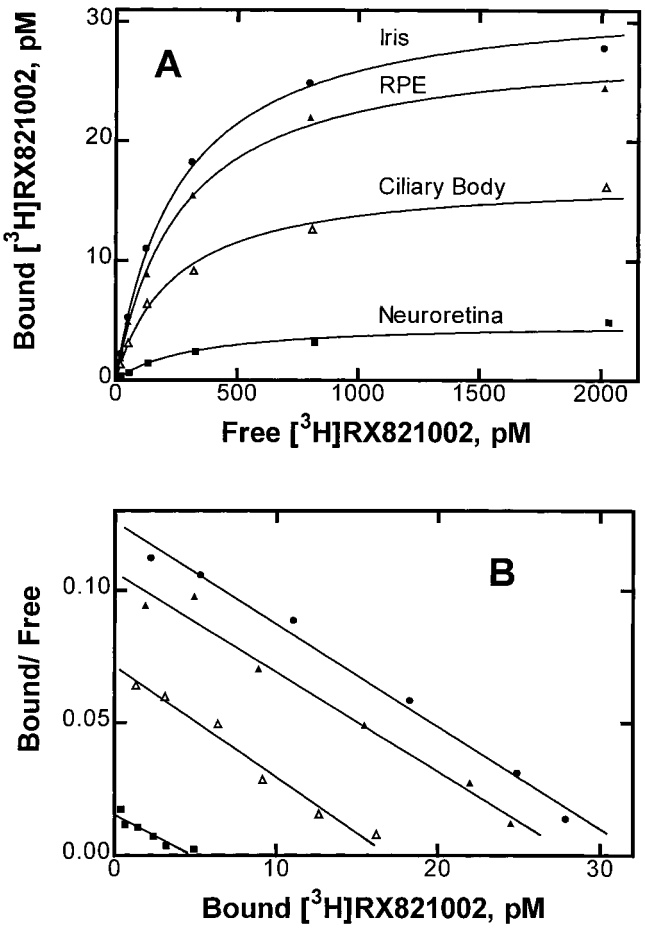

Figure 1. Saturation radioligand-binding experiments of α_2 adrenergic receptors. Membrane preparations from four human ocular tissues were incubated with various concentrations of [^{3}H]RX821002 (Free) and the specific binding (Bound) determined. Specific binding was calculated as the difference between the total binding and the nonspecific binding (100 μM norepinephrine). The results presented are from a single experiment, and the mean ± SEM of four similar experiments are presented in Table 1. (**A**) The results as a hyperbolic saturation curve, determined by nonlinear regression. (**B**) Data have been transformed to a Rosenthal plot that linearizes the data.[25]

for the other three tissues (Fig. 3). In all three cases, the correlation coefficients were close to 1.0, indicating that the same subtype is present in all four tissues. Similarly, the pK_i values for the ciliary body were correlated with the pK_i values obtained previously for the three cloned human subtypes (Fig. 4). The values for the ciliary body correlated highly with those for the human α_{2A} subtype ($r = 0.97$) but poorly with the α_{2B} ($r = 0.22$) and the α_{2C} ($r = 0.50$) subtypes. Similar results were obtained for the other three human ocular tissues (Table 4).

Table 1. Affinity (K_d) and Density (B_{max}) of α_2 Adrenergic Receptor Sites Determined by [^{3}H]RX821002 Binding

Tissue	K_d	B_{max}
Ciliary Body	0.225 ± 0.011	87 ± 7
RPE–choriocapillaris	0.225 ± 0.009	95 ± 6
Iris	0.220 ± 0.012	440 ± 50
Neurosensory retina	0.320 ± 0.003	14 ± 2

Saturation radioligand-binding experiments were used to determine K_d (in nanomolar) and density B_{max} (in femtomoles per milligram protein) in membrane preparations of various human ocular tissues. The results are the means ± SEM of four experiments.

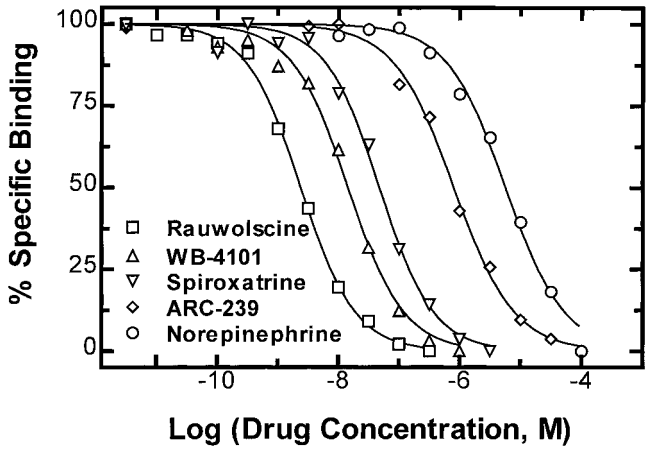

Figure 2. Inhibition radioligand-binding experiments of ciliary body α_2 adrenergic receptors. Membrane preparations of human ciliary body were incubated with various concentrations of the indicated adrenergic agents and with 0.24 nM [^{3}H]RX821002. The data are presented as the percentage of specific binding in the absence of any inhibitor. Specific binding was calculated as the difference between total binding and nonspecific binding. Typical levels of total binding and nonspecific binding in cpm were: 1000 and 200 (iris); 550 and 120 (ciliary body); 750 and 150 (RPE–choriocapillaris); 120 and 35 (neurosensory retina). The IC_{50} values, determined by nonlinear regression, were used to calculate K_i values. The results presented are from a single experiment, and the mean ± SEM of three similar experiments are presented in Table 2.

Although these data clearly indicate that the α_{2A} subtype is the predominant α_2 adrenergic receptor subtype in human ocular tissues, the possibility of a low density of an additional α_2 subtype cannot be excluded. In the porcine neurosensory retina, for example, 85% of the receptors are α_{2A}, but 15% are α_{2C}.[18] Furthermore, the α_{2C} subtype is the main α_2 receptor in a human retinoblastoma cell line (Y79).[27] These observations prompted a more careful evaluation of the data. The K_d value for [^{3}H]RX821002 was somewhat higher in the neurosensory retina than in the other tissues (Table 1). Similarly, in the neurosensory retina the slope factors for many of the antagonists were less than 1.0. Spiroxatrine has a 40-fold higher affinity for the human α_{2C} subtype, compared with the α_{2A}, and thus is a good antagonist for detecting a minor amount of α_{2C} in the presence of the α_{2A} subtype. If a measurable amount of α_{2C} were present in the neurosensory retina, then the spiroxatrine inhibition data should fit a two-site model better than a one-site model. In four of six inhibition experiments in the neurosensory retina, the data fit a two-site model significantly better than a one-site model ($P < 0.05$). For the four experiments that modeled better as two sites, the higher affinity site had a median effective concentration (EC_{50}) of 1.4 nM and accounted for 56% of the receptors, whereas the lower affinity site had an EC_{50} of 72 nM. When the data for all six experiments were combined and fit as a single curve, similar results were obtained (Fig. 5). Thus, it appears likely that the

TABLE 2. Affinities (K_i) of Adrenergic Drugs of Ocular α_2 Adrenergic Receptors as Determined by [^{3}H]RX821002 Binding

	Ciliary Body				RPE-Choriocapillaris				Iris				Neurosensory Retina			
Drug	K_i	SEM	Slope	*n*	K_i	SEM	Slope	*n*	K_i	SEM	Slope	*n*	K_i	SEM	Slope	*n*
Rauwolscine	0.99	0.06	1.02	3	1.12	0.06	1.03	3	1.07	0.11	1.01	3	0.43	0.07	0.80	2
Oxymetazoline	4.5	0.4	1.15	3	3.1	0.2	0.95	4	3.1	0.2	0.96	2	6.7	0.8	0.82	3
WB4101	6.6	0.1	0.99	3	7.1	0.2	0.99	3	13	2	1.29	3	3.0	0.0	0.80	2
Spiroxatrine	26	2	1.13	3	25	1	1.10	6	42	2	1.08	2	4.2	0.4	0.81	6
ARC-239	499	21	1.09	3	492	23	1.01	3	530	42	1.08	3	284	23	1.00	3
Prazosin	1419	66	1.37	3	2319	180	1.56	3	851	157	1.21	3	177	14	0.73	2
Norepinephrine	2944	128	0.71	3	4147	278	0.87	3	3360	143	0.91	3	2468	161	0.78	2

Inhibition radioligand-binding experiments were used to determine IC_{50} values in membrane preparations of various human ocular tissues. K_i in nanomolar was calculated from IC_{50}. The slope is the pseudo Hill coefficient. The results are the means ± SEM of *n* experiments.

human neurosensory retina contains a significant amount of both the α_{2A} and α_{2C} subtypes.

DISCUSSION

Alpha-2 adrenergic agents, such as brimonidine and apraclonidine, effectively lower intraocular pressure, although the mechanisms involved are not yet well understood. Three presumed sites of action for these agonists are the ciliary nonpigmented epithelium (reduction of aqueous humor production), ciliary muscle (increase in uveoscleral outflow facility), and the trabecular meshwork (increase in trabecular outflow). In addition, in some species a central site of action for α_2 agonists is also probable. Brimonidine and apraclonidine are equally efficacious in decreasing aqueous humor production,[28] presumably by acting on the nonpigmented epithelium of the ciliary body. However, brimonidine[7] and oxymetazoline[29] also appear to increase uveoscleral outflow, whereas apraclonidine increases outflow through the trabecular meshwork.[6] One potential explanation for these differences is that the agonists have differential potencies at the three α_2 adrenergic receptor subtypes, and that these subtypes are differentially located in the relevant ocular tissues.

Several techniques have been used to define the localization of α_2 adrenergic receptors in the human eye. Autoradiographic studies found high levels of α_2 adrenergic receptors in the iris epithelium and ciliary epithelium, as well as in the ciliary muscle, retina, and RPE.[30] In these studies, the total pool of α_2 receptors was visualized, but the individual receptor subtypes were not considered. In the human ciliary body, immunofluorescence labeling indicates the presence of the α_{2B} and α_{2C} subtypes, but not the α_{2A} subtype.[20] In contrast to these immunofluorescence results, our radioligand-binding data indicate that the α_{2A} is the main, if not the only, subtype present in the human ciliary body, iris, and RPE–choriocapillaris. The neurosensory retina appears to contain mostly α_{2A} and perhaps some α_{2C}. This conclusion is based on a comparison of K_i values in the ocular tissues with previous data from our laboratory with the cloned human subtypes expressed in COS cells.[26] Our conclusion that the α_{2A} is the major subtype in human ocular tissues is consistent with radioligand-binding studies in the cow, rabbit, and pig, which also identify the α_{2A} subtype as the main ocular subtype.[16,18,19]

TABLE 3. Relative Affinities of Adrenergic Antagonists for Human α_2 Adrenergic Receptor Subtypes

Antagonist	α_{2A}	α_{2B}	α_{2C}
Rauwolscine	1.0	1.2	0.41
Oxymetazoline	1.0	230	9.4
WB4101	1.0	4.7	0.44
Spiroxatrine	1.0	0.03	0.02
ARC-239	1.0	0.02	0.03
Prazosin	1.0	0.10	0.04

The affinities (K_i values) for the α_{2B} and α_{2C} subtypes were divided by that for the α_{2A} to determine relative affinities. Thus, a number higher than 1.0 indicates that the subtype has a lower affinity (higher K_i) than the α_{2A}, whereas a number less than 1.0 indicates a higher affinity. The data are for COS cells transfected with the three human subtypes.[26]

In contrast to our results from the radioligand-binding technique are the results obtained using PCR and immunofluorescence techniques. Two studies have used PCR to determine the absence or presence of mRNA encoding the three α_2 adrenergic receptor subtypes in the human eye. In a transformed cell line of human nonpigmented epithelium, PCR studies indicate the presence of mRNA for the α_{2B} and α_{2C} subtypes, but not the α_{2A}.[21] In a second, similar study published thus far only in abstract form, the PCR technique indicated the presence of only the α_{2B} subtype in a transformed cell line of human nonpigmented epithelium and in the human ciliary body.[21] Both studies also examined rabbit iris–ciliary body. The former study found evidence for mRNA for all three subtypes, whereas the latter study found only the α_{2A} and α_{2B} subtypes. The single study using the immunofluorescence technique suggested the presence of the α_{2B} and α_{2C} subtypes, but not the α_{2A}, in the human ciliary body.[20] By contrast all three subtypes were found in the rabbit iris–ciliary body. Thus, although these two techniques appear to differ on exactly which subtypes are present in human and rabbit ocular tissues, they agree that the α_{2A} subtype is absent in human tissues studied. This conclusion is not supported by the results of the radioligand-binding studies reported here.

It is of interest that the immunofluorescence technique identifies all three subtypes in the rabbit ciliary body,[20] whereas the radioligand-binding approach finds only the α_{2A} subtype.[19] Because immunofluorescence is not a quantitative approach, it may be that the other two subtypes are present in such low concentrations in the rabbit ciliary body that they are not detected by the radioligand-binding technique, or that they are not functional (i.e., unable to bind ligand) proteins. That

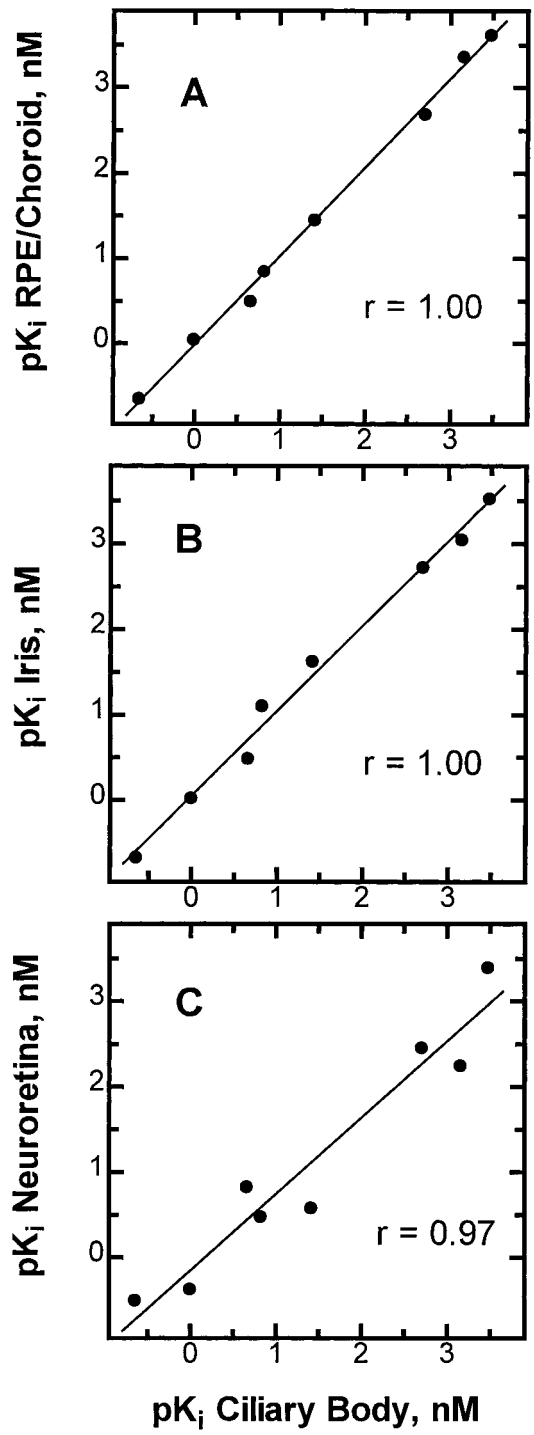

FIGURE 3. The correlation of pK_i values of adrenergic antagonists for human ciliary body with the human RPE–choriocapillaris (**A**), iris (**B**), and neurosensory retina (**C**). The data are taken from Tables 1 and 2. The correlation coefficients (r) are also shown. A summary of the correlation coefficients is presented in Table 4.

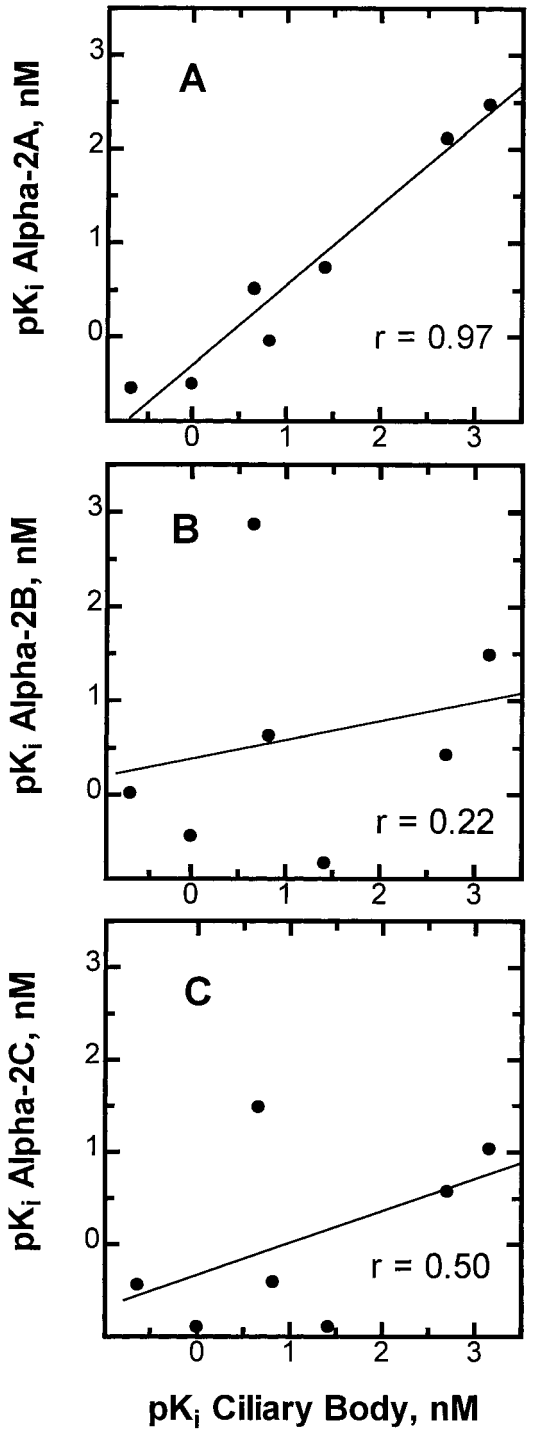

FIGURE 4. The correlation of pK_i values (logarithm of the K_i or K_d values) of adrenergic antagonists for ciliary body with the cloned human α_2 adrenergic receptor subtypes α_{2A} (**A**), α_{2B} (**B**), and α_{2C} (**C**). The data are taken from Tables 1 and 2 and from Berlie et al.[16] The correlation coefficients (r), are also shown. A summary of the correlation coefficients for all four human ocular tissues with the three cloned human α_2 adrenergic receptor subtypes is shown in Table 4.

there was no immunofluorescence detection of the α_{2A} subtype in the human ciliary body is of concern, because this is the major subtype detected by the radioligand technique in all four species investigated to date: human, cow, pig, and rabbit. Furthermore, the cultured human trabecular meshwork cells express only the α_{2A} subtype.[31] As Huang et al.[20] point out, the α_{2A} subtype may well exist in the human eye but was not detected in their immunofluorescence experiments, perhaps because the antibody used was not sufficiently sensitive. However, the PCR studies should have detected the mRNA for the

TABLE 4. Correlation Coefficients (r) from Correlations of pK_i Values Between Human Ocular Tissues and the Cloned Human α_2 Adrenergic Receptor Subtypes

	Ciliary Body	Cloned Subtypes		
		α_{2A}	α_{2B}	α_{2C}
Ciliary Body	—	0.974	0.22	0.50
RPE–choriocapillaris	0.998	0.967	0.19	0.46
Iris	0.995	0.945	0.15	0.41
Neurosensory retina	0.965	0.974	0.39	0.66

The data are taken from Tables 1 and 2 and from Berlie et al.[16] The correlation plots for ciliary body with the other ocular subtypes and with the cloned human subtypes are presented in Figures 3 and 4, respectively.

α_{2A} subtype if it were present. Similarly, it is possible that the α_{2B} and α_{2C} subtypes are present at low density in the human ciliary body, and thus were not detected by the radioligand technique because of the presence of a much higher density of the α_{2A} subtype. Another potential resolution to the difference in conclusions reached by the two techniques is one of receptor subtype localization, because the radioligand-binding technique detects the binding in the tissue as a whole, whereas the immunofluorescence experiments detect receptors only in a small area. Thus, the α_{2A} subtype could be the major subtype in the ciliary body but did not happen to be in the specific place studied in the immunofluorescence experiments. Conversely, the α_{2B} and α_{2C} subtypes may be localized to the specific places studied in the immunofluorescence experiments, but not sufficiently widely distributed to be detected by the radioligand-binding technique.

Based on the data in Figure 5, it appears probable that some α_{2C} (or possibly α_{2B}) receptors are present in the neurosensory retina. A firm conclusion cannot be drawn, however, because the data were significantly better fit by a two-site

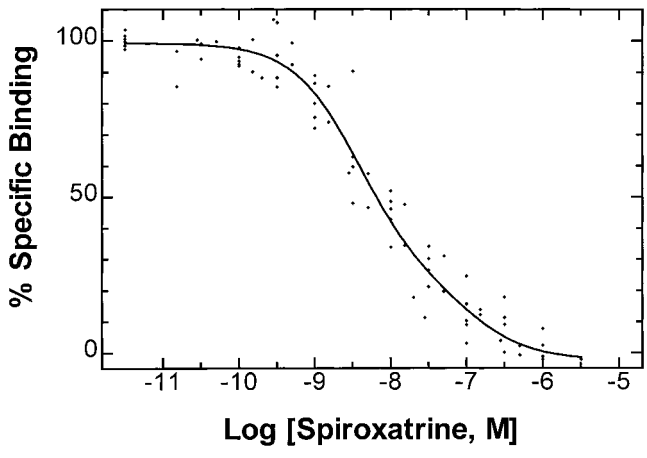

FIGURE 5. Inhibition of [^{3}H]RX821002 binding to the α_2 adrenergic receptors in the neurosensory retina by spiroxatrine. The data shown are the merged data from six experiments. Each data point is the mean of duplicate determinations. A two-site model fit the data significantly better than a one-site model ($F = 5.0$; $P = 0.009$). The higher affinity site had an EC_{50} of 3.7 nM and accounted for 74% of the receptors, whereas the lower affinity site had an EC_{50} of 104 nM.

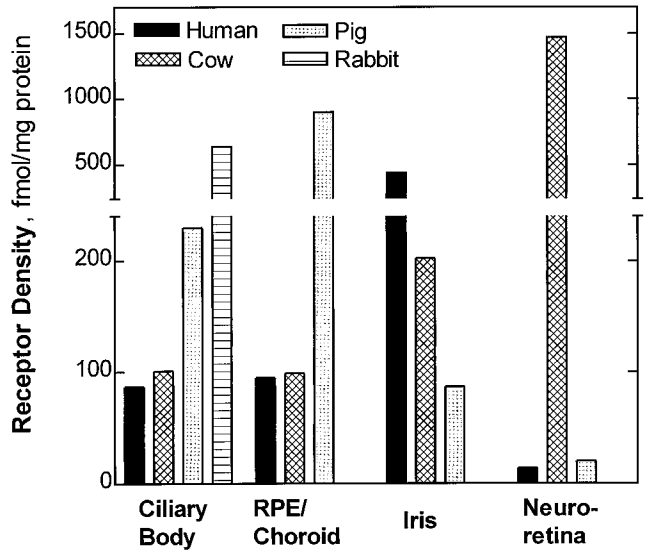

FIGURE 6. The density (B_{max}) of ocular α_2 adrenergic receptors in various species. The data for the human are from Table 1, the data for the cow, pig, and rabbit (ciliary body was the only tissue studied in rabbit) are from Bylund et al.,[17] Wikberg–Matsson et al.,[18] and Jin et al.,[19] respectively.

model in only four of the six individual experiments. In addition, the IC_{50} values for spiroxatrine derived from Figure 5 (3.7 μM and 104 μM) do not agree well with the K_i values determined for the human clones (5.5, 0.19, 0.13 μM) for the α_{2A}, α_{2B}, and α_{2C} subtypes, respectively.[26] The low density of α_2 adrenergic receptors in the neurosensory retina and the limited availability of human tissue make it difficult to resolve this issue clearly.

The density of α_2 adrenergic receptors has now been determined in one or more ocular tissues in four species, as is summarized in Fig. 6. It is remarkable that the relative density in the four tissues is different in each species and that the highest density for each tissue is found in a different species. For example, in the cow, the receptor density in the neurosensory retina is approximately 100 times higher than in the human. The significance of this marked species variability is unknown at the present time. Furthermore, it must be emphasized that receptor density does not necessarily relate to either its physiological or pharmacologic importance.

The pineal gland and the neurosensory retina share many similarities including light sensitivity and embryonic origins. In the pineal gland, the β adrenergic receptor shows similar species variations in density. The sheep pineal has a very high density of β adrenergic receptors (4400 femtomoles/mg protein[32]), the rat is approximately 10 times lower (550 femtomoles/mg protein[33]), and the human (35 femtomoles/mg protein[34]) and hamster (55 femtomoles/mg protein[33]) are 10 times lower yet. By contrast, the α_2 receptor density is relatively constant in the three species that have been examined to date: human, 63 femtomoles/mg protein (David Bylund, unpublished); cow, 71 femtomoles/mg protein[35]; and rat, 69 femtomoles/mg protein.[36]

Some α_2 adrenergic agents, including those used in the treatment of glaucoma, also bind to the nonadrenergic imidazoline sites. The I_1 imidazoline site may have a role in regulating blood pressure,[37] but its role, if any, in regulating

intraocular pressure is unknown.[38,39] Our studies do not address this issue because the radioligand that we chose ([^{3}HRX821002) has a low affinity for imidazoline sites[40] and thus would not bind to those sites under the conditions of our assay. In addition, norepinephrine, which does not bind to imidazoline sites, was used to define nonspecific binding in our studies, thereby eliminating any contribution of imidazoline sites to our data.

Alpha-2 adrenergic agonists are increasingly used in glaucoma therapy. There is some evidence to suggest that these agents may have some direct neuroprotective effect on the optic nerve in addition to the protective effect of reduced intraocular pressure.[41,42] However, long-term studies are needed to evaluate the extent to which α_2 adrenergic agents preserve visual function. Attempts to design new α_2 agents with increased specificity and thus fewer side effects will be strengthened by a better understanding of the α_2 adrenergic receptor subtype(s) mediating the ocular hypotensive effects of these agents.

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