Characterization of Alpha-2D Adrenergic Receptor Subtypes in Bovine Ocular Tissue Homogenates

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ABSTRACT

The alpha-2 adrenergic receptors are known to be present in the mammalian eye and to mediate the effects of alpha-2 agonists used in the treatment of glaucoma. Little is known, however, regarding the relative densities of the three alpha-2 subtypes in the various tissues of the eye. We used receptor binding experiments with the radioligand \([^3H]RX821002\) to characterize the alpha-2 adrenergic receptors in three tissues of the bovine eye, the ciliary body, retinal pigment epithelium/choriocapillaris and iris. The \(K_D\) values in the three tissues were similar (0.12–0.14 nM), and the \(B_{\text{max}}\) values ranged from 100 fmol/mg of protein for the ciliary body and retinal pigment epithelium/choriocapillaris to 200 fmol/mg of protein for the iris. The pharmacological characteristics of the alpha-2 receptors in all three tissues of the bovine eye, as assessed by competition studies, were essentially identical and were similar to the characteristics of the alpha-2A/D receptors of the bovine neurosensory retina. The correlation coefficients between the logarithms of the \(K_D\) values for the three tissues and the neurosensory retina for nine adrenergic agents were .98 to .99. We conclude that the alpha-2 adrenergic receptors in the ciliary body, iris and retinal pigment epithelium/choriocapillaris of the bovine eye are mainly alpha-2D.

Three major types or subfamilies of adrenergic receptors have been identified: alpha-1, alpha-2 and beta. Within each of these three main types of adrenergic receptors, three or more subtypes have been defined (Bylund, 1988, 1992). Based on pharmacological characteristics and molecular cloning, the alpha-2 adrenergic receptor subfamily currently includes three genetic and four pharmacological subtypes: alpha-2A, alpha-2B, alpha-2C and alpha-2D (Bylund et al., 1994). The alpha-2A adrenergic receptor subtype, for which prazosin and ARC-239 have relatively low affinity, is found in the human platelet and the HT29 cell (Bylund et al., 1988). The gene for this receptor has been cloned from the human (Kobilka et al., 1987), and orthologous clones have been found in the pig (Guyer et al., 1990), rat (Lanier et al., 1991), mouse (Link et al., 1992) and guinea pig (Svensson et al., 1996). The second subtype, alpha-2B, which has been identified in neonatal rat lung and the NG108 cell line (Bylund et al., 1988), has been cloned from the rat (Zeng et al., 1990), human (Lomasney et al., 1990; Weinshank et al., 1990), mouse (Chruscinska et al., 1992) and guinea pig (Svensson et al., 1996) and has a relatively high affinity for prazosin and ARC-239. A third subtype, alpha-2C, has been identified in an opossum kidney cell line (Murphy and Bylund, 1988), and its cDNA has been identified by molecular cloning (Blaxall et al., 1994). Species orthologs have been cloned from the human (Regan et al., 1988), rat (Lanier et al., 1991), mouse (Link et al., 1992) and guinea pig (Svensson et al., 1996). Although the alpha-2C subtype also has relatively high affinity for prazosin and ARC-239, its overall pharmacological profile is clearly different from that of alpha-2B. A fourth pharmacological subtype, alpha-2D, has been identified in the rat salivary gland (Michel et al., 1989) and bovine pineal (Simonneaux et al., 1991). This pharmacological subtype has been cloned from the rat (Lanier et al., 1991) and mouse (Link et al., 1992). On the basis of predicted amino acid sequence, the alpha-2D appears to be a species ortholog of the human alpha-2A. Thus, only three subtypes occur in any given species: alpha-2A/D, alpha-2B and alpha-2C.

The actions of norepinephrine, an important neuroregulator that controls many physiological functions in the eye, are mediated by adrenergic receptors. Numerous agents have been developed that interact with adrenergic receptors, many of which are used for diagnostic and therapeutic purposes in ophthalmology. 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. Adrenergic drugs are effective ocular hypotensive agents. Currently, beta adrenergic antagonists are the most commonly used drugs for the medical treatment of glaucoma (Quigley, 1993). The alpha-2 adrenergic agonists are also used for...
reducing intraocular pressure (Chacko and Camras, 1994), although their mechanism of action is not clear (Serle, 1994; Toris et al., 1995a, 1995b). The development of subtype-selective \( \alpha \)-2 adrenergic agents for topical application is considered desirable to reduce both systemic and ocular side effects. An understanding of the distribution of \( \alpha \)-2 receptor subtypes in the eye would be useful in designing new drugs with greater effectiveness and fewer adverse effects.

The \( \alpha \)-2 adrenergic receptors have been identified in some ocular tissues, including the rabbit iris-ciliary body (Jin et al., 1994; Mittag and Tormay, 1985) and the bovine retina (Bittiger et al., 1986; Convets et al., 1987; Osborne, 1982; Van Liefde et al., 1993) by radioligand binding studies and the rabbit retina by using the inhibition of cyclic AMP production as the assay (Osborne, 1991). Autoradiographic studies have indicated the presence of \( \alpha \)-2 receptors in rat, rabbit and human eye (Elena et al., 1989; Matsuo and Cynader, 1992; Zarbin et al., 1986). This work has clearly established the presence of \( \alpha \)-2 receptors in ocular tissues. However, little work has been done on the more important issue of the identification and localization of the three \( \alpha \)-2 receptor subtypes in ocular tissues from various species. Antibodies selective for the subtypes have been used in an attempt to localize the subtypes in the ciliary body of the human and rabbit eye (Huang et al., 1995). These studies found evidence for all three subtypes in the rabbit ciliary body but for only \( \alpha \)-2B and \( \alpha \)-2C in the human ciliary body (Huang et al., 1995). Previous work from our laboratory using the radioligand binding assay has established that the bovine neurosensory retina contains \( \alpha \)-2A/D but not \( \alpha \)-2B or \( \alpha \)-2C adrenergic receptors (Berlie et al., 1995). The presence of the \( \alpha \)-2D subtype in the bovine has been recently confirmed by molecular techniques (Venkataraman et al., 1996). We present an evaluation of the characteristics of the \( \alpha \)-2 adrenergic receptor subtypes in three additional tissues of the bovine eye: the ciliary body, RPE/choriocapillaris and iris. On the basis of receptor binding experiments using the \( \alpha \)-2 antagonist radioligand \(^3\text{H}\)RX821002, we conclude that the \( \alpha \)-2 adrenergic receptors in these tissues of the bovine eye are mainly \( \alpha \)-2D.

**Materials and Methods**

**Drugs and chemicals.** \(^3\text{H}\)RX821002 (specific activity, 55 Ci/mmol) was obtained from Amersham International (London, UK). Rauwolscine, WB 4101 and spiroxatrine were purchased from Research Biochemicals, Inc. (Natick, MA). Oxyamphetamine was obtained from Sigma Chemical Co. (St. Louis, MO). The following were gifts: prazosin from Pfizer, Inc. (Groton, CT), ARC-239 from Boehringer-Ingelheim (Ridgefield, CT) and phentolamine from Ciba-Geigy Corp. (Suffern, NY). Prazosin was prepared in methanol, spiroxatrine was prepared in 80% dimethylsulfoxide/20% 1 M HCl, SK&F 104078 was prepared in 50% ethanol and all other drugs were prepared in 5 mM HCl. Drugs were prepared as 5 or 10 mM stock solutions and diluted in 5 mM HCl.

**Tissue preparation.** Bovine eyes (Pel-Freez Biologicals, Rogers, AR) were thawed and cut coronally 7 to 8 mm posterior to the limbus while bathed in 50 mM Tris buffer at 4°C. The vitreous and lens were discarded, and the iris was separated from its attachment on the ciliary body. The remnants of neurosensory retina, RPE/choriocapillaris, were trimmed from the anterior portion of the bisected globe, and the ciliary body was dislodged from the scleral spur. The RPE/choriocapillaris were detached from the posterior pole after the neurosensory retina was removed. The various tissues were suspended in 25 ml of ice-cold 50 mM Tris-HCl, pH 8, and homogenized with a Polytron (model PT 10-35; Brinkman, Westbury, NY). The homogenate was filtered through a 53-µm nylon mesh and centrifuged at 1,400 rpm for 10 min. The supernatant was transferred to another tube, recentrifuged at 20,000 rpm for 10 min and 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 (Berlie et al., 1995; Bylund et al., 1988; Depree et al., 1996). Briefly, saturation experiments were performed using two sets of duplicate tubes that contained 970 µl of membrane suspension and 20 µl of \(^3\text{H}\)RX821002 (final concentration, 0.24 ± 0.04 nM). The protein concentration was adjusted to ensure that the specifically bound radioligand was <10% of the total added radioligand. One set contained 10 µl of 100 µM (-)norepinephrine to determine nonspecific binding. Specific binding was calculated as the difference between total and nonspecific binding. After a 30-min incubation at room temperature, the suspensions were filtered through GF/B glass-fiber filter strips (Whatman, Clifton, NJ) that had been soaked overnight in 0.2% 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 of 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 \( B_{\text{max}} \) values were calculated from nonlinear regression of bound vs. free ligand concentrations. \( K_d \) values are geometric mean values, and \( B_{\text{max}} \) values are arithmetic mean values. Protein concentrations were determined according to the method of Bradford (1976) with bovine serum albumin used as the standard.

For inhibition experiments, 20 µl of a fixed concentration of radioligand \(^3\text{H}\)RX821002 (−0.2 nM, which is close to 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 above for saturation experiments. Competition binding data were analyzed with the Prism program (GraphPAD, San Diego, CA) to determine IC_{50} values assuming a one-site model, and the pseudo-Hill slope was determined from fitting the data to the four-parameter logistic equation. IC_{50} values were converted to \( K_d \) values according to the method of Cheng and Prusoff (1973) and are presented as geometric mean values.

**Results**

The \( \alpha \)-2 adrenergic receptors in the ciliary body, iris and RPE/choriocapillaris of the bovine eye were characterized by saturation binding using \(^3\text{H}\)RX821002 as the radioligand (Berlie et al., 1995; Galitzky et al., 1990; O’Rourke et al., 1994b). \(^3\text{H}\)RX821002 has similar and high affinities for all four pharmacological subtypes of the \( \alpha \)-2 adrenergic receptor (O’Rourke et al., 1994a, 1994b; Renouard et al., 1994). As shown in figure 1, binding is saturable and of high affinity (\( K_d \sim 0.1 \) nM) in all three tissues. The nonspecific binding is relatively low (20–30% at the \( K_d \) concentrations). When the data are transformed according to the Rosenthal procedure (Rosenthal, 1967), they fall on a straight line, indicating that a single class of binding sites is being labeled. The \( K_d \) and \( B_{\text{max}} \) values for four saturation experiments are given in table 1. The \( B_{\text{max}} \) values for these three bovine eye tissues are 5- to 10-fold lower than that found in the bovine neurosensory retina (Berlie et al., 1995). The pharmacological characteristics of the \( \alpha \)-2 adren-
TABLE 1

Affinity ($K_d$) and density ($B_{max}$) of $[^3]$H$RX821002$ binding to alpha-2 adrenergic receptors in bovine eye tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K_d$ (^a)</th>
<th>$B_{max}$ (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliary body</td>
<td>0.136 ± 0.005</td>
<td>101 ± 17</td>
</tr>
<tr>
<td>Iris</td>
<td>0.116 ± 0.006</td>
<td>202 ± 26</td>
</tr>
<tr>
<td>RPE/choriocapillaris</td>
<td>0.134 ± 0.015</td>
<td>99 ± 13</td>
</tr>
<tr>
<td>Neurosensory retina(^b)</td>
<td>0.163</td>
<td>1470</td>
</tr>
</tbody>
</table>

\(^a\) The $K_d$ values are geometric means and the $B_{max}$ values are arithmetic means of four experiments.

\(^b\) Data from Berlie et al. (1995).

The mean values for four experiments are given in Table 1.

**Ocular Alpha-2D Adrenergic Receptors**

Adrenergic receptors in the ciliary body, iris and RPE/choriocapillaris of the bovine eye were assessed by competition radioligand binding studies. Representative curves of the inhibition of $[^3]$H$RX821002$ binding in membrane preparations by rauwolscine, ARC-239 and norepinephrine are shown in figure 2. Table 2 gives the mean $K_d$ values with pseudo-Hill slopes for eight adrenergic antagonists and for norepinephrine. These are a subset of the antagonists that we used previously to characterize the alpha-2A, alpha-2B, alpha-2C and alpha-2D adrenergic receptor subtypes in a variety of tissues (Bylund et al., 1988, 1992; O’Rourke et al., 1994a, 1994b). For purposes of comparison, table 2 includes data for the neurosensory retina, which we have previously shown to contain exclusively the alpha-2B subtype (Berlie et al., 1995), as well as the data for ciliary body, iris and RPE/choriocapillaris. For none of the eight antagonists in the three tissues were the pseudo-Hill slopes significantly <1.0, which is consistent with the conclusion that only a single subtype of alpha-2 adrenergic receptor is present in these bovine eye tissues. Prazosin and ARC-239 are alpha-1-selective antagonists that also differentiate alpha-2A/D from alpha-2B and alpha-2C subtypes. The low affinity of prazosin (2–3 $\mu$M) and ARC-239 (0.5 $\mu$M) indicates that the predominant subtype is the alpha-2A/D rather than the alpha-2B or alpha-2C (table 3). The relatively high affinity of the receptors in the bovine eye tissues for oxymetazoline (20 nM) also eliminates the alpha-2B subtype. Similarly, the affinity of spiroxatrine is consistent with alpha-2A/D rather than the alpha-2B or alpha-2C subtypes. The affinity of rauwolscine (10 nM) is also consistent with the conclusion that only a single subtype of alpha-2 adrenergic receptor is present in the bovine neurosensory retina and bovine pineal gland but clearly different from the human alpha-2A, alpha-2B and alpha-2C subtypes, as determined previously using the human clones transfected into COS cells (table 3).

Rather than comparing affinities for two tissues or receptor subtypes according to one drug at a time, it is often helpful to consider all the drugs in a single comparison by correlating the negative logarithms of $K_d$ values ($pK_d$ values). Figure 3 presents the correlation of the $pK_d$ values for the four bovine eye tissues with our published data for the alpha-2D receptor of the bovine pineal gland. The correlation between each of the four eye tissues and the bovine pineal is excellent (correlation coefficients, $r = .97–1.00$), indicating that the pharmacological characteristics of the alpha-2 receptors in these bovine tissues are identical. These results indicate that in the bovine ciliary body, iris and RPE/choriocapillaris as well as in the neurosensory retina (Berlie et al., 1995), the predominant alpha-2 adrenergic receptor subtype is alpha-2D.

Fig. 1. Saturation of $[^3]$H$RX821002$ binding in the bovine eye tissues. A, Various concentrations of $[^3]$H$RX821002$ (total $[^3]$H$RX821002$) were incubated with membranes prepared from the iris (▲), ciliary body (●), RPE (□) or RPE/choriocapillaris (○). The total binding (closed symbols) and the nonspecific binding (open symbols) as determined with the use of $10^{-4}$ M norepinephrine are shown. B, Specific binding as calculated as the difference between total and nonspecific binding. C, Linear Rosenthal transformation (Rosenthal, 1967) of the specific binding data plotted as bound $[^3]$H$RX821002$ divided by free $[^3]$H$RX821002$ vs. bound $[^3]$H$RX821002$ converted to units of pM. For this experiment, the $K_d$ values as calculated by nonlinear regression analysis were 0.09, 0.10 and 0.09 nM and the $B_{max}$ values were 163, 91 and 75 fmol/mg of protein for the iris, ciliary body and RPE/choriocapillaris, respectively. The mean values for four experiments are given in table 1.
from the four bovine ocular tissues used in this study. In contrast, the rat alpha-2D pKᵢ values are similar to the bovine eye tissues (r = .96–.97), supporting the conclusion that these ocular tissues are alpha-2D. The correlation coefficients for the human alpha-2A and bovine ocular tissues (.86–.89) are much higher than those found with the human alpha-2B and alpha-2C subtypes (.31–.45) but somewhat lower than those with the rat alpha-2D clone (.97–1.00). This is as expected because the alpha-2A and alpha-2D are orthologous subtypes and have similar correlation coefficients, as have been documented in previous studies (O’Rourke et al., 1994b).

**Discussion**

The mechanisms through which alpha-2 adrenergic agents lower intraocular pressure are not 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 trabecular meshwork (increase in trabecular outflow). Recent evidence suggests that alpha-2 agonists may have differential effects at these sites of action. In animal studies, although both apraclonidine and brimonidine have been found to reduce intraocular pressure in part by reducing aqueous flow, brimonidine increases uveoscleral outflow facility, whereas apraclonidine does not (Serle et al., 1991a, 1991b). Similar conclusions have recently been reached for human eyes. Both alpha-2 agonists appear to lower intraocular pressure in part by decreasing aqueous humor production, presumably by acting on the nonpigmented epithelium of the ciliary body. However, brimonidine (Toris et al., 1995a) and oxymetazoline (Wang et al., 1993) appear to increase uveoscleral outflow, whereas apraclonidine increases outflow through the trabecular meshwork (Toris et al., 1995b). To understand the mechanisms of these agents and the differences among them, an understanding of the distribution of the subtype in various eye tissues is needed.

Several techniques have been used to localize alpha-2 adrenergic receptors in the eye. Initial radioligand binding studies indicated that the majority of the alpha adrenergic receptors in the rabbit iris-ciliary body were of the alpha-2 type (Mittag and Tormay, 1985). The alpha-2 adrenergic receptors were also demonstrated to exist in the bovine retina (Bittiger et al., 1980; Van Liefde et al., 1993). An autoradiographic study of alpha-2 adrenergic receptor localization found that alpha-2 adrenergic receptors were most dense in the inner plexiform layer in rats and might be found in the inner nuclear and ganglion cell layer (Zarbin et al., 1986). A similar study found that alpha-2 adrenergic receptors were localized to ocular muscles, ciliary processes and retina in rat and rabbit eye (Elena et al., 1989). Autoradiographic studies in the human eye found alpha-2 adrenergic receptors at high levels in the iris epithelium and ciliary epithelium, as well in the ciliary muscle, retina and retinal pigment epithelium (Matsuo and Cynader, 1992).

More recent efforts have been directed at identifying which alpha-2 adrenergic receptor subtypes are present in the eye. Of the four pharmacological alpha-2 subtypes, only three appear to be present in a given species. The alpha-2A adrenergic receptor is present in humans, rabbits and pigs, whereas alpha-2D subtype is present in the bovine, rat,

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**Fig. 2.** Inhibition of [³H]RX821002 binding by alpha-2 adrenergic antagonists. Various concentrations of rauwolscine (A), ARC-239 (B) or norepinephrine (C), as indicated on the abscissa, were incubated with 0.3 nM [³H]RX821002 and with membranes prepared from the iris (▲), ciliary body (■) or RPE/choriocapillaris (○). The total binding is plotted on the ordinate. The binding in the absence of any inhibiting compound is indicated by data points on the left (at an inhibitor concentration of −11). Norepinephrine at 10⁻⁴ M was included in all experiments to determine nonspecific binding and is included in the curves as the −4 data points. Values are mean ± S.E.M. of duplicate determinations from single experiments. Mean Kᵢ values for three or four experiments are given in table 2.

We also used correlation analyses to compare the pKᵢ values in bovine eye tissues with our published data for the human alpha-2A, alpha-2B and alpha-2C clones, as well as the rat alpha-2D clone (table 4). For the alpha-2B and alpha-2C subtypes of the alpha-2 adrenergic receptor, the correlation coefficients are poor (r = .31–.45), indicating that the characteristics of these receptors are significantly different...
mouse and guinea pig. Because the \( \alpha_2 \)-A and \( \alpha_2 \)-D pharmacological subtypes are species orthologs and thus mutually exclusive in the same species, they are sometimes referred to as the \( \alpha_2 \)-A/D subtype. The affinities of drugs inhibiting the binding of both the agonist \( [125I] \) p-iodo-epinephrine and the antagonist \( [3H] \) rauwolscine are consistent with the conclusion that the majority of the \( \alpha_2 \)-D receptors in the rabbit iris-ciliary body are of the \( \alpha_2 \)-A subtype (Jin et al., 1994). Immunofluorescence microscopy with antibodies to each of the three human \( \alpha_2 \)-subtypes indicates the presence of not only the \( \alpha_2 \)-A subtype but also the \( \alpha_2 \)-B and \( \alpha_2 \)-C subtypes in rabbit ciliary body (Huang et al., 1995). In the bovine retina, radioligand binding studies with \( [3H] \) RX821002 and \( [3H] \) rauwolscine have demonstrated the presence of the \( \alpha_2 \)-A/D receptor and excluded the presence of even a minor population of the \( \alpha_2 \)-B and \( \alpha_2 \)-C subtypes (Berlie et al., 1995). The presence of the \( \alpha_2 \)-D subtype in the bovine retina has been recently confirmed by molecular techniques (Venkataraman et al., 1996).

Our data indicate that the \( \alpha_2 \)-A/D is the main, if not the only, subtype present in the bovine ciliary body, iris, RPE/choriocapillaris and retina. This conclusion is based on a comparison of \( K_e \) values in the ocular tissues with previous data from our laboratory. Some of these earlier experiments were performed with glycylglycine buffer, whereas the current data were obtained using a phosphate buffer. In a recent study, we documented that buffer composition can affect the affinity of antagonists as determined in radioligand binding experiments (Deupree et al., 1996). We demonstrate, however, that the affinity of all antagonists is affected in a consistent fashion, and thus correlation analysis of \( pK_a \) values remains a valid approach for identifying \( \alpha_2 \)-D receptor subtypes. Our conclusion that the \( \alpha_2 \)-A/D is the major subtype in bovine ocular tissues is consistent with radioligand binding studies in the rabbit and pig, which also identify the \( \alpha_2 \)-A subtype as the main subtype (Jin et al., 1994; Wikberg-Matsson et al., 1996). In the human ciliary body, immunofluorescence labeling indicated the presence of \( \alpha_2 \)-B and \( \alpha_2 \)-C subtypes but not the \( \alpha_2 \)-A subtype (Huang et al., 1995). It is of interest that the immunofluorescence is not a quantitative approach, it may be that the other two subtypes are present in such low concentrations that

### Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ciliary body</th>
<th>Iris</th>
<th>RPE/choriocapillaris</th>
<th>Neuroretina</th>
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<tbody>
<tr>
<td></td>
<td>( K_e )</td>
<td>Slope</td>
<td>( K_e )</td>
<td>Slope</td>
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<tr>
<td>RX821002</td>
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<td>4</td>
<td>0.12 ( \pm ) 0.01</td>
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<td>4</td>
<td>10.3 ( \pm ) 0.6</td>
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<tr>
<td>Phentolamine</td>
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<td>4</td>
<td>12.6 ( \pm ) 0.6</td>
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<tr>
<td>Spironolactone</td>
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<td>4</td>
<td>17.4 ( \pm ) 0.9</td>
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</tr>
<tr>
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<td>4</td>
<td>36 ( \pm ) 3</td>
<td>4</td>
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<tr>
<td>ARC-239</td>
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<td>4</td>
<td>1.04 ( \pm ) 459</td>
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<tr>
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<td>1870 ( \pm ) 142</td>
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<td>1.58 ( \pm ) 3149</td>
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<tr>
<td>Norepinephrine</td>
<td>4804 ( \pm ) 318</td>
<td>3</td>
<td>0.89 ( \pm ) 5870</td>
<td>609</td>
</tr>
</tbody>
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### Table 3

<table>
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<tr>
<th>Drug</th>
<th>Bovine eye tissues</th>
<th>Bovine pineal</th>
<th>Human clones</th>
<th>Rat clone</th>
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<td></td>
<td>Ciliary Body</td>
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<td>RPE</td>
<td>Neuroretina</td>
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<tr>
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<td>0.13 ( \pm ) 0.02</td>
<td>0.16 ( \pm ) 0.02</td>
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<tr>
<td>Phentolamine</td>
<td>12.0 ( \pm ) 0.4</td>
<td>0.12 ( \pm ) 0.01</td>
<td>0.13 ( \pm ) 0.02</td>
<td>0.16 ( \pm ) 0.02</td>
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<tr>
<td>WB 4101</td>
<td>39 ( \pm ) 6</td>
<td>0.12 ( \pm ) 0.01</td>
<td>0.13 ( \pm ) 0.02</td>
<td>0.16 ( \pm ) 0.02</td>
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<tr>
<td>Rauwolscine</td>
<td>11.4 ( \pm ) 0.5</td>
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<td>8.8 ( \pm ) 0.1</td>
<td>6.1 ( \pm ) 0.1</td>
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<tr>
<td>Spironolactone</td>
<td>20.0 ( \pm ) 1.5</td>
<td>17.4 ( \pm ) 0.9</td>
<td>16.2 ( \pm ) 0.2</td>
<td>17.2 ( \pm ) 2.8</td>
</tr>
<tr>
<td>Prazosin</td>
<td>551 ( \pm ) 64</td>
<td>459 ( \pm ) 17</td>
<td>440 ( \pm ) 50</td>
<td>367 ( \pm ) 34</td>
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<tr>
<td>Norepinephrine</td>
<td>4804 ( \pm ) 318</td>
<td>5870 ( \pm ) 609</td>
<td>1787 ( \pm ) 119</td>
<td>1469 ( \pm ) 305</td>
</tr>
</tbody>
</table>

\( a \) Data from O’Rourke et al. (1994a) and Simonneaux et al. (1991).
\( b \) Data from Bylund et al. (1992) and O’Rourke et al. (1994a).
they are not detected by the radioligand binding technique or that they are not functional proteins. The lack of immunofluorescence to the alpha-2A in the human is of concern because this is the major subtype detected by the radioligand technique in all three species investigated to date: rabbit, porcine and bovine. As Huang et al. (1995) point out, the alpha-2A subtype may well exist in the human but was not detected in their immunofluorescence experiments, perhaps because the antibody used was not sufficiently sensitive. It will be important to determine whether this apparent discrepancy is really due to a species difference or the immunofluorescence technique is providing misleading data. Thus, the alpha-2 subtypes in human ocular tissues need to be identified by the radioligand binding technique.

The alpha-2 adrenergic agonists are increasingly used as adjunctive agents with beta adrenergic blockers in stepwise glaucoma therapy. The ocular hypotensive effects of some adrenergic drugs may not always correlate with the protection of the optic nerve, possibly because of a reduction in posterior segment blood flow. However, the vasoconstrictive side effects of apraclonidine are seen mainly in the anterior segment and apparently do not affect blood flow to the optic nerve. Nevertheless, long-term studies are needed to evaluate the extent to which apraclonidine and other alpha-2 adrenergic agents preserve visual function. An understanding of the alpha-2 adrenergic receptor subtype mediating the ocular hypotensive effects of alpha-2 adrenergic receptor agonists will be important in designing new alpha-2 agents with increased specificity and reduced side effects.

References


