Potent α_{2A}-Adrenoceptor–Mediated Vasoconstriction by Brimonidine in Porcine Ciliary Arteries

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Purpose. An investigation into whether α_2-adrenoceptor agonists induce contractions in the porcine ciliary arteries and to characterize the functional receptor subtype mediating these responses.

Methods. Isolated arteries from the intraocular part of the porcine ciliary artery were suspended in microvascular myographs for isometric tension recording. The segments were contracted with the α_2-adrenoceptor agonists brimonidine, apraclonidine, and oxymetazoline. To determine which subtypes of the α_2-adrenoceptor mediate this contraction, antagonists subselective for the different α_2-adrenoceptors were added to the vessel bath before concentration–response curves for brimonidine were obtained. The following α_2-adrenoceptor antagonists were applied: BRL44408 (α_{2A}-selective), ARC239 (α_{2B}- and α_{2C}-selective), and prazosin (α_{2B}- and α_{2C}-selective).

Results. The α_2-adrenoceptor agonists induced vasoconstriction in the porcine ciliary artery with the following potency order (EC_{50}) expressed in nanomolar: brimonidine 2.11, oxymetazoline 5.26, and apraclonidine 13.0. As a reference, noradrenaline was tested, and its EC_{50} was determined to be 247 nM in the ciliary artery. In the porcine ciliary arteries BRL44408, ARC239, and prazosin caused concentration-dependent and parallel rightward shifts of the concentration–response curves for brimonidine. Schild analyses for the antagonists against brimonidine yielded regression lines with slopes of unity and functional antagonist potencies (pK_B) for BRL44408 (7.8), ARC 239 (5.8) and for prazosin (6.0) suggesting the presence of functional α_{2A}-adrenoceptors. Moreover, there was a good correlation of pK_B with ligand-binding affinity (pK_i) of the α_{2A}-adrenoceptor in the porcine eye tissue.

Conclusions. The α_2-adrenoceptor agonists brimonidine, apraclonidine, and oxymetazoline are potent vasoconstrictors in the porcine ciliary artery. In the present work, it was shown for the first time that the α_{2A}-adrenoceptor subtype mediates this contraction. (*Invest Ophthalmol Vis Sci.* 2001;42: 2049–2055)

The α_2-adrenoceptor agonists brimonidine and apraclonidine are both powerful ocular hypotensive agents when applied topically. Brimonidine is established for treatment of primary open-angle glaucoma in patients with high intraocular pressure (IOP). Apraclonidine is mainly used to prevent increased IOP after argon laser or YAG laser treatment in the anterior segment of the eye.[1–3] Both apraclonidine and brimonidine lower the IOP by decreasing aqueous flow.[4–6] The mechanism of action of these drugs is thought to be by activation of the α_2-adrenoceptors in the ciliary body, which decreases cyclic adenosine monophosphate (cAMP) levels, causing a decrease of the aqueous humor production.[7] Although the main mechanism behind the IOP reduction of apraclonidine and brimonidine is a reduction of flow,[5,8,9] it has been suggested that they may also have some effect on outflow,[10,11] but the detailed mechanisms have not yet been completely clarified.

The posterior ciliary arteries of the eye are innervated by sympathetic nerve fibers and supply blood to the optic nerve, the choroid, the iris, and the ciliary body.[12–15] It is generally accepted that postjunctional α_1- and α_2-adrenoceptors coexist in the systemic vasculature.[16,17] The α_1-adrenoceptors cause vasoconstriction, whereas α_2-adrenoceptors mediate vasodilation in large conductive arteries,[18] but vasopressor α_2-adrenoceptors are well known in the resistance vasculature of both humans and animals.[19–21] In the eye, functional studies with different in vitro techniques have demonstrated α_2-adrenoceptors in the vascular bed. It has been shown by using the microsphere method that α_2-adrenoceptor agonists decrease blood flow in the rabbit choroid and the ciliary body.[22,23] The α_2-adrenoceptors were verified in the bovine ciliary artery, where isolated segments of the intraocular part of the artery were studied on a small-vessel myograph.[24]

On the basis of pharmacologic and molecular cloning evidence, α_2-adrenoceptors have been divided into three subtypes: α_{2A}, α_{2B}, and α_{2C}.[25–27] All three subtypes have been cloned from the human, but only the α_{2A} subtype has been cloned in the pig.[28] We have investigated by pharmacologic characterization α_2-adrenoceptor subtypes from the iris, the ciliary body, the retina, and the choroid of the porcine eye.[29] By using radioligand binding, we identified dense populations of the α_{2A}-adrenoceptors in the choroid (900 femtomoles/mg) and the ciliary body (220 femtomoles/mg).

Functional studies of the different α_2-adrenoceptor subtypes have, to our knowledge, never been demonstrated in isolated segments of ocular arteries or veins. The posterior ciliary arteries supply blood to the choroid and the ciliary body of the eye. The purpose of the present study was to investigate whether some of the α_2-adrenoceptor agonists, used in the clinical practice, induce vasoconstriction in the porcine ciliary arteries and to characterize the α_2-adrenoceptor subtypes involved in these responses by use of selective antagonists: for the α_{2A}-adrenoceptor, BRL44408, and for the α_{2B}- and α_{2C}-adrenoceptors, ARC239 and prazosin.

Methods

Preparation of Blood Vessels

Fresh porcine eyes were obtained from the local abattoir. The ciliary arteries, located intraocularly before they penetrate the ciliary body, were identified, quickly dissected, and placed in Ca^{2+}-free physiological salt solution (PSS) at 4°C. Endothelium-intact ring segments (~2 mm) were mounted on 40-μm wires in a small-vessel myograph for

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isometric recordings (J. P. Trading, Aarhus, Denmark).[30] The experiments were performed in PSS of the following composition (in mM): 119 NaCl, 4.7 KCl, 1.5 $CaCl_2$, 1.17 $MgSO_4$, 1.18 KH_2PO_4, 25 $NaHCO_3$, 0.027 EDTA, and 6 glucose. The preparations were allowed to equilibrate in oxygenated (95% O_2 and 5% CO_2) PSS at 37°C (pH 7.4), for approximately 30 minutes. The level of optimal passive tension for the porcine ciliary arteries was determined in preliminary experiments ($n = 3$) and defined as that tension at which the contraction to 125 mM KCl was maximal. The relationship between resting wall tension and the internal circumference was then determined, and the internal circumference, L_{60}, corresponding to a transmural pressure of 60 mm Hg for a relaxed vessel, was calculated. A maximum level of 60 mm Hg was chosen, because higher pressure exposure destroyed the vessels. The vessels were set to the internal circumference L_1, arrived at by $L_1 = 0.9 \times L_{60}$. The effective internal lumen diameter was determined as $l_1 = L_1/\pi$.[31] The final resting tension after normalization was found to be between 1.5 and 2.5 mN.

The vessels were used either immediately or saved for the next day (and used within 36 hours). The preparations were kept in PSS in the refrigerator until used. There was no difference in response to α_2-adrenoceptor agonists between these preparations and fresh preparations that were used immediately after dissection.

Experimental Procedure

After normalization, the contractile ability of the vessels was tested by stimulating the arterial rings with 125 mM K-PSS (equivalent to PSS but with NaCl replaced with KCl on an equimolar basis, giving a final concentration of 125 mM K^+), which was continued until reproducible responses were recorded. After washout, the preparation was exposed to noradrenaline (10^{-6} M) and allowed to contract for approximately 5 minutes. The PSS contained propranolol (10^{-6} M) to inhibit possible activity from β-adrenergic receptors and indomethacin (2.8×10^{-6} M) to prevent synthesis of endogenous prostaglandins.

Responses to α_2-Adrenoceptor Agonists. Cumulative concentration–response curves for brimonidine, apraclonidine, and oxymetazoline were constructed, and the agonists were added to the baths cumulatively in half-log increments. Two ring segments of the same vessels were used in parallel at the same time: A concentration–response curve for brimonidine was performed on one segment and a concentration–response curve for brimonidine in the presence of antagonist was performed on the other segment. Unfortunately, it was not possible to repeat the experiment in the same vessel, because the contractions induced by the α_2-adrenoceptor agonists were reduced in a second exposure, excluding the repetition of the concentration–response curve for any of the α_2-adrenoceptor agonists. In the experiments with oxymetazoline the tissues were incubated with 1×10^{-6} M rauwolscine for 5 minutes to protect the α_2-adrenoceptor before adding 3×10^{-6} M phenoxybenzamine for 20 minutes to alkylate the α_1-adrenoceptors, according to the method of MacLennan et al.[32] After washing every 5 minutes for at least 60 minutes the contraction of the ciliary arteries were tested with phenylephrine (1×10^{-6} M). If the vessel responded to phenylephrine, it was excluded from the experiment. Concentration–response curves were then determined with oxymetazoline.

Responses to Brimonidine in the Presence of Antagonists. The effect of different α_2-adrenoceptor subselective antagonists on the concentration–response curves for brimonidine was evaluated. In these experiments the following antagonists were used: BRL44408 which is selective for the α_{2A}-adrenoceptor; ARC239, which shows low affinity for the α_{2A}-adrenoceptor and high affinity for the α_{2B}- and α_{2C}-adrenoceptors in the pig[29,33]; and prazosin, which shows low affinity for the α_{2A}-adrenoceptor and high affinity for the α_{2B}- and α_{2C}-adrenoceptors.[26] The concentrations of antagonists 10^{-4} M; and BRL44408, 10^{-8} to 10^{-6} M. As a control, another segment of the same vessel or a segment from a parallel vessel of the porcine ciliary artery from the same eye was used. The antagonist was added 30 minutes before the cumulative agonist concentration curves were determined.

Drugs

Indomethacin, (−)-noradrenaline bitartrate, papaverine hydrochloride, L-phenylephrine hydrochloride, and propranolol were purchased from Sigma Chemical Co. (St. Louis, MO). Oxymetazoline hydrochloride, *p*-aminoclonidine (apraclonidine), phenoxybenzamine hydrochloride, prazosin hydrochloride, rauwolscine hydrochloride, and [5-bromo-*N*-(dihydro-1*H*-imidazol-2-yl)]-6-quinoxalinamine (UK14304, brimonidine) were from Research Biochemicals International (Natick, MA). 2-[(4,5-Dihydro-1*H*-imidazol-2-yl) methyl]-2,3-dihydro-1-methyl-1*H*-isoindole (BRL44408) was from Tocris Cockson Ltd, Bristol, UK. 2-[2-(4-(2-Methoxyphenyl) piperazin-1-yl)ethyl]-4,4-dimethyl-1,3(2*H*,4*H*)-isoquinolindione (ARC239) was a gift from Karl Thomae GmbH, (Biberach, Germany).

Analysis of Data

The concentration–response curves were fitted to the Hill equation and calculated by nonlinear regression on a computer (Prism; GraphPad Software, San Diego, CA). Sensitivities to drugs were calculated on the basis of data from individual vessels and are expressed as EC_{50}—that is, the agonist concentration needed to produce 50% of the maximal response. If antagonists produced parallel displacements of agonist concentration–response curves, Schild analysis was constructed by use of least-squares linear regression of log (CR − 1) against log antagonist concentration, where CR is the concentration ratio of the agonist in the absence and presence of antagonist.[34] Concentration ratios were calculated at the EC_{50} level. Provided that the regression of the Schild plot is linear and that the slope is not significantly different from unity, pA_2, which is the intercept along the abscissa scale of the Schild plot, is equal to the negative logarithm of the equilibrium dissociation constant for the antagonist: $pA_2 = -\log K_B = pK_B$. The slope was also constrained to unity and more precise value of pK_B was calculated. The solver function of the statistical analysis software (Excel; Microsoft, Redmond, WA) was used to fit the model of linear regression.

Results

The experiments were performed on ciliary arteries with an internal diameter of 246 ± 27 μM (measured from 10 vessels). A concentration of 125 mM K-PSS induced a contraction of 2.4 ± 0.02 Nm ($n = 220$) in these arteries.

Agonist Studies

As shown in Table 1 the rank order of potency of the agonists based on the EC_{50} values was brimonidine > oxymetazoline > apraclonidine ≫ noradrenaline. All agonists produced monophasic concentration–response curves in the porcine ciliary artery (Figs. 1, 2). In the experiments with oxymetazoline the tissues were first treated with 3×10^{-6} M phenoxybenzamine in the presence of 1×10^{-6} M rauwolscine to inactivate the α_1-adrenoceptors (see the Methods section). The same method was also used in some pilot experiments with the porcine ciliary arteries to evaluate the effect of brimonidine in the presence of phenoxybenzamine. The mean of the calculated EC_{50} ($n = 4$) from the concentration–response curves for brimonidine with phenoxybenzamine were $6.3 \pm 1.0 \times 10^{-9}$ M and the maximal response (*T*) was 2.9 ± 0.6 Nm. The maximal response of phenylephrine (1×10^{-6} M) before adding phenoxybenzamine was 2.4 ± 0.8 Nm, and after phenoxybenzamine, phenylephrine (1×10^{-6} M) merely raised

TABLE 1. Potencies of Various Agonists in the Intraocular Part of the Porcine Ciliary Artery in the Presence of Propranolol (1×10^{-6} M) and Indomethacin (2.8×10^{-6} M)

Agonist	n	EC_{50} ($\times 10^{-9}$ M)	Slope	T (Nm^{-1})
Noradrenaline	6	247 ± 104*	1.0 ± 0.13	3.3 ± 0.8
Brimonidine	26	2.11 ± 0.21	1.28 ± 0.08	3.1 ± 0.4
Oxymetazoline	12	5.26 ± 1.11	1.67 ± 0.25	2.2 ± 0.4
Apraclonidine	12	13.0 ± 6.67	1.55 ± 0.17	3.0 ± 0.4

Values are mean ± SEM. n, number of vessel segments. The slope is determined from the Hill equation. EC_{50} is the concentration of agonist required to produce half-maximal contraction in the response obtained at the highest concentration of agonist applied. T (tension) describes the maximal response obtained with the respective substance.

* Denotes a significant-difference parameter of $P < 0.001$ versus brimonidine, paraminoclonidine, and oxymetazoline (Bonferroni test). In the experiments with oxymetazoline the vessels were incubated with 3×10^{-6} M phenoxybenzamine.

performed in exactly the same way but without incubating the vessel segments with phenoxybenzamine. The mean EC_{50} values of the control sepcimens ($n = 4$) were $6.4 \pm 2.1 \times 10^{-9}$ M, and the maximal response was 2.5 ± 0.5 Nm. The conclusion drawn from these results was that incubation with phenoxybenzamine changed neither the EC_{50} nor the effect of response in the porcine ciliary arteries. Therefore, experiments with brimonidine were performed without prior incubation of phenoxybenzamine. All agonists induced comparable maximal responses in the arterial segments of the ciliary artery (Table 1).

Antagonist Studies

To identify the subtypes of α_2-adrenoceptors mediating the vasoconstrictive response of the porcine ciliary artery, concentration–response curves for brimonidine were performed in the presence of α_2-adrenoceptor subtype–selective antagonists. Increasing concentrations of the α_{2A}-selective antagonist BRL44408 (10^{-8}, 3×10^{-8}, 10^{-7}, 3×10^{-7}, and 10^{-6} M), caused parallel rightward shifts for the brimonidine concentration–response curves without affecting the maximum response, indicating a competitive antagonism. Analysis of the data by Schild regression gave a slope (0.90 ± 0.10) that was not significantly different from unity and pA_2 of 7.85 ± 0.12 (Figs. 3A, 3D). The antagonist ARC239 (concentrations: 10^{-6}, 3×10^{-6}, 10^{-5}, 3×10^{-5}, and 10^{-4} M), produced rightward shifts of the concentration–response curves for brimonidine, with no depression of the maximum response. Analysis of the data by Schild regression gave a slope that was not significantly different from unity (0.98 ± 0.10) and pA_2 of 5.86 ± 0.12 (Figs. 3B, 3D). The antagonist prazosin (concentrations: 10^{-6}, 3×10^{-6}, and 10^{-5}) produced rightward shifts of the concentration–response curves for brimonidine, without reducing the maximum responses over the ranges used for analysis. Analysis of the data by Schild regression gave a slope that was not different from unity (1.0 ± 0.10) and pA_2 of 6.02 ± 0.06 (Figs. 3C, 3D). The Schild plots for all antagonists were constrained to unity giving pK_B of ARC239 (5.84 ± 0.12), BRL44408 (7.77 ± 0.12), and prazosin (6.02 ± 0.07). The values for pA_2 and pK_B obtained from the analysis of the concentration–response curves are given in Table 2.

DISCUSSION

The first interesting finding of the present study is the clear vasoconstrictors in the intraocular part of the porcine ciliary artery. It has been shown that apraclonidine and brimonidine, both of which are used clinically, have a 10- to 100-fold higher potency than noradrenaline. The agonist oxymetazoline was included in the study, because it has previously been shown to be α_{2A}-adrenoceptor selective in porcine tissues.[33] Because oxymetazoline has affinity for α_1- as well as α_2-adrenoceptors in the pig,[35] the α_1-adrenoceptors were inactivated by treatment of the vessels with phenoxybenzamine (3×10^{-6} M) in the presence of rauwolscine (1×10^{-6} M) according to MacLennan et al.[32] The choice of brimonidine as agonist in the antagonist experiments was based on the higher selectivity for α_2-adrenoceptor versus α_1-adrenoceptor (790-fold), compared with apraclonidine (100-fold) in binding assays.[36,37]

The presence of α_2-adrenoceptors in the ocular vessels of both the animal and the human is not a clear-cut fact, however. In vitro studies by Nyborg and Nielsen,[24] who used the myograph technique on isolated vessels, have verified α_2-adrenoceptors in the intraocular part of the bovine ciliary artery. However, Yu et al.[38] found only α_1-adrenoceptors in the extraocular part of the human ciliary arteries. Retinal vessels have a smaller diameter (30–35 μm) than the ciliary artery and consequently are technically more difficult to study in vitro. Therefore, most studies are performed with noninvasive techniques. However, Spada et al.[39] studied the effect of α_2-adrenoceptor agonists on human retinal vessels transplanted into hamster cheek pouch membrane, and vasoconstriction was observed with apraclonidine but not with brimonidine, which is more α_2-adrenoceptor selective, suggesting that the α_2-adrenoceptors do not act as vasoconstrictors there.

Several studies in human ocular vessels have been performed with noninvasive techniques such as color laser Doppler. Topically applied brimonidine has not shown any effect on the flow velocity in various ocular or retrobulbar vessels measured with this technique.[40,41] Still, because it is not known to what extent the topically applied drug reaches therapeutic concentrations in the retina or the retrobulbar tissues, these results do not exclude the presence of α_2-adrenoceptors in those vessels.

The variability in myogenic tone of the vessel segments could be another explanation for the variable response evoked by α_2-adrenoceptors in vitro in eye arteries. Dunn et al.[42] suggest that the α_2-adrenoceptors are only activated in the presence of a small increase in tone of the vessels. In the work by Nyborg and Nielsen[24] the vessels were precontracted with 30 mM K^+ or prostaglandin $F_{2\alpha}$ to raise the myogenic tone before adding the α_2-adrenoceptor agonist, which was not the case in the study by Yu et al.[38] In the porcine ciliary arteries, raising basal tone by increasing the extracellular K^+ concentration did not change the contractile responses induced by brimonidine, compared with responses obtained in physiological buffer (Wikberg-Matsson, unpublished observation, 2001). Therefore, we did not precontract the vessels before the α_2-adrenoceptor agonist was added.

The regional differences of α_2-adrenoceptor distribution throughout the different parts of the vascular bed is a well known fact.[43] One possible reason that α_2-adrenoceptor agonists induce a powerful vasoconstriction in the isolated ciliary arteries and not in the retinal vessels is that the ciliary arteries contain higher amounts of α_2-adrenoceptors than do other smaller vessels of the eye. Earlier studies support this theory: Resistance arteries (to which the ciliary arteries belong) show well-pronounced contractions in response to α_2-adrenoceptor stimulation, and there is also a correlation between the diameter of the vessel and the response by α_2-adrenoceptor agonists, both in humans and animals.[20,21,44]

In summary, the information on α_2-adrenoceptors in the

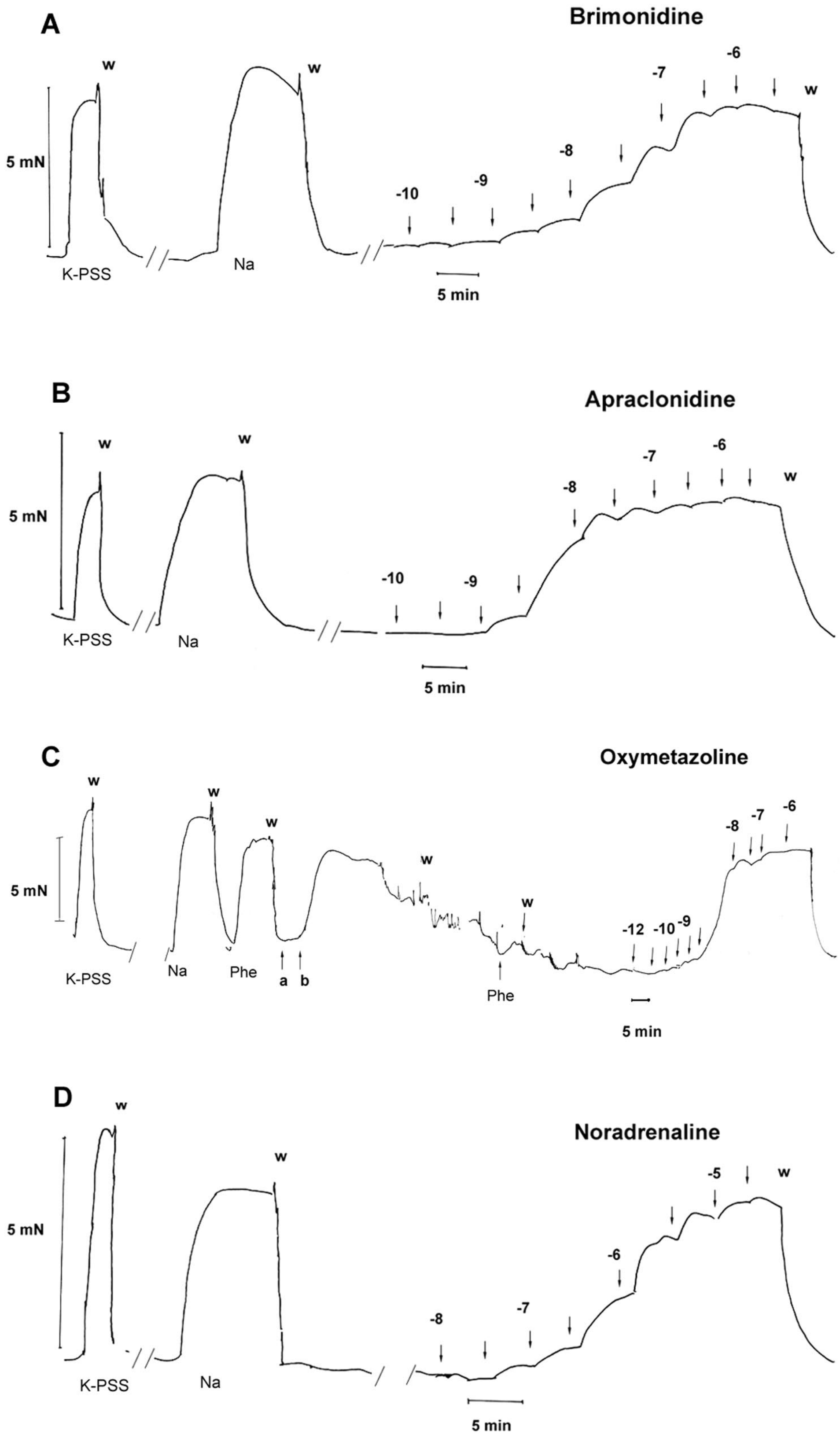

FIGURE 1. Original trace recordings showing the effect of (**A**) brimonidine, (**B**) apraclonidine, (**C**) oxymetazoline, and (**D**) noradrenaline (Na) at increasing concentrations (in half-log units indicated by *arrows*) of the agonists in the porcine ciliary artery. The contractility of the vessels are first tested with 125 mM K-PSS and 1×10^{-6} M noradrenaline. The experiments were performed in the presence of 10^{-6} M propranolol and 2.8×10^{-6} M indomethacin. In the experiment with oxymetazoline (**C**), 3×10^{-6} M phenoxybenzamine (b) was added 5 minutes after 1×10^{-6} M rauwolscine (a), and the contractility of the vessel was also tested with 1×10^{-6} M phenylephrine (Phe) before and after the treatment with phenoxybenzamine. w, wash.

several reasons. There are methodological aspects (i.e., it is difficult to compare the in vitro techniques with the noninvasive techniques), the distribution of the α_2-adrenoceptors in the vascular beds vary, and the distribution of α_2-adrenocep-

The second important finding of the present study was that the α_{2A}-adrenoceptors mediate vasoconstriction in the examined intraocular part of the porcine ciliary artery. This conclusion is based on the fact that the selective α_{2A}-adrenoceptor

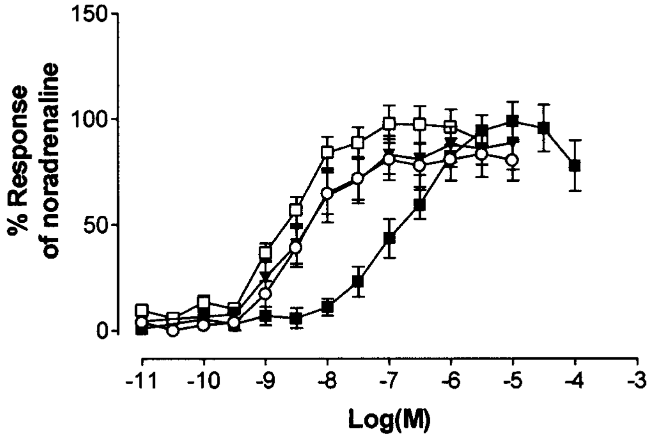

FIGURE 2. Cumulative concentration–response curves for the vasoconstrictive effects of brimonidine (□), apraclonidine (▼), oxymetazoline (○), and noradrenaline (■) in the presence of 1×10^{-6} M propranolol and 2.8×10^{-6} M indomethacin. Values are means ± SEM (n = 6–23 preparations). The concentration–response curves for oxymetazoline were obtained after incubation with phenoxybenzamine (3×10^{-6} M).

displacement of the concentration–response curve for brimonidine. Significant parallel displacement occurred at high concentrations of ARC239 and prazosin, both of which are antagonists with low affinity for α_{2A}-adrenoceptor and high affinity for the α_{2B}- and α_{2C}-adrenoceptor.

In two previous studies the α_2-adrenoceptor subtypes were characterized pharmacologically in major organs including eye tissues of the pig.[29,33] By using radioligand binding, we identified dense populations of α_{2A}-adrenoceptors in the choroid (900 femtomoles/mg) and ciliary body (270 femtomoles/mg), but less density in the iris (87 femtomoles/mg). Furthermore, drug affinities for selective antagonists were evaluated. Among these, BRL44408 was shown to be selective for the pig α_{2A}-adrenoceptor, whereas ARC239 showed low affinity for the α_{2A}-adrenoceptor and high for the α_{2B}- and α_{2C}-adrenoceptors in the pig. Prazosin which was included in the present study has been shown by other authors to have low affinity for the α_{2A}-adrenoceptor and high for α_{2B}- and α_{2C}-adrenoceptors.[26,45] The K_d values (in this study recalculated to pK_i) obtained from the ligand binding studies give an accurate guide to the affinities of the antagonists at the respective subtypes of receptor. Given in Table 2 are the pK_B values obtained from concentration–response curves with subselective antagonists in the present study and pK_i values of the affinities obtained from binding experiments in the earlier studies. The good correlation of pK_i and the pK_B implies that the α_{2A}-adrenoceptor is responsible for the contraction induced by α_2-agonists.

Our conclusion that the α_{2A}-adrenoceptor mediates contraction in the porcine ciliary artery is not a great surprise in the view of the earlier study of porcine tissue homogenates of the iris, choroid, and ciliary body which also demonstrated only the α_{2A}-adrenoceptor. From the pig retina, however, both the α_{2A}- and α_{2C}-adrenoceptors were detected in lower densities (20 and 3.6 femtomoles/mg, respectively).[29] Radioligand binding has also been used to characterize α_2-adrenoceptor subtypes in other species: in the ciliary body of the rabbit[46] and in the ciliary body, iris, choroid, and retina of the cow.[47,48] Comparable to the binding studies in animals, a study of human ocular tissue homogenates showed only the α_{2A}-adrenoceptor in the human iris, ciliary body, and choroid.[49] According to the results in both animals and humans, binding studies indicate that the predominant subtype is the α_{2A} subtype in the richly vascularized tissues of the eye, such as the choroid and the ciliary body. This suggests that the human ciliary vessels also contain α_{2A}-adrenoceptors.

In contrast to these binding data, immunofluorescence labeling of the human ciliary body indicates the presence of α_{2B}- and α_{2C}-adrenoceptor subtypes, but not the α_{2A} subtype.[50] 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.[50]

Which of the three α_2-adrenoceptor subtypes is coupled to the inhibition of aqueous humor production in the human eye is not known, however. Furthermore, it remains to be evaluated whether the α_{2A}-adrenoceptors are involved in the vaso-

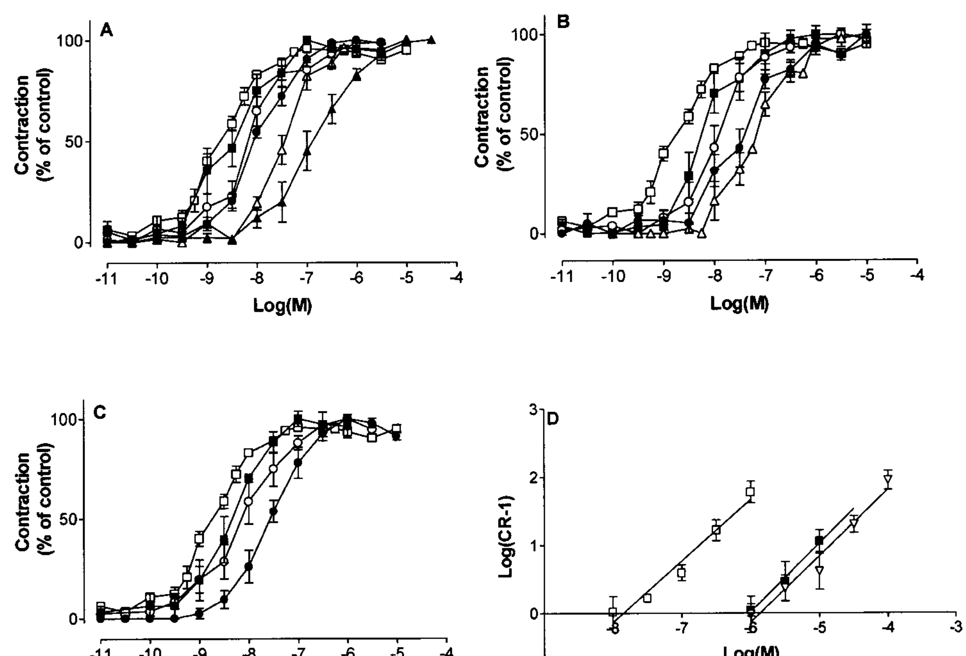

FIGURE 3. Cumulative concentration–response curves for brimonidine (□) and presence of **(A)** BRL44408 in concentrations of 10^{-8} M (■), 3×10^{-8} M (○), 10^{-7} M (●), 3×10^{-7} M (△), and 10^{-6} M (▲); **(B)** ARC239 at 3×10^{-6} M (■), 10^{-5} M (○), 3×10^{-5} M (●), and 10^{-4} M (△); **(C)** prazosin, at 10^{-6} M (■), 3×10^{-6} M (○), and 10^{-5} M (●); and **(D)** the Schild regression for the effect of BRL44408 (□), ARC239 (■), and prazosin (▽) on concentration–response curves of brimonidine in the porcine ciliary artery. The experiments were performed in the presence of 10^{-6} M propranolol and 2.8×10^{-6} M indomethacin. The results are means ± SEM of four to

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