Alpha-1 Adrenergic Receptors on Rabbit Retinal Pigment Epithelium

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Retinal pigment epithelium (RPE)-choroid-sclera preparations from black, dutch-belted rabbits were sealed in an Ussing chamber. The RPE-generated trans-RPE voltage (V_e) and electrical resistance (R) were monitored. Epinephrine (an alpha and beta adrenergic agonist) reduced V_e by as much as 39% without affecting R. A response to epinephrine was noted at concentrations as low as 1.4 × 10^-7 M. Phenylephrine (an alpha adrenergic agonist) had essentially the same effect as epinephrine at identical concentrations. Clonidine (an alpha-2 adrenergic agonist) had a very slight effect but only at 10^-4 M. Isoproterenol (a beta adrenergic agonist) had no apparent effect upon V_e or R. The RPE response to epinephrine and phenylephrine was blocked by the alpha adrenergic antagonist phentolamine and by the alpha-1 adrenergic antagonist prazosin but not by the alpha-2 adrenergic antagonist yohimbine or by the beta adrenergic antagonist propranolol. Dibutyryl cyclic AMP (in the presence and absence of IBMX) and cyclic GMP (as cGMP and in the dibutyryl form) had no apparent effect upon V_e or R. These results indicate that rabbit RPE possesses an alpha-1 adrenoreceptor which, when stimulated, substantially reduces the RPE generated trans-RPE electrical current. Invest Ophthalmol Vis Sci 29:737-741, 1988

The retinal pigment epithelium (RPE) is an epithelial layer that lies between the photoreceptors and the rich blood supply of the choriocapillaris. An important role of the RPE is to maintain the appropriate extracellular environment in the outer retina necessary for normal photoreceptor function.1 The trans-epithelial transport properties of the RPE have been studied in vitro in several laboratories.2-4 In addition to determining the components of RPE active transport, it has been important to understand how this transport is regulated. Cyclic AMP (cAMP) has been reported to slow bullfrog RPE-mediated fluid movement5 and ion transport6 in vitro. In vivo rabbit experiments have suggested that cAMP slows RPE-mediated fluid movement while cyclic GMP (cGMP) accelerates this fluid movement.7

We have recently developed an in vitro rabbit RPE-choroid-sclera preparation that is well suited for studying the effects of pharmacologic agents (applied to the RPE apical membrane) on RPE-generated trans-RPE electrical current.8 This electrical current, more commonly called the “short circuit current” (I_sc), has been related to RPE transport in several species.5-6 Since RPE-mediated fluid movement in rabbits is dependent upon active transport,9 it seemed important to determine the effects of cAMP and cGMP on rabbit RPE I_sc. Three of the four known adrenergic receptor types use cAMP as their “second messenger.”10 Therefore, we also sought to determine whether rabbit RPE possesses adrenergic receptors that might modify intracellular cyclic nucleotide levels and serve in vivo as regulators of RPE transport. The experiments described herein show that dibutyryl cAMP (in the presence and absence of IBMX) and cGMP (as cGMP and in the dibutyryl form) do not affect I_sc. However, the experiments do show that rabbit RPE possesses an alpha-1 adrenergic receptor that, when stimulated, substantially reduces the RPE-generated trans-RPE electrical current.

Materials and Methods

Rabbit retinal pigment epithelium (RPE)-choroid-sclera explants were sealed within an Ussing chamber using techniques that we have previously described.8 We have adhered to the principles of the ARVO Resolution on the Use of Animals in Research. Briefly, black dutch-belted rabbits were anesthetized with xylazine (27 mg/kg IM, Rompun®, Miles Laboratories, Elkhart, IN) and ketamine (130 mg/kg IM, Vetalar®, Parke-Davis, Morris Plains, NJ).11 For one set of experiments, pentobarbital (36 mg/kg subcutaneously) was used instead of xylazine.9 The retina was separated from the RPE by making total retinal detachments in vivo using a technique previously described.12 Pupillary dilation (required to make the...
Epinephrine at $1.4 \times 10^{-4}$ M reduced the short circuit current ($I_{sc}$) of preparations by 39%. Epinephrine has both alpha and beta adrenergic activity. Residual detachments) was obtained with one drop of 1% cyclopentolate (AK-Pentolate®, Akorn Inc., Abita Springs, LA). The rabbits' eyes were then enucleated and RPE-choroid-sclera explants were obtained and sealed in an acrylic chamber such that the explants separated two otherwise electrically isolated saline solutions. Electronics within the chamber allowed us to measure the spontaneous transepithelial voltage ($V_e$) and electrical resistance ($R$) of the preparations. The RPE-generated trans-RPE electrical current or "short circuit current" ($I_{sc}$) was calculated from $V_e$ and $R$ using Ohm's Law. Previous work with these preparations has shown that all of the $V_e$ and essentially all of the $R$ can be attributed to the RPE.\textsuperscript{9}

The bathing media consisted of (in millimoles per liter): 143 Na, 3.6 K, 1.2 Ca, 2.5 Mg, 125 Cl, 23 HCO$_3$, 2.5 SO$_4$, 0.5 PO$_4$, 10 glucose. Water-saturated 5% CO$_2$–95% O$_2$ was bubbled into each side of the chamber to provide mixing and to maintain a pH of 7.4 ($\pm$0.1). The chamber was surrounded by a water jacket and kept at 38–39°C.

The pharmacologic agents employed in these experiments were epinephrine, phenylephrine, clonidine, isoproterenol, propranolol, phentolamine, yohimbine, prazosin, N$_6,2'$-O-dibutyryladenosine 3':5'-cyclic monophosphate (dibutyryl cAMP), N$_2,2'$-O-dibutyrylguanosine 3':5'-cyclic monophosphate (dibutyryl cGMP), guanosine 3':5'-cyclic monophosphate (cGMP), and 3-isobutyl-1-methyl-xanthine (IBMX). Except for isoproterenol (Elkins-Sinn, Cherry Hill, NJ), phentolamine (Regitine® IV, CIBA, Inc., Summit, NJ), propranolol (Inderal® IV, Ayerst, New York, NY), and prazosin (Minipress®, Pfizer, New York, NY), these agents were obtained from Sigma Chemical Co. (St. Louis, MO).

Experiments consisted of sealing the RPE-choriocapillaris explants within the Ussing chamber and taking baseline measurements. The desired drug, typically in 100 µl volumes, was then injected into one (or both) sides of the chamber and its effect upon $V_e$, $R$, and $I_{sc}$ determined. Except where specifically noted, agents were added to both sides of the preparation.

Results

Typical RPE-choriocapillaris preparations produced a spontaneous transepithelial voltage ($V_e$) of 12.5 mV (apical side positive) and an electrical resistance ($R$) of 350 ohm-cm$^2$ that gradually fell during the course of the experiments. RPE-choriocapillaris preparations typically lose $V_e$ and $R$ with time,\textsuperscript{4,8} presumably because the conditions in the Ussing chamber do not perfectly replicate the in vivo state as a consequence of which the preparations deteriorate.

When epinephrine, an adrenergic agonist with both alpha and beta activity,\textsuperscript{13} was added to both sides of the preparation, there was a marked fall in $V_e$ and calculated short circuit current ($I_{sc}$) with no effect upon $R$. At epinephrine concentrations of $1.4 \times 10^{-4}$ M, $I_{sc}$ fell by 39% ($\pm$6% SD, n = 5). A typical experiment is illustrated in Figure 1. When lower concentrations of epinephrine were used, there were smaller reductions in $V_e$ and $I_{sc}$ with no apparent effect upon $R$. At epinephrine concentrations less than $10^{-4}$ M, the $V_e$ and $I_{sc}$ spontaneously recovered even though the epinephrine was not washed out of the chamber. This occasionally occurred when the epinephrine concentration exceeded $10^{-4}$ M. Figure 2 shows a typical response of the RPE to increasing epinephrine concentrations ranging from $1.4 \times 10^{-7}$ M to $1.4 \times 10^{-4}$ M. Epinephrine was also effective when applied to only the apical side of the RPE (n = 3) but there was no apparent effect when epinephrine was
applied to only the scleral side of the preparation (n = 3).

When isoproterenol, an agent with predominantly beta adrenergic agonist activity, was added to both sides of the preparation, there was no apparent response at concentrations of 2.5 × 10⁻⁵ M (n = 3). To confirm this lack of beta adrenergic activity, the RPE preparations were incubated in 10⁻⁶ M propranolol, a potent beta adrenergic antagonist, and then exposed to epinephrine. The fall in I(s) in response to epinephrine in these preparations incubated in propranolol (40% ± 8% SD, n = 3) was essentially identical to that noted when tissues were exposed to epinephrine without propranolol. Figure 3 shows an experiment where propranolol did not block the epinephrine response.

When the preparations were exposed to phenylephrine, an adrenergic agent with predominantly alpha agonist activity,⁴ the responses were similar to those seen with epinephrine. After exposure to phenylephrine, further stimulation with epinephrine produced only a small change in V(e) and I(s) (Fig. 4). At 1.4 × 10⁻⁴ M, the average fall in I(s) was 32% (±10% SD, n = 4).

Clonidine, an adrenergic agent with predominantly alpha-2 agonist activity,⁴ had very little effect upon the preparations. Clonidine concentrations of 10⁻⁴ M were necessary before an effect could be seen. Since xylazine, the agent used to anesthetize the rabbits, has alpha-2 adrenergic agonist activity,⁴ it was possible that this anesthesia could have prestimulated RPE alpha-2 adrenoreceptors. Consequently, three clonidine experiments were performed under the pentobarbital and ketamine anesthesia used in earlier experiments.⁹ With pentobarbital substituted for xylazine, there could be no prestimulation of alpha-2 adrenoreceptors. Figure 5 shows a typical clonidine experiment where pentobarbital and ketamine rather than xylazine and ketamine were used for anesthesia. There was no apparent difference in responses to clonidine when xylazine was used for anesthesia (n = 3) and when xylazine was not used (n = 3).

The effect of epinephrine was completely blocked by 10⁻³ M phentolamine, a potent alpha adrenergic antagonist⁴ (n = 3). Figure 6 shows a typical experiment where phentolamine completely blocked the effect of 10⁻⁴ M epinephrine. Yohimbine, an adrenergic agent with predominantly alpha-2 antagonist activity,⁴,¹⁵ at 10⁻⁴ M did not affect the RPE response to 10⁻⁴ M epinephrine (n = 3). This is illustrated in Figure 7. Prazosin, an alpha-1 adrenergic antagonist,⁴ at 10⁻⁵ M markedly attenuated the RPE response to epinephrine (n = 10) and to phenylephrine (n = 7). Figure 8 shows the RPE response to epinephrine blocked by prazosin.

Dibutyryl cAMP at 10⁻³ M had no apparent effect
PHENTOLAMINE

![Graph showing effect of phentolamine on RPE response to epinephrine]

Fig. 6. Phentolamine (an alpha adrenergic antagonist) blocked the RPE response to epinephrine.

PRAZOSIN

![Graph showing effect of prazosin on RPE response to epinephrine]

Fig. 8. Prazosin (an alpha-1 adrenergic antagonist) blocked the RPE response to epinephrine and to phenylephrine.

Discussion

The results of these experiments clearly indicate that the retinal pigment epithelium (RPE) of rabbits has a receptor for epinephrine that can influence RPE-generated trans-RPE electrical currents. Epinephrine has both alpha and beta adrenergic activity and 1.4 \times 10^{-4} M epinephrine reduced the calculated short circuit current ($I_{sc}$) by 39%.

Epinephrine was effective only when applied to the apical side of the RPE. This, however, is only weak evidence that the epinephrine receptors are localized to the RPE apical membrane because the intact choroid and sclera may have prevented epinephrine applied to the scleral side of the preparation from reaching the RPE basolateral membrane.

It was interesting to note that the RPE recovered from the effects of lower concentrations of epinephrine even though the epinephrine was not removed from the chamber (Fig. 2). There are at least two possible explanations for this. First, epinephrine is quickly inactivated by oxygenation and may simply have become oxidized by the 95% O2 that we bubbled through the chamber. Second, the RPE may have internalized the epinephrine receptors or in some other way the receptors may have become less responsive to epinephrine.

The epinephrine receptor clearly is not a beta adrenergic receptor because it is neither stimulated by the beta adrenergic agonist, isoproterenol, nor...
blocked by the beta adrenergic antagonist, propranolol. It is possible that the isoproterenol concentration (2.5 \times 10^{-3} \text{ M}) was not sufficiently high to rule out the presence of a beta adrenoreceptor. However, were a beta adrenoreceptor present, at least a portion of the epinephrine response should have been blocked by propranolol. Furthermore, the RPE responded to phenylephrine, an alpha adrenergic agonist, in essentially the same way it responded to epinephrine. Since phenylephrine has predominantly alpha-1 adrenergic activity,\textsuperscript{14} this suggests that the epinephrine receptor is an alpha-1 adrenoreceptor. The experiments where clonidine, an alpha-2 adrenergic agonist,\textsuperscript{14} failed to affect RPE electrical activity except in high concentrations support this hypothesis. (At these high concentrations, clonidine presumably acted as both an alpha-2 and an alpha-1 adrenergic agonist.) Phentolamine blocks both alpha-1 and alpha-2 adrenoreceptors\textsuperscript{14} and completely blocked the RPE response to epinephrine. Yohimbine, an alpha-2 adrenergic antagonist,\textsuperscript{14,15} appeared to have no effect upon the RPE response to epinephrine. Prazosin (an alpha-1 adrenergic antagonist)\textsuperscript{14} blocked the RPE response to epinephrine and to phenylephrine. These experiments with alpha adrenergic antagonists confirm that the RPE epinephrine receptor is the alpha-1 subtype.

The "second messenger" for alpha-1 adrenoreceptor responses appears to be intracellular calcium and, in many cases, increased phosphoinositol hydrolysis.\textsuperscript{10,19,20} In contrast, the "second messenger" for alpha-2 adrenoreceptors and for both beta-1 and beta-2 adrenoreceptors is cyclic AMP.\textsuperscript{10} It has been reported that exogenously applied cyclic AMP and promoters of intracellular cyclic AMP formation decrease bullfrog RPE-mediated fluid movement and ion transport.\textsuperscript{6} Bullfrog RPE-mediated fluid movement is thought to occur via an active transport process.\textsuperscript{21} Cyclic AMP has also been reported to slow (and cyclic GMP accelerate) resorption of fluid from the subretinal space in rabbits\textsuperscript{7} which is dependent upon RPE active transport process.\textsuperscript{9} We could detect no effect of exogenously applied dibutylryl cAMP (in the presence or absence of IBMX) or cyclic GMP (as cGMP or in the dibutylryl form) on $i_{sc}$. It appears that the alpha-1 receptor that we have identified acts independently of cyclic AMP, as has been reported for alpha-1 adrenoreceptors from other tissues.\textsuperscript{10,19,20}

Further work is required to understand the biologic effect and significance of this alpha-1 adrenoreceptor on rabbit RPE. If a similar receptor is present on human RPE, it may have important therapeutic implications.

**Key words:** alpha-1 adrenoreceptor, epinephrine, prazosin, rabbit, retinal pigment epithelium

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**References**