Dopamine Modulation of Active Ion Transport in Rabbit Corneal Epithelium

Craig E. Crosson, Roger W. Beuerman, and Stephen D. Klyce

The addition of micromolar quantities of dopamine stimulated ion transport in the isolated rabbit corneal epithelium. This response was blocked by pretreatment with the dopamine antagonist, haloperidol, and by the elimination of Cl− from the bathing solutions. The α-adrenergic antagonist, timolol, was also a potent inhibitor of the epithelial response to dopamine. The presence of the serotonin antagonist, methysergide, or the dopamine β-hydroxylase inhibitor, FLA-63, did not significantly alter the corneal response to dopamine. Following superior cervical ganglionectomy, the epithelial response to dopamine was abolished. These findings are consistent with the idea that Cl− secretion in the rabbit corneal epithelium can be modulated by preterminal dopamine receptors located on the sympathetic nerve fibers; therefore, dopamine stimulation appears to be a serial process mediated by the release of norepinephrine from sympathetic nerve terminals in the epithelium. Invest Ophthalmol Vis Sci 25:1240–1245, 1984

In the cornea, epithelial ion transport is stimulated by neurotransmitters normally associated with sympathetic innervation. Histochemical studies have shown catecholaminergic, serotonergic, and substance P containing fibers in the epithelium.1–3 Activation of β-adrenergic receptors elevates corneal short-circuit current by an increase in Cl− conductance at the apical membrane.4 Stimulation of specific serotonin receptors also has been shown to modulate Cl− secretion.7 In each case, the epithelial response is mediated by the intracellular second messenger, cAMP.5,6 Catecholamines also have an antimitogenic effect on the corneal epithelium.7

Dopamine is a naturally occurring catecholamine, which has been shown to function as a neurotransmitter in the central nervous system.8 Recent pharmacologic evidence has demonstrated that a number of tissues receiving autonomic innervation also possess specific dopaminergic receptors.9–13 In ocular tissues, light-activated dopaminergic neurons may exist in the retinas of several animals.14,15 In addition, dopamine is known to have a hypotensive effect on intraocular pressure.16,17 Therefore, the present study was designed to determine whether dopamine may have a role in the modulation of corneal epithelial electrophysiology. Results from this study indicate that activation of specific dopamine receptors in the corneal epithelium stimulates Cl− secretion, and this activation requires normal sympathetic innervation.

Materials and Methods

This study was conducted in compliance with the ARVO Resolution on the Use of Animals in Research.

Adult New Zealand white rabbits, weighing 3–4 kg, were killed by intracardial injection of sodium pentobarbital. The eyes were removed, and corneas isolated and mounted in modified Lucite chambers as previously described.7 The tissues were incubated at 35°C.

Electrophysiology

Normal Ringer's solution used in this study contained the following: 99.7 mM NaCl, 3.6 mM KCl, 6.98 mM Na2SO4, 20 mM NaHCO3, 0.6 mM K2HPO4, 25 mM HEPES/Na, 1.4 mM Ca-gluconate, 0.61 mM MgSO4, and 26 mM glucose. This normal Ringer's solution was bubbled with 95% O2/5% CO2 maintaining a constant pH of 7.4. In experiments employing Cl− and HCO3−-free Ringer's, replacements of Na2SO4 for NaCl and NaHCO3, 0.6 mM K2HPO4, 25 mM HEPES/Na, 1.4 mM Ca-gluconate, 0.61 mM MgSO4, and 26 mM glucose. This normal Ringer's solution was bubbled with 95% O2/5% CO2 maintaining a constant pH of 7.4. In experiments employing Cl− and HCO3−-free Ringer's, replacements of Na2SO4 for NaCl and NaHCO3, and K-gluconate for KCl were made. Chloride and HCO3−-free solutions pH 7.4, were bubbled with air, and normal osmolality was maintained at 305 mOsm by the addition of sucrose. To reduce dopamine oxidation...
or metabolism by monoamine oxidase, all Ringer's solutions contained 1 mM reduced glutathione and $10^{-4}$ M nialamide. Neither the addition of nialamide nor reduced glutathione alone alter significantly the electrical parameters of the corneal epithelium.\textsuperscript{2,18}

All experiments were performed under short-circuit conditions. Corneal short-circuit current (SCC) and transcorneal potential were measured with the use of dual voltage/current clamps (D-Lee Inc., Sunnyvale, CA). Voltage clamps were interrupted at 1-min intervals for 3 sec to measure the resting epithelial potential difference. Corneal resistance was calculated from Ohm's Law.

**Pharmacologic Agents**

In paired experiments, the following pharmacologic agents were used to assess the role of dopamine in the modulation of ion transport in the corneal epithelium: 3-hydroxytyramine hydrochloride (dopamine, Sigma; $10^{-6}$, $10^{-5}$ M), ± butaclamol (Ayerst Laboratories; $10^{-5}$ M), haloperidol in citric acid buffer (McNeil Pharmaceutical; $10^{-5}$ M), methysergide bimaleate (Sandoz Pharmaceutical; $10^{-5}$ M), timolol maleate (Merck, Sharp and Dohme; $10^{-5}$ M), FLA-63 in acetic acid buffer (bis [4-methyl-l-homopipеразинилтхиоформамил] дисульфид; Astra Pharmaceutical; $10^{-6}$ M), 1-epinephrine bitartrate (Sigma; $10^{-5}$ M). Concentrated stock solutions were made just prior to their use, and small aliquots of drug or vehicle were added to the bathing solutions on each side of the cornea. Unless noted, all drugs were dissolved in the appropriate Ringer's solution.

**Superior Cervical Ganglionectomy**

Using a ventral approach, superior cervical ganglia were exposed surgically, pre- and postganglionic axons were severed, and the entire ganglia removed. Two weeks following unilateral ganglionectomy, corneal pairs were isolated and mounted in the incubation chambers. Earlier studies have shown that 2 weeks is sufficient for complete degeneration of vegetative innervation in the cornea.\textsuperscript{19}

**Statistics**

All values are expressed as mean ± standard error of the mean. Student's t-test, for paired observations, was used for data analysis.

**Results**

The effects of dopamine on SCC and potentials from one experiment are presented in Figure 1. Addition of $10^{-6}$ M or $10^{-5}$ M dopamine increased corneal SCC. Following dopamine stimulation, the cornea was still very sensitive to epinephrine. Although both dopamine and epinephrine treatments resulted in an increase in SCC, there were temporal differences in the response characteristics. Epinephrine elevated the SCC within 30-60 sec. In contrast, the onset of the dopamine response was much slower, requiring several minutes.

Table 1 summarizes the epithelial electrophysiologic response to dopamine. Mean corneal potential difference, SCC, and resistance prior to treatment were comparable with those found in earlier studies.\textsuperscript{4-7} Dopamine ($10^{-6}$ M and $10^{-5}$ M) significantly increased SCC (26% and 51%). Concurrently, epithelial resistance was decreased by 32% or 41% with the addition of $10^{-6}$ M or $10^{-5}$ M dopamine, respectively. Because of the time required to assess the dopamine response, small decreases in mean epithelial potential difference probably represent normal declines in vitro potential differences during incubation. Although the endothelium was left intact in the majority of the experiments, its removal in selected cases did not significantly alter the electrophysiologic measurements for untreated tissue or the corneal response to dopamine.

In paired experiments, pretreatment with the dopamine antagonist, haloperidol, at $10^{-5}$ M blocked the dopamine response.

**Table 1. Effect of dopamine on corneal electrical parameters**

<table>
<thead>
<tr>
<th>Condition</th>
<th>SCC ($\mu$A/cm$^2$)</th>
<th>Potential (mV)</th>
<th>Resistance (k$\Omega$·cm$^2$)</th>
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<tbody>
<tr>
<td>Basal</td>
<td>2.7 ± .17</td>
<td>24 ± 1.3</td>
<td>8.8 ± .53</td>
</tr>
<tr>
<td>Dopamine ($10^{-6}$ M)</td>
<td>3.4 ± .22$^*$</td>
<td>21 ± 1.3*</td>
<td>6.4 ± .48$^*$</td>
</tr>
<tr>
<td>Dopamine ($10^{-5}$ M)</td>
<td>4.1 ± .24$^+$</td>
<td>20 ± 1.4†</td>
<td>5.0 ± .34$^+$</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N = 25). $^*$P < 0.05; $^+$P < 0.01; $^§P < 0.001.
the increase in SCC induced by $10^{-6}$ M dopamine, while the SCC response to $10^{-5}$ M dopamine was reduced by 66% ($P < 0.05$) (Fig. 2, Table 2). Corneas pretreated with haloperidol still responded to epinephrine stimulation (Fig. 2). In preliminary experiments, pretreatment with the putative dopamine antagonist, $\pm$ butaclamol ($10^{-5}$ M), alone increased the epithelial SCC by 82%. This action of butaclamol is difficult to explain. However, the magnitude of the response argues against a nonspecific action by butaclamol and precluded its use in the assessment of the corneal response to the dopamine.

The serotonin antagonist, methysergide, and the dopamine $\beta$-hydroxylase inhibitor, FLA-63, were not effective in significantly altering the dopamine-induced increase in SCC, compared with controls (Table 2). The increase in responsiveness of FLA-63 control corneas to dopamine may reflect changes induced by the addition of the acetic acid vehicle. Following pretreatment of the corneas with the $\beta$-adrenergic antagonist, timolol, dopamine failed to significantly alter the epithelial SCC (Fig. 3, Table 2). This inhibition was unexpected since $\beta$-adrenergic antagonists are considered to be ineffective as dopamine antagonists.20

Two weeks following superior cervical ganglionectomy, the spontaneous SCC in the ipsilateral cornea was significantly elevated over contralateral control corneas (Fig. 4). As presented in Table 3 and Figure 4, the epithelial response to $10^{-6}$ M dopamine was abolished, and the increase in SCC after the addition of $10^{-5}$ M dopamine was reduced by 65% ($P < 0.01$). The response to epinephrine was unaltered in the ipsilateral corneas, when compared with controls (Figure 4).

Removal of $Cl^-$ or $Cl^-$ and $HCO_3^-$ from the bathing solution resulted in a significant decrease in the basal SCC. Corneas exposed to $Cl^-$-free Ringer's solution did not respond to dopamine stimulation. However, the addition of $10^{-5}$ M epinephrine resulted in a small increase in the epithelial SCC. In $Cl^-$ and $HCO_3^-$-free bathing solutions, corneas were again unresponsive to dopamine; however, the addition of $10^{-5}$ M epinephrine still elicited a small response similar to that in $Cl^-$-free Ringer's solution (Fig. 5, Table 4). In a series of additional experiments using $Cl^-$-free Ringer's solution, the additional replacement of Na ions in the apical bathing solution blocked the epithelial response to epinephrine (unpublished results). This suggests that in the absence of $Cl^-$, epinephrine ($10^{-5}$ M) stimulates Na$^+$ absorption across the corneal epithelium.

### Discussion

Specific dopamine receptors have been identified in several tissues outside of the central nervous system.

<table>
<thead>
<tr>
<th>Table 2. Effect of pharmacologic antagonists on the dopamine-stimulated increase in corneal SCC</th>
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<tr>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Treated</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>$P$ value</td>
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Values are mean ± SEM of dopamine (DA)-induced increase in short-circuit current ($\mu$A/cm$^2$) following the addition of specific antagonists or control vehicle.
In the kidney and intestine, stimulation of these receptors has been shown to regulate membrane ion transport. In addition, dopamine modulates sympathetic nerve activity via preterminal and postsynaptic neuronal receptors.

Dopaminergic neurons have been identified in the sympathetic ganglia, kidney, and paw pad of the dog. Histochemical studies also have shown that mast cells contain large amounts of dopamine, independent of the neuronal dopamine pools. The source of dopamine in the corneal epithelium may be the catecholaminergic neurons or the mast cells that invade the central cornea in response to trauma. However, under normal conditions, mast cells are absent from the central cornea.

This study demonstrates that the addition of micromolar quantities of dopamine result in a significant increase in the corneal SCC over basal levels. This increase in SCC can be blocked by the removal of Cl⁻ from the bathing solutions. Therefore, it appears that dopamine elevates corneal SCC by activating Cl⁻ secretion in a manner similar to epinephrine and serotonin.

The time course of the corneal response to dopamine is similar to that of serotonin, but is slower than that of epinephrine. This suggests that dopamine must diffuse deeper into the corneal epithelium than epinephrine to initiate a response at the receptor locus. On the basis of response time, dopamine receptors appear to be physically separated from the β-adrenergic receptors activated by exogenous epinephrine. In addition, haloperidol inhibits the dopamine response while sparing the responsiveness to epinephrine. These results support the idea that dopamine stimulates ion transport by activating specific dopamine receptors.

High concentrations of dopamine (>10⁻⁶ M) are only partially inhibited by haloperidol. This may indicate that dopamine cross-reacts with other receptors in the epithelium, or that the receptor affinity for haloperidol is low. Previous studies have demonstrated that the activation of β-adrenergic or serotonin receptors stimulates Cl⁻ secretion. However, α-adrenergic receptors are apparently not involved in the regulation of ion transport in the mammalian corneal epithelium, since epinephrine stimulation is not altered by the presence of an α-adrenergic antagonist (phentolamine), and specific α-adrenergic agonists (phenylephrine) failed to alter corneal SCC (unpublished results). The corneal response to dopamine, although blocked by the β-adrenergic antagonist, timolol, differs significantly from the epinephrine activation of the β-adrenergic receptors. First, the dopamine response is reduced following superior cervical ganglionectomy. This is in sharp contrast to the supersensitivity exhibited by β-adrenergic receptors

### Table 3. Effect of superior cervical ganglionectomy on the dopamine-stimulated increase in corneal SCC

<table>
<thead>
<tr>
<th></th>
<th>Dopamine (10⁻⁴ M)</th>
<th>Dopamine (10⁻⁵ M)</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.60 ± 0.32</td>
<td>2.3 ± 0.33</td>
</tr>
<tr>
<td>Denervated</td>
<td>0.05 ± 0.10</td>
<td>0.8 ± 0.30</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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</table>

Values are mean ± SEM (N = 6) of dopamine-induced increase in corneal short-circuit current (µA/cm²).

### Table 4. Effect of chloride- and bicarbonate-free solution on the dopamine-stimulated increase in corneal SCC

<table>
<thead>
<tr>
<th></th>
<th>Dopamine (10⁻⁴ M)</th>
<th>Dopamine (10⁻⁵ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.78 ± 0.24</td>
<td>1.44 ± 0.44</td>
</tr>
<tr>
<td>Cl⁻⁻ and HCO₃⁻⁻ free</td>
<td>0.02 ± 0.05</td>
<td>0.06 ± 0.07</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N = 5) of dopamine-induced increase in short-circuit current (µA/cm²).
after superior cervical ganglionectomy. Second, the time course of dopamine response is much slower than that of epinephrine. Pretreatment with the serotonin receptor antagonist, methysergide, did not significantly alter dopamine stimulation of corneal SCC.

Dopamine is also the precursor to norepinephrine in catecholamine biosynthesis. The enzyme responsible for this conversion—dopamine β-hydroxylase—is located in synaptic vesicles of autonomic neurons. Recent studies have suggested that significant levels of dopamine β-hydroxylase are also present in the aqueous humor. Therefore, dopamine may activate β-adrenergic receptors by conversion to norepinephrine. Pretreatment of isolated corneas with dopamine β-hydroxylase inhibitor, FLA-63, failed to reduce significantly the increase in SCC induced by dopamine. This indicates that dopamine at 10^-6 M does not act at β-adrenergic or serotonin receptors, nor is there any significant metabolic conversion. This provides further support for the presence of a specific dopamine receptor. However, the small response to 10^-5 M dopamine following haloperidol pretreatment or ganglionectomy suggests that at high concentrations (>10^-6 M) dopamine may also cross-react with other receptors in the epithelium.

Dopamine has been shown to modulate neuronal activity in sympathetic ganglia and sympathetic fibers associated with the cardiovascular system. The corneal epithelium is innervated by sympathetic fibers from the superior cervical ganglion. Therefore, it is possible that dopamine action in the corneal epithelium results in the release of norepinephrine from sympathetic nerve terminals. This type of serial mechanism would explain the potent antagonism of the dopamine response by the β-adrenergic receptor antagonist, timolol. Following superior cervical ganglionectomy, basal SCC in the ipsilateral cornea was not altered markedly in the ipsilateral corneas, compared with controls. This abolition of the epithelial response to dopamine following sympathectomy also supports the idea that dopamine receptors are located on the sympathetic nerve fiber and not on the epithelial cell surface. Therefore, we argue that dopamine stimulation causes the release of norepinephrine from sympathetic nerve terminals.

In summary, we suggest that dopamine modulates Cl^- secretion primarily by the activation of specific dopamine receptors. The results are consistent with the idea that dopamine receptors are preterminal receptors on sympathetic nerve fibers and are not located on the epithelial cells. As a result, the corneal response to dopamine stimulation appears to be a serial process mediated by the release of norepinephrine from sympathetic nerve terminals.

Key words: cornea, dopamine, chloride, transport, sympathetic innervation, epinephrine, haloperidol, timolol

Acknowledgments

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References