Alteration of Corneal Epithelial Ion Transport by Sympathectomy

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The cornea is dually innervated, receiving afferent nerves from the trigeminal ganglion and efferent nerves from the superior cervical ganglion. This study examines the specific effects of superior cervical ganglionectomy (SCGX) on the in vitro ion transport characteristics of the rabbit corneal epithelium. Two weeks after SCGX, epithelial Cl^--dependent transport and total ionic conductance were increased in comparison to values obtained in paired control eyes. This increased transport level appeared to be independent of membrane receptor activity as demonstrated by lack of responsiveness to α-adrenergic, β-adrenergic, serotonergic, dopaminergic, nicotinic cholinergic, or muscarinic cholinergic blockade. Nevertheless, SCGX produced a supersensitivity to epinephrine-stimulated transport as measured by the responsiveness of the ion transport current. Furthermore, SCGX abolished the responsiveness of the epithelium to serotonin. On the basis of these and earlier findings, the authors conclude that corneal sympathetic innervation influences membrane and receptor properties. Autonomic neurotrophic effects in the corneal epithelium include suppression of apical membrane Cl^- permeability and of β-adrenoreceptor sensitivity to biogenic amines. It is proposed that the corneal serotonergic receptors that activate Cl^- transport lie on the sympathetic nerve terminals and stimulate this transport process by causing the neural release of a catecholamine. Invest Ophthalmol Vis Sci 26: 434–442, 1985

The corneal epithelium is a metabolically active stratified layer that serves as a barrier to both the ocular penetration of tear-borne infectious and noxious agents and to the accumulation of fluid from the tears. In addition, the cells secrete ions in a manner that augments the endothelial function in the regulation of corneal hydration. The cornea is dually innervated by autonomic and sensory fibers that have been implicated in the maintenance of normal epithelial function.[1,2]

The secretory function of the epithelium is linked to the active Cl^- transport process, which has been examined in detail in the rabbit. Catecholamine activation of β-adrenoreceptors in the epithelium elevates cellular cyclic AMP[3] which leads to an increase in Cl^- permeability of the surface epithelial membranes.[4] Serotonin,[5] and recently dopamine,[6] also have been shown to stimulate Cl^- transport; however, their action, as well as that of epinephrine, is effectively blocked by timolol, a β_1, β_2-adrenergic receptor antagonist not previously shown to cross- with other receptor types.

The superior cervical ganglion innervates the nea[7,8] and is a likely source of the neuroregula responsible for modulating epithelial Cl^- trans and other cellular processes. This paper investig the effects of superior cervical ganglionectomy on the transport properties of the corneal epithelium in the rabbit model. The results leave little doubt that sympathetic innervation is essential for the maintenance of normal cellular processes in the cornea, and support a scheme whereby serotonin acts on preterminal nerve receptors as a neuromodulator rather than a neurohormone.

Portions of this work were reported at the annual meeting of the Association for Research in Vision and Ophthalmology, May 1–6, 1983, Sarasota, Florida.[9]

Materials and Methods

Animal care and treatment in this investigation were in compliance with the ARVO Resolution on the Use of Animals in Research. Adult New Zealand White rabbits, 3–5 kg in body weight and of both sexes, were tranquilized with 27.5 mg of thorazine, and anesthesia was induced by the intravenous injection of ketamine hydrochloride (50–75 mg/kg). The ventral approach was used to remove one superior cervical ganglion in each animal. The surgical tech-

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nique involved severing the pre- and postganglion nerve trunks, and extricating the entire ganglion. Differential pupil asymmetry and light response were verified in every case. After a 2-week interval, a time sufficient for the complete degeneration of corneal sympathetic nerves (unpublished data), the operated animals were killed with an overdose of sodium pentobarbital and the corneal pairs rapidly excised and mounted in modified Ussing-style chambers.[3] Statistical measures were obtained with the use of Student's t-test for paired data. Statistical data are reported as means and standard errors.

The normal Ringer's solution used in this study contained: 99.7 mM NaCl, 3.7 mM KCl, 26 mM glucose, 6.92 mM Na_2SO_4, 20 mM $NaHCO_3$, 0.6 mM K_2HPO_4, 25 mM HEPES/Na, 1.4 mM Ca-gluconate, 0.61 mM $MgSO_4$, and 10^{-4} M nialamide. Where Cl^- and HCO_3^--free media were used, 3.7 mM K-gluconate and 67.2 mM Na_2SO_4 were substituted for NaCl, KCl, and $NaHCO_3$ with 59.8 mM sucrose added to maintain a total osmolarity of 305 mOsm. Where Na^+, Cl^-, and HCO_3^--free media were used, 3.7 mM K-gluconate and 72 mM Tris/SO_4 were substituted for NaCl, KCl, Na_2SO_4, HEPES/Na and $NaHCO_3$. The pH of the solutions was 7.5 ± 0.1 and the temperature of the isolated corneas was maintained at 35°C.

The following pharmacologic agents were used: 10^{-4} M nialamide; 10^{-4} M serotonin; 10^{-9} to 10^{-5} M l-epinephrine bitartrate; 10^{-4} M atropine; 10^{-4} M curare (all from Sigma Chemical Co.); 10^{-4} M methysergide maleate (Sandoz Pharmaceuticals); 10^{-5} timolol maleate (Merck, Sharp and Dohme); 10^{-4} phentolamine (Ciba Pharmaceutical Co.); and 10^{-5} M haloperidol (McNeil Pharmaceutical).

After mounting corneas in Lucite chambers, corneal short-circuit current (SCC) and potential (PD) were measured with dual automatic current/voltage clamps (D. Lee, Inc., Sunnyvale, CA) as previously described[3] and recorded on chart paper. Resistances (R) were calculated from the ratio of corneal PD and SCC. The corneal epithelium generates 95–98% of the total corneal potential difference and accounts for more than 99% of the overall corneal electrical resistance.[10] Hence, the transcorneal measurements reported here reflect predominantly epithelial transport characteristics. This was confirmed in several experiments in which the endothelium and Descemet's membrane were removed prior to mounting the cornea in the chamber.

Results

Compared with normally innervated control corneas, corneas obtained after superior cervical ganglionectomy showed altered corneal epithelial ion transport characteristics with respect to untreated levels, as well as differences in the response to serotonin, epinephrine, and timolol (Figure 1). The most apparent effects of the denervation were an elevation of the basal level of SCC and the elimination of the normal response to serotonin. The unstimulated SCC was increased by 2.0 ± 0.1 $\mu A/cm^2$ ($P < .0001$) in denervated corneas in comparison to normally innervated corneas (Figure 2). This differential in SCC remained throughout the subsequent serial additions of pharmacological agents. Whereas 10^{-4} M serotonin stimulated the SCC by 0.5 ± 0.2 $\mu A/cm^2$ ($P = .03$) in the control corneas, there was no statistically significant response to serotonin in the denervated corneas (0.1 ± 0.2 $\mu A/cm^2$; $P = 0.73$). It should be noted that the control response to serotonin (0.5 $\mu A/cm^2$) was considerably less in this series compared to our earlier measurements (1.9 $\mu A/cm^2$).[5] However, variability in the corneal response to serotonin among different populations of rabbits does exist.[5] Whether

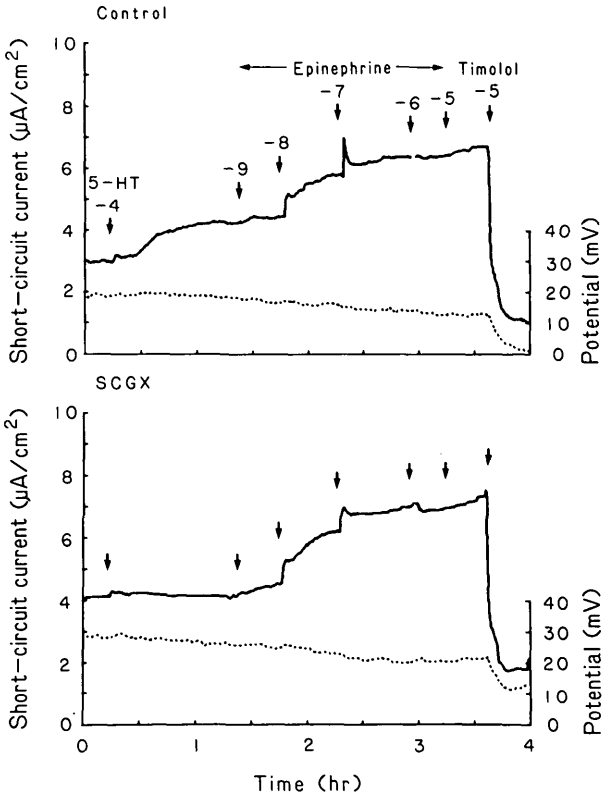

Fig. 1. An example of the effect of unilateral sympathectomy (SCGX) on the corneal epithelial response to serotonin (5-HT), epinephrine, and timolol. The control cornea is from the contralateral eye. Drug concentrations are given as the $\log_{10}$ of the molarity. The continuous lines in this and subsequent figures represent the short-circuit current, whereas the interrupted lines indicate corneal resting potential. The graphs exclude an initial 60–90 min period of incubation, which is required to achieve steady state conditions.

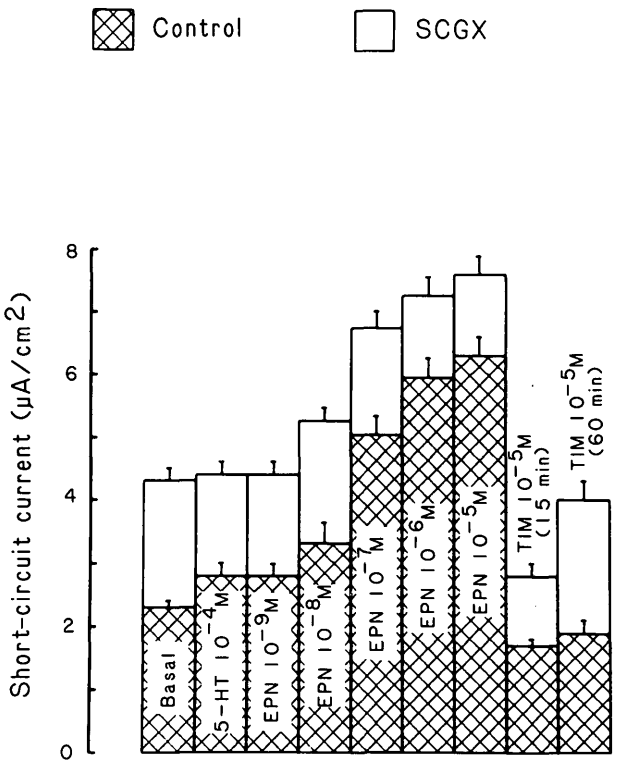

Fig. 2. Short-circuit current means and standard errors of 14 paired experiments of the type illustrated in Figure 1. The SCC of the sympathectomized cornea is significantly greater than that of the controls in each test period. EPN: epinephrine, TIM: timolol. Note the transient effect of timolol in the sympathectomized cornea.

these differences are due to dietary supplements (such as steroids used by certain animal suppliers), seasonal variations, or different strains of rabbits is unknown at this time.

The SCC in normal and denervated corneas showed dose-dependent responses to a concentration series of exogenous epinephrine (Figs. 1, 2). In control corneas, 10^{-5} M timolol effectively blocked the serotonin- and epinephrine-induced increase in SCC. However, in the denervated cornea, the increase in the basal SCC was blocked only transiently by timolol (Fig. 3). After timolol treatment, the SCC rebounded in the denervated cornea and, after 1 hr, the difference between basal and stimulated levels was not statistically significant (0.3 ± 0.4 $\mu A/cm^2$; $P = 0.49$). The mechanism underlying the transient nature of the timolol inhibition following epinephrine pretreatment in the denervated cornea with respect to the basal level of SCC is not understood. It should be noted that timolol has no effect on the SCC of denervated corneas prior to epinephrine stimulation (see below).

The effect of sympathetic denervation on corneal potential difference was analyzed (Fig. 3). Epithelial PD was significantly greater ($P < .005$ for all periods) for the denervated corneas compared with controls. In terms of epithelial resistance, ganglionectomy significantly altered only the basal value (Fig. 4), where the average resistance was reduced by 2.8 ± 0.7 $k\Omega cm^2$ ($P < .002$).

As noted above, the SCC response of denervated and normally innervated corneas appeared similar for a graded series of exogenous epinephrine concentrations. However, when these responses were analyzed in a dose/response fashion, normalized to the maximum response to 10^{-5} M epinephrine, an increased responsiveness to epinephrine in the denervated cornea could be demonstrated by a shift in the response relationship (Fig. 5). The ED_{50} for the denervated cornea was 4.5×10^{-8} M, whereas the value for the control corneas was 7.2×10^{-8} M.

The elevation of the basal SCC by sympathetic denervation could be a consequence of supersensitivity of membrane receptors to circulating endogenous tissue hormones or neuromodulators. However, no significant reduction in basal epithelial SCC could be produced in either denervated or control corneas by β- or α-adrenergic receptor antagonists (timolol and phentolamine), by serotonergic blockade (methysergide), by dopaminergic blockade (haloperidol), or by muscarinic or nicotinic cholinergic receptor antago-

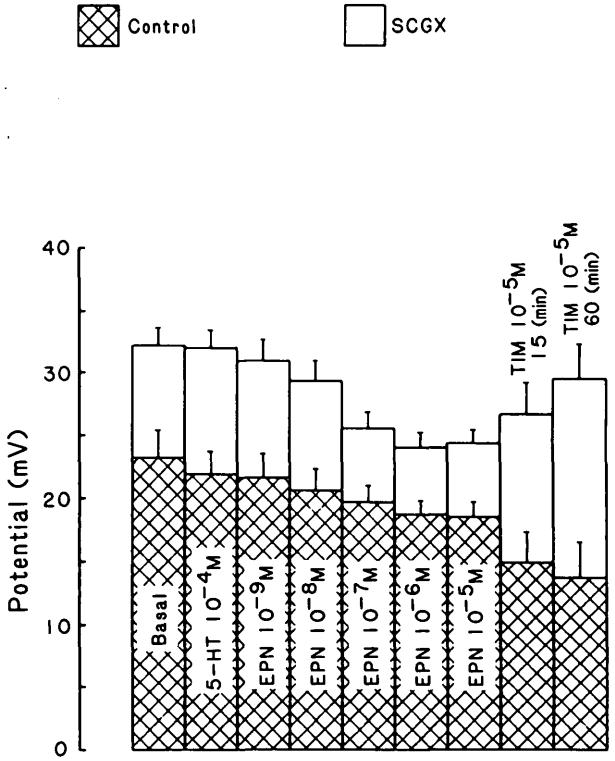

Fig. 3. Corneal potential means and standard errors of 14 paired corneas (cf, Fig. 1). The potential is significantly greater for the sympathectomized corneas in each test period.

nism (atropine or curare) (Fig. 6). Indomethacin, an inhibitor of prostaglandin synthetase, at a concentration of 10^{-5} M, also had no effect on the increased basal SCC. Hence it was concluded that denervation did not lead to the increased level of basal SCC via increased receptor sensitivity to endogenous hormones or neuromodulators.

It has been shown previously that epinephrine[3]- and serotonin[5]-stimulated corneal epithelial SCC is a result of increased Cl^- secretion. In the present studies we eliminated the possible contribution of Cl^- transport by perfusion with Cl^--free Ringer's solution for more than 90 min, a period sufficient to deplete the cells of chloride[4] (Fig. 7). Replacement of Cl^- essentially reduced the SCCs of both the denervated and control corneas to the same value (Fig. 7). However, in the absence of Cl^-, epinephrine was still able to stimulate the transport current, albeit to a lesser degree. This small response was completely eliminated by replacing tear side Na^+ with an impermeant cation (Fig. 8). In separate experiments (not illustrated) this small stimulation of SCC in Cl^--depleted corneas also was elicited with 10^{-3} M dibutyryl cyclic AMP and was also reversed by Na^+-free Ringer's added to the tear side bathing solution.

Timolol quite effectively inhibits the SCC stimulated by β-adrenoreceptor agonists, as well as that

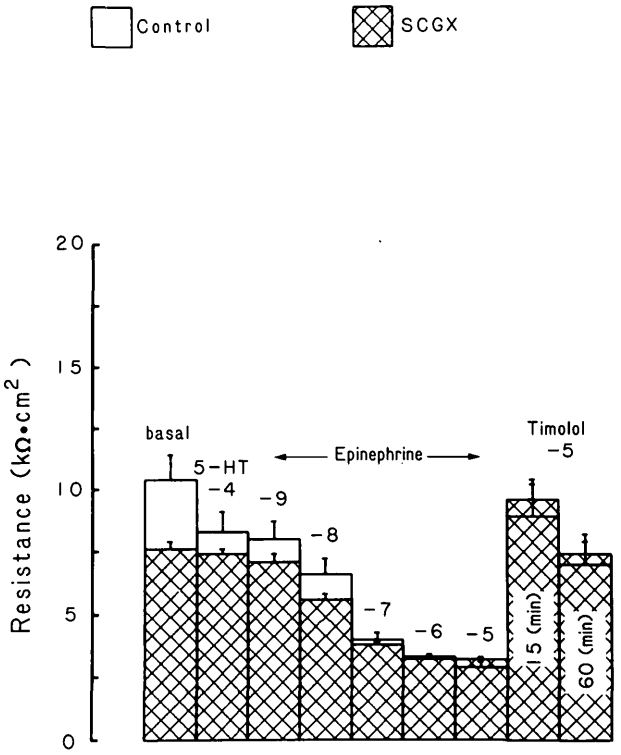

Fig. 4. Corneal resistance means and standard errors (cf, Fig. 1). The only significant difference in resistance between control and SCGX corneas occurred in the period of monitoring basal values.

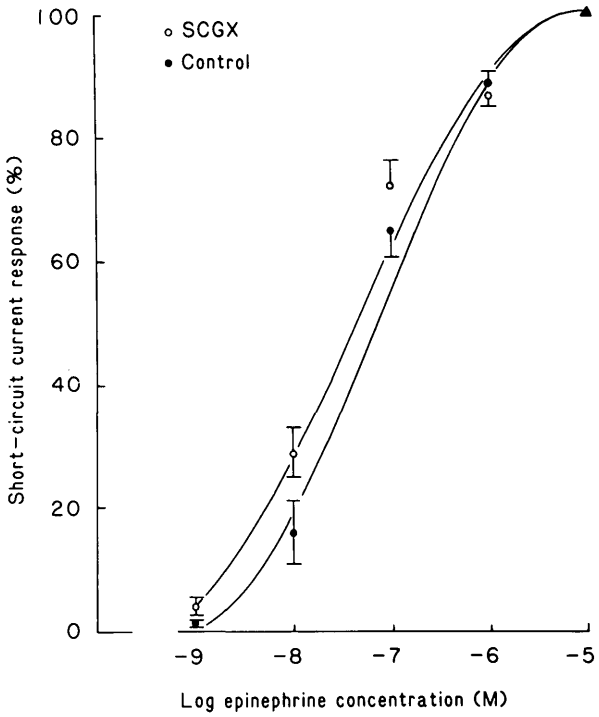

Fig. 5. Dose-response relationship of the SCC response to epinephrine for 14 paired corneas. The increase in SCC to 10^{-5} M epinephrine was taken as 100% response. The data were fit with a linear least squares method applied to an angular transform ($\sin^{-1}[\sqrt{\text{response}/100}]$) of the percent response as a function of the log of the epinephrine concentration.

evoked by serotonin[5] and dopamine.[6] To account for this, we entertained two hypotheses in the action of timolol: (1) that timolol inhibits serotonin and dopamine because the agonist effects of these agents are a process in series with the activation of the β-adrenergic receptors; and (2) that timolol has an additional effect on an intermediate process between the adenylate cyclase mediated increase in cyclic AMP[5] and the end response of increased apical membrane Cl^- conductance.[11,12] The latter hypothesis was rejected on the basis of the observation that the normally innervated cornea was fully responsive to exogenous dibutyryl cyclic AMP following β-adrenoreceptor blockade with timolol (Fig. 9). However, this evidence supports the hypothesis that serotonin acts in a serial fashion in the stimulation of β-adrenoreceptors in the activation of Cl^- secretion by the corneal epithelium (see below).

Discussion

Stimulation of Transport in Cl^--Depleted Corneas

The elevation of the basal SCC in unstimulated denervated corneas is totally dependent on the presence of Cl^- in the bathing solution (Fig. 7). However, the SCC of normally innervated and denervated

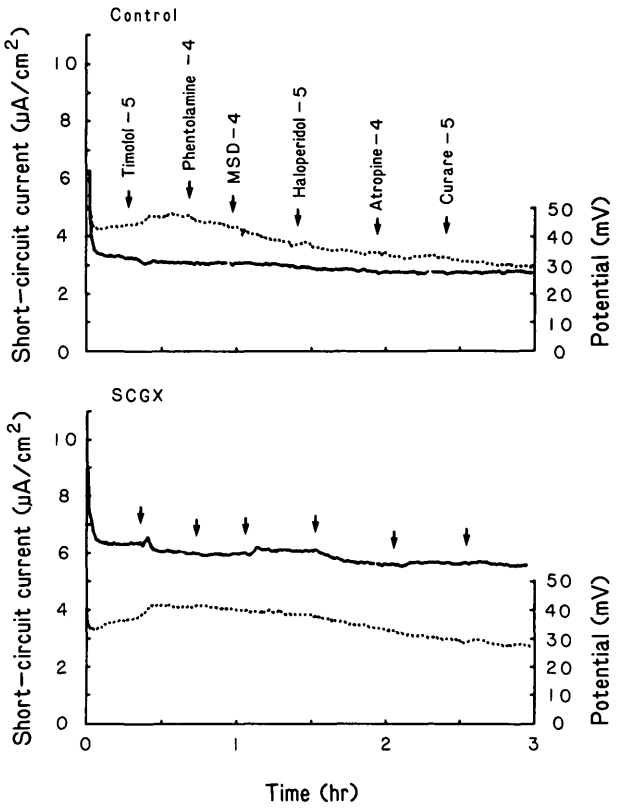

Fig. 6. The lack of effect of several membrane receptor antagonists on epithelial SCC and PD for sympathectomized and control corneal pair. MSD: methysergide. The $\log_{10}$ values of the substance concentrations (M) are given.

tissues was slightly responsive to both epinephrine and dibutyryl cyclic AMP, and these responses could be abolished by bathing the tear side with Na^+-free solution. A small increase in SCC due to epinephrine in Cl^--free Ringer's has been shown previously (cf, Fig. 10 in reference 3), but in this early work it was supposed that this minor response was due to residual epithelial cell Cl^-. However, the time course for Cl^- depletion in these cells has subsequently been measured,[4] and in the current experiments these findings have been utilized to ensure adequate Cl^- depletion. Hence, an effect of cellular cyclic AMP on the transport of an ion other than Cl^- in this tissue is postulated. Since HCO_3^- also was removed along with Cl^- in these experiments, and these anions constitute the majority of permeable anions in the tissue, the involvement of a cation in this response was indicated. Increased Na^+ or K^+ absorption (tears to stroma) could be responsible for the SCC responding to epinephrine in anion depleted tissue. However, K^+ transport is in the secretory direction across the corneal epithelium in both the rabbit[13] and the toad.[14] Na^+ transport is normally absorptive and removing Na^+ from the supply side (tears) eliminated the response. Hence, it is likely that increased levels of cell cyclic AMP stimulate Na^+ transport, but only when the epithelium is depleted of permeable anions. Whether the stimulation is a result of leakage of Na^+ through the apical cyclic AMP-dependent Cl^- channels, an effect on apical membrane Na^+ conductance or the Na^+ pump at the basolateral membrane, or some other cause is unknown at this time. However, this phenomenon is unmasked only in the absence of Cl^-. In normal Ringer's all of the SCC stimulated by dibutyryl cyclic AMP can be accounted for by net Cl^- secretion; no change in net Na^+ transport can be demonstrated.[3] Nevertheless, the phenomenon is interesting and future studies might use this observation to understand more fully the details of ion transport in the corneal epithelium.

Locus of 5-HT Receptors in Cl^- Transport Pathway

There are several lines of evidence that suggest that serotonin receptors in the cornea and other tissues might be preterminal rather than on the epithelial cells, as originally hypothesized.[5] The potent β_1, β_2-

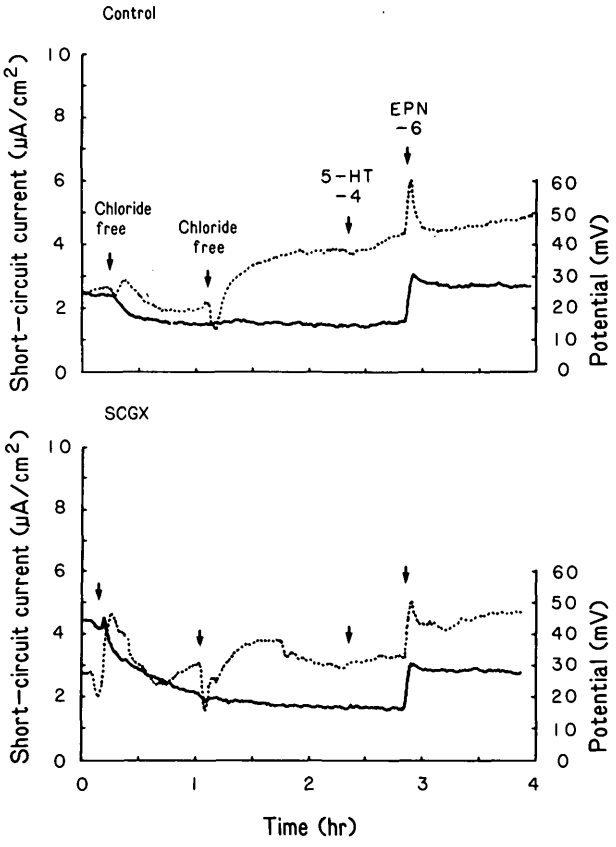

Fig. 7. Cl^--free Ringer's fluid reduces SCC in both control and sympathectomized corneas. Where indicated by *chloride-free*, about seven times the volume of each hemichamber was exchanged with Cl^--free Ringer's by perfusion. The response to serotonin was abolished; however, a concomitant residual subnormal response to epinephrine was observed despite the lack of Cl^-.

adrenergic receptor antagonist, timolol, has been shown not only to block β-adrenergic agonist activation of Cl transport by the corneal epithelium but also to practically eliminate the responsiveness of this pathway to both serotonin[5] and dopamine.[6] Whereas timolol is nonselective in terms of its binding to both classes of β-adrenoreceptors, it is specific in its action as a β-adrenoreceptor blocker. Hence, in the cornea one might suspect that the dominant functional response to activation of serotonin (and dopamine) receptors involves a pathway, which is in series with the β-adrenergic receptor. Timolol also blocks dopaminergic functions in aqueous humor formation,[15] and while it has been postulated[16] that this action is linked to β-adrenergic receptor blockade, the evidence is, at best, indirect. Details of the events that occur between cell cyclic AMP elevation in the corneal epithelium and the increase in apical membrane Cl^- conductance are not known. This uncertainty raises the possibility of some unknown action of timolol in that sequence. Yet in this work, timolol was found to have no effect on the corneal response to exogenous dibutyryl cyclic AMP (Fig. 9), which lends further support to the hypothesis that serotonin acts in a serial fashion in the activation of β-adrenoreceptors in this tissue.

Perhaps the strongest evidence for the preterminal locus of serotonin receptors is the fact that serotonin responsiveness of the epithelial Cl^- transport is essentially absent after sympathectomy, as reported here. Pure subcultures of corneal epithelial cells also lose their responsiveness to serotonin, whereas their sensitivity to β-adrenergic agonists remains intact.[17] It should be noted that sympathectomy does not alter corneal responsiveness to serotonin with respect to total cyclic AMP content nor does timolol block the synthesis of cyclic AMP in whole incubated corneas stimulated by serotonin.[18] However, cyclic AMP is an ubiquitous second messenger in cells, and it is held that specific cellular functional responses are initiated by changes in compartmentalized fractions of cyclic AMP.[19] Therefore, measurements of whole tissue cyclic AMP or even pure cell extracts in response to a given agonist may not parallel the specific effects of this agonist on a localized cell membrane function. In terms of Cl^- transport stimulation in the corneal epithelium, β-adrenergic and serotonergic responses are specific and local; each agonist has the singular process-controlling effect of an increase in apical epithelial membrane Cl^- conductance.[4,11]

It should be noted that the isolated cornea in the Lucite chamber has lost all connection with the neuronal cell bodies that are the source of the corneal nerve terminals. We suggest that for the short-term

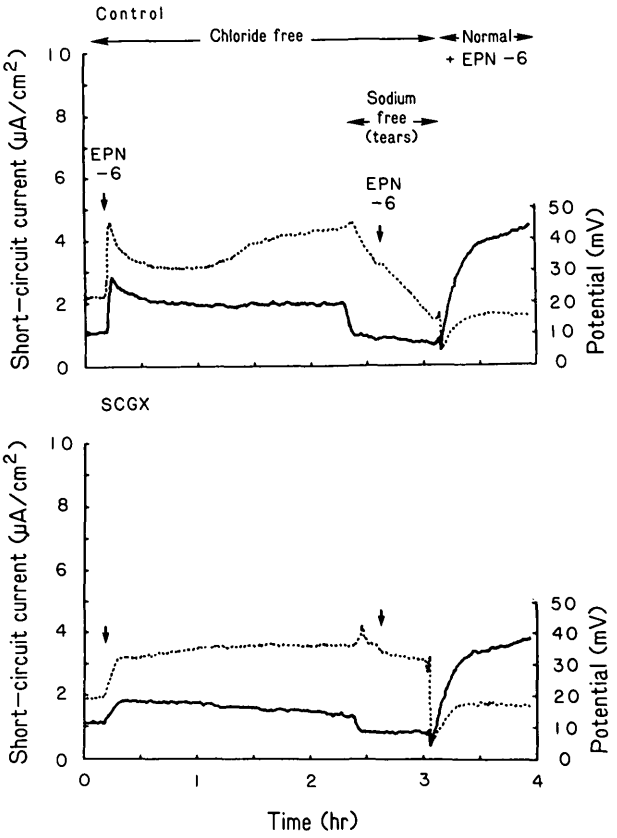

Fig. 8. The remaining response to epinephrine in Cl^--free Ringer's was inhibited by replacing Na^+ in the tear side bathing solution with an impermeant cation (TRIS). The return to normal Ringer's containing Na^+ and Cl^- restored the responsiveness to epinephrine.

experiments reported here, the nerve terminals can survive this insult, since they are mechanically sealed at the corneal periphery by the chamber. In fact, the

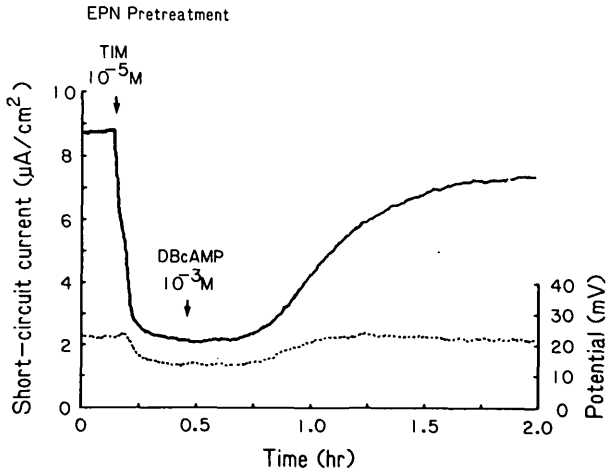

Fig. 9. Effect of timolol and dibutyryl cyclic AMP on a normally innervated corneal pretreated with 10^{-5} M epinephrine. The normal response to dibutyryl cyclic AMP in the presence of timolol lessens the possibility that timolol will interfere with events in the cyclic AMP/membrane conductance increase in this pathway.

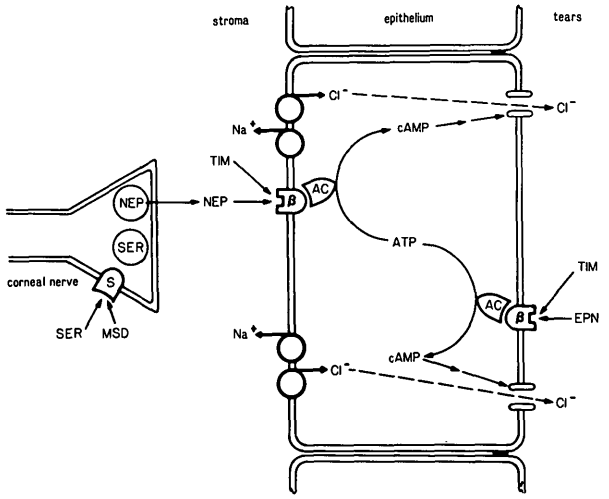

Fig. 10. Proposed model for the mechanism by which serotonin stimulates Cl^- transport across the rabbit corneal epithelium. Abbreviations: SER: serotonin, MSD: methysergide, S: serotonergic receptor, NEP: norepinephrine, TIM: timolol, β: β-adrenoreceptor, AC: adenylate cyclase, and EPN: epinephrine. Note that this scheme is consistent with the experimental observation that both sympathectomy and timolol treatment block epithelial responsiveness to serotonin. It is unknown whether serotonin and β-adrenergic agonists reside in the same or separate terminals. See text for additional details.

terminals are hours distant from the cell body in the normal animal in terms of replenishment of molecular constituents by axoplasmic transport. Hence, whatever needs are ordinarily met by transport from the nerve cell bodies are likely provided for by sufficient reserves for normal serotonin receptor and neuroregulator release functions during the experimental period.

Additional support for the preterminal locus of serotonin receptors in the cornea comes from studies of the mammalian intestinal epithelium. Ion and fluid secretion is stimulated in the intestine by exogenous serotonin,[20] yet the serotonin receptors are not located on the epithelial membranes themselves,[21] allowing for the possibility of the existence of preterminal receptor sites in that tissue as well.

Taken together, the above arguments strongly support the preterminal locus for the serotonin receptor. The best supporting evidence for the locus of corneal serotonin receptors linked to epithelial Cl^- secretion would be isolation or histochemical demonstration, but this proof awaits future studies.

Serotonin as a True Neuromodulator in Cornea

Serotonin fibers have been demonstrated in the subepithelial stroma of the rabbit,[5] rat,[22] guinea pig,[23] and cow,[24] and serotonin has been identified in the superior cervical ganglion of the rat.[25] Hence, serotonin appears to be a neuromodulator in corneal sympathetic nerves. Preganglionic stimulation of corneal fibers in the in vivo rabbit evokes epithelial ion conductance responses consistent with the in vitro stimulation of epithelial Cl^- transport.[26] Although the time course of the response is similar to that induced by exogenous serotonin, it is much slower than that evoked by exogenous β-adrenergic agonists. However, the question remains whether the sympathetic terminals, shown histochemically to be in part serotonergic, are distinct from those that presumably release norepinephrine to activate epithelial β-adrenoreceptors. The presence of preterminal serotonin receptors on sympathetic fibers also could provide a means for mast-like corneal cells, which invade the tissue following a wound, to activate the sympathetic terminals. Although corneal sympathetic nerves also modulate epithelial growth characteristics,[1,27] the measurement of ion transport and permeability remains one of the most sensitive methods to detect functional responses of epithelia to pharmacological modulation.

Receptor and Extrareceptor Alterations by Sympathectomy

Following sympathectomy, corneas displayed a supersensitivity to the β-adrenergic agonist epinephrine possibly induced by a reduction in the normal catecholamine supply to the cornea. Supersensitivity has been noted previously in the rabbit eye following the application of topical timolol.[18] Additionally, reduced sensitivity can be produced with topical epinephrine.[28] The fact that sympathectomy produces supersensitivity, as shown in this paper, argues for the role of a β-adrenergic agonist as a normal neuroregulatory substance in the cornea.

Sympathectomy also altered the basal ion transport pattern across the epithelium. Corneal SCC, which was nearly doubled, was almost entirely Cl^--dependent. Furthermore, none of the basal increase could be inhibited with adrenergic, serotonergic, dopaminergic, or cholinergic antagonists. Whereas the basal corneal cyclic AMP content is not measurably altered by sympathectomy,[18] changes in a compartmentalized fraction cannot be ruled out. Alternatively, the increase in basal SCC could be caused by increased apical membrane Cl^- channel density. Microelectrode studies showed that sympathectomy increased the apical membrane Cl^- conductance and depolarized the apical membrane resting potential.[11] This postneuronal, extrareceptor increase in basal Cl^- transport following sympathectomy apparently involves the control of apical membrane Cl^- permeability. The conductance of the apical membrane, which is the rate limiting step in the vectorial transport of Cl^-, is increased not only by sympathetic nerve activity and

exogenous biogenic amines in the normal cornea but also by denervation. The increased membrane conductance following denervation is an anomaly we can't explain. However, we suggest that corneal sympathetic nerves exert a trophic action whereby apical membrane Cl^- permeability is suppressed.

Model for the Action of Serotonin Epithelial Cl^- Secretion

The postulated scheme of serotonin action in the corneal epithelium is depicted in Figure 10. Serotonin, derived from activity in corneal serotonin fibers, binds to preterminal serotonin receptors causing the release of norepinephrine or other β-adrenoreceptor neuroregulatory substances. It is unknown at this time whether there are different classes of terminals involved, or whether individual corneal sympathetic nerves contain both serotonin and a β-adrenergic agonist. According to our scheme, methysergide and lysergic acid diethylamide would inhibit the serotonin response by binding to the preterminal receptors.[5,29] Norepinephrine or other β-adrenoreceptor agonists released by the sympathetic nerves in response to serotonin would activate epithelial membrane β-adrenergic receptors situated in the deeper epithelium, which in turn would activate adenylate cyclase to increase cell levels of cyclic AMP. Additionally, β-adrenoreceptors located in the outer epithelial membranes could be activated by exogenous epinephrine in the tears, but the physiologic significance of this route of activation has not been established. Both routes of β-adrenergic receptor activation would be inhibited by timolol, which also would block any corneal response to serotonin since in this scheme, serotonin would be part of a serial process leading to the release of a β-adrenergic agonist. From thermodynamic considerations,[30] active Cl^- and Na^+ pumps operate in the deeper epithelial membranes, which serve as the metabolically linked driving forces for the well-documented transepithelial Cl^- secretion and Na^+ absorption.[3,31] The apical epithelial membrane has a very low ionic permeability[10] and is normally the rate-limiting step in terms of transepithelial transport.[4] Artificially increasing outer epithelial membrane Na^+ permeability with Amphotericin B or with Ag^+ increases Na^+ absorptive transport severalfold and promotes corneal swelling.[13] Activation of the β-adrenoreceptors, either by neural activity[1,26] or by the application of physiologic doses of β-adrenergic agonists, elevates cell levels of cyclic AMP with a concomitant increase in the permeability of the outer epithelial membranes that is specific for chloride.[4] Thus the net rate of Cl^- transport from stroma to tears is increased as Cl^- moves passively down its electrochemical potential gradient across an increased number of Cl^- conductance sites. Increased Cl^- secretion can dominate net electrolyte transport across the epithelium with a subsequent isotonic secretion of fluid[31,32] to augment the endothelium in the control of corneal hydration.

Key words: denervation, rabbit, cornea, epithelium, Cl^- transport

Acknowledgments

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