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CELLULAR MODE OF SEROTONIN ACTION ON Cl^- TRANSPORT IN THE RABBIT CORNEAL EPITHELIUM

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The present work examines serotonin-induced changes in cell potential difference and barrier resistances in the corneal epithelium in vitro using voltage-measuring microelectrodes and related techniques. Component resistances were determined using voltage and resistance profiles of the epithelium before and during the serotonin response. Serotonin, added to the stromal side of the cornea in the presence of nialamide, markedly reduced transcorneal and apical membrane resistances, while basal barrier resistance increased slightly and shunt resistance was unchanged. The marked drop in apical membrane resistance after serotonin treatment reflects an increase in apical membrane chloride permeability, inasmuch as the serotonin-stimulated short-circuit current is indistinguishable from the increase in net chloride flux. Prolonged (more than 1 h) exposure of corneas to serotonin markedly depolarized the epithelial cells and reduced the voltage divider ratio from 12.3 ± 2.1 to 1.5 ± 0.5, while not significantly affecting the stimulated short-circuit current. These later effects suggest changes in epithelial ion distribution during long periods of stimulation by serotonin.

Introduction

The unstimulated corneal epithelium of the rabbit has a transepithelial potential of 20–35 mV (tears-side negative), a net secretory transport of chloride ion and a net absorptive transport of sodium ion [1,2]. Transcorneal resistance is 6.0–12.0 $k\Omega \cdot cm^2$, and the shunt resistance is 12–16 $k\Omega \cdot cm^2$, indicating that the corneal epithelium may be considered as a 'tight' Cl-secreting epithelium [3]. Because the epithelium is a functional syncytium of similar cells that are readily impaled for intracellular recording using microelectrodes [1,3,4], this tissue provides a convenient general model for the study of cellular mechanisms in the control of chloride secretory transport.

There is considerable evidence to suggest involvement of serotonergic and dopaminergic actions on chloride transport in the corneal epithelium. Serotonin has been identified analytically in the cornea and histochemically in corneal nerves; stimulation of the tissue with this neurotransmitter increases cyclic AMP content and chloride secretion in the cornea [5,6]. The effects of serotonin appear to be specific to chloride transport, as the substitution of sulphate for chloride eliminates the rise in corneal short-circuit current and the fall in corneal resistance [5]. Also, there is no apparent effect of serotonin on transcorneal sodium fluxes (when the tissue is bathed in NaCl-containing Ringer's solution) and the serotonin-stimulated short-circuit current is not significantly different from the change in net chloride flux [6]. Recent experiments suggest the existence of two separate

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

and distinct receptor populations: one, the β-adrenergic receptor, is near or on the apical surface of the cornea [4], and another (for serotonin) may be situated deeper in the epithelium [5,6].

The corneal response to serotonin is less marked than that to epinephrine [4–6], is of long duration and reversal of the serotonin effect can be elicited rapidly using the serotonin receptor antagonist, methysergide [5]. These properties of the serotonin response allow the investigation of cellular events in the stimulation of chloride transport in the corneal epithelium. Of particular relevance to the present study, the voltage profiles and component resistances (that of the apical membrane, basal barrier, and shunt) may now be determined for the cornea under chronic stimulation of chloride transport. The primary aim of the present work is to determine, using intracellular voltage and resistance measurements, the membrane location of the serotonin response. Parts of this work have been reported previously in abstract form [7].

Methods

Adult New Zealand White rabbits (3.5–4.5 kg) were killed by an overdose of sodium pentobarbital administered via an ear vein. Eyes were enucleated and the corneas were mounted in an Ussing-style membrane chamber [4] and bathed on both sides with Hepes-buffered Ringer's solution with 10^{-4} M nialamide added [5]. Nialamide, an inhibitor of monoamine oxidase, has been shown previously to potentiate the serotonin effect [5]. The cornea in vitro was allowed to equilibrate for at least 1 h before the addition of serotonin. To treat the tissue with serotonin, the aqueous-side perfusion was switched to Ringer's with 10^{-4} M nialamide and 10^{-4} M serotonin added. This serotonin treatment is the maximally effective dose; threshold for the serotonin response in the presence of nialamide is about $5 \cdot 10^{-6}$ M [5]. During the development of the serotonin response, the effects on the resting voltage profile, the voltage divider ratio and transepithelial voltage, resistance and potential were monitored.

Intracellular measurements

The configuration of the apparatus and the recording methods were the same as those used previously [3]. Complete voltage and resistance profiles of the epithelium were made by advancing the microelectrode stepwise and impaling sequentially the superficial, wing and basal cell layers. The measured parameters included V_{ts}, transcorneal potential (tears-side grounded), V_{tc}, the apical membrane potential, V_{cs}, the basal barrier potential, V_{wb}, an intra-epithelial drop in the resting potential between the wing cell layer and the basal cell layer (as a result of incomplete coupling between these cell layers, cf. Ref. 1) and the voltage deflections at the primary barriers (dV_{tc}, dV_{cs} and dV_{ts} for the apical barrier, basal barrier and whole cornea, respectively). The voltage deflections were elicited by 1.0 s 5.0 $\mu A \cdot cm^{-2}$ square pulses (I_e) applied transcorneally, and were used to calculate R_{ts} and R_{tc}. The voltage divider ratio ($\alpha = R'_a/R'_b$) was measured as dV_{tc}/dV_{cs}. In the same manner as Klyce [1] and Marshall and Klyce [3], estimates were made of the component resistances of the epithelium (R'_a, R'_b and R_{sh}, which correspond to apical membrane, basal barrier and shunt resistances, respectively). The relations used [1] were:

$$R_{ts} = dV_{ts}/I_e \tag{1}$$

$$V_{wb} = V_{cs} - V_{tc} - V_{ts} \tag{2}$$

$$R_{tc} = dV_{tc}/I_e \tag{3}$$

$$E_a = [V_{tc} \cdot R_{ts} + (V_{ts} - V_{cs}) R_{tc}]/[R_{ts} - R_{tc}] \tag{4}$$

$$R'_a = [(V_{tc} - E_a)(V_{cs} - E_a) R_{ts}]/[(V_{cs} - E_a - V_{ts}) V_{ts}] \tag{5}$$

$$R'_b = [(R_{ts} - R_{tc})/R_{tc}] \cdot R'_a \tag{6}$$

and

$$R_{sh} = [R_{ts} \cdot (R'_a + R'_b)]/[R'_a + R'_b - R_{ts}] \tag{7}$$

where V_{ts}, V_{tc}, V_{cs} and V_{wb} are expressed for convenience as absolute values (the cell interior is negative with respect to the tears and to the stromal bathing solutions). In this way, for a single voltage and resistance profile of the epithelium either before or during the serotonin effect, the component resistances could be estimated as could any effects of serotonin on these calculated parameters. This technique (cf. Ref. 1 for details) yields results similar to those derived from the perturbation

technique of Reuss and Finn [8], in a study that included both methods and that used the rabbit corneal epithelium [3]. Data are presented as the means ± S.E.; comparisons between means used either paired or unpaired *t*-tests, where appropriate.

Results

A moderate, progressive depolarization of V_{tc} and V_{cs} follows the addition of serotonin (Table I). This is accompanied by a reduction in the transcorneal resistance (Table III). The pronounced reduction in the voltage divider ratio and marked drop in apical barrier resistance (R_{tc}) are suggestive of a reduction in apical membrane resistance (R'_a), given that basal barrier resistance increased only slightly and that the shunt resistance is finite (Table II). Depolarization of V_{tc} and V_{cs} after serotonin addition started about 5 min after initiation of the effect on R_{ts}, suggesting that the increase in conductive chloride transport is the primary effect and that the depolarization is a secondary response, possibly involving changes in intracellular ion activity (vide infra). The voltage profile in the corneal epithelium 1 h after serotonin addition is compared to the initial resting potentials (Table I). Although V_{tc} and V_{cs} were both reduced by 10–15 mV, V_{ts} was not significantly changed. The latter result is in agreement with our previous transcorneal recordings after serotonin treatment [5]. Corneas that were not treated with serotonin show little change in resting potential profiles over the 3–4 h duration of in vitro incubation [1].

TABLE I

RESTING POTENTIALS ACROSS APICAL AND BASAL BARRIERS (V_{tc} AND V_{cs}, RESPECTIVELY) AND TRANSEPITHELIAL POTENTIAL (V_{ts}) BEFORE AND 1 h AFTER SEROTONIN TREATMENT

Results are means ± S.E.; potentials expressed as absolute values, in mV. *P* values are from paired, two-tailed *t*-test; $n = 11$.

Period	V_{tc}	V_{cs}	V_{ts}
Control	42.9 ± 2.5	75.2 ± 1.7	26.4 ± 2.7
Serotonin	31.4 ± 2.3	58.9 ± 3.6	23.0 ± 3.1
P	< 0.01	< 0.01	N.S.

TABLE II

VOLTAGE DEFLECTIONS (dV_{tc} AND dV_{cs}) AND VOLTAGE DIVIDER RATIO (α) IN RESPONSE TO 5.0 $\mu A \cdot cm^{-2}$ TRANSCORNEAL PULSES BEFORE AND 1 h AFTER SEROTONIN TREATMENT

Results are means ± S.E., with voltage deflections in mV. *P* values are from paired, two-tailed *t*-test; $n = 11$.

Period	dV_{tc}	dV_{cs}	α
Control	43.8 ± 2.4	5.2 ± 1.1	12.3 ± 2.1
Serotonin	15.1 ± 2.0	14.0 ± 2.0	1.5 ± 0.5
P	< 0.001	< 0.01	< 0.001

Because of the possibility of alterations in intracellular ion distribution during the serotonin response and as the development of the effect was slower than that of other perturbing agents (e.g., epinephrine and silver ion, cf. Ref. 3), the perturbation method for the estimation of component resistances [3,8] was not used. The perturbation method requires that the epithelial ion distribution should remain in steady-state during the perturbation [8], but with marked or prolonged stimulation of chloride transport we cannot ensure that this condition is met [3]. Also, the perturbation method assumes perforce that only one resistor of the equivalent circuit is affected by the perturbing agent. For serotonin, this could not be determined a priori. Instead, to determine the membrane location of the serotonin effect, the resistances of the apical membrane, basal barrier and shunt were estimated using voltage and resistance profiles (cf. Methods).

Serotonin markedly reduced apical membrane resistance and transcorneal resistance, consistent with the hypothesis that serotonin increases apical membrane permeability (Table III). The estimates for shunt resistance were highly variable and, while the control values were often lower than those after serotonin, the two means are not significantly different. The apparent increase in basal barrier resistance after serotonin treatment (Table III) likely is not a primary effect that would increase transcellular chloride transport, as the transcellular path for chloride secretion is conductive. Because the stimulation in chloride transport accounts entirely for the change in short-circuit current after serotonin treatment [5], the reduction in api-

TABLE III

EFFECT OF SEROTONIN ON RESISTANCES (IN $k\Omega \cdot cm^2$) OF THE APICAL MEMBRANE (R'_a), BASAL BARRIER (R_b), SHUNT (R_{sh}) AND WHOLE CORNEA (R_{ts}) AND ON VOLTAGE DIVIDER RATIO (α) IN THE RABBIT CORNEAL EPITHELIUM

Results are means ± one S.E.; $n = 11$.

Treatment	R'_a	R_b	R_{sh}	R_{ts}	α
Control	21.3 ± 2.6	1.0 ± 0.2	21.3 ± 4.2	9.8 ± 0.5	12.3 ± 2.1
Serotonin	3.9 ± 1.0	2.8 ± 0.4	28.2 ± 3.5	5.8 ± 0.5	1.5 ± 0.5
P	< 0.001	< 0.01	N.S.	< 0.001	< 0.01

cal membrane resistance would appear to be the main effect of serotonin and the conductance change apparently reflects an increase in chloride permeability at this location.

Discussion

The present study indicates that the primary effect of serotonin is to stimulate chloride secretion in the rabbit cornea via an increase in apical membrane chloride conductance. In this way, and because Cl^- intracellular activity is above its electrochemical equilibrium potential [4,10], the chloride exit rate across the apical barrier would be enhanced. As has been shown elsewhere [4,10], the active step for chloride secretion in this tissue is almost certainly located at the basal barrier (basolateral membrane) of the epithelial cells. It is unlikely that the basal entry of Cl^- limits the secretion rate for this ion in resting corneas. Rather, under resting conditions, the high apical membrane resistance appears to be rate-limiting. Inasmuch as the serotonin-enhanced short-circuit current is maintained at a consistently high level, and is much lower than the sustained stimulation that is possible using theophilline or epinephrine [2], it would appear that Cl^- secretion in serotonin-stimulated corneas is still rate-limited by the apical membrane Cl^- conductance. Given the above scheme for sustained stimulation of Cl^- secretion by serotonin, it seems that the depolarization (Table I) seen under these conditions represents a reduction in intracellular Cl^- activity or an accumulation of intracellular Na^+. These proposed mechanisms will need to be confirmed using intracellular ion activity measurements (see Ref. 10) during stimulation of Cl^- secretion.

The mean values for R'_a, R_{sh} and R_{ts} (Table III) are higher than those reported by us previously [3] and by others [10]; this apparently reflects a slight improvement in the mounting procedure and/or in the cellular impalements. In agreement with previous work [3], the apical membrane resistance is high and approximately equal to that of the shunt in the unstimulated corneal epithelium.

The increase in basal barrier resistance (Table II) may be a secondary effect if, as presented above, there is significant depletion of intracellular chloride after marked stimulation of chloride transport by serotonin. Hence the primary effect of serotonin appears to be a reduction in apical membrane resistance, rather than an effect on the basal barrier or shunt.

The mode of serotonin action in the corneal epithelium is in many respects similar to that of epinephrine. Klyce and Wong [4] demonstrated that the stimulation of β-adrenergic receptors results in a rapid increase in the conductance of the apical (tears-side) membrane to chloride, a result that accounts for the increased chloride secretory flux after epinephrine treatment, or after exposure to phosphodiesterase inhibitors and cyclic AMP [2]. Neufeld et al. [6] observed increases in corneal cyclic AMP content in response to β-adrenergic receptor stimulation, and the time course and dose response of changes in cyclic AMP content paralleled the electrophysiological responses [5]. Other experiments have indicated the presence of a sparse population of adrenergic neurons that arise from the superior cervical ganglion and that terminate subjacent to the corneal epithelium [1]. These neurons would appear to be the source of physiologically important release of norepinephrine, although such release per se has not been demon-

strated. Hence chloride secretion by the corneal epithelium may be controlled adrenergically through norepinephrine stimulation of β-adrenergic receptors which leads (via an intracellular messenger, cyclic AMP) to enhancement of chloride conductance of the apical membrane.

Although serotonin reduces apical membrane Cl^- resistance via a mechanism involving cyclic AMP [5], as does epinephrine [4], there are two distinct receptor populations that mediate the responses. Epinephrine acts from the tears side in a few seconds [4], while serotonin acts more slowly (3–7 min) and only from the stromal side of the cornea [5], suggesting that the population of β-adrenergic receptors is located on the apical surface, while the serotonin receptors may be located deeper in the epithelium. Also, pharmacological considerations [5,6] demonstrate that both serotonergic and β-adrenergic receptors are present. The importance of dually controlled chloride transport is not clear at present, although such control involving catecholamines and serotonin in stimulus-release coupling has been observed in other systems [11]. Recent evidence [12] suggests that the serotonin receptor is associated with sympathetic fibers in the epithelium, as the serotonin response is abolished by ablation of the superior cervical ganglion.

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