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EFFECTS OF TRYPTAMINE ON ACTIVE SODIUM AND CHLORIDE TRANSPORT IN THE ISOLATED BULLFROG CORNEA

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Summary

The effects of the serotonin analogue, tryptamine, on the active transepithelial transport of Na^+ and Cl^- in the in vitro bullfrog cornea were studied. Tryptamine, 1 mM, inhibited both the short-circuit current (I_{sc}) and potential difference (PD) of corneas transporting either Na^+ alone or both Na^+ and Cl^-. The electrical resistance, R, increased in all cases. Both unidirectional Na^+ and Cl^- fluxes were decreased by tryptamine and these changes accounted for the inhibitory effects on the I_{sc}. The effects of tryptamine were considered along with those of 2 mM theophylline and 0.1 mM ouabain. Tryptamine inhibited the I_{sc} and both undirectional Cl^- fluxes which were previously stimulated by theophylline. Theophyline addition, after tryptamine preincubation, increased the Cl^- undirectional fluxes but did not restore the inhibited I_{sc}. The inhibitory effects of tryptamine on active Na^+ and Cl^- transport were different from those of ouabain. While both drugs inhibited the forward Na^+ and Cl^- fluxes, their backfluxes decreased with tryptamine and increased with ouabain. The addition to the bathing solution of tryptamine after ouabain preincubation reduced the ouabain-increased backward Cl^- flux and further increased the electrical resistance. These results are analyzed in terms of an electrical model from which it appears that tryptamine's mechanism of action was to decrease cellular permeability to the transepithelial movement of Na^+ and Cl^-.

Introduction

The hydration and transparency of the rabbit cornea is controlled by a fluid pump located at its endothelial limiting layer [1]. In addition, there is evidence indicating that the epithelial layer may also play an active role in the maintenance of deturgescence [2]. In the frog cornea, the epithelium has been shown to play a role in controlling the hydration of the cornea and a coupling

between fluid movement and active Cl^- transport has been suggested [3,4].

The epithelium of the frog cornea contains pump mechanisms for the active extrusion of Cl^- into the tears and Na^+ into the stroma. Under control conditions, about 90% of the short-circuit current (I_{sc}) can be accounted for by the net movement of Cl^-. The rate of Na^+ transport is small because of the low Na^+ permeability of the tear-side membrane of the epithelial cells. Amphothericin B increases the permeability of this membrane to a number of ions one of which is Na^+, and this increase in Na^+ permeability stimulates net Na^+ transport [5]. The maintenance of Na^+ and Cl^- transport appears to be dependent on the activity of $(Na^+ + K^+)$-ATPase and the presence of Na^+ in the bathing solution [6,7]. Cl^- transport is specifically stimulated by the catecholamines (epinephrine and norepinephrine), the β-agonist isoproteronol [8], theophylline, cyclic AMP [9,10], the Ca^{2+} ionophore A23187 [11] and ascorbic acid [12]. The loop diuretics furosemide, bumetanide and ethacrynic acid are specific inhibitors of active Cl^- transport [13,14].

The purpose of this study was to consider the effects of the serotonin analogue, tryptamine, on unidirectional Na^+ and Cl^- fluxes across the isolated bullfrog cornea in conjunction with the effects of the drug on the tissue's electrical parameters. In an attempt to understand tryptamine's mechanism of action and the interrelationships between Na^+ and Cl^- transport, the effects of the drug were considered in combination with either theophylline, ouabain, or amphotericin B.

Materials and Methods

Corneas of the bullfrog, *Rana catesbeiana*, were dissected and mounted as a membrane between two halves of a Lucite chamber following a procedure previously described [6]. Each side of the chamber was filled with 5 ml of a Ringer's solution whose composition has been previously described [5]. In the experiments where a Cl^--free solution was used, each mol of Cl^- was replaced by 0.5 mol of SO_4^{2-} and the difference in osmolality was compensated for with sucrose. The solutions bathing the cornea were bubbled with air. The following drugs were used: tryptamine · HCl, theophylline and ouabain (Sigma Chemical Co., St. Louis, Mo.) and amphotericin B (Squibb, New York). It was found that 10^{-3} M tryptamine had similar effects on the electrical parameters regardless of the bathing solution into which it was placed. Therefore, unless otherwise noted, the effects of tryptamine, theophylline and ouabain result from these drugs being added to the endothelial bathing solution.

Transcorneal potential difference (PD) was monitored and current was sent across the cornea by means of agar-Ringer-filled polyethylene tubing. PD bridges were kept close to the corneal surfaces so that the solution resistance was negligible. The PD bridges were connected to the measuring and recording equipment through calomel cells (Keithley 200B millivoltmeters and Heath EU 20 recorders). Short-circuit current (I_{sc}) was obtained and measured with an automatic voltage clamp apparatus. Resistance was determined by periodically measuring the additional current necessary to further depolarize the membrane by 22.5 mV from the short-circuited condition. The obtained resistance agreed fairly well with that determined by the ratio PD/I_{sc}.

Unidirectional Cl^- fluxes were measured by adding about 3—5 μCi of Cl^- to one chamber and taking subsequent periodic samples from the opposite compartment. The surface area of the cornea exposed to the solution was 0.5 cm^2. Specific activity on the labeled side remained constant throughout the experiment, and the activity on the label-free side was always about 0.1% of that present on the labeled side. The samples were mixed with a modified Bray's solution and counted with a Packard Tricarb liquid scintillation spectrometer.

In the experiments where unidirectional Na^+ and Cl^- fluxes were measured simultaneously, ^{24}Na and ^{36}Cl were used. One set of corneas was used to measure Na^+ and Cl^- fluxes in one direction and another set to measure the fluxes in the opposite direction. The specific activity on the labeled side was kept constant and the activity on the label-free side was always 1% or less of that in the labeled compartment. ^{24}Na samples were immediately counted with a Packard autogamma spectrometer and fluxes were calculated after correction for decay. The same samples were counted 2 weeks later with a Packard Tricarb liquid scintillation spectrometer to determine ^{36}Cl activity after ^{24}Na had decayed to less than 1% of its initial activity.

Results

(A) Effects of tryptamine on sodium transport

The effects of tryptamine on unidirectional Na^+ fluxes and electrical parameters were studied under four different experimental conditions: (1) corneas bathed in Na_2SO_4-Ringer; (2) corneas bathed in Na_2SO_4 Ringer whose Na^+ transport rates had been stimulated with 10^{-5} M amphotericin B; (3) corneas bathed in NaCl Ringer; (4) corneas bathed in NaCl Ringer whose Na^+ transport rates had been stimulated with amphotericin B.

Condition 1. As shown previously the I_{sc} was small (less than 1.5 $\mu A/cm^2$) and unidirectional Na^+ fluxes are almost the same in both directions [15]. Values obtained from ten experiments in which the Na^+ forward fluxes were measured are summarized in the top panel of Table I. The effect was gradual and the full effect of tryptamine as shown in the table was only attained 60 min after its addition. Tryptamine increased the resistance in every experiment and decreased the Na^+ flux in seven cases. On the average, the increase in resistance was 0.6 $k\Omega \cdot cm^2$ which is equivalent to a reduction in ionic conductance of 0.0474 $m\Omega^{-1}/cm^2$ (cf. Table I).

It is of interest to consider what the predicted decrease in Na^+ flux would be from the partial conductance equation with such a decrease in membrane conductance [16]. From this equation,

$$G_i = \phi_i z^2 F^2 / RT$$

where G_i, the partial ionic conductance, is expressed in Ω^{-1}/cm^2, ϕ, is the flux in equiv./$s \cdot cm^2$ and z, F, R and T have their usual meanings, a reduction in measured ionic conductance of 0.0747 $m\Omega^{-1}/cm^2$ corresponds to a decrease in ionic flux of 0.045 μequiv./$h \cdot cm^2$. The average decrease of the experimentally measured Na^+ fluxes was 0.046 μequiv./$h \cdot cm^2$ suggesting that most of the resistance change was due to a reduction in Na^+ flux.

In a separate series of nine experiments, the effect of 10^{-3} M tryptamine was

TABLE I

EFFECTS OF 1 mM TRYPTAMINE ON THE ELECTRICAL RESISTANCE AND UNIDIRECTIONAL Na^+ FLUXES ACROSS ISOLATED BULLFROG CORNEAS BATHED IN Na_2SO_4-RINGER

Resistance ($k\Omega \cdot cm^2$) ($n = 10$)				Forward Na^+ flux (μequiv./h $\cdot$ cm^2)		
	Control (C)	Tryptamine (T)	(T—C) *	Control (C)	Tryptamine (T)	(C—T) *
Mean	3.27	3.87	0.60 **	0.302	0.256	0.046
±S.E.	0.72	0.96	0.27	0.041	0.031	0.016
Resistance ($k\Omega \cdot cm^2$) ($n = 9$)				Backward Na^+ flux (μequiv./h $\cdot$ cm^2)		
	Control (C)	Tryptamine (T)	(T—C) *	Control (C)	Tryptamine (T)	(C—T) *
Mean	4.09	5.00	0.91 ***	0.206	0.181	0.025
±S.E.	0.87	1.12	0.27	0.029	0.028	0.006

* The differences are statistically significant as paired data for $P < 0.01$.

** Average change in resistance. The average change in conductance can be calculated as:

$$\frac{3.87 - 3.27}{3.87 \times 3.27} = 0.0474 \text{ m}\Omega^{-1}/\text{cm}^2.$$

*** Average change in resistance. The average change in conductance can be calculated as:

$$\frac{5.00 - 4.09}{5.00 \times 4.09} = 0.0445 \text{ m}\Omega^{-1}/\text{cm}^2.$$

determined on the backward Na^+ flux and electrical resistance. The results are shown in the bottom panel of Table I. A small but statistically significant reduction in the Na^+ flux (0.025 μequiv./h $\cdot$ cm^2) was observed. The resistance increased on the average 0.91 $k\Omega \cdot cm^2$ which corresponded again to a reduction in ionic conductance of 0.0445 $m\Omega^{-1}/cm^2$; remarkably similar to the conductance change in the forward flux experiments. However, the observed decrease in Na^+ backflux was smaller than that calculated from the change in ionic conductance.

Condition 2. Corneas were bathed in Na_2SO_4 Ringer and their Na^+ transport rates were stimulated by adding amphotericin B at a final concentration of 10^{-5} M to the epithelial bathing solution. As shown previously [5], amphotericin B increased the I_{sc} (representing net Na^+ transport) to a stable mean value of about 17 $\mu A/cm^2$. After the I_{sc}, electrical resistance and either forward or backward Na^+ fluxes were measured during four or five 30-min periods, tryptamine was added for a final concentration of 10^{-3} M and measurements were continued for five additional 30-min periods. 60—90 min after the addition of tryptamine the electrical parameters and unidirectional fluxes had reached new stable values. These results are summarized in Table II. In the two groups shown, amphotericin B reduced the electrical resistance to 2.68 and 2.48 $k\Omega \cdot cm^2$ (compared to the control values in Na_2SO_4 of 3.27 and 4.09 $k\Omega \cdot cm^2$ shown in Table I).

Tryptamine increased the electrical resistance to 4.33 and 4.68 $k\Omega \cdot cm^2$; a 62 and 89% increase, respectively. Forward and backward Na^+ fluxes that had been increased by amphotericin B to 1.99 and 0.38 μequiv./h $\cdot$ cm^2 were

TABLE II

EFFECTS OF 1 mM TRYPTAMINE ON THE ELECTRICAL RESISTANCE, Na^+ FLUXES AND SHORT-CIRCUIT CURRENT ACROSS ISOLATED BULLFROG CORNEAS STIMULATED BY AMPHOTERICIN B AND BATHED IN Na_2SO_4-RINGER

Values in the same horizontal line are from the same experiments before and after tryptamine. The differences are statistically significant as paired data for $P < 0.01$ for all parameters.

Resistance ($k\Omega \cdot cm^2$) ($n = 7$)			Forward Na^+ flux (μequiv./h $\cdot$ cm^2)		I_{sc} (μequiv./h $\cdot$ cm^2)	
	Amphotericin B (A)	A plus tryptamine	Amphotericin B (A)	A plus tryptamine	Amphotericin B (A)	A plus tryptamine
Mean	2.68	4.33	1.99	0.90	0.63	0.21
±S.E.	0.19	0.54	0.46	0.16	0.10	0.03
Resistance ($k\Omega \cdot cm^2$) ($n = 8$)			Backward Na^+ flux (μequiv./h $\cdot$ cm^2)		I_{sc} (μequiv./h $\cdot$ cm^2)	
	Amphotericin B (A)	A plus tryptamine	Amphotericin B (A)	A plus tryptamine	Amphotericin B (A)	A plus tryptamine
Mean	2.48	4.68	0.38	0.25	0.78	0.22
±S.E.	0.37	1.03	0.06	0.04	0.14	0.04

reduced to 0.90 and 0.25 μequiv./h $\cdot$ cm^2, respectively. This represented a 60% reduction in net Na^+ flux. Likewise the I_{sc} was reduced on the average by 70%. As seen previously [5], the net Na^+ transport did not correspond to the I_{sc} in amphotericin-stimulated corneas. This discrepancy persisted after inhibition by tryptamine.

Condition 3. As shown before Na^+ fluxes are similar when the corneas are bathed either in Cl^--rich or Cl^--free solution [5]. It was of interest to see whether tryptamine would inhibit Na^+ fluxes across corneas bathed in NaCl Ringer. The results are shown in Table III. Tryptamine inhibited the forward Na^+ flux in all cases and the mean decline was 0.076 μequiv./h $\cdot$ cm^2. The resistance increased in every case and on the average from 1.65 to 2.91 $k\Omega \cdot$

TABLE III

EFFECTS OF 1 mM TRYPTAMINE ON ELECTRICAL RESISTANCE, FORWARD Na^+ FLUX AND SHORT-CIRCUIT CURRENT ACROSS ISOLATED BULLFROG CORNEAS BATHED IN NaCl-RINGER

Values are from the same experiments before and after tryptamine. The differences are statistically significant as paired data for $P < 0.01$ for all parameters.

	Resistance ($k\Omega \cdot cm^2$) ($n = 9$)		Forward Na^+ flux (μequiv./h $\cdot$ cm^2) ($n = 9$)		I_{sc} (μequiv./h $\cdot$ cm^2) ($n = 9$)	
	Control	Tryptamine	Control	Tryptamine	Control	Tryptamine
Mean	1.65	2.91	0.29	0.22	0.56	0.08
±S.E.	0.14	0.28	0.04	0.03	0.02	0.02

TABLE IV

EFFECTS OF 1 mM TRYPTAMINE ON THE ELECTRICAL RESISTANCE, FORWARD Na^+ FLUX AND SHORT-CIRCUIT CURRENT ACROSS ISOLATED BULLFROG CORNEAS STIMULATED BY AMPHOTERICIN B AND BATHED IN NaCl-RINGER

The paired differences for the same parameters were statistically significant for $P < 0.01$.

	n	Amphotericin B (A) (mean ± S.E.)	A plus tryptamine (mean ± S.E.)
Resistance ($k\Omega \cdot cm^2$)	8	0.53 ± 0.04	0.93 ± 0.12
Forward Na^+ flux ($\mu equiv./h \cdot cm^2$)	6	2.54 ± 0.09	1.29 ± 0.12
I_{sc} ($\mu equiv./h \cdot cm^2$)	8	0.97 ± 0.04	0.36 ± 0.03

cm^2 which corresponds to a decrease in conductance of 0.262 $m\Omega^{-1}/cm^2$. Although the changes in Na^+ fluxes in NaCl Ringer were similar to those in Na_2SO_4 Ringer (0.076 and 0.046 $\mu equiv./h \cdot cm^2$, respectively), the changes in conductance were much larger (0.262 and 0.047 $m\Omega^{-1}/cm^2$, respectively) suggesting that tryptamine was also altering the permeability of the cornea to Cl^-. The effect of tryptamine on the backward Na^+ fluxes was not determined in this condition.

Condition 4. Corneas bathed in NaCl Ringer were treated with amphotericin B which increased the I_{sc} and reduced the electrical resistance. In this condition, 10^{-3} M tryptamine was added to determine its effects on resistance, I_{sc} and forward Na^+ flux. The results are shown in Table IV. The forward Na^+ flux was reduced by tryptamine while the low electrical resistance in this condition was increased by 75%. This represented a decrease in conductance of 0.813 $m\Omega^{-1}/cm^2$, the largest change of all four conditions studied, suggesting again that tryptamine decreased both Na^+ and Cl^- permeability. The I_{sc} representing net Na^+ and Cl^- transport was reduced to 36% of the amphotericin B-stimulated control value. The effect of tryptamine on the backward Na^+ flux was not studied.

(B) Effects of tryptamine on chloride transport; Interactions with theophylline and ouabain

The effects of tryptamine on unidirectional Cl^- fluxes and electrical parameters were studied under three different experimental conditions: (5) corneas bathed in NaCl Ringer, (6) combined effects with theophylline, (7) combined effects with ouabain.

Condition 5. As shown in Table V under control conditions, the net Cl^- flux c alculated from the unidirectional fluxes is nearly equal to the I_{sc}, 0.52 $\mu equiv./h \cdot cm^2$. Tryptamine reduced both unidirectional Cl^- fluxes, the forward flux proportionally more. The I_{sc} was reduced by 81%. A good match between net Cl^- flux and I_{sc} persisted after tryptamine had inhibited Cl^- transport. In both cases, the resistance increased to values similar to those observed in condition 3 (cf. Table III).

Condition 6. Theophylline is known to stimulate active Cl^- transport [9]. This occurs apparently as a consequence of an increase in Cl^- permeability: both indirectional Cl^- fluxes are increased by theophylline and the electrical

TABLE V

EFFECTS OF 1 mM TRYPTAMINE ON UNIDIRECTIONAL Cl^- FLUXES, SHORT-CIRCUIT CURRENT AND ELECTRICAL RESISTANCE ACROSS ISOLATED BULLFROG CORNEAS BATHED IN NaCl-RINGER

The paired differences for the same parameters were statistically significant for $P < 0.01$.

	n	Control (mean ± S.E.)	Tryptamine (mean ± S.E.)
Forward Cl^- flux (μequiv./h · cm^2)	13	0.68 ± 0.03	0.24 ± 0.02
Backward Cl^- flux (μequiv./h · cm^2)	14	0.19 ± 0.03	0.14 ± 0.02
I_{sc} (μequiv./h · cm^2)	27	0.52 ± 0.03	0.10 ± 0.01
Resistance (kΩ · cm^2)	27	1.78 ± 0.12	2.91 ± 0.42

resistance is reduced. In this respect, the effects of theophylline seem to be opposite to those of tryptamine. It was of interest to examine the combined effects of these drugs when they were added successively. The effects of the theophylline-tryptamine sequence, where tryptamine was added to the epithelial bathing solution and theophylline to the endothelial bathing solution, on the unidirectional Cl^- fluxes and electrical parameters are shown in Table VI. Theophylline increased the I_{sc} and both unidirectional Cl^- fluxes while the electrical resistance decreased. Tryptamine had an opposing effect on these parameters. The effects of the opposite sequence of drug addition are shown in Table VII. In spite of preincubation with tryptamine, theophylline increased both unidirectional Cl^- fluxes and decreased the electrical resistance. However, despite the increase in unidirectional Cl^- fluxes, the I_{sc} continued to be inhibited in the presence of theophylline. As with the experiments shown in Table VI, since forward and backward Cl^- fluxes were measured in a different and relatively small set of corneas, there is a poor correlation between net Cl^- flux and the I_{sc}.

Condition 7. Ouabain inhibits active Cl^- transport across the frog corneal epithelium [6]. The decrease in net Cl^- flux is brought about by a reduction of the forward flux associated with an increase in the backward flux. In this respect, ouabain is different from the other Cl^- transport inhibitors; furosemide

TABLE VI

COMBINED EFFECTS OF 2 mM THEOPHYLLINE AND 1 mM TRYPTAMINE ON UNIDIRECTIONAL Cl^- FLUXES, I_{sc} AND ELECTRICAL RESISTANCE ACROSS ISOLATED BULLFROG CORNEAS BATHED IN NaCl-RINGER

The paired differences for the pairs "control-theophylline" and "theophylline-plus-tryptamine" were statistically significant for $P < 0.01$ for all parameters.

	n	Control (mean ± S.E.)	Theophylline (T) (mean ± S.E.)	T plus tryptamine (mean ± S.E.)
Forward Cl^- flux (μequiv./h · cm^2)	4	0.66 ± 0.05	1.03 ± 0.05	0.55 ± 0.03
Backward Cl^- flux (μequiv./h · cm^2)	4	0.18 ± 0.01	0.32 ± 0.02	0.22 ± 0.02
I_{sc} (μequiv./h · cm^2)	8	0.60 ± 0.05	0.96 ± 0.06	0.23 ± 0.03
Resistance (kΩ · cm^2)	8	1.93 ± 0.14	1.32 ± 0.07	1.67 ± 0.17

TABLE VII

COMBINED EFFECTS OF 1 mM TRYPTAMINE AND 2 mM THEOPHYLLINE ON UNIDIRECTIONAL Cl^- FLUXES' I_{sc} AND ELECTRICAL RESISTANCE ACROSS ISOLATED BULLFROG CORNEAS BATHED IN NaCl-RINGER

The paired differences for the pairs "control-theophylline" and "theophylline-plus-tryptamine" were statistically significant for $P < 0.01$ for all parameters, except for the pair marked with asterisks.

	n	Control (mean ± S.E.)	Tryptamine (T) (mean ± S.E.)	T plus theophylline (mean ± S.E.)
Forward Cl^- flux (μequiv./h · cm^2)	6	0.68 ± 0.05	0.39 ± 0.05	0.51 ± 0.04
Backward Cl^- flux (μequiv./h · cm^2)	6	0.30 ± 0.02	0.18 ± 0.01	0.29 ± 0.02
I_{sc} (μequiv./h · cm^2)	12	0.55 ± 0.04	0.13 ± 0.01 *	0.16 ± 0.02 *
Resistance (kΩ · cm^2)	12	1.78 ± 0.11	2.98 ± 0.27	2.36 ± 0.14

TABLE VIII

COMBINED EFFECTS OF 0.1 mM OUABAIN AND 1 mM TRYPTAMINE ON BACKWARD Cl^- FLUX, I_{sc} AND ELECTRICAL RESISTANCE ACROSS ISOLATED BULLFROG CORNEAS BATHED IN NaCl-RINGER

The paired differences for the pairs "control-ouabain" and "ouabain-plus-tryptamine" were statistically significant for $P < 0.01$ for all parameters.

	n	Control (mean ± S.E.)	Ouabain (O) (mean ± S.E.)	O plus tryptamine (mean ± S.E.)
Backward Cl^- flux (μequiv./h · cm^2)	6	0.27 ± 0.04	0.51 ± 0.04	0.29 ± 0.04
I_{sc} (μequiv./h · cm^2)	6	0.67 ± 0.05	0.14 ± 0.01	0.01 ± 0.02
Resistance (kΩ · cm^2)	6	1.60 ± 0.07	1.95 ± 0.16	n.d.

n.d., not determined.

[13], ethacrynic acid [13], bumetanide [14] and tryptamine which reduce backward Cl^- fluxes. It was then of interest to compare the successive effects of ouabain and tryptamine on the backward Cl^- fluxes and electrical parameters. These results are shown in Table VIII. Ouabain increased the backward Cl^- flux while reducing the I_{sc} to a small value. Tryptamine further reduced the I_{sc} to near zero and decreased the ouabain increased backward Cl^- flux as well as decreasing further the forward Cl^- flux. The resistance that was moderately increased by ouabain was further increased by tryptamine. These effects suggest that ouabain and tryptamine inhibit active Cl^- transport by acting on different components of the Cl^- transport mechanism.

Discussion

Two active transport systems have been described in the frog corneal epithelium: a Cl^- transport directed outwards towards the tears which normally accounts for about 90% of the I_{sc} and a Na^+ transport system directed inwards whose rate is greatly stimulated by the polyene antibiotic, amphothericin B [5]. Previous work in our laboratory suggests that there is a separation between the two pathways for Na^+ and Cl^- transport: Na^+ transport occurs in the absence of Cl^- in the bathing solution [5,15] and various pharmacological

agents selectively stimulate or inhibit Cl^- transport [8—14]. However, there are two kinds of experimental evidence suggesting an association between the two transport systems: Cl^- transport is dependent on the presence of Na^+ in the endothelial bathing solution [7] and both Na^+ and Cl^- transport are inhibited by ouabain [6]. Tryptamine, like ouabain, inhibits both Na^+ and Cl^- transport. There are, however, clear differences between the effect of ouabain and tryptamine. Ouabain has less of an effect on the electrical resistance than tryptamine and the inhibitory effect of ouabain is associated with an increase of both Na^+ and Cl^- backwards fluxes whereas tryptamine reduces these fluxes. Ouabain reduced oxygen consumption 34%, whereas the reduction by tryptamine was only 26% [17]. However, the tryptamine effect is larger than the 16% reduction in oxygen consumption produced by specific inhibitors of Cl^- transport [17].

The effects of tryptamine on ionic pathways can be analyzed in terms of an electrical model. Fig. 1 shows such a model for a cornea bathed in Cl^--free solution. It is assumed that only Na^+ moves transcellularly. R_{sNa} and R_{Na} are the resistances to Na^+ of the opposing cell membranes. Rp represents a paracellular pathway for Na^+ and possibly other ions in the solution. E_{Na} is the EMF of the Na^+ pump. Under control conditions, the unidirectional Na^+ fluxes are similar and the Na^+-originated I_{sc} is small suggesting that most of the Na^+ flux proceeds via paracellular pathways. The equivalent resistance of the parallel pathway to Na^+ can be calculated from the unidirectional Na^+ fluxes by using the partial conductance equation as shown before. From the average backward Na^+ flux (Table I), a value for R_{pNa} of 4.58 k$\Omega \cdot cm^2$ is calculated. Since the total resistance for that group of corneas was 4.09 k$\Omega \cdot cm^2$, a resistance equal to 38.22 k$\Omega \cdot cm^2$ must be placed in parallel to R_{pNa} to obtain the 4.09 k$\Omega \cdot$

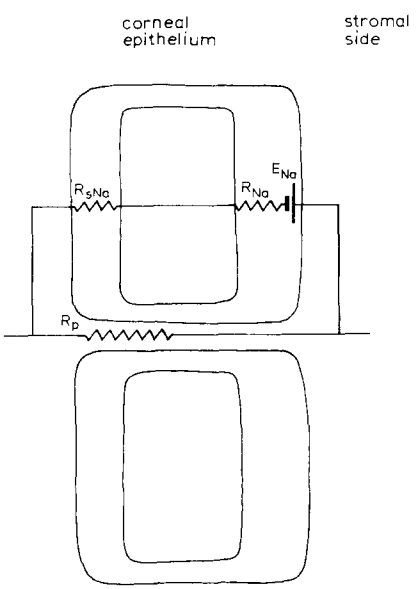

Fig. 1. Electrical model for one cell layer of the corneal epithelium bathed in Na_2SO_4-Ringer. For simplicity, the electrical equivalent is drawn for only one cell and one intercellular channel.

cm^2 value. 38.22 $k\Omega \cdot cm^2$ could represent parallel leaks to ions other than Na^+, the transcellular resistance to Na ($R_{sNa} + R_{Na}$), or both. Thus, the minimum value for $R_{sNa} + R_{Na}$ is 28.22 $k\Omega \cdot cm^2$. If a fraction of the Na^+ backflux is transcellular, R_{pNa} (part of Rp) will be larger and the minimum value for $R_{sNa} + R_{Na}$ will be proportionally smaller. The use of the Na^+ forward flux (from Table I) for calculating R_{pNa} would be inaccurate since perhaps 12% (i.e. I_{sc}/forward Na^+ flux) of the forward flux is probably transcellular.

Tryptamine decreased the forward Na^+ flux and slightly the backward flux while increasing the electrical resistance. This could be interpreted in two ways: (a) that tryptamine changes the permeability of the intercellular pathways possibly due to cell swelling or (b) that tryptamine increases only $R_{sNa} + R_{Na}$. If the first possibility was true, both unidirectional fluxes should change equally, which is not the case. The second alternative assumes that Rp does not change. Thus, the correct value to be assumed for Rp_{Na} is that calculated from the remaining Na^+ backflux after tryptamine incubation (0.181 μequiv./h $\cdot$ cm^2) which is 5.22 $k\Omega \cdot cm^2$. The total electrical resistances before and after tryptamine incubation were 4.09 and 5.00 $k\Omega \cdot cm^2$, respectively. Thus, 18.9 and 118.6 $k\Omega \cdot cm^2$ are the required values for $R_{sNa} + R_{Na}$ which must be placed in parallel with 5.22 $k\Omega \cdot cm^2$ to conform with the resistances measured before and after tryptamine incubation. It can be interpreted that the effect of tryptamine was to increase the resistance of a cellular Na^+ pathway from 18.9 to 118.6 $k\Omega \cdot cm^2$.

The rate-limiting step for transepithelial Na^+ transport appears to be R_{sNa}. The stimulation of net transepithelial Na^+ transport by the addition of amphotericin B to the epithelial bathing solution probably results from reducing the value of R_{sNa} [5]. Tryptamine increased the electrical resistance of corneas bathed in Na_2SO_4 Ringer to similar values regardless of whether the corneas had been treated with amphotericin B. This effect of tryptamine suggests that it affects R_{Na} rather than R_{sNa}. Additional evidence indicating an effect of tryptamine on a site other than R_{sNa}, where amphotericin B presumably acts, is that amphotericin B had no stimulatory effect on the I_{sc} in corneas that had been preincubated with tryptamine.

The effect of tryptamine on the Na^+ forward fluxes when the corneas were bathed in NaCl Ringer is similar to that in Cl^--free Ringer. The change in resistance, however, corresponds to a conductance decrease of 0.262 $m\Omega^{-1}/cm^2$ which suggests that tryptamine inhibits transcellular Cl^- pathways. Additional evidence for this conclusion stems from the much larger conductance change observed in corneas transporting Cl^- whose Na^+ transport rates had been stimulated by amphotericin B (0.811 $m\Omega^{-1}/cm^2$) than in similarly treated corneas transporting only Na^+ (0.142 $m\Omega^{-1}/cm^2$). That tryptamine increases transepithelial corneal resistance to Cl^- movement was directly demonstrated by the effect of this drug on unidirectional Cl^- fluxes as seen in Table V.

As with Na^+ transport, the effect of tryptamine on Cl^- transport appears to result from increasing the resistance of the Cl^- transcellular pathway. This is shown by the fact that the I_{sc} was inhibited and the effect on the forward Cl^- flux was larger than that on the backward Cl^- flux.

The effect of tryptamine on Cl^- transport can also be analyzed in terms of an electrical model which is shown in Fig. 2. The model is the same as that

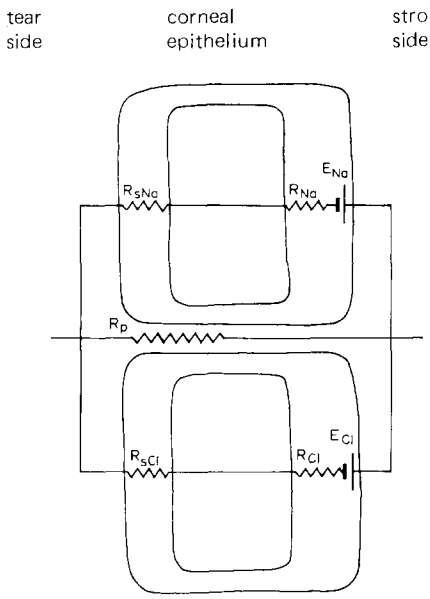

Fig. 2. Electrical model for one cell layer of the corneal epithelium bathed in NaCl-Ringer. For simplicity, the electrical equivalents of the Na^+ and Cl^- transport systems are drawn in different cells.

shown for Na^+ and for simplicity the Cl^- transport system is shown to be located in a different cell. The position assignment for E_{Cl} in the bullfrog cornea epithelium is similarly arbitrary. Theophylline increases Cl^- permeability and active Cl^- transport [9]. It appears that theophylline and tryptamine affect different components of the transcellular Cl^- pathway (i.e. R_{sCl} or R_{Cl}) since the inhibitory effect of tryptamine on the I_{sc} was not counteracted by theophylline; a stimulant of both unidirectional Cl^- fluxes (cf. Tables VI and VII).

The mechanisms whereby tryptamine and ouabain inhibit active transport appear to be different. The inhibitory effect of ouabain on Na^+ transport is associated with an increase of the backward flux and little effect on the electrical resistance [5]. A similar mode of action is observed on Cl^- transport [6]. When oubain and tryptamine are used in succession (experiments in Table VIII) their different mode of action is clearly shown by their effects on the backward flux and resistance.

It has been suggested [6] that ouabain selectively inhibits a component which in electrical terms is represented as the EMF of the transport system. Unidirectional ionic fluxes across a pathway represented by a simple resistor will be equal in both directions. If an EMF, E, is added in series, the measured resistance, R, will not change but a net flow equal to E/R will be created. The net flow will be the result of an increased flux in one direction and a decreased flux in the opposite direction. The new fluxes will be equal to the product of the flux, J_0, (when $E = 0$), times a factor $(EF/RT)/(1 - e^{-EF/RT})$ where F, R, and T have their usual meanings and the sign of E determines whether the flux will increase or decrease. If the value of E is then reduced towards zero, the larger flux will decrease and the smaller flux will increase. The predictions of

the model are consistent with the experimental effects of ouabain on these fluxes. The effect of tryptamine, on the other hand, appears to be more ascribable to increasing transcellular resistance (i.e. R_{sCl} or R_{Cl}) since tryptamine markedly increased the electrical resistance and decreased the backward flux. Thus, the most plausible explanation for the tryptamine effect on active Na^+ and Cl^- transport is that the drug decreases Na^+ and Cl^- membrane permeability but sufficient information is not available to determine how this is done.

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