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INHIBITION OF IONIC TRANSPORT AND ATPase ACTIVITIES BY SEROTONIN ANALOGUES IN THE ISOLATED TOAD LENS

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Summary

The effects of serotonin and five other indoles were tested on the electrical parameters and ionic transport in the isolated toad lens. Serotonin, tryptophan and 5-hydroxy-L-tryptophan did not affect the electrical parameters of the lens at concentrations as high as 1 mM. Tryptamine, 5-methyltryptamine and 5-methoxytryptamine had dual effects: 1 mM in the posterior bathing solution depressed the potential difference of the posterior face of the lens, which resulted in an increase in the translenticular potential difference and short-circuit current; 1 mM in the anterior solution (in contact with the lens epithelium) produced a quick and pronounced reduction of the potential difference of the anterior face. This resulted in a 90—100% decline of the translenticular short-circuit current. Serotonin and tryptamine were then tested for their effect on the ATPases of lens epithelium. Both amines inhibited the enzymes with tryptamine at 5 mM completely inhibiting all ATPase activity. Since tryptophan is transported from the aqueous humor into the lens and may be converted by lens enzymes to serotonin and tryptamine, these findings may have physiological implications in cataractogenesis.

Introduction

The maintenance of the normal hydration and transparency of the lens is dependent upon a proper electrolyte balance regulated by the (Na, K)-pump. Kinsey [1] proposed a pump-leak system, whereby the (Na, K)-pump is located on the anterior (epithelial) surface of the lens, and Na^+ and K^+ diffuse passively

through the posterior surface. Disruption in the pump-leak system can produce opacities by osmotic swelling and subsequent structural disruption. Duncan and Croghan [2] have suggested that perhaps the universal cause of cataracts is an increased permeability to Na^+. Kinoshita [3] has shown that in many experimental cataracts a striking change in hydration results from an imbalance of the pump-leak system. Russell et al. [4] have also shown that in the congenital cataract of Nakano mice, a specific inhibitor of $(Na^+ + K^+)$-ATPase initiated the pump-leak imbalance.

Serotonin is an indole found throughout the central nervous system and the gastrointestinal tract. Its pharmacological effects include mediation of transsynaptic conduction and stimulation of smooth muscle. It has recently been found that palytoxin, a powerful toxin isolated from *Palythoa caribaeorum*, is a strong inhibitor of the $(Na^+ + K^+)$-ATPase found in the electric organ of *Electrophorus electricus.* The toxic and the $(Na^+ + K^+)$-ATPase inhibitory effects of palytoxin were separable into different components, and the inhibitor was found to be serotonin [5]. In addition, other indole derivatives have been shown to inhibit $(Na^+ + K^+)$-ATPase from several tissues [6].

The effects of serotonin and its analogue, tryptamine, on ionic transport across the isolated frog cornea have recently been studied [7]. Although serotonin itself showed no effect, tryptamine was found to have a powerful inhibitory action on active Cl^- and Na^+ transport. Thus, it was of interest to determine the effect of tryptamine and other indoles on ionic transport and ATPases of the lens. In addition, Cotlier and Sharma [8] have recently reported that increased levels of plasma tryptophan are found in patients with senile cataracts, suggesting a relationship between tryptophan metabolism and cataractogenesis.

In this study, we report the effects of serotonin and its analogues, tryptophan, 5-hydroxy-L-tryptophan, 5-methyltryptamine, methoxytryptamine and tryptamine, on the electrical parameters of the isolated toad lens. We also report the effects of serotonin and tryptamine on the ATPases of toad lens epithelium.

Methods

Electrophysiological measurements

Isolated lenses from toads (*Bufo marinus*) were used in this study. Electrical potential difference (V), short-circuit current (I_{sc}) and resistance (R) were measured with the lens placed in a glass chamber so that the anterior and posterior surfaces were isolated and bathed by separate solutions. A fine-tip glass electode (tip diameter about 80 μm) was inserted into the lens through its equator, so that the V values of the anterior (V_a) and the posterior surfaces (V_p) could be measured (for details see Ref. 9). The V values were recorded by means of agar bridges connected through calomel cells to a Keithley 173 digital multimeter and a Heath recorder. Current across the lens was sent by means of another pair of agar bridges in contact with the bathing solutions and connected to an automatic voltage-clamp device. By clamping the translenticular voltage (V_t) or V_a to zero, $I_{sc,t}$ and $I_{sc,a}$ were measured. I across the posterior surface ($I_{sc,p}$) and resistances of the anterior (R_a) and posterior surfaces (R_p) were calculated from a current-voltage plot, as described previously [10].

Biochemical methods

Toad lenses were totally excised by opening the removed eye and carefully cutting around the zonules. Throughout this dissection the lenses were maintained in an ice-cold phosphate-free Ringer solution. A light equatorial incision on the freshly excised lens enabled the capsule, with the adhering epithelial layer, to be peeled away from the cortex. The cortex was separated from its nucleus by teasing with forceps. Capsules and cortices obtained from this procedure were stored separately in liquid N_2 until enough material could be pooled for assay.

Homogenization in ice-cold 0.25 M sucrose, 20 mM Tris (pH 7.5) buffer of the capsular (epithelial) and cortex material from approx. 40 lenses was performed in a hand-held glass mortar and pestle with 10—20 strokes. The postnuclear homogenate was fractionated into 8000, 50 000 and 100 000 $\times g$ pellets. All pellets were resuspended and treated for 15 min at 15° C with 0.05% sodium deoxycholate, 2 mM Tris, 0.25 M sucrose at pH 7.0. Each fraction was then recentrifuged at 100 000 $\times g \cdot 90$ min. The deoxycholate-treated pellets were then resuspended in the homogenization medium. The above treatment was our implementation of various procedures given by Jorgensen and Skou [11].

The protein content of each fraction was determined by using the method of Lowry et al. [12]. Each fraction was then assayed for total Mg^{2+}-dependent ATPase, and 5'-nucleotidase (used as a plasma membrane marker). The total Mg^{2+}-dependent ATPase activity in each fraction was tested for its sensitivity to ouabain, serotonin, tryptamine and various combinations of these substances, as given in Table I.

Appropriate volumes of resuspended pellet were added to each tube. Tubes with epithelial fractions were adjusted to contain 0.5—1 μg of protein, whereas assay of cortex fractions contained 20—40 μg of protein per tube. Each tube was brought to a final volume of 0.5 ml with H_2O. The concentrations given in tubes A—H are essentially those of Bonting [13]. Tubes H and J were used as a test for nonspecific phosphorylase activity, which was found to be less than 5%

TABLE I

ASSAY MEDIA FOR ATPase AND 5'-NUCLEOTIDASE

Tubes A—H, pH 7.5; tubes I and J, pH 8.5. All cations were added as the chloride salts. All concentrations are given as millimolar (mM).

	A	B	C	D	E	F	G	H	I	J
Mg^{2+}	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.01	0.01
Na^+	60.0	—	60.0	60.0	60.0	60.0	60.0	60.0	—	—
K^+	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	—	—
EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	—	—
Tris	92.0	151.0	92.0	92.0	92.0	92.0	92.0	92.0	0.1	0.1
Ouabain	—	—	1.0	—	1.0	—	1.0	—	—	—
Tryptamine	—	—	—	1.0	1.0	—	—	—	—	—
Serotonin	—	—	—	—	—	1.0	1.0	—	—	—
ATP	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	—	—
AMP	—	—	—	—	—	—	—	—	3.0	—
ADP or 3'-AMP	—	—	—	—	—	—	—	1.0	—	1.0

of the total ATPase activity. Tube I was used as an assay for 5'-nucleotidase and contains our modification of several slightly different assays for that enzyme [14,15]. Incubations were performed for 1 h at 25°C and the reaction stopped by the addition of color reagent. The low amount of protein in each tube did not require its removal by centrifugation.

The P_i liberated by the enzymatic reactions was assayed by a method recently developed in our laboratory [16]. After the incubation period, 800 μl of a solution containing ammonium molybdate, malachite green and sterox (see Ref. 16 for details) were added to each tube and mixed. Within 1 min, 100 μl of a 34% citrate solution were added and mixed. The final solution was then read at 660 nm in a Beckman DB spectrophotometer. All solutions were read against references containing the same concentration of adenosine nucleotides as the incubation tubes. Enzyme activity is expressed as μmol P_i released/h per mg protein.

Materials

The drugs used in this study: 5-hydroxytryptamine hydrochloride, 5-hydroxytryptamine creatine sulfate complex, DL-tryptophan, 5-hydroxy-L-tryptophan, 5-methyltryptamine hydrochloride, 5-methoxytryptamine and tryptamine hydrochloride were from Sigma Chemical Co. (St. Louis, MO).

Results

Electrophysiological results

Three of the six drugs tested — serotonin (hydrochloride and creatine sulfate complex), DL-tryptophan and 5-hydroxy-L-tryptophan — showed no effect when added to either side of the lens at concentrations up to 10^{-3} M.

The effects of the other three drugs used at concentrations of 10^{-4} and 10^{-3} M were very similar to one another. A typical trace showing the effect of 10^{-4} M tryptamine on the anterior side is shown in Fig. 1. In general, the actions of the three active drugs may be described as follows: concentrations of 10^{-4} M on the posterior side had very little or no effect at all on any parameters; the same concentration on the anterior side reduced the $I_{sc,a}$ and $I_{sc,t}$ by about 15 and 5%, respectively, produced a variable effect on $I_{sc,p}$, and left V_a and V_p virtually unchanged. Taken together, these results indicate that the alteration of these parameters was due to an increase in the resistances R_a and R_p.

Concentrations of 10^{-3} M on the posterior side produced changes on the electrical indicators of both surfaces of the lens. A trace of the effect of methyltryptamine is illustrated in Fig. 2. The effects of the three effective drugs are shown in Table II. As can be seen, both $I_{sc,a}$ and $I_{sc,p}$ declined, the inhibition of $I_{sc,p}$ having been usually larger. V_a was only slightly affected, whereas V_p was clearly depressed. As a result of these changes, V_t and $I_{sc,t}$ increased. The effect of the resistances was variable, but in general R_a increased while R_p decreased.

The changes produced by 10^{-3} M concentrations of any of the effective drugs in the anterior bathing solution were substantial. These are also tabulated in Table II. As can be seen in both the table and in Fig. 3, the decline of $I_{sc,t}$

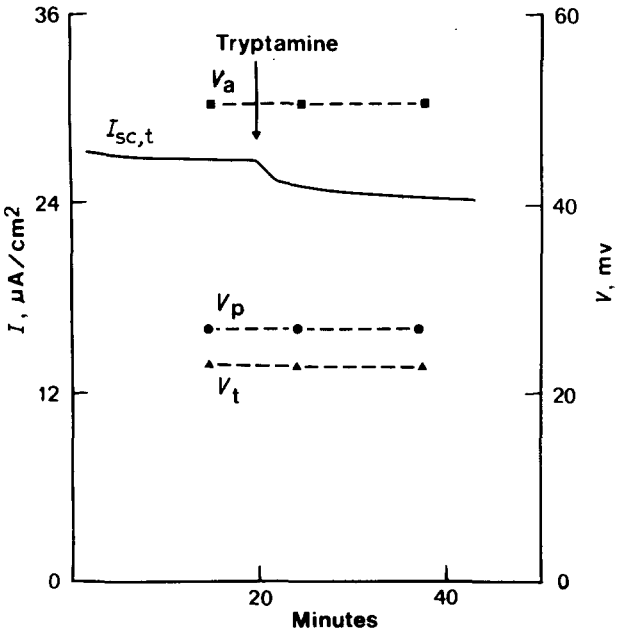

Fig. 1. Effect of 10^{-4} M tryptamine hydrochloride added to the solution bathing the anterior side of an isolated toad lens. $I_{sc,t}$ was continuously recorded.

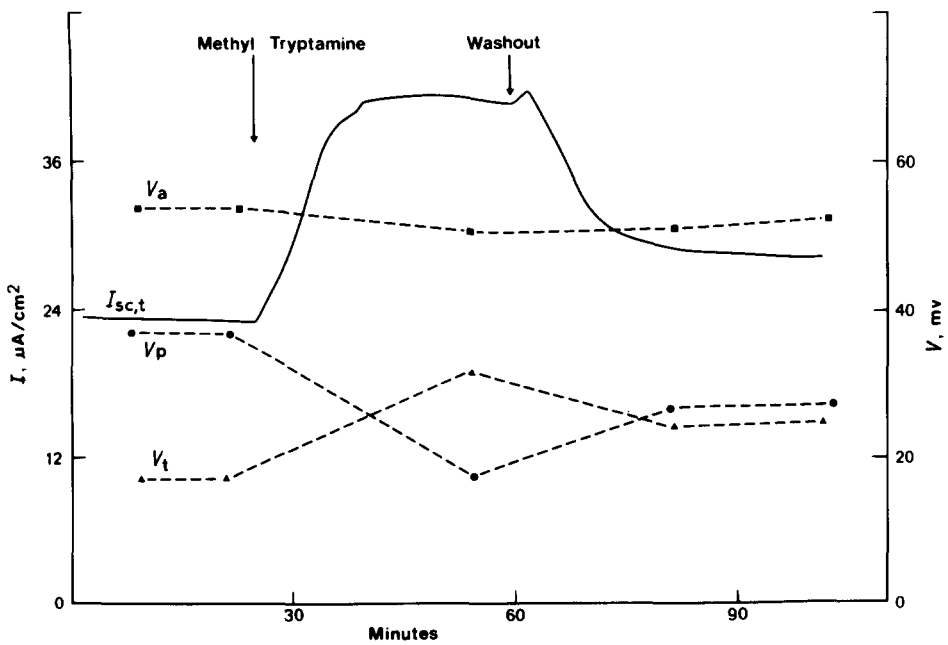

Fig. 2. Effects of 10^{-3} M methyltryptamine added to the solution bathing the posterior side of an isolated toad lens. $I_{sc,t}$ was continuously recorded. A washout restored the electrical parameters to near their control values.

TABLE II

EFFECTS OF TRYPTAMINE, 5-METHOXYTRYPTAMINE AND 5-METHYLTRYPTAMINE ON THE ELECTRICAL PARAMETERS OF THE ISOLATED TOAD LENS

	n	V (mV)		
		V_a	V_p	V_t
Control values	23	57.1 ± 3.2	34.1 ± 2.8	22.1 ± 2.3
Changes after addition to posterior solution		(V experimental—V control)		
10^{-3} M tryptamine	4	−1.3 ± 0.8	−10.5 ± 1.6 *	9.0 ± 1.5 *
10^{-3} M 5-methoxytryptamine	4	−3.0 ± 1.7	−17.7 ± 2.4 *	15.3 ± 1.8 *
10^{-3} M 5-methyltryptamine	4	−0.8 ± 0.6	−10.7 ± 1.6 *	10.7 ± 1.4 *
Changes after addition to anterior solution ***				
10^{-3} M tryptamine	4	−28.5 ± 5.3 *	−6.7 ± 1.8 *	−21.2 ± 3.6 *
10^{-3} M 5-methoxytryptamine	3	−31.0 ± 5.9 *	−2.0 ± 0.3 *	−26.0 ± 4.2 *
10^{-3} M 5-methyltryptamine	4	−36.0 ± 1.9 *	−5.3 ± 0.5 *	−30.6 ± 2.8 *

* The difference between experimental and corresponding control, as paired data, was statistically significant for $P < 0.005$.

** The mean difference with respect to a ratio 1.0 (no effect), as paired data, was statistically significant for $P < 0.005$.

*** Values obtained after 20 min of addition of the drug.

upon addition was both immediate and pronounced. The values in Table II were computed 20 min after addition of the drug, for in most cases the drug had exerted its full effect by that time. V_a decreased by 50% upon addition of both tryptamine and 5-methoxytryptamine, and by 65% after addition of 5-methyltryptamine. V_p was slightly, yet significantly, reduced. In many cases,

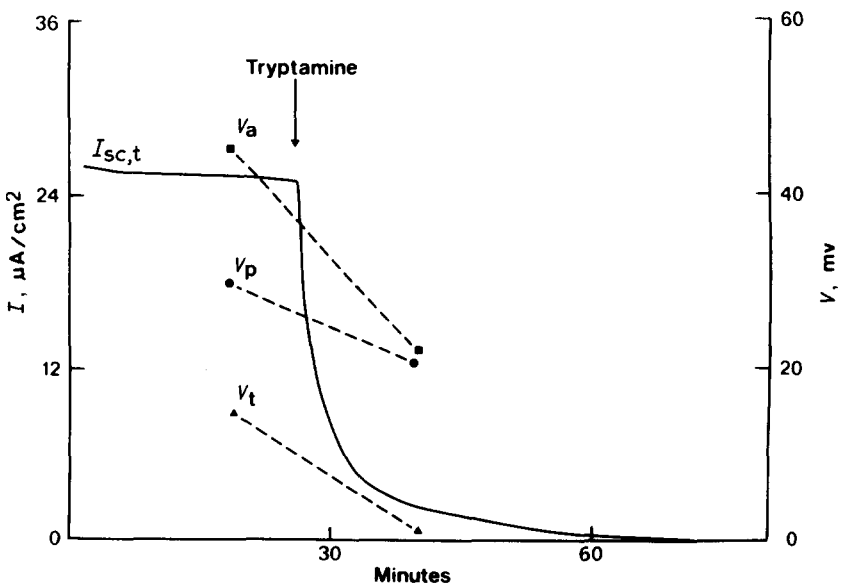

Fig. 3. Effects of 10^{-3} M tryptamine hydrochloride in the anterior bathing solution on I and V values of an isolated toad lens. $I_{sc,t}$ was continuously recorded.

I ($\mu A/cm^2$)			R ($k\Omega \cdot cm^2$)		
$I_{sc,a}$	$I_{sc,p}$	$I_{sc,t}$	R_a	R_p	R_t
139 ± 12	61 ± 11	22 ± 3	0.43 ± 0.06	0.69 ± 0.12	1.10 ± 0.16
(I experimental/I control)			(R experimental/R control)		
0.87 ± 0.03 **	0.62 ± 0.03 **	1.31 ± 0.06 **	1.10 ± 0.02 **	1.11 ± 0.08	1.10 ± 0.08
0.83 ± 0.03 **	0.70 ± 0.03 **	1.79 ± 0.07 **	1.07 ± 0.01 **	0.92 ± 0.06	0.99 ± 0.03
0.95 ± 0.04 **	0.62 ± 0.03 **	1.64 ± 0.07 **	1.06 ± 0.01 **	0.85 ± 0.07 **	0.92 ± 0.04 **
0.46 ± 0.02 **	1.22 ± 0.08	0.07 ± 0.06 **	1.05 ± 0.08	0.65 ± 0.05	0.84 ± 0.06
0.48 ± 0.02 **	0.66 ± 0.07	0.13 ± 0.06 **	1.08 ± 0.07	1.53 ± 0.08 **	1.36 ± 0.08
0.43 ± 0.03 **	1.84 ± 0.15	0.00 ± 0.04 **	1.17 ± 0.08	1.35 ± 0.09	1.18 ± 0.08

V_a fell below the value of V_p so that V_t became negative. $I_{sc,a}$ was drastically reduced, whereas the effect on $I_{sc,p}$ was variable. $I_{sc,t}$, which indicated activity of the (Na, K)-pump, was depressed to near-zero values, suggesting that the drug on the anterior side inhibits the pump at the lens epithelium. There was a slight increase in R_a and a variable effect on R_p.

It should also be noted that all effects mentioned in this paper were reversible, as lenses recovered to about 80% of their control values when the drug was washed out within 15 min of addition.

Biochemical results

Table III shows the distribution of ATPases and 5′-nucleotidase in various fractions of lens epithelium. It was found that the lens cortex contains very little ATPase activity in general and virtually no ($Na^+ + K^+$)-ATPase activity in particular. It can be seen that while the epithelium has a relatively high activity of ATPases, the activities are primarily limited to the 8000 and 50 000 × g pellets. Because of the lack of activity in all cortex and the 100 000 × g epithelial fractions, further studies were limited to the 8000 and 50 000 × g epithelial fractions. It should be mentioned that we subfractionated the homogenate into 8000 and 50 000 × g fractions, instead of the more common 10 000 and 20 000 × g fractions, in the hope of eliminating the mitochondrial enzymes (Mg^{2+}-ATPase) and bringing into the post 8000 × g pellet as much ($Na^+ + K^+$)-ATPase-containing membrane material as possible. This procedure worked very well for us in corneal epithelium; however, as noted in Table III, even though the 50 000 × g lens epithelial pellet contains 55% of the 5′-nucleotidase activity compared to 34% for the 8000 × g pellet, the ($Na^+ + K^+$)-ATPase is evenly distributed between the two pellets.

Table IV shows the effects of the indoles, tryptamine and serotonin, on the

TABLE III

DISTRIBUTION OF ATPase AND 5'-NUCLEOTIDASE IN VARIOUS FRACTIONS OF TOAD LENS EPITHELIUM

Each value is the mean ± S.E. Each experiment was performed in duplicate. The Na^+-sensitive values were calculated as the difference between specific activities in Na^+-rich and Na^+-free media (media A and B in Table I). Ouabain-sensitive values were calculated as the difference between total activity and activity in the presence of 1 mM ouabain.

Fraction	(*n*)	Specific activity (μmol P_i/h per mg protein)	% of total
8000 × *g*			
Total ATPases	4	7.43 ± 0.63	100
Na^+-senstive	3	4.41 ± 0.95	59
Ouabain-sensitive	4	4.87 ± 0.91	66
5'-Nucleotidase	2	1.39 ± 0.01	—
50 000 × *g*			
Total ATPases	5	9.06 ± 0.36	100
Na^+-sensitive	4	5.24 ± 0.29	58
Ouabain-sensitive	5	5.76 ± 0.53	64
5'-Nucleotidase	3	2.23 ± 0.41	—
100 000 × *g*			
Total ATPases	2	1.24 ± 0.75	100
Na^+-sensitive	2	0.03 ± 0.04	2
Ouabain-sensitive	2	0.03 ± 0.03	2
5'-Nucleotidase	2	0.42 ± 0.04	—

ATPase activity of the 8000 and 50 000 × *g* epithelial fraction. As can be noted in the 8000 × *g* pellet, the activity of the tryptamine plus ouabain-inhibitable enzyme is larger than the ouabain-sensitive enzyme, indicating that tryptamine is also inhibiting the non-(Na^+ + K^+)-dependent, Mg^{2+}-dependent ATPase (Mg^{2+}-ATPase). When this amount (6.08 − 4.87 = 1.21) is subtracted from the activity of the tryptamine-sensitive enzyme (3.46 − 1.21 = 2.25), the activity of the

TABLE IV

EFFECT OF TRYPTAMINE AND SEROTONIN ON ATPases OF 8000 AND 50 000 × *g* FRACTIONS OF TOAD LENS EPITHELIUM

Each value is the mean ± S.E. in μmol P_i/h per mg protein. Each experiment was performed in duplicate. The specific activity for each inhibitor is the fraction of enzyme sensitive to the inhibitor and was calculated as the difference between the total activity and the activity obtained for each condition.

	8000 × *g*			50 000 × *g*		
	n	Specific activity	% of total	*n*	Specific activity	% of total
Total ATPases	4	7.43 ± 0.63	100	5	9.06 ± 0.36	100
Ouabain (1 mM)	4	4.87 ± 0.91 *	66	5	5.76 ± 0.53 *	64
Tryptamine (1 mM)	4	3.46 ± 0.64 *	47	4	3.57 ± 0.19 *	39
Tryptamine and ouabain	4	6.08 ± 0.90 *	82	4	6.54 ± 0.29 *	72
Serotonin (1 mM)	2	2.12 ± 0.47 *	29	2	1.72 ± 0.77	19
Serotonin and ouabain	2	5.89 ± 0.73 *	79	2	6.99 ± 0.99 *	77

* Values significantly different from zero change in total activity for $P < 0.05$.

TABLE V

EFFECT OF DIFFERENT CONCENTRATIONS OF TRYPTAMINE ON ATPases OF TOAD LENS EPITHELIUM

Each value is the mean ± S.E. in μmol P_i/h per mg protein. Each experiment was performed in duplicate. The specific activity for each concentration of inhibitor(s) is the fraction of enzyme sensitive to that condition and was calculated as the difference between the total activity and the activity obtained for each condition.

Concentration (M) ($X10^{-3}$)	8000 X *g*			50 000 X *g*		
	n	Specific activity	% of total	*n*	Specific activity	% of total
Total ATPases	4	7.43 ± 0.63	100	5	9.06 ± 0.36	100
1.0 Ouabain	4	4.87 ± 0.95	66	5	5.76 ± 0.53	64
0.1 Tryptamine	3	1.11 ± 0.43	15	3	1.38 ± 0.25	15
0.1 Tryptamine and 1.0 ouabain	3	5.22 ± 1.12	70	3	5.77 ± 0.29	64
0.5 Tryptamine	3	2.18 ± 0.71	29	3	2.25 ± 0.05	25
0.5 Tryptamine and 1.0 ouabain	3	5.58 ± 1.11	75	3	6.08 ± 0.42	67
1.0 Tryptamine	4	3.46 ± 0.64	47	4	3.57 ± 0.19	39
1.0 Tryptamine and 1.0 ouabain	4	6.08 ± 0.90	82	4	6.54 ± 0.28	72
5.0 Tryptamine	4	7.43 ± 0.63	100	5	9.06 ± 0.36	100
5.0 Tryptamine and 1.0 ouabain	4	7.43 ± 0.63	100	5	9.06 ± 0.36	100

($Na^+ + K^+$)-ATPase which is 1 mM tryptamine-sensitive is obtained. This manipulation indicates that 1 mM tryptamine inhibits 46% (2.25/4.87) of the ($Na^+ + K^+$)-ATPase in the 8000 X *g* pellet. The same manipulation with virtually the same results can be performed on the 50 000 X *g* pellet. Serotonin has a similar, but smaller, inhibitory effect on both the total and ($Na^+ + K^+$)-ATPase activities.

The lack of complete inhibition of ($Na^+ + K^+$)-ATPase by the indoles suggested that 1 mM was not enough for complete inhibition. Subsequently, the effect of different concentrations of tryptamine on ATPase activity was tested. The results in Table V indicate that tryptamine is a general ATPase inhibitor of which the effect is concentration dependent. Table VI illustrates the percentages of both ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase which are inhibited by various concentrations of tryptamine. The pattern of inhibition of the two enzymes is similar, and at 5 mM all ATPase activity is completely inhibited.

TABLE VI

PERCENT INHIBITION OF ($Na^+ + K^+$)-ATPase AND Mg^{2+}-ATPase ACTIVITIES INHIBITED BY INCREASING CONCENTRATIONS OF TRYPTAMINE IN TOAD LENS EPITHELIUM

Values are expressed as percentages.

Tryptamine (mM)	8000 X *g*		50 000 X *g*	
	($Na^+ + K^+$)	Mg^{2+}	($Na^+ + K^+$)	Mg^{2+}
0.1	16	14	24	1
0.5	30	28	34	10
1.0	46	47	48	24
5.0	100	100	100	100

Discussion

The activity of the (Na, K)-pump at the lens epithelium results in an electrical asymmetry that can be detected in the isolated lens as a translenticular potential difference and as a translenticular short-circuit current [10,17]. We have also previously shown that $I_{sc,t}$ can be completely inhibited by anterior addition of iodoacetate and ouabain over a period of 5 and 12 h, respectively [18]. In contrast, 10^{-3} M tryptamine, 5-methyltryptamine and 5-methoxytryptamine in the anterior bathing solution produced complete inhibition of $I_{sc,t}$ in less than 30 min. The fact that the most dramatic effect of these drugs was produced when added to the anterior side suggests an action on the (Na, K)-pump in the epithelial cells. This was confirmed by the biochemical data. However, their influence was not limited to the pump, since tryptamine inhibited Mg^{2+}-ATPase, and addition of the effective drugs to the posterior surface increased the $I_{sc,t}$ by reducing the V_p. This can be interpreted as an effect on the resistance of the cortical fiber membranes. It can also be inferred that the drug-induced effects were not due to any structural damage, as they were reversible after the drug was washed out.

Reinach et al. [19] have shown that tryptamine inhibits oxygen consumption in the bullfrog cornea. Reinach and Candia [7,20] have also shown that tryptamine inhibits active transepithelial Na^+ and Cl^- transport as does ouabain. They speculated that the tryptamine effect on the cornea was different from that of ouabain and suggested that tryptamine may be altering ionic pathways; however, the data presented here indicate a direct influence of tryptamine on the ATPases of the epithelial cell. They also show that tryptamine is affecting both the non-($Na^+ + K^+$)-dependent, Mg^{2+}-dependent ATPase(s) and the ouabain-sensitive ATPase. Furthermore, the effect is concentration dependent.

It should be noted that serotonin and 0.1 mM tryptamine had little or no effect on the pump, as measured by the electrical parameters, although they inhibited the isolated enzymes. A possible explanation is that the lens capsule or other restricting barriers impede the diffusion of these drugs to their site of action when added to the bathing medium of the intact lens. Similarly, ouabain, which inhibits the enzyme, has a delayed and incomplete effect on the electrical parameters [18].

Addition of 0.1 mM tryptamine to the anterior surface slightly increased R_a and R_p; however, 1 mM tryptamine increased R_a but decreased R_p. Since lens resistance may be voltage dependent, this discrepancy could be due to the marked decrease in V produced by 1 mM tryptamine.

Our results concerning the very low levels of ($Na^+ + K^+$)-ATPase in the cortex are essentially in agreement with those of Bonting [21] and Palva and Palkama [22]. Neville et al. [23] also reported low cortex concentrations of ATPase, but the authors note that the total amount may be considerable because of the bulk of the cortex. Our attempt to separate the plasma membrane fragments from the classical mitochondrial fraction (even though lens tissue has a low mitochondrial density) was unsuccessful, for our homogenization of lens tissue always produced membrane fragments that precipitated with the $8000 \times g$, as well as the $50\,000 \times g$ pellet. This is not unusual, for it is known that upon cell disruption, the plasma membrane can assume a wide variety of sizes and densi-

ties. Subsequently, simple centrifugation techniques preclude a separation of plasma membrane with high yield and purity [24].

Both the electrophysiological and biochemical data indicate that tryptamine and some of its analogues are strong inhibitors of ATPases and ion transport. Thus, the presence of serotonin and tryptamine in the lens may conceivably result in inhibition of pump mechanisms regulating hydration and lens transparency.

Tryptophan is present in the aqueous humor and it is actively transported into the lens [25—27]. Tryptophan can be hydroxylated into 5-hydroxytryptophan and then decarboxylated into serotonin. Alternatively, tryptophan can be directly decarboxylated into tryptamine by the same decarboxylase [28, 29]. While there is little direct evidence for these interconversions in the lens, it is not unreasonable to suggest that they do occur. For example, it has been shown that *p*-chlorophenylalanine, a potent inhibitor of tryptophan hydroxylase, induces cataracts in rats [30—32]. *p*-Chlorophenylalanine, by blocking the serotonin pathway, may direct more tryptophan to be directly decarboxylated into tryptamine. It has also been suggested that the behavioral alterations produced by *p*-chlorophenylalanine may be due to the elevation of tryptamine rather than to a decrease in serotonin levels [33]. Cotlier and Sharma [8] have found elevated levels of plasma tryptophan (bound and free) in cataract patients. They suggest that tryptophan or its metabolites may play a role in cataract formation. Our present findings are consistent with this hypothesis.

Acknowledgments

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