Biochimica et Biophysica Acta, 413 (1975) 277–282

BBA 77127

EFFECT OF COLCHICINE ON THE OSMOTIC WATER FLOW ACROSS THE TOAD URINARY BLADDER

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(Received May 20th, 1975)

SUMMARY

Osmotic water movement across the toad urinary bladder in response to both vasopressin and cyclic AMP was inhibited by 10^{-5} to 10^{-4} M colchicine on the serosal but not on the mucosal side. This inhibitory effect was found to be time- and dose-dependent. Colchicine alone did not change basal osmotic flow and a baseline of the short-circuit current (I_{sc}) and also did not affect a vasopressin-induced rise of the I_{sc}. The inhibitory effect was not prevented by the addition of pyruvate. The osmotic water movement produced by 360 mM Urea (mucosal), 360 mM mannitol (serosal) or 2 μg/ml amphotericin B (mucosal), was not affected by 10^{-4} M colchicine. These results suggest that colchicine inhibits some biological process subsequent to the formation of cyclic AMP except a directional cytoplasmic streaming process where microtubules may be involved.

INTRODUCTION

Vasopressin promotes transcellular water movement across the epithelium of the distal part of mammalian nephrons, and across amphibian skin and bladder. The hormone enhances the permeability of the membrane at the luminal surface of these responsive epithelial cells via an increase in intracellular cyclic AMP [1, 2]. However, the molecular mechanisms of the enhancement of permeability induced by the nucleotide are still unknown.

In 1973 Taylor et al. [3] proved the possibility that the microtubule and microfilament system or fibrous structures within the cells are involved in the cellular action of vasopressin on steps subsequent to cyclic AMP formation. Thus colchicine and other agents which exert disruptive effects [4, 5] on these organelles inhibited the hydroosmotic response to both vasopressin and cyclic AMP in the toad bladder.

In the present study, using the bladder of the toad, *Bufo bufo japonicus*, we confirmed the above effects of colchicine and also investigated the effect on increased permeability to water induced by hypertonic solution of urea and mannitol, and by

amphotericin B, the modes of action of which differ from that of vasopressin and cyclic AMP.

MATERIALS AND METHODS

The toads (*B. bufo japonicus*) were kept on straw moistened with tap water. Urinary bladders were removed from pithed toads and placed in amphibian Ringer's solution composed of 90 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, 10 mM glucose, and aerated with 97 % O_2/3 % CO_2, final pH = 7.6, osmolality = 230 mOsm/kg H_2O.

The osmotic flow of water (net water flux) across the bladder was measured gravimetrically by the technique of Bentley [6] in which paired hemilobes were each attached as a sac, mucosal side inwards, to the end of a glass cannula, filled with 3 ml of Ringer's solution diluted 1 : 5 with water and bathed in 20 ml of Ringer's solution on the serosal surface. At the outset of the experiment one sac from each bladder was designated as the control lobe, with the other member of the pair serving as the experimental lobe. About 30 min later the solutions on both sides of the bladder were replaced with fresh solution and net water flux, which is equivalent to weight loss of sac and contents, was measured every 20 min until the end of experiments and expressed as μl/min/sac. Following 20 to 40 min equilibration the serosal or the mucosal side of experimental lobes was exposed to colchicine for appropriate period. Then, net water flux over a 40 min period beginning usually 20 min after the addition of test agents was measured.

The electrical potential difference (PD) and short-circuit current (I_{sc}) across the bladder were measured according to the technique of Ussing and Zerahn [7]. The details of the method have been given in a previous paper [8]. The effect of colchicine on the I_{sc} was expressed as the ratio of the values after (4 h later) and before exposure to colchicine divided by the same ratio for the spontaneous changes in the paired control area. The effect of the agent on the response to vasopressin was expressed as the ratio of the peak values after (usually 15–20 min later) and before the addition of vasopressin in the same manner.

Vasopressin used was commercial Pitressin (Parke Davis and Co.), and colchicine purchased from Wako Pure Chemical Industry and amphotericin B from E. R. Squibb and Sons, Inc.. The data were expressed as mean$\pm$standard error and statistical analysis for paired experiments was done using the Student "*t*" test.

RESULTS

Effect of colchicine on the osmotic water flow and I_{sc} induced by vasopressin and cyclic AMP

The serosal surface of experimental lobes was exposed to 10^{-5} or 10^{-4} M colchicine for 1, 2 and 4 h and the hydro-osmotic responses to vasopressin and cyclic AMP were examined. Incubation with 10^{-5} M colchicine for 1 and 2 h had no effect. The other data are indicated in Table I. Colchicine alone did not exert any effect on the osmotic flow of water, but the agent added to only the serosal side inhibited the responses to vasopressin and cyclic AMP time- and dose-dependently.

TABLE I

EFFECT OF COLCHICINE ON THE PERMEABILITY TO WATER

After the equilibration period, 10^{-5} or 10^{-4} M colchicine was added to the serosal medium bathing the experimental lobe. Following each pre-incubation period with colchicine, 5 munits/ml vasopressin or 2 mM cyclic AMP was added to the serosal medium bathing both lobes. Net water flux over a 40-min period beginning 20 min after the addition of these agents was used to estimate the response to each agent. Δ = Flux(exptl)–Flux(cont.); n.s., not significant.

	n	period***	Net water flux (μl/min/sac)			*p*
			control lobe	Δ	% inhibition	
10^{-5} M Colchicine						
Alone	7	4	1.0±0.2	0.1±0.2		n.s.
Vasopressin	7	4	10.7±1.0	−2.5±0.8	23	<0.02
Cyclic AMP	7	4	8.0±1.2	−1.7±0.4	21	<0.01
10^{-4} M Colchicine						
Alone	7	1	1.3±0.3	−0.1±0.3		n.s.
Vasopressin	6	1	12.9±1.2	−3.9±1.3	30	<0.05
Cyclic AMP	6	1	7.3±1.9	−2.0±0.5	27	<0.02
Alone	6	4	1.0±0.1	0.0±0.1		n.s.
Vasopressin	6	4	11.2±2.1	−4.0±1.4	36	<0.05
Cyclic AMP	7	4	9.9±1.1	−3.9±0.5	39	<0.001
Vasopressin*	6	4	13.6±0.9	0.8±0.6		n.s.
Cyclic AMP**	7	4	12.1±1.6	−5.4±0.6	45	<0.001

* Colchicine was added to the mucosal medium bathing the experimental lobe.
** 5mM pyruvate was added to the serosal medium bathing both lobes.
*** Pre-incubation period with colchicine (h).

TABLE II

EFFECT OF COLCHICINE ON SHORT CIRCUIT CURRENT

After the equilibration period (a, b), the serosal surface of the experimental area was exposed to 10^{-4} M colchicine for 4 h (d). Then, in 7 out of 10 experiments (e, f) 25 munits/ml vasopressin was added to the serosal medium bathing both areas and the peak values of I_{sc} were measured (g, h). *P* is the probability of the ratio differing from 1.0. n.s., not significant.

	n	I_{sc} (μA/3.5 cm^2)		Ratio	*P*
		a	c		
Control	10	31.9± 8.6	27.0± 7.5		
		b	d	0.98±0.06†	n.s.
Experiment	10	38.1± 7.3	31.1± 6.3*		
		e	g		
Control	7	32.4±10.2	74.1±13.6**		
		f	h	0.93±0.08††	n.s.
Experiment	7	34.4± 8.1*	78.6±13.9***		

* Colchicine.
** Vasopressin.
*** Colchicine plus vasopressin.
† (d/b)/(c/a).
†† (h/f)/(g/e).

In leucocytes colchicine is known to inhibit glycolysis [9]. However, pyruvate did not prevent the inhibition of the response to cyclic AMP.

As shown in Table II, 4 h incubation of the bladder with 10^{-4} M colchicine did not affect the baseline of the I_{sc} and the elevation of the I_{sc} stimulated by vasopressin.

Effect of colchicine on the osmotic flow enhanced by hypertonicity of the bathing medium

It has been reported that hypertonicity of the medium bathing the bladder may alter the permeability to water and this effect was dependent upon the specific solute employed and its site of addition [10]. A marked osmotic water flow from the serosal to the mucosal side was observed despite the absence of vasopressin, when the mucosal surface was exposed to hypertonic Ringer's solution with solutes such as urea. Furthermore, an increased osmotic flow of water in the opposite direction was found when the serosal medium was made hypertonic with mannitol [10].

Paired hemilobes were filled with 3 ml of full-strength Ringer's solution and bathed in 20 ml of the same solution. The serosal surface of the experimental lobe being exposed to 10^{-4} M colchicine, both lobes were pre-incubated for 4 h. Then, the solutions bathing the mucosal surface of both lobes were replaced with 3 ml of the Ringer's solution containing 360 mM urea and osmotic flow was measured every 20 min. Net water fluxes in control and experimental lobes were 8.3 ± 0.6 and 8.2 ± 1.0 μl/min/sac ($n = 7$), respectively. In another experiment the solutions bathing the serosal surface were replaced with 20 ml of the Ringer's solution containing 360 mM mannitol (the control lobe) and 360 mM mannitol plus 10^{-4} M colchicine (the experimental lobe). The fluxes in control and experimental lobes were 7.0 ± 0.2 and 7.0 ± 0.5 μl/min/sac ($n = 7$), respectively. From the above results it is clear that increased osmotic flow induced by the mucosal (urea) or the serosal (mannitol) hypertonicity was not influenced by the presence of colchicine.

Effect of colchicine on the osmotic flow enhanced by amphotericin B

Amphotericin B added to the solution bathing the mucosal surface of the bladder is known to enhance the passive permeabilities to water and urea, and although not always, to cause an increase in osmotic flow of water [11, 12].

Therefore, the experimental lobe was pre-incubated for 4 h with 10^{-4} M colchicine. Then, the solutions inside the control and the experimental lobes were replaced with 1 : 5 diluted Ringer's solution containing 2 μg/ml amphotericin B. Net water fluxes in control and experimental lobes after this treatment were 11.4 ± 1.3 and 11.3 ± 1.3 μl/min/sac ($n = 7$), respectively.

DISCUSSION

It has been reported by Taylor et al. [3] that colchicine on the serosal side inhibited the osmotic water movement in response to both vasopressin and cyclic AMP without affecting the basal movement across the bladder, but did not influence not only the baseline of the short-circuit current, but also the rise in response to vasopressin. These facts have been confirmed by the present study using another species of toad, *B. bufo japonicus*. In addition we observed that the prior exposure of the mucosal surface to 10^{-4} M colchicine for 4 h did not induce any effect on the

hydro-osmotic response to 5 munits/ml vasopressin, and the addition of 5 mM pyruvate to the serosal bathing medium did not prevent the inhibitory effect of colchicine on the osmotic flow in response to 2 mM cyclic AMP. These results suggest that colchicine inhibits the osmotic water movement across the bladder acting only from the serosal side not via a suppression of glycolysis in the epithelial cells, but via an interference with some subsequent process after the formation of cyclic AMP.

As is well known, colchicine exerts disruptive effects on microtubules in vivo [4] and interacts with tubulin or microtubule subunit protein in vitro [13, 14]. The binding of colchicine to the tubulin extracted from porcine brain, has been found to approach a maximum value in about 4 h [15]. This time-dependent course seems to be similar to that of the inhibitory effect of 10^{-5} to 10^{-4} M colchicine on the response to vasopressin (Table I). From these observations it seems likely that the inhibition of colchicine on vasopressin-induced osmotic flow of water is due to its interaction with microtubule subunit protein, as has been reported by Taylor et al. [3].

However, biological roles of microtubules in the epithelial cells of the bladder are still unknown. One possibility that has been suggested by Taylor et al. [3] is that microtubules may participate in the action of vasopressin on the water movement through its involvement in the mechanism of release of secretion granules [16] at the mucosal surface of the granular epithelial cells. Another possibility is that microtubules may be involved in a directional cytoplasmic streaming process and may facilitate transcellular water movement subsequent to a vasopressin-induced permeability change in the mucosal membrane of the epithelial cell. If a role of microtubules is ascribed to the latter, the osmotic water movement produced by the hypertonicity of the bathing solutions [10] and amphotericin B [11, 12] which enhance the osmotic movement through a configuration or a destructive change in the mucosal barrier via some cyclic AMP independent processes, must also be inhibited by colchicine as well as in the cases of vasopressin and cyclic AMP. However, no effect of colchicine was found on increased osmotic flows induced by the mucosal hypertonicity with urea, the serosal hypertonicity with mannitol, and the addition of amphotericin B to the mucosal bathing solution. Therefore, it can be concluded that microtubules are not involved in a facilitation of transverse water flow in the epithelial cells. In addition we obtained indirect evidence supporting the proposal [10] that the osmotic flow produced by hypertonic environments may be secondary to configuration changes in the mucosal barrier induced by physical rather than biochemical forces. Because, if the effect of hypertonicity on the water permeability is mediated by an elevated intracellular cyclic AMP concentration, it might be inhibited by colchicine.

From the above it is clear that colchicine inhibits some biological processes subsequent to the formation of cyclic AMP except a directional cytoplasmic streaming process where microtubules may be involved. However, exact action-site of colchicine is still unknown.

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