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THE EFFECTS OF LITHIUM ON THE PERMEABILITY OF AN EPITHELIAL MEMBRANE, THE TOAD URINARY BLADDER

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SUMMARY

1. Li^+ reduces the short-circuit current (Na^+ transport) across the toad urinary bladder *in vitro* when present at the serosal (blood) or mucosal (urine) side. The latter may reflect an action in the mammalian renal tubule consistent with the diuresis which initially accompanies administration of Li_2CO_3.

2. The increase of short-circuit current seen in the presence of vasopressin is reduced by Li^+. However, the natriferic response to cyclic AMP is also prevented while that to theophylline is unaffected. Thus Li^+ does not appear to be acting by inhibiting adenyl cyclase.

3. The effect of amphotericin B on short-circuit current was undiminished by Li^+.

4. The vasopressin stimulated increase in osmotic water transfer across the toad bladder was unchanged by the presence of Li^+.

INTRODUCTION

The administration of Li_2CO_3 to patients suffering from manic-depressive disease is associated with an altered electrolyte metabolism[1]. In the first 1–2 days after administering this salt there is a profound increase in urinary Na^+ and water excretion (see for instance refs 1–3). The precise cause of the initial diuretic phase is not clear though it presumably involves a decline in renal tubular Na^+ reabsorption along with an associated indirect, or even a direct hormonally mediated reduction in water retention. The latter possibility has been indicated from the experiments of Harris and Jenner[4] who showed that the antidiuretic action of vasopressin in rats was reduced following the infusion of Li^+. The precise nature of this effect is unknown but Li^+ inhibits adenyl cyclase *in vitro*[5] and activation of this enzyme is involved in the action of vasopressin[6]. Thus Li^+ could be acting by inhibiting tissue adenyl cyclase.

An *in vitro* preparation of the urinary bladder of toads has for many years provided a method for studying processes which take place across the renal tubule including its permeability to sodium and water and the action of vasopressin, aldosterone and various diuretics (see for instance refs 6–11). Thus the effects of Li^+

on this preparation may provide information relevant to its action on the mammalian kidney. We have measured the effects of Li^+ on Na^+ transport across this tissue and the actions of vasopressin on both this process and osmotic water absorption.

METHODS

Toads, *Bufo marinus*, were obtained from the Lemberger Company of Oshkosh, Wisc. and kept in the Laboratory at 21–23° C on a bed of damp earth.

Toad urinary bladder preparation

This has been described in detail previously[8,12]. Each lobe of the bladder was dissected from toads that had been pithed. One such lobe served as a control preparation for experimental observations on the other. Each lobe was tied onto the end of a piece of glass tubing to make a sac with the mucosal (urine) side facing inwards. The sac was filled with 1.5 ml of Ringer's solution (composition (mM): NaCl, 111; KCl, 3.35; $CaCl_2$, 2.70; $NaHCO_3$, 4.0 and glucose 5.5) or a modification of this. It was immersed in a test tube so that the serosal side was also bathed with this solution (20 ml) which was aerated.

Measurement of short-circuit current (Na^+ transport)

Short-circuit current across the toad urinary bladder reflects active Na^+ transport from the mucosa to serosa under a variety of conditions (see for instance refs 8 and 11). The electrical potential difference across the bladder was measured with the aid of a pair of agar–KCL bridges connected to a voltmeter (Keithley Model 200B) through a pair of calomel cells. The short-circuit current was applied from a battery connected to each side of the tissue with a similar pair of bridges and through Ag–AgCl cells. Water movement was measured by weighing the preparation to 0.1 mg on a highly damped balance.

Drugs and solutions

The vasopressin was 8-arginine vasopressin kindly given to us by Parke, Davis and Co. The theophylline was from the Sigma Chemical Company and adenosine 3′,5′-monophosphate (cyclic AMP) from Calbiochem. These drugs were added to the Ringer's solution bathing the serosal side of the tissue and where necessary the Na^+ present was adjusted so as to maintain the osmotic concentration. Amphotericin B (Squibb) was added to the solution at the mucosal side. Choline chloride was chosen as the salt to replace Na^+ as for comparison with Li^+. This is widely used for such substitutions but we cannot exclude the possibility that it may exert an effect on its own. When Li^+ or choline were used these replaced equivalent amounts of Na^+. For measurements of the short-circuit current the solutions on each side of the tissue were osmotically equal; thus if 40 mM of Li^+ was tested on serosal or mucosal side the solutions were kept isotonic with NaCl. For measurement of water movement an osmotic gradient must be present with the mucosal solution hypotonic to that at the serosal side. In these experiments 11 mM Li^+ on the mucosal side represented a 10-fold dilution of a Ringer's solution containing 111 mM Li^+ (instead of Na^+) with distilled water and 40 mM represented a 1:2.78 dilution. The concentrations of the other ions present were unchanged.

RESULTS

Effects of Li^+ on short-circuit current (Na^+ transport)

The effects of substitution of Li^+ for Na^+ in the Ringer's solutions bathing each side of the toad urinary bladder preparation were measured. The short-circuit current has been shown to reflect active Na^+ transport from the mucosal (urinary) to serosal (blood) side of this preparation. It may also reflect some Li^+ transport but this is small and seen only for very short periods of time[13]. If this were occurring in our experiments the observed decline in short-circuit current would reflect an even greater decrease in Na^+ transport than indicated.

Replacement of 40 mM Na^+ by 40 mM Li^+ in the Ringer's solution bathing both sides of the toad bladder preparations resulted in a prompt decline in the short-circuit current from 368 to 143 μA per 100 mg tissue in 30 min (mean difference 225 ± 63, 5 experiments). In control experiments using choline instead of Li^+ to replace Na^+ the short-circuit current rose slightly from 246 to 268 μA per 100 mg (mean difference 22 ± 11, 5 experiments). When vasopressin (10 units/ml) was added to the 40 mM Li^+–Ringer's solution at the serosal side the increase in short-circuit current recorded after 15 min (invariably a maximal one) was 62 ± 35 μA per 100 mg compared to 281 ± 63 μA per 100 mg in the controls with the usual amount of Na^+ present. The substitution of choline instead of Li^+ for the Na^+ did not significantly change the response to vasopressin.

Further experiments were carried out using lower concentrations of Li^+ and with replacement at either side separately. Li^+ (but not choline) at a concentration of 40 mM was found to reduce the short-circuit current across the toad bladder when present at either the serosal or mucosal side of the tissue (Table I). The latter observation was particularly interesting as while therapeutic plasma levels of Li^+ do not exceed about 2 mM those in the urine and renal papilla may be much higher[14]. When lower concentrations of Li^+ were tested the decline in the short-circuit current was much less though with 11 mM Li^+ small declines as compared to the choline controls, were still apparent (Table I). More prolonged incubation may possibly show a greater effect but variations in the tissues condition makes such changes difficult to evaluate. When 40 mM Li^+ was present at the mucosal side of the tissue the decline in short-circuit current was greatest in the first 30 min, amounting to $41 \pm 6.4\%$[9] of the initial level while in the next 30 min a further decline of only $11 \pm 1.7\%$ was observed.

As the short-circuit current was reasonably stable after 30 min this time was chosen to further test the effects of vasopressin on the short-circuit currents (natriferic effect). Li^+, 40 mM, on either the mucosal or serosal side of the bladder caused a considerable decline in the natriferic effect as compared to choline controls (Table I). However, the initial baseline level of the short-circuit current was lower in the presence of the Li^+, so when the response was expressed as a percent change of the initial level it was reduced much less. When examined in the latter manner a significant change was not apparent when the Li^+ concentration was 11 mM on either side. However, at a concentration of 40 mM, Li^+ reduces the natriferic response to vasopressin independently of its effect on the normal rate of Na^+ transport.

The effects of vasopressin on water and Na^+ permeability are mediated by the enzyme adenyl cyclase[6] which promotes the formation of cyclic AMP. It was thus of interest to see what effects the latter nucleotide had on the short-circuit current

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TABLE I

EFFECTS OF Li^+ ON THE SHORT-CIRCUIT CURRENT (Na^+ TRANSPORT) ACROSS THE TOAD URINARY BLADDER *in vitro* AND THE ACTION OF VASOPRESSIN

Results are as means and mean differences ± S.E. Number of experiments in parentheses. Not significant = n.s.

	μA/100 mg wet wt tissue									
	Initial	*30 min Li⁺ or choline*	*Difference*	*P*	*Vasopressin (10 munits/ml)*					
					Initial Li⁺ or choline	*15 min*	*Difference*	*P*	*IV as % III*	*P*
	(I)	*(II)*	*(II–I)*		*(III)*	*(IV)*	*(IV–III)*			
Serosal side										
Li^+, 11 mM (8)	145	114	−31 ± 5.6	<0.001	114	291	177 ± 32.0	<0.001	274 ± 38.5	n.s.
Choline, 11 mM (8)	130	152	22 ± 9.9		152	493	341 ± 12.8		335 ± 18.2	
Li^+, 40 mM (8)	159	99	−60 ± 10.6	<0.001	99	183	84 ± 24.9	<0.001	185 ± 20.0	<0.01
Choline, 40 mM (8)	173	164	9 ± 5.1		164	524	360 ± 58.0		330 ± 30.2	
Mucosal side										
Li^+, 11 mM (8)	108	94	−14 ± 10.4	<0.01	94	432	338 ± 43.5	n.s.	483 ± 49.8	n.s.
Choline, 11 mM (8)	138	179	41 ± 12.9		179	570	391 ± 37.8		386 ± 70.7	
Li^+, 40 mM (7)	214	140	−74 ± 21.5	<0.001	140	186	46 ± 13.3	<0.001	134 ± 10.2	<0.001
Choline, 40 mM (7)	155	216	61 ± 14.7		216	491	275 ± 49.1		225 ± 13.4	

in the presence of Li^+. In addition xanthines, such as theophylline are thought to reduce the action of phosphodiesterase, an enzyme responsible for the endogenous degradation of cyclic AMP. While the absolute increase in the short-circuit current in the presence of theophylline was less with Li^+ than choline present this apparently reflects an inhibition of the Na^+ transport mechanism itself, as in terms of the initial short-circuit current, the responses were similar (Table II). Li^+ greatly reduced the response to cyclic AMP both in terms of direct increase in the short-circuit current and that relative to the initial level. Neither of these results are consistent with an inhibition of adenyl cyclase.

TABLE II

EFFECTS OF CYCLIC AMP, THEOPHYLLINE AND AMPHOTERICIN B ON THE SHORT-CIRCUIT CURRENT (Na^+ TRANSPORT) ACROSS THE TOAD URINARY BLADDER *in vitro* IN THE PRESENCE AND ABSENCE OF Li^+

Results are as means and mean differences ± S.E. of 8 experiments on paired bladder lobes. The tissues were preincubated for 30 min with the Li^+ or choline solutions. Not significant = n.s.

	μA/100 mg wet wt tissue					
	Initial (I)	*II*	*Difference (I–II)*	*P*	*II as % of I*	*P*
Cyclic AMP, 5 mM		15 min exposure				
Li^+, 40 mM, mucosa	108	148	40 ± 4.6	<0.001	139 ± 6.5	<0.001
Choline, 40 mM, mucosa	240	547	307 ± 26.6		229 ± 11.1	
Theophylline, 20 mM		20 min exposure				
Li^+, 40 mM, mucosa	105	233	128 ± 25.2	n.s.	219 ± 15.3	n.s.
Choline, 40 mM, mucosa	207	419	212 ± 31.2		202 ± 9.1	
Amphotericin B (12.5 μg/ml)		10 min exposure				
Li^+, 40 mM, mucosa	96	183	87 ± 18.4	n.s.	203 ± 28.6	<0.05
Choline, 40 mM, mucosa	207	256	49 ± 13.4		131 ± 10.3	

Amphotericin B stimulates the short-circuit current and Na^+ transport across the toad bladder by increasing the permeability of the mucosal surface[15]. Thus it was of interest to know if the action of this drug like that of vasopressin and cyclic AMP (which have a similar site of action) was also reduced by Li^+. When Li^+ (40 mM) was present at the mucosal side of the tissue the response to amphotericin B was undiminished as compared to the control preparation where choline was present instead (Table II). This could be consistent with either an antagonism to a mucosal site of action of Li^+ or an enhanced entry of Na^+ overcoming an inhibition of the 'pump'.

Effects of Li^+ on the hydro-osmotic response of the toad bladder to vasopressin

Vasopressin, like in the renal tubule, facilitates osmotic movement of water (hydroosmotic response) across the toad bladder. In view of the observation of Harris and Jenner[4] showing inhibition of the antidiuretic effect of vasopressin in rats infused with Li^+, the demonstration by Dousa and Hechter[5] that renal adenyl cyclase may be inhibited by Li^+ *in vitro*, and our own measurement of a decreased natriferic response, it was of interest to examine the action of this ion on the hydroosmotic response of the toad bladder.

The results are summarized in Table III. We could find no evidence indicating that Li^+ inhibits the hydro-osmotic response. Even when nearly all (111 mM) the Na^+ was replaced by Li^+ at the serosal side and 40 mM Li^+ was present at the mucosal side no significant decline in the response was observed which could be attributed to the presence of Li^+. Removal of Na^+ *per se* may result in a slightly reduced response as when choline was substituted for Na^+.

TABLE III

EFFECTS OF Li^+ ON THE HYDROOSMOTIC (WATER TRANSPORT) RESPONSE OF THE TOAD BLADDER TO VASOPRESSIN

Results are as means and mean differences ± S.E. Number of experiments in parentheses. In Period I normal Na^+–Ringer's solution was present, substitution was made after this, the tissue being preincubated in the Li^+ or choline solutions for 30 min before adding the vasopressin.

	Water movement (mg/30 min)					
	Vasopressin (10 munits/ml)			*Vasopressin (1 munit/ml)*		
	Period I	*Period II*	*Difference I–II*	*Period I*	*Period II*	*Difference I–II*
Mucosal side						
Li^+, 11 mM (6)	653	403	250 ± 27	310	398	−88 ± 34
Choline, 11 mM (6)	594	421	173 ± 32	316	362	−46 ± 27
Li^+, 40 mM (9)	489	440	49 ± 34	313	266	47 ± 38
Choline, 40 mM (9)	509	454	55 ± 17	319	311	8 ± 30
Serosal side						
Li^+, 11 mM (6)	—	—	—	316	330	14 ± 36
Choline, 11 mM (6)	—	—	—	348	374	26 ± 31
Li^+, 40 mM (9)	645	597	48 ± 54	323	307	16 ± 39
Choline, 40 mM (9)	595	558	37 ± 50	343	302	41 ± 37
Li^+, 111 mM serosa and 40 mM mucosa (8)	592	320	272 ± 38	—	—	—
Choline, 111 mM serosa and 40 mM mucosa (8)	547	357	190 ± 30	—	—	—

In the absence of vasopressin Li^+ has little effect on osmotic water transfer. Thus in bladders containing 3 ml of 40 mM Li^+ and bathed in 111 mM Li^+–Ringer's solution water movement increased from 28 μl in 30 min (with Na^+ present) to 64 μl in 30 min (mean difference 36 ± 7, 8 experiments). When the tissue was restored to Na^+–Ringer's solution the water movement returned towards normal. When 40 mM Li^+ was present at the mucosal side alone the increase amounted to 13 ± 5 μl in 30 min[8]. These changes are too small to influence the interpretation of the results with vasopressin.

DISCUSSION

In 1955, Zerahn[16] showed that Li^+ decreased Na^+ transport and the short-circuit current across the frog skin. Li^+ was also found to produce a decline in the short-circuit current (Na^+ transport) across the toad urinary bladder when present on either the serosal or mucosal surface. As the concentrations required to produce

this effect are much higher than observed therapeutically in the plasma the latter observation is particularly interesting. It suggests the possibility that this ion may reduce renal tubular Na^+ reabsorption when present in the urine where it may attain higher concentrations than in the blood. Such an effect could account for the initial saluresis observed when manic-depressive patients are given Li_2CO_3.

Interactions of Li^+ and vasopressin are of particular interest. Thus Harris and Jenner[4] have shown that the antidiuretic effect of vasopressin in rats is reduced by infusion of Li^+. As renal adenyl cyclase has also been shown to be inhibited by Li^+, albeit at high concentrations[5], this may afford an explanation for its antagonism to the hormone. However, in the present experiments there was no evidence indicating that this was occurring. The hydro-osmotic effect of vasopressin was unchanged even by very high concentrations of Li^+, though the natriferic effect was reduced. As Li^+ also blocked the natriferic effct of cyclic AMP and had no effect on that of theophylline this is also not consistent with an inhibition of adenyl cyclase. In view of the observation that the response to cyclic AMP was prevented by Li^+ it is somewhat surprising to find that to theophylline was unchanged. Although it is often considered that theophylline acts by increasing accumulation of endogenous cyclic AMP there is also evidence that it may have a separate action[17]. The present observation would be consistent with this.

The precise mechanism by which Li^+ inhibits Na^+ transport and the natriferic responses to vasopressin and cyclic AMP is not clear. Competition between Na^+ and Li^+ for essential sites is most likely and this could result in an inhibition of the Na^+ "pump", either directly or by interfering with the access of Na^+ into the cell. As vasopressin acts to increase the Na^+ transfer across the mucosal surface of the cell the latter possibility is attractive, though alternatively an increased entry of Li^+ could be initiated by the hormone which may further inhibit the "pump". However, when the mucosal barrier was breached with amphotericin B an inhibition was not seen. This drug increases the permeability of the mucosal side of the toad bladder to Na^+[15] so that its effect would be consistent with the overcoming of an antagonism between Li^+ and Na^+ at this site. However, direct determination of Na^+ fluxes in the tissue will be necessary to resolve this question.

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REFERENCES

1 L. Baer, S. R. Platman and R. R. Fieve, *Arch. Gen. Psychiat.*, 22 (1970) 108.
2 J. P. Tupin, G. K. Schlagenhauf and D. L. Creson, *Am. J. Psychiat.*, 125 (1968) 536.
3 D. L. Murphy, F. K. Goodwin and W. E. Bunney, *Lancet*, 2 (1969) 458.
4 C. A. Harris and F. A. Jenner, *J. Physiol. (London)*, 200 (1969) 59P.
5 T. Dousa and O. Hechter, *Life Sci.*, 9 (1970) 765.
6 J. Orloff and J. S. Handler, *Am. J. Med.*, 42 (1967) 757.
7 A. Leaf, J. Anderson and L. B. Page, *J. Gen. Physiol.*, 41 (1958) 657.
8 P. J. Bentley, *J. Endocrinol.*, 17 (1958) 201.
9 P. J. Bentley, *J. Physiol. (London)*, 195 (1968) 317.
10 A. Leaf, *Ergeb. Physiol.*, 56 (1965) 216.
11 J. Crabbe, *J. Clin. Invest.*, 40 (1961) 2103.

12 P. J. Bentley, *J. Endocrinol.*, 21 (1960) 161.
13 F. C. Herrera, R. Egea and A. M. Herrera, *Am. J. Physiol.*, 220 (1971) 1501.
14 S. Solomon, *Proc. Soc. Exp. Biol. (N.Y.)*, 125 (1967) 1183.
15 N. S. Lichtenstein and A. Leaf, *J. Clin. Invest.*, 44 (1965) 1328.
16 K. Zerahn, *Acta Physiol. Scand.*, 33 (1955) 347.
17 A. W. Cuthbert and E. Painter, *J. Physiol. (London)*, 199 (1968) 593.