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Some aspects of the inhibition of the action of antidiuretic hormone by lithium ions in the rat kidney and bladder of the toad *Bufo marinus*

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Summary

1. The effect of intravenous infusions of various ions on the antidiuretic action of antidiuretic hormone has been studied in rats.

2. Lithium (13 mmol/l.) reversibly inhibits the antidiuretic responses. Similar concentrations of potassium, rubidium, strontium, magnesium, choline and calcium do not. Lithium has a similar effect on the antidiuretic activity of oxytocin.

3. The inhibition is not simply related to blood nor whole body lithium concentrations.

4. Lithium (2 mmol/l.) in contact with the serosal surface also inhibits the transport of water facilitated by either 0·5 U/l. antidiuretic hormone or 1·1 mmol/l. cyclic adenosine monophosphate in the isolated toad bladder.

5. Choline (2 mmol/l.) on the serosal surface also inhibits the transport of water facilitated by vasopressin in the toad bladder.

Introduction

The frequent use of lithium salts in the treatment of affective psychoses (Schou, 1969) has stimulated renewed interest in the pharmacology of this ion. This paper deals with the effect of lithium on the action of antidiuretic hormone (ADH). Goodwin & Jenner (1967) have presented evidence that at least in some recurrent psychoses there are large changes in the antidiuretic activity of the urine occurring simultaneously with the changing mental state. Angrist, Gershon, Levitan & Blumberg (1970) have reported the antidiuretic hormone resistance of the polyuria caused by lithium in some patients. Schou (1958) and Thomsen (1971) have shown that the polyuria more easily induced in rats by lithium is also resistant to the action of antidiuretic hormone. It is therefore possible that interference with the action of antidiuretic hormone by lithium may have clinical relevance.

This work is also a study of the mode of action of antidiuretic hormone by studying the conditions in which it is inhibited.

Methods

Rats

Male albino Wistar rats (0·13–0·15 kg) were anaesthetized with intraperitoneal pentobarbitone sodium (Nembutal, Evans, 0·7 ml/kg b.w. of a solution containing 60 g/l.).

A tracheotomy was performed followed by cannulation of the bladder, the right external jugular vein and in some experiments the left carotid artery. Anaesthesia was then maintained and water loading achieved using a 3·3 μl/s (0·2 ml/min) intravenous infusion of modified Czaczkes, Kleeman & Koenig (1964) solution: 51 mmol/l. sodium chloride, 1·67% w/v glucose and 2% v/v ethanol (the control solution). In various experiments the infusion was later further modified by replacing or omitting some of the sodium chloride (the test solution) but the rate of administration was always the same. The urine flow rate was continuously monitored by a drop counter. Preparations were ready for use when there was a constant diuresis.

The preparation was therefore similar to that used by Bisset (1962) to assay antidiuretic hormone. Antidiuretic hormone (Pitressin, Parke, Davis & Co.) or oxytocin (Pitocin, Parke, Davis & Co.) diluted in 0·9% saline (0·05–0·25 ml) was given into the infusion into the jugular vein to test the responses. The antidiuretic responses (R) were calculated according to the formula $R = \frac{100\,(a-b)}{a}$, where a= the volume of urine passed in the 10 min before the injection and b=the volume produced in the period 1–11 min after the injection. The first 60 s after an injection was ignored as there is usually a non-specific diuresis due to the injection. This is in fact Goodwin's (1966) modification of the calculation suggested by Dettelbach (1958) and Bisset (1962). Assays were repeated until a repeatable response to antidiuretic hormone or oxytocin was observed. Then the infusion was changed to include a test ion in place of some sodium and further responses to the injected hormones were studied. The mean percentage inhibition (I) was calculated as follows:

$$I = 100\left(1 - \left(\frac{\text{Mean } R \text{ during the test infusion}}{\text{Mean } R \text{ during the control infusion}}\right)\right)$$

After the test infusion the control was often used again.

In some experiments timed serial 1·0 ml urine samples were collected. Terminal blood was obtained directly from the heart. Blood pressure was monitored using a Statham pressure transducer (Bell & Howell Ltd., type 4-327 003) and heparinized cannula in the carotid artery.

Studies of renal electrolytes were made using the method of Atherton, Hai & Thomas (1968). Calculations of intracellular lithium were based on the assumption that lithium is equally distributed in the various cells of the body and accepting figures for the intra- and extracellular spaces of the rat given by Spector (1956).

Toads

Toads (*Bufo marinus*) were double pithed. Each lobe of the bladder was removed and quickly placed in Bentley Ringer (Bentley (1958): NaCl, 111 mmol/l.; $CaCl_2$, 3·35 mmol/l.; $NaHCO_3$, 2·38 mmol and glucose, 5·50 mmol). The lobes were tied on to glass tubing which fitted into a rubber stopper from a boiling tube. The lobe was then filled (usually 1–2 ml) with Bentley Ringer diluted to one-fifth of the above concentrations and suspended in the boiling tube by replacing the rubber stopper. The tube contained 30 ml of undiluted Bentley Ringer. The whole preparation was maintained at 298 $\pm$ 1° (24·85° C) in a water bath. The Bentley Ringer was constantly aerated by water vapour, saturated air being pumped

through the solution. The procedure followed, including washing and emptying between assays, was as recommended by Bentley (1958) and included a test period of 40 min to demonstrate the absence of leaks. The preparation was accepted as satisfactory if the weight change was less than 50 mg. Further periods were used to study the effects of alterations of the fluid on the mucosal or serosal surface on the passage of water facilitated by (a) antidiuretic hormone and (b) adenosine 3′,5′-monophosphate (cyclic AMP) in the serosal fluid. This was achieved by comparing periods when the test solution and cyclic AMP or antidiuretic hormone were present, with periods when the control solution and antidiuretic hormone of cyclic AMP were present. The mean percentage inhibition of a response was calculated from the mean responses with a control solution compared to the mean responses with the test solution.

Chemical and statistical

Lithium was estimated by atomic absorption spectroscopy, sodium and potassium by flame photometry, and glucose was estimated using the Nelson (1944) method.

P values were calculated using Student's *t* test, after satisfying the conditions as suggested by Snedecor (1962).

Results

Figure 1a shows a typical recording of urine flow from an experiment in which the intravenous infusion into a rat of the control solution was changed to one containing 13 mmol/l. lithium. The test solution was, however, equimolar with the control, as it contained 13 mmol/l. less sodium. Figure 1b shows a typical tracing in which the infusion of the 13 mmol/l. lithium solution was again followed by the control solution. It can be seen that during lithium infusion a clear inhibition of the antidiuresis due to antidiuretic hormone develops. This is detectable after 10 min and it reaches a plateau after 30 minutes. It takes a similar period to reverse the effect.

Figure 2 shows the time course of the inhibition followed in eight studies and the limited degree of variability is illustrated. Inhibition of the antidiuretic effect of 80 μunits of antidiuretic hormone by the infusion of 13 mmol/l. lithium varied from 24 to 85% but always occurred. Studies with 25 mmol/l. or 13 mmol/l. of lithium produced indistinguishable results. The urine flow rate was not altered in a predictable way by lithium alone, and the effect of lithium was only apparent when antidiuretic hormone was also administered. No consistent changes in blood pressure occurred in similar experiments with the same dose of lithium.

The total urinary osmotic pressure due to the sodium, potassium and lithium is higher during the lithium infusion than during the initial control period and hence the kidney must work against an increased osmotic load to produce the antidiuresis after antidiuretic hormone. However, when the infusion is changed back to the original solution again, the total ionic concentrations in the urine are still higher but the inhibition has been reversed. Hence, the inhibition by lithium of the antidiuretic effect of antidiuretic hormone is not due to changes in the osmotic pressure of the major electrolytes in the urine.

Studies of renal cortical, medullary and papillary electrolytes were performed on six rats. After a period of stable diuresis, three rats received the control solution for 90 min ; the other three were treated similarly but received the control solution

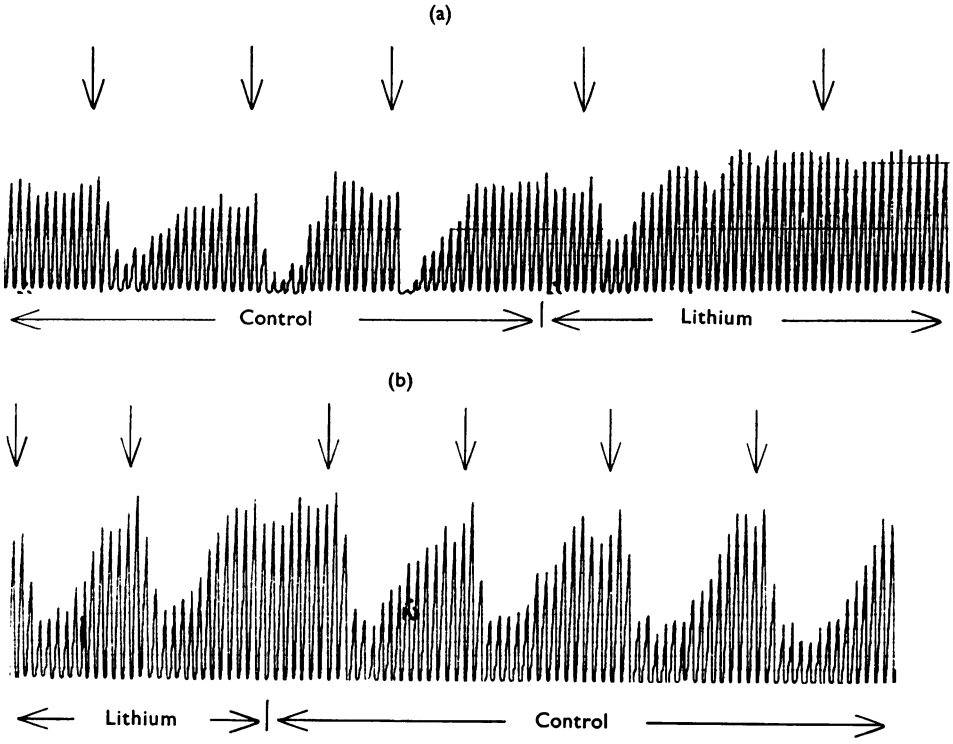

FIG. 1. Typical tracings from experiments on the antidiuretic responses to antidiuretic hormone (ADH). The height of each line represents the volume of urine passed in 60 s, the arrows indicate the time at which 80 μU of ADH had been administered intravenously. Under each tracing the constant infusion solution (3·3 μl/s) is shown. The control solution was modified from Czaczkes *et al.* (1964), the other solution had 13 mmol/l. of sodium replaced by equimolar lithium. (a) Shows the development of inhibition ; (b) its reversal in another animal, more sensitive to ADH and showing a clear but less striking inhibition due to lithium.

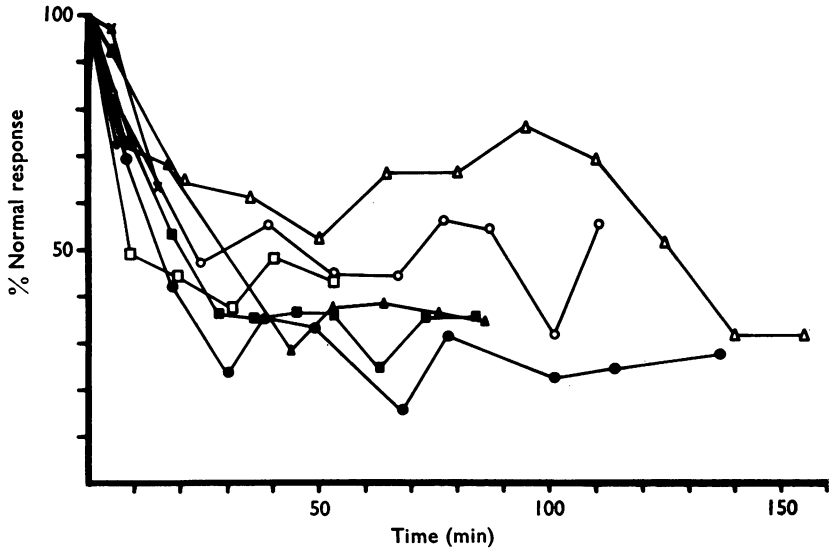

FIG. 2. The course of the inhibition of the antidiuretic effect of 80 μU ADH by the infusion of a solution containing 13 mmol/l. of lithium is shown against the time for which the infusion has been maintained. The mean responses due to ADH during the periods of infusion with the control solution are defined at 100% normal response for that preparation. Each response R during the lithium infusion has then been plotted as a percentage of the normal response for that animal.

for 60 min and then the 13 mmol/l. lithium solution for 30 minutes. Table 1 summarizes the results. They only show statistically significant differences between the control and lithium treated rats for sodium and potassium in the inner medulla of the kidney where the concentrations are decreased in the lithium treated rats. These observations would be consistent with the hypothesis that lithium reduces the osmotic pressure in this possibly relevant area of the kidney. However, the data are too few to allow a firm conclusion. With the tissue dilutions used in these studies lithium ions were not detectable.

In our studies, the solution of Czaczkes *et al.* (1964) caused glycosuria in some rats. In the original paper, the authors state that glycosuria is not produced. They did, however, recommend an infusion rate of 0·12 ml/minute. The possibility of glycosuria was not taken into account in our initial experiments. It did however occur in five of twelve rats studied for this effect. The glycosuria was not affected by lithium. The inhibition of antidiuretic hormone was always present in all the rats studied and when tested was always reversible. Hence this oversight does not seem to detract from the significance of the result.

Studies were made using 13 mmol/l. of either potassium ($n=12$), choline ($n=10$), calcium ($n=23$), magnesium ($n=14$), rubidium ($n=28$), strontium ($n=28$), or caesium ($n=28$), instead of lithium in otherwise identical experiments on the antidiuretic effect of 80 μU antidiuretic hormone. Except for somewhat contradictory results using caesium, infusion of no other solution produced any results reminiscent of the inhibition produced by lithium ions in this preparation. Lithium was still clearly effective in the actual preparations used; it was administered after a period with the control solution at the end of the experiments to demonstrate that they were (1) still functioning normally and (2) capable of showing the inhibition due to lithium.

Most ions produced no obvious effect during their administration. After the infusion of strontium and rubidium, however, the kidney's sensitivity to ADH had increased. The effect for each of these ions was significant at $P<0·005$ level and the results were repeatable.

The rapid development and reversal of the inhibition of the antidiuretic effect of antidiuretic hormone by lithium seemed unlike an enzyme effect. A study was therefore made of the whole body lithium (the input minus the amount excreted) throughout the same experiment ($n=13$). Three groups of rats were also killed for serum lithium estimations: (A) after 20 min of lithium infusion when the inhibition should have been 80% ($n=4$); (B) after 30 min of lithium infusion when the in-

TABLE 1. *Range of concentrations of sodium, potassium, urea and ammonium (mmol/kg of tissue water) in the kidneys of rats which have received intravenous infusions of the control solution or of a solution containing* 13 *mmol/l. LiCl in place of* 13 *mmol/l. of NaCl*

	Sodium		Potassium		Urea		Ammonium	
Part of kidney	Control	Li treated	Control	Li treated	Control	Li treated	Control	Li treated
Papillary tip	116–137	107–141	57–60	55–62	24–33	24–32	24–30	20–28
Papillary base	114–122	94–119	49–59	48–51	26–31	28–33	13–18	14–18
Inner medulla	128–142	104–107	50–53	40–49	23–31	23–32	8–21	13–16
Outer medulla	98–113	90–105	62–67	49–64	12–17	13–16	9–22	16–24
Inner cortex	81–85	74–85	65–73	64–71	5–12	13–20	17–30	22–28
Outer cortex	76–83	73–79	64–72	63–65	6–13	10–16	17–31	18–22

The mean differences are only significant for sodium and potassium in the inner medulla ($P<0·01$ in both cases).

hibition would have been maximal ($n=5$); (C) 40 min after reverting to the control solution in rats which had received lithium for 60 min but would now have been expected to show no inhibition ($n=4$). These rats did not in fact receive antidiuretic hormone. Figure 3 shows the results, plotted against time, of the serum values, the whole body concentrations and the percentage inhibition when tested. Clearly, neither the total body level nor the serum level is critical. Eighty per cent inhibition can occur with a lower total body lithium and a similar serum concentration to that found when the inhibition has been reversed. The calculated average whole body intracellular concentrations at the time of taking the above serum samples are 0·23–0·50, 0·40–0·80 and 1·4–2·12 mmol/l. respectively. Hence this measure is also not critical.

As oxytocin has an antidiuretic effect, it was thought to be of interest to see whether this effect was also inhibited by lithium ions. Table 2 shows the results from studies on seven rats. Including all the results the mean inhibition was 26%. Applying Student's t test, this is statistically significant ($P<0·05$). In the three animals in which it was tested, the effects of lithium on the antidiuretic effect of oxytocin were reversed by perfusion with the original solution as can also be seen in Table 2.

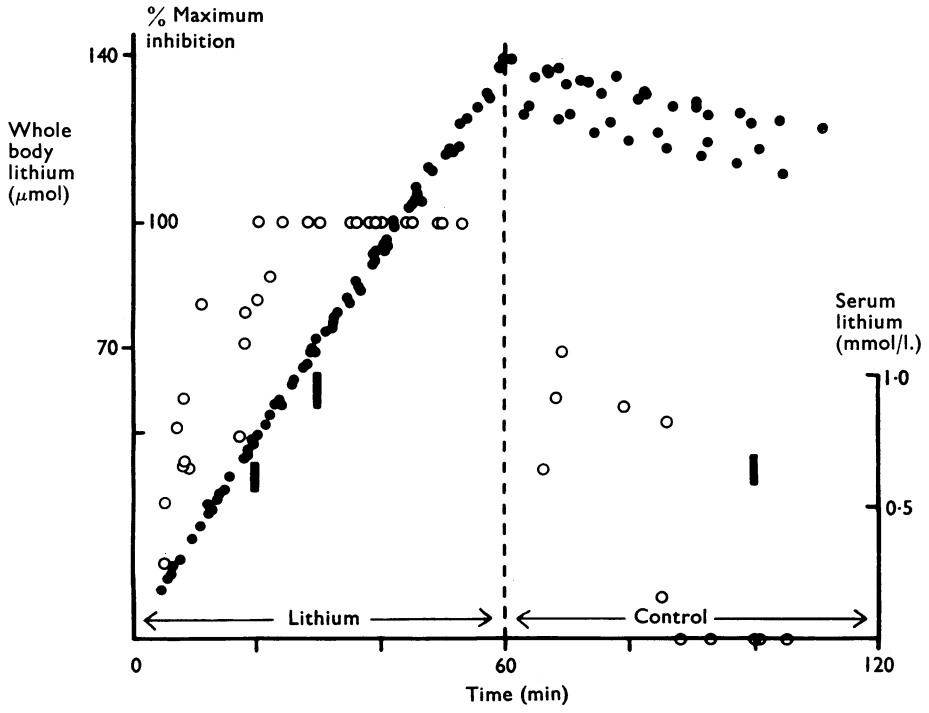

FIG. 3. (○), Percentage inhibition of the antidiuretic response to 80 μU of ADH in six different rats plotted against time. The bars represent the range of serum values at the three times at which they were measured. (●), Total body lithium against time in six animals. The time for which the animal received lithium is separated from the time for which it received the control solution by the vertical line. The figure shows that the inhibition may be fully reversed at similar serum and higher whole body lithium concentrations than can produce 80% inhibition.

Presumably the effect of lithium ions on the response to oxytocin is similar to the effect on the action of antidiuretic hormone, but it is less striking and there is clearly a lower percentage inhibition. This reduced inhibition is not easy to explain if oxytocin in larger doses than antidiuretic hormone is thought to act by stimulating the antidiuretic hormone sensitive adenylcyclase to release enough cyclic AMP to produce the same degree of effect, and if lithium acts on the effect of cyclic AMP. (Oxytocin (5 mU) and antidiuretic hormone (80 μU) have similar antidiuretic effects.)

Toad bladder experiments

Table 3 presents the results from studies on the effect of lithium, potassium and choline on the facilitation by antidiuretic hormone or cyclic AMP of water transport by the toad bladder. In each case the test ion has replaced an equimolar amount of sodium from the control solution. As can be seen, 2 mmol/l. lithium or choline on the serosal surface cause a significant inhibition of the effect. That choline causes inhibition shows how careful one must be in assuming it is biologically inactive (see

TABLE 2. *Antidiuretic responses to intravenous oxytocin (5 mU) during the infusion of the control solution, the lithium (13 mmol/l.) solution, and finally after reverting to the control*

Rat (no. of assays control-Li-control)	Average response with control solution	Average response with lithium infusion	% Inhibition	Average response after reverting to control solution
A (4,4,0)	54	29	47	—
B (4,4,0)	30	30	0	—
C (4,4,0)	17	10	39	—
D (3,3,0)	26	29	−10	—
E (3,2,2)	29	12	60	33
F (3,3,3)	49	40	18	56
G (5,4,3)	35	25	29	36
			Av. 26% $P<0.05$	

The inhibition due to lithium is presented comparing the responses during lithium infusion with those from the preceding period when the control solution was being given.

TABLE 3. *Effects of various alterations of the solutions bathing the toad bladder sacs on the water transport facilitated by either 500 μU/ml of ADH or 1·1 mmol/l. of cyclic AMP*

No. of bladders used	1st solution	2nd solution	Mean inhibition %	$P<$
	Studies on the inhibition of the effect of 500 μU/ml ADH			
11	Control	26 mmol/l. Li	69	0·0005
3	Control	2 mmol/l. Li	74	0·0025
2	2 mmol/l. Li	Control	56	0·05
2	Control	2 mmol/l. Li (mucosal)	−6	N.S.
2	Control	2 mmol/l. NaCl (deficient)	−28	N.S.
2	2 mmol/l. NaCl (deficient)	Control	−13	N.S.
11	Control	2 mmol/l. K	25	N.S.
4	2 mmol/l. K	Control	12	N.S.
11	Control	2 mmol/l. choline	28	0·005
	Studies on the inhibition of the effect of 1·1 mmol/l. cyclic AMP			
9	Control	2 mmol/l. Li	46	0·005

Unless stated the solution altered is on the serosal surface. In each case the bladder is bathed in two solutions (a) the control and (b) the test solution. Inhibition is the reduction of the effect of the test compared to the control solution, negative values indicate stimulation.

also Hodgkin & Martin (1965), Lingjaerde (1969), Martin (1968), Sanford & Smyth (1969), Sung & Johnstone (1969), who have all presented evidence of biological effects of or on choline). It should be noted that the inhibition due to 2 and 26 mmol/l. lithium are very similar and of a different order from that produced by choline. Changes in potassium and sodium concentrations had no statistically significant effect on the system. In two experiments reversal of inhibition by lithium could be shown when the lithium solution preceded the normal Bentley Ringer. Two further experiments showed no significant inhibition if lithium was applied to the mucosal surface.

Using a solution of 1·1 mmol/l. cyclic AMP instead of ADH, a 2 mmol/l. lithium solution caused a mean percentage inhibition of 46 ($46 \pm 6{\cdot}3$ S.E. ($n=9$)). Hence, in this preparation lithium ions inhibit the stimulating effect on water transport of cyclic AMP.

Discussion

The results presented show that lithium ions inhibit the antidiuretic action of antidiuretic hormone in the rat kidney, and that they inhibit the action of antidiuretic hormone and of cyclic AMP on the toad's bladder. These results are consistent with the hypothesis that cyclic AMP mediates the action of antidiuretic hormone (Orloff & Handler (1967), Sutherland & Robison (1966)). In terms of some current hypotheses (of a series mucosal barrier in the granular cells, Dibona, Civan & Leaf (1969), and a serosal pump, Anderson & Ussing (1957)) it might be postulated that lithium acts on the water porous mucosal membrane at which site antidiuretic hormone may also act (Crabbé, de Weer & Scarlata (1969)).

It is difficult to understand how this effect can be achieved if the absolute concentration of lithium is not relevant in the blood or the cells. Unfortunately, no direct measurements of the relevant intracellular concentrations have been made. Thomsen, however, has given us unpublished data on his rats showing, as we had predicted, high renal lithium concentrations (0·75–1 mmol/l.) 40 min after ending lithium infusions in his large 0·4 kg rats. At this time, antidiuretic hormone inhibition would not occur, however the exact location of the lithium is difficult to determine.

In the renal electrolyte studies presented in this paper, there is some evidence to suggest that lithium caused osmotic pressure changes in the inner medulla. These changes may affect the action of antidiuretic hormone in the kidney but would not obviously add much towards an explanation of the effects of lithium on the amphibian tissue. The two effects may, of course, be unrelated, as may the effect of cyclic AMP and antidiuretic hormone in the toad bladder (see, for example, Cuthbert & Painter, 1968).

The studies of the ionic specificity of lithium for the inhibition in the rat presented here and by Harris & Jenner (1969) can be criticized in the absence of knowledge of the serum concentrations. In all situations in which the body counteracts the serum rise by extrarenal mechanisms, the kidney is not directly tested. Potassium serum concentrations, for example, were not significantly changed by the infusion of 13 mmol/l. potassium and potassium caused no inhibition of vasopressin in the rat kidney. In the toad bladder, 2 mmol/l. potassium tended to cause inhibition but this did not achieve statistical significance. Bentley (1959) with somewhat similar concentrations of potassium reports significant inhibition of pituitrin in the toad

bladder. He found this also with 3 mmol/l. magnesium, barium or manganese, and 6 mmol/l. calcium or strontium. He also showed inhibition with 50% replacement of sodium by choline in the serosal fluid. We have reported this using only 2 mmol/l. choline and shown that it is not due to a reduced sodium concentration. It may therefore be true that the amphibian system is very sensitive to many cations other than sodium in the serosal fluid. More work is required to show whether this is also true in the kidney.

The relevance of these studies to either the clinically encountered toxic or therapeutic effects of lithium is also at least open to doubt. Both clinical effects take some time to develop whereas our study on rats and toads is on acute effects. The possibility that under equilibrium conditions lithium may not inhibit the action of antidiuretic hormone in studies on rats described here might suggest that another action is necessary to explain the antidiuretic hormone resistant, diabetes insipidus-like state which can arise clinically.

The polyuria in the clinical situation is also not necessarily a purely renal phenomenon. Smith, Balagura & Lubran (1971) for example have presented evidence that the polydipsia induced by intragastric lithium in the rat is a reaction to a direct effect of lithium on the rat's lateral hypothalamus.

Clinically, lithium causes a diuresis on the first day of treatment (Crammer, 1971). This is followed by a normal urine output; the persistent polyuria which is only occasionally severe develops later. Clearly, the hypothalamus or posterior pituitary could compensate and then become exhausted. However, a great deal more work needs to be done to answer all these questions.

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