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J Clin Invest. 1985;75(6):1869-1879. <https://doi.org/10.1172/JCI111901>.

Research Article

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Pathogenesis of Nephrogenic Diabetes Insipidus Due to Chronic Administration of Lithium in Rats

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Abstract

A polyuric syndrome with nephrogenic diabetes insipidus (NDI) is a frequent consequence of prolonged administration of lithium (Li) salts. Studies in the past, mainly the acute and in vitro experiments, indicated that Li ions can inhibit hydroosmotic effect of [8-arginine]vasopressin (AVP) at the step of cAMP generation in vitro. However, the pathogenesis of the NDI due to chronic oral administration of low therapeutic doses of Li salts is not yet clarified. We conducted a comprehensive study to clarify the mechanism by which Li administered orally for several weeks induces polyuria and NDI in rats. Albino rats consuming a diet which contained Li (60 mmol/kg) for 4 wk developed marked polyuria and polydipsia; at the end of 4 wk the plasma Li was 0.7 ± 0.09 mM (mean \pm SEM; $n = 36$). Li-treated rats had a significantly decreased (-33%) tissue osmolality in papilla and greatly reduced cortico-papillary gradient of urea (cortex—43%; medulla—64%; papilla—74%). Plasma urea was significantly ($P < 0.001$) lower in Li-treated rats (5.4 ± 0.2 mM) compared with controls (6.8 ± 0.3 mM). Medullary collecting tubules (MCT) and papillary collecting ducts (PCD) microdissected from Li-treated animals had higher content of protein than MCT and PCD from the control rats. The cAMP accumulation in response to AVP added in vitro was significantly ($\Delta = -60\%$) reduced. Also, the cAMP accumulation in MCT and PCD after incubation with forskolin was markedly lower in Li-treated rats. Addition of 0.5 mM 1-methyl,3-isobutyl-xanthine did not restore the cAMP accumulation in response to AVP and forskolin in MCT from Li-treated animals. In collecting tubule segments from polyuric rats with hypothalamic diabetes insipidus (Brattleboro homozygotes) the AVP-dependent cAMP accumulation was not diminished. The activity of adenylate cyclase (AdC) in MCT of Li-treated rats, both the basal and the activity stimulated by AVP, forskolin, or fluoride, was significantly ($\Delta \cong -30\%$) reduced, while the activity of cAMP phosphodiesterase (cAMP-

PDIE) in the same segment showed no significant difference from the controls. Also, the content of ATP in MCT microdissected from Li-treated rats and incubated in vitro did not differ from controls. The rate of [14 C]succinate oxidation to $^{14}\text{CO}_2$ in MAL was inhibited (-77%) by 1 mM furosemide, which indicates that this metabolic process is coupled with NaCl cotransport in MAL. The rate of $^{14}\text{CO}_2$ production from [14 C]succinate in MAL was not significantly different between control and Li-treated rats. In MCT of control rats, the rate of [14 C]succinate oxidation was ~ 3 times lower than in MAL. The rate of $^{14}\text{CO}_2$ production from [14 C]succinate in MCT of Li-treated rats was significantly ($\Delta +33\%$) higher than in MCT dissected from control rats. Based on these results, we conclude that at least two factors play an important role in the pathogenesis of NDI consequent to chronic oral administration of Li: (a) decreased ability of MCT and PCD to generate and accumulate cAMP in response to stimulation by AVP; this defect is primarily due to diminished activity of AdC in these tubular segments caused by prolonged exposure to Li; and (b) lower osmolality of renal papillary tissue, due primarily to depletion of urea, which decreases osmotic driving force for water reabsorption in collecting tubules. On the other hand, NaCl reabsorption in MAL is apparently not affected by chronic Li treatment.

Introduction

The polyuric syndrome that develops as a consequence of chronic administration of lithium (Li) salts is the most frequent and often troubling complication in the treatment of manic-depressive disease with this antidepressant drug (1-6). The major component of this polyuric syndrome is a [8-arginine]vasopressin (AVP)-resistant urinary concentrating defect—the nephrogenic diabetes insipidus (NDI) (2, 5, 6). In spite of numerous clinical and experimental studies in the past several decades, the cellular mechanisms in the pathogenesis of NDI caused by chronic Li treatment remain to be clarified.

Most of the past studies aimed to investigate pathogenesis of Li-induced NDI, conducted on mammalian kidney and amphibian epithelia (1-6), examined the effects of the acute Li addition in vitro, or acute Li infusion in vivo on the AVP

This paper was presented at the 16th Annual Meeting of the American Society of Nephrology, 1983, Wash. DC (abstract in *Kidney Int.* 1984; 25:297).

Dr. Christensen, visiting scientist from the University of Copenhagen, Denmark, was a recipient of a Fulbright Scholarship and was supported by the Danish Medical Research Council. Dr. Kusano and Dr. Murayama were recipients of the Research Fellowship from the American Heart Association, Minnesota Affiliate. Address correspondence and reprint requests to Dr. Dousa, 921 Guggenheim Building, Mayo Clinic.

Received for publication 16 August 1984 and in revised form 19 February 1985.

J. Clin. Invest.

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0021-9738/85/06/1869/11 \$1.00

Volume 75, June 1985, 1869-1879

1. *Abbreviations used in this paper:* AdC, adenylate cyclase; AVP, [8-arginine]vasopressin; b wt, body weight; cAMP-PDIE, cyclic AMP phosphodiesterase; CCT, cortical collecting tubule; Cr, creatinine; GFR, glomerular filtration rate; HHDI, hereditary hypothalamic diabetes insipidus; KRB, Krebs-ringer buffer; Li-rat(s), rats chronically treated with Li; MAL, medullary thick ascending limb of Henle's loop; MCT, medullary collecting tubule; MIX, 1-methyl,3-isobutyl-xanthine; NDI, nephrogenic diabetes insipidus; PCD, papillary collecting duct; P_{cr} , plasma creatinine; P_{Li} , plasma Li levels; SDH, succinate dehydrogenase; U_{osm} , urinary osmolality; $U_{osm} \times V$, total solute excretion; $U_{urea} \times V$, total urea output.

dependent, cyclic 3',5'-AMP (cAMP)-mediated transport processes (7–13). Such studies suggested that Li ion inhibits the increase of water permeability of AVP mainly, albeit not exclusively (7, 8, 14), at the step of the AVP-dependent generation of cAMP (2–6, 9–12). Most renal studies of the AVP-dependent cAMP system have been conducted mainly on kidney tissue preparations (9–12) that contain at least two types of tubular segments that are both endowed with AVP-dependent cAMP metabolism (3, 15), specifically medullary collecting tubules (MCT) and the medullary thick ascending limb of Henle's loop (MAL).

Moreover, the effects of Li on renal components of urinary concentrating mechanism were examined, usually, only after short periods (1 wk) of treatment and/or with Li salt administered parenterally (2, 8, 16, 17). While results of the past studies suggested in principle the mode of Li action, these experimental models are quite remote from the conditions under which the NDI develops after chronic oral consumption of relatively small amounts of Li, as it is employed in management of manic-depressive psychosis (1, 2, 5, 10, 12).

In view of these considerations, we analyzed the animal model of Li-induced polyuria, which closely duplicates renal effects of chronic Li administration encountered in humans (17–21). In this model, the Li is administered to rats orally for at least several weeks in solid food, simultaneously with ad lib. intake of 0.9% NaCl (18–20). This regimen prevents intermittent sharp increases in plasma Li above the therapeutic range (17, 22) and prevents sodium depletion that leads to acute Li nephrotoxicity (19, 20, 22).

Our present results show that chronic oral administration of Li impairs the AVP-dependent cAMP metabolism in collecting tubule system at the step of AVP-sensitive adenylate cyclase (AdC), and reduces cortico-papillary gradient of solutes in renal tissue, which is due to severe depletion of urea.

Methods

Experiments were conducted on adult male albino rats of either Sprague-Dawley or Wistar strains, both purchased from Charles River Breeding Laboratories, Wilmington, MA. Results of the studies on these two strains of rats were virtually identical and data are presented interchangeably. The rats were kept in an air-conditioned room (21°–25°C) with a free access to (free choice to drink) both distilled water and solution of 0.9% NaCl (18–21). Likewise, they had ad lib. access to standard rat diet (Purina Laboratory Rat Chow, Ralston Purina Co., St. Louis, MO) with or without admixed LiCl. Rats of Long-Evans strain and adult male homozygotes of Brattleboro strain with hereditary hypothalamic diabetes insipidus (HHDI) were purchased from Blue Spruce Farms, Altamont, NY.

Experimental design. As a general rule, rats treated with Li (referred to from here on as Li-rats) and the corresponding controls were always handled and analyzed using paired design (23–25). The control and Li-treated animals entered and completed the treatment regimen *pari passu*, were killed on the same day, and the blood, urine, and tissues were collected and analyzed simultaneously (23–25). Likewise, specific tubule segments were microdissected from the kidneys of the control and Li-rats on the same day using the same solutions, hormones, drugs, and other chemicals (23–25).

Typically, 4–6 rats, weighing 200–230 g, were entered into the protocol as a control group (2–3 rats), which received standard chow diet, and as an experimental group (2–3 rats), which received the same chow but with added Li to the final content of 60 mmol LiCl/kg (19–21). The Li-containing diet was prepared by adding 1 M LiCl and an approximate amount of water to powdered standard rat chow to give wet mash, which was subsequently dried in the oven at 60°C.

Intake of fluids, distilled water, and 0.9% NaCl solution was recorded 4 d within 1 wk and the body weight once a week. After 24 d, the rats were placed in individual metabolic cages and the urine was collected between 9 a.m. and 12 a.m. (20). After a total period of 26–29 d of the above-mentioned dietary regimen, the rats (control and experimental) were anesthetized with intraperitoneal sodium pentobarbital, and under anesthesia a sample of blood was taken from the jugular vein. The abdomen was opened under anesthesia and a sample of urine was collected from the urinary bladder by syringe. The right kidney was ligated and removed for analysis of tissue solutes. The left kidney was prepared for perfusion and microdissection of tubules, as described below.

Analysis of solutes in tissue and fluids (23). The kidneys were rapidly removed under anesthesia. The cortex, outer (red) medulla, and papilla were separated by razor blade and rapidly frozen by clamping with stainless steel tongs precooled by immersion in liquid N₂. Deep-frozen tissue was transferred to polyethylene (12 × 75 mm) test tubes filled with liquid N₂ and surrounded by dry ice. The tubes with tissues in the dry-frozen state were weighed with a Sartorius microbalance to determine the wet weight. After overnight lyophilization, tubes were then weighed again for the determination of dry weight (23).

Lyophilized dry tissue was extracted for determination of solutes using, in principle, the method of Appleboom (26), as in our previous study (23). Water content in samples was expressed as percentage of wet weight. Total tissue osmolality was calculated as follows (23): total osmolality [mosM/kg H₂O] = [urea] + 2[Na⁺] + 2[K⁺]. Na⁺ and K⁺ were determined by atomic adsorption photometer (Instrument Laboratory 951, Elk Grove Village, IL) and urea by the colorimetric method using Harleco kits (23). Plasma Li was determined by flame emission spectrophotometry, after precipitation of proteins with 5% trichloroacetic acid. Urine osmolality (U_{osm}) was measured with a Fiske osmometer (Fiske Assoc., Burlington, MA). Creatinine (Cr) was determined colorimetrically (27).

Microdissection and analysis of tubules. Tubular segments were dissected from control and Li-treated rats, always on the same day, using the procedure described in our previous studies (23–25, 28–30). Briefly, under light anesthesia with pentobarbital (6 mg/100 g body weight [b wt]), the aorta was cannulated retrogradely with polyethylene PE-100 tubing (Clay Adams Div. of Becton, Dickinson & Co., Parsippany, NJ), and the tip was placed just distal to the branching of the left renal artery. Composition of all solutions is described at the end of the Methods section. The kidney was perfused to complete blanching with 5–10 ml of perfusion solution, and subsequently followed by 20 ml of heparinized (heparin concentration 20 U. S. Pharmacopeia U/ml) collagenase medium for ~5 min. After the perfusion, the kidney was quickly removed and sliced with a razor blade (23–25). The slices were then incubated in aerated collagenase medium at 35°C for 35–45 min. For dissection of papillary collecting ducts (PCD), the tissue strips were incubated in a collagenase medium for 60 min at 35°C (25, 29). The slices were then thoroughly rinsed in ice-cold microdissection medium and transferred to Petri dishes for microdissection (23–25). Microdissection and all subsequent procedures were performed at 0°–4°C. Segments of MAL and MCT were carefully teased out from the inner stripe of the outer medulla, PCD from inner medulla (papilla), or cortical collecting tubules (CCT) from cortex, with sharpened steel needles using a stereomicroscope (magnification of 30). Segments were identified using the established criteria, as in our previous studies (23–25, 28–30). Dissected segments were transferred onto small discs (circa 3 mm²) of glass coverslip. The total length of tubules in the sample was then determined. Samples were placed on the stage of microscope with a drawing attachment (camera lucida) and inspected under 100 magnification (23, 25). Tubules in the sample and in the 1-mm calibration grid were quickly drawn on white paper with a marker. Total tubular length was measured using a Dietzger planimeter from drawn pictures of samples. The measured samples were kept at 0°–4°C before incubations, when the assay was performed immediately, or before quick freezing for storage at –80°C.

Determination of tubule diameter. Since chronic treatment with Li can produce morphologically detectable enlargement of collecting tubules (19, 21), we determined the outer diameter of dissected tubules from photographs. Samples containing several freshly microdissected tubules were photographed under microscope at low magnification and the negatives were projected on a screen with an overhead projector. The diameter was measured in millimeters with a ruler and calculated into micrometers by measuring a 1-mm grid on photographs taken at the same magnification. The outer diameters of examined segments of MCT from Li-treated rats ($53.0 \pm 2.2 \mu\text{m}$, mean \pm SEM from eight rats; total number of samples $n = 224$) was significantly ($P < 0.001$, t test; $+22\%$, $P < 0.005$, paired t test) wider than the diameter of MCT microdissected from corresponding controls ($43.4 \pm 1.4 \mu\text{m}$, mean \pm SEM from eight rats; total number of samples $n = 189$).

Enzyme assays. Assays for AdC and cAMP-phosphodiesterase (cAMP-PDIE) were performed on permeabilized (disrupted) tubular segments (23–25, 28–30). The microdissection medium was replaced with $0.25 \mu\text{l}$ of hypoosmotic medium; samples were then frozen rapidly on dry ice and stored at -80°C overnight. Samples were allowed to thaw at 4°C before being assayed for enzyme activities.

The AdC activity was measured according to the method described in detail previously (23–25, 28–30). Tubular samples (1–3 mm) were incubated for 30 min at 30°C in $2.5 \mu\text{l}$ consisting of 0.25 mM [α - ^{32}P]ATP (4×10^6 cpm/sample), 1 mM cAMP, 3.8 mM MgCl_2 , 0.25 mM EDTA, 100 mM Tris HCl, 20 mM Cr phosphate, and 1 mg/ml Cr kinase (pH 7.4), with NaCl and urea added in a 1:2 M ratio to adjust final osmolality to 600 mosM (23, 29). After stopping the reaction (23–25, 30), the cAMP was isolated according to the method of Salomon et al. (31); the AdC activity was expressed as picomoles cAMP per 30 min/ μg of protein.

The activity of cAMP-PDIE. Tubular segments (0.5–1.5 mm length) were incubated for 10 min at 37°C in $2.5 \mu\text{l}$ consisting of 10^{-6} M [^3H]cAMP, 10 mM MgSO_4 , 0.1 mM EDTA, 50 mM Tris-HCl (pH 8.0) (23–25, 29, 32), with added NaCl and urea in a 1:2 M ratio to adjust the osmolality of medium to 600 mosM. The reaction was stopped by freezing on dry ice and further analyzed as described in our previous studies (23–25, 32). Activity of cAMP-PDIE was expressed in femtomoles cAMP hydrolyzed per minute per microgram of protein (32).

Accumulation of cAMP in MCT, PCD, and CCT was measured on freshly microdissected tubules, as described in detail previously (23–25, 29–30). For these incubations, ~ 3.5 – 8.5 mm of tubule length for basal cAMP accumulation and 0.5 – 5 mm for AVP-stimulated or forskolin-stimulated cAMP accumulation were required. After measurement of tubule length, microdissection medium was aspirated off and replaced with $2.5 \mu\text{l}$ of modified Krebs-ringer buffer (KRB) (for composition, see below) with or without test agents and hormone. After 10 min incubation at 30°C , the reaction was stopped by placing the slides on dry ice. Samples were transferred into test tubes with $100 \mu\text{l}$ of 50 mM Na acetate and placed for 3 min in a boiling water bath (23–25, 30). The cAMP content was determined by radioimmunoassay as described in every detail previously (23–25, 28–30). The content of cAMP in the sample (tubules and incubation medium together) is expressed in femtomoles per micrograms of tubular protein (24, 28, 30), unless specified otherwise.

Determination of ATP. The ATP content in dissected intact tubules was measured with a micro-bioluminescence assay using luciferin-luciferase system, which is described in detail elsewhere (33). Briefly, freshly microdissected tubules (1–2 mm) were first incubated for 20 min at 30°C in $2.5 \mu\text{l}$ of KRB to stabilize tubules metabolically and to normalize assay conditions for all samples (33). Samples were then frozen on dry ice, transferred to glass test tubes containing $15 \mu\text{l}$ of 0.02 M Tris/ MgSO_4 buffer (Picocheck buffer, Packard 6016820, Packard Instrument Co., Downers Grove, IL) pH 7.75. An equal volume of an ATP-extracting reagent for somatic cells (Picoex S; Packard 6016740, Packard Instrument Co.) was added and mixed. Then $40 \mu\text{l}$ of enzyme reagent (Picozyme F; Packard 6016710, Packard Instrument Co.) were added into a 6×50 -mm glass tube and the background light (enzyme

blank) was counted three times for 12 s for each sample (33). ATP standards or tubular extracts ($10 \mu\text{l}$) were rapidly injected directly into the tube containing enzyme, and light production was measured on Picolite model 6100 luminometer (Packard Instrument Co.). An average of three values was used for calculations. Enzyme blanks were subtracted and the standards and unknown extracts were treated in the same way (33). Results are expressed as femtomoles ATP per microgram of tubular protein.

NaCl transport-coupled $^{14}\text{CO}_2$ production from 1,4- ^{14}C succinate in isolated tubules. $^{14}\text{CO}_2$ production from 1,4- ^{14}C succinate was measured, in principle, according to the method of Le Bouffant et al. (34) modified for MAL in our previous study (23), with several further modifications. We used [^{14}C]succinate rather than [^{14}C]lactate (23), in view of the recent observations that activity of succinate dehydrogenase (SDH) determined histochemically was altered in some tubular segments of Li-rats (21). Moreover, we observed in preliminary studies that the rate of $^{14}\text{CO}_2$ production was greater with use of [^{14}C]succinate than with [^{14}C]lactate (23). The principle of the method consists in the continuous trapping, by simple diffusion into KOH, of the metabolic product $^{14}\text{CO}_2$ resulting from oxidative metabolism of ^{14}C -radiolabeled metabolic substrate by isolated tubules incubated in a bicarbonate-free solution (23, 34). Freshly dissected MAL or MCT from both control and Li-treated rats were transferred onto a round coverslip placed on the concave bacteriological slide (23) and the length of tubule segments was measured. At the beginning of the assay, the microdissection medium was replaced with 1.0 – $1.5 \mu\text{l}$ of metabolic medium, which contained 1,4- ^{14}C succinate (total composition given below) with or without added drugs. A 2 - μl droplet of 0.3 M KOH was placed on a filter disc that was situated on another coverslip in the same concave of bacteriological slide (23). Then the sealed chamber (23) was incubated at 35°C for 80 min. Preliminary experiments showed that, as with use of [^{14}C]lactate (23), the rate of $^{14}\text{CO}_2$ production from 1,4- ^{14}C succinate was linear with time for this period in both MAL and MCT. Likewise, $^{14}\text{CO}_2$ production from 1,4- ^{14}C succinate was proportional to length of tubules in the sample up to 3 mm . The reaction was stopped by placing the slides with samples on dry ice, and ^{14}C -radioactivity on coverslips with 0.3 M KOH was measured by liquid scintillation counting (23). The rate of $^{14}\text{CO}_2$ production is expressed as picomoles of $^{14}\text{CO}_2$ generated from 1,4- ^{14}C succinate per 80 min/ μg of tubule protein.

In our previous study we confirmed, using several criteria, that metabolism of [^{14}C]lactate in our system is coupled to continuing NaCl cotransport in MAL (23). Inclusion of loop diuretic furosemide or replacement of NaCl in the metabolic medium with equimolar LiCl resulted in marked suppression of [^{14}C]lactate oxidation (23). Likewise, in the present study, the $^{14}\text{CO}_2$ formation from 1,4- ^{14}C succinate in MAL ($1,084 \pm 216 \text{ fmol } ^{14}\text{CO}_2/\mu\text{g protein}/80 \text{ min}$; mean \pm SEM, $n = 4$ experiments) was strongly inhibited ($\Delta -77 \pm 4\%$, mean \pm SEM; $P < 0.001$, paired t test) by addition of 10^{-3} M furosemide ($236 \pm 52 \text{ fmol } ^{14}\text{CO}_2/\mu\text{g protein}/80 \text{ min}$; mean \pm SEM, $n = 4$ experiments). Also, replacement of NaCl in metabolic medium by NaI caused marked decrease (-51%) of $^{14}\text{CO}_2$ production from 1,4- ^{14}C succinate.

Determination of protein content in tubules. To examine whether the protein content per millimeter of tubular length in MCT, MAL, or PCD is affected by chronic Li administration, the samples of microdissected segments, total length (~ 5 – 10 mm) solubilized in $25 \mu\text{l}$ of 1% sodium dodecyl sulphate and then assayed with use of Lowry's method (35) as adapted by us (24, 28, 33) for measurements of microdissected tubule samples (Table I).

Expression and evaluation of results. The biochemical parameters of microdissected nephron segments are expressed per millimeter of tubular length, or per total protein content, calculated from the typical protein content per length unit (millimeter) of the given segment (24, 30), and per the actual length of the tubules measured in each individual sample (24, 28). Since in the distal nephron segments of rats chronically treated with lithium (Li-rats) the protein content was markedly higher (Table I) than in corresponding segments from controls, the metabolic parameters in tubules from Li-rats and controls

Table I. Protein Content in Segments of MCT, MAL, and PCD from Control Rats and Li-treated Rats

Segment	N*	$\mu\text{g Protein/mm tubule length}$		P value‡
		Controls	Li-treated	
MCT	6	0.102±0.004§ (50)	0.156±0.004 (62)	<0.001
MAL	4	0.113±0.003 (43)	0.143±0.005 (36)	<0.001
PCD	3	0.127±0.004 (24)	0.140±0.005 (30)	NS

For details, see Methods.

* Denotes number of paired experiments.

‡ For significance of difference from controls (*t* test). All values of Li-treated rats were significantly higher ($P < 0.05$ or higher degree of significance, paired *t* test) when experiments were evaluated on paired basis.

§ Denotes mean±SEM; total number of samples in parentheses.

were expressed relative to protein content in the sample (24, 28, 29), determined as described above (Table I).

As outlined above, experimental rats, i.e., Li-rats (or HHDI Brattleboro homozygote rats), and corresponding control rats entered and finished the experimental protocol simultaneously, and also all analyses were performed on paired basis (23–25). Statistical evaluation was made with use of the *t* test, as specified in Results. Values $P > 0.05$ were considered nonsignificant (NS).

Solutions and materials. The microdissection medium was a modified Hank's solution as used in our previous studies (23–25, 28–30) (in final concentration): 127 mM NaCl, 5 mM MgSO₄, 0.33 mM Na₂HPO₄, 1.0 mM MgCl₂, 20 mM Tris-HCl, and 0.25 mM CaCl₂, pH 7.4. The composition of the collagenase medium was identical to that of the microdissection medium, except that the CaCl₂ concentration was 1 mM and it included collagenase (0.1% wt/vol), bovine serum albumin (0.1% wt/vol), and hyaluronidase (0.1% wt/vol). The hypoosmotic medium contained 1 mM MgCl₂, 0.25 mM EDTA, 0.1% bovine serum albumin, and 1 mM Tris-HCl (pH 7.4). Modified Krebs-ringer buffer contained 140 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 0.8 mM CaCl₂, 10 mM sodium acetate, 10 mM glucose, 20 mM Tris,

and 2.0 mM NaH₂PO₄, pH 7.4. Metabolic medium for ¹⁴CO₂ production contained 140 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 1.8 mM CaCl₂, 10 mM Na acetate, 5 mM glucose, 20 mM Tris, 2.0 mM NaH₂PO₄, and 5 mM of 1,4-¹⁴C-labeled-succinate (3×10^5 cpm/sample), and pH was 7.4. Metabolic medium, KRB, and enzyme assay solutions were adjusted to final osmolality of 600 mosM by addition of NaCl and urea in molar ratio 1:2 (23, 29). In experiments with use of forskolin (38), the stock solution of forskolin was made in ethyl alcohol (EtOH). We established that 0.1% EtOH does not interfere with assays and analyses; nevertheless, in all experiments with use of forskolin, 0.1% EtOH was included to all samples (38).

Bovine serum albumin, rattlesnake venom (*Crotalus atrox*, collagenase type I, 150 U/mg), hyaluronidase (type 1-5, 500 NF U/mg), cAMP, and furosemide, as well as other biochemicals were purchased from Sigma Chemical Co., St. Louis, MO. Sodium heparin (Panheparin 1,000 U. S. Pharmacopeia U/ml) was purchased from Abbot Laboratories, Chemical Div., North Chicago, IL; [α -³²P]ATP (37.6 Ci/mmol) was purchased from ICN Nutritional Biochemicals, Cleveland, OH; [³H]cAMP (32 Ci/mmol) and 1,4-[¹⁴C]succinate (53 Ci/mmol) were also from ICN Nutritional Biochemicals. Synthetic AVP (385 U/mg), 1-methyl,3-isobutyl-xanthine (MIX), and forskolin were purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA. Radioimmunoassay kits for the measurement of cAMP content were purchased from Schwarz/Mann Div., Becton-Dickinson & Co., Orangeburg, NY. These and all other compounds and reagents were of the highest quality grade available and were purchased from standard suppliers.

Results

Fluid and solute balance. The rats consuming chronically food containing Li developed polydipsia and polyuria, which reached plateau on the second week of the experiment. In parallel with increased water intake, the Li-rats also increased consumption of 0.9% NaCl solution offered to both groups of animals ad lib. On the third week of the experiment, the Li-treated rats consumed ~9 times more NaCl in 0.9% solution than the corresponding control rats. Therefore, about one-half of increment in fluid intake (Table II) was due to increase drinking of distilled water (increase $\Delta +42.5$ ml/100 g b wt/24 h) while another half was due to increased drinking of isotonic saline

Table II. Body Fluids, Solutes, and Excretion Data from Control Rats and from Li-treated Rats*

	Controls	Li-treated	P value‡
Total water intake (ml/100 g b wt/24 h)	17.00±1.00§ (40)	106.00±5.00 (40)	<0.001
Intake of 0.9% saline (ml/100 g b wt/24 h)	5.6±0.8 (40)	52.1±6.2 (40)	<0.001
Urine flow (ml/100 g b wt/60 min)	0.40±0.03 (38)	2.16±0.19 (39)	<0.001
U _{osm} (mosmol/kg H ₂ O)	905.00±53.00 (38)	254.00±18.00 (39)	<0.001
Total solute excretion (U _{osm} × V) ($\mu\text{osmol}/100 \text{ g b wt}/60 \text{ min}$)	324.00±21.00 (38)	454.00±28.00 (39)	<0.001
Total urea excretion (U _{urea} × V) ($\mu\text{mol}/100 \text{ g b wt}/60 \text{ min}$)	144.00±8.00 (35)	157.00±8.00 (36)	NS

* After 3 wk of experimental regimen. Urine was collected in 3-h periods, 9 a.m.–12 a.m. For other details, see Methods. ‡ For significance of difference from controls (*t* test). § Values are mean±SEM; number of animals in parentheses.

($\Delta +46.5$ ml/100 g b wt/24 h). Li-rats showed steady but slower growth, so that at the end of the study their body weight was $\sim 20\%$ lower compared with the controls (Table III). The increased water intake (Table II) ($\Delta +524\%$) in Li-rats was accompanied by a corresponding extent of increase in the urine flow ($\Delta +440\%$). The Li-rats had markedly (~ 3 times) lower U_{osm} ; urine was hypotonic to plasma (Table II) and showed an increase in the total solute excretion ($U_{osm} \times V$) of rather small extent ($\Delta +40\%$); the total urea output ($U_{urea} \times V$) was slightly higher, but the difference did not reach statistical significance (Table II).

The urinary concentrating ability was briefly tested by subjecting several control rats and Li-rats to a dehydration test until 8% loss of the body weight (20). Under these conditions, on the third week of the experiment, the maximum U_{osm} achieved in Li rats ($U_{osm} = 679 \pm 91$ mosM; mean \pm SEM, $n = 3$) was similar as when tested after 4 wk of the Li treatment ($U_{osm} = 751 \pm 123$ mosM; mean \pm SEM, $n = 3$), which was a value far lower than achieved in the corresponding control rats ($U_{osm} = 2,177 \pm 225$ mosM; mean \pm SEM, $n = 3$). At the end of the experiments, control and Li-rats showed a similar difference in U_{osm} in the samples collected from the urinary bladder (Table III) as in previous measurements (Table II). These data show that after the third week of treatment with Li, the experimental rats were already in established steady state, as indicated by several parameters of fluid and solute metabolism (Tables II and III).

The plasma Li levels (P_{Li}) achieved in this experimental model were well below 1 mM (Table III). As indicated in previous reports (19, 20), chronically Li-treated rats show no impairment in glomerular filtration rate (GFR). This is in agreement with findings in the present study, where plasma creatinine (P_{cr}) at the end of the experiment in the Li-rats ($P_{cr} = 0.62 \pm 0.03$ mg/dl; mean \pm SEM, $n = 6$) was identical to values

in the control animals ($P_{cr} = 0.63 \pm 0.04$ mg/dl; mean \pm SEM, $n = 5$). On the other hand, the Li-rats showed a consistent significant decrease ($\Delta -21\%$) in the plasma urea (P_{urea}) levels (Table III). The renal clearance of urea was assessed only approximately; it was calculated from values of urinary excretion ($U_{urea} \times V$; Table II) and P_{urea} values (Table III) of the same animal, and expressed in milliliters per 100 g b wt/60 min. Expressed in this way, the clearance of urea in Li-rats (29.2 ± 1.9 ; mean \pm SEM, $n = 31$) was significantly ($P < 0.02$, t test) higher than in controls (21.9 ± 1.9 ; mean \pm SEM, $n = 27$).

Tissue solutes. The content of water and major solutes was analyzed in the three major zones of kidney tissue, i.e., in cortex, medulla, and papilla, at the end of the experimental period (Table IV). Control rats and Li-rats did not differ in the net weight of kidney (Table III) or in the content of water and K^+ in the three zones of kidney, with the single exception that the content of K^+ was detectably higher ($\Delta +14\%$) in the medulla of Li-rats. The control rats and Li-rats did not differ in cortical and medullary content of Na^+ ; it was only in the papilla of Li-rats that the Na^+ content was slightly lower ($\Delta -15\%$) than in controls. On the other hand, Li-rats differed from controls in urea content of all the three analyzed zones of the kidney. The most profound decline in urea content ($\Delta -74\%$) was observed in the papilla of Li-rats (Table IV). Also, the steepness of the cortico-papillary gradient of urea, expressed as percentage ($\Delta\%$) difference of solute content in the medulla or papilla from the solute content in cortex of the same kidney, taken as 100%, was markedly diminished (Table IV). The above-mentioned differences in individual solutes were reflected also in a well-pronounced decrease ($\Delta -33\%$) in total tissue solutes in papilla of Li-rats (Table IV).

The basal accumulation of cAMP in the intact tubules did not differ between MCT from control and MCT from Li-rats (Table V). On the other hand, when incubated in the presence of AVP alone, the cAMP accumulation in MCT of Li-rats was markedly diminished (Table V). The AVP-stimulated accumulation of cAMP in MCT in Li-treated rats was lower at both the maximum and submaximum stimulatory doses of AVP (Fig. 1). Forskolin (10^{-4} M) stimulated the cAMP to a greater degree than the maximum stimulatory dose (10^{-6} M) of AVP (Table V). The forskolin-stimulated cAMP accumulation in MCT, and cAMP accumulation in the presence of AVP with forskolin, added together were also markedly lower in MCT of Li-rats than in controls (Table V).

In our previous studies (28, 32), as well as in the preliminary experiments (data not shown), we established that the addition of 5×10^{-4} M MIX causes more than -90% inhibition of cAMP-PDIE, both in the MCT from the control and from the Li-rats. When incubated in the presence of 5×10^{-4} M MIX (Table V), the extent of cAMP accumulation in MCT incubated with AVP alone, or with AVP plus forskolin, was also significantly lower in Li-rats than in controls (Table V). The relative extent of differences between control and Li-rats with stimulants of cAMP generation alone ($\Delta\%$ decrease of 50–68%; Table V) was more pronounced than in experiments conducted in the presence of MIX (decrease $<40\%$; Table V). Similar differences in cAMP accumulation between the control and Li-rats were also observed when PCD were incubated with AVP alone, or to a lesser degree, with AVP plus MIX, and with forskolin alone (Table VI). Since Li accumulates in the rat kidney along the cortico-papillary gradient, reaching a concentration <7 mmol/kg H_2O in the papilla (8, 36), we examined whether the

Table III. Plasma, Urine, and Kidney Size Data from the Control Rats and Li-treated Rats at the End of the Experiment*

	Control	Li-treated	P value \ddagger
Body weight (g)	387.00 \pm 3.00§ (40)	311.00 \pm 4.00 (40)	<0.001
Net kidney weight (g)	1.42 \pm 0.03 (16)	1.36 \pm 0.05 (15)	NS
Kidney weight relative to total b wt (mg wt/100 g b wt)	377.00 \pm 4.00 (16)	435.00 \pm 13.00 (15)	<0.005
P_{Li} (mM)	—	0.70 \pm 0.04 (36)	—
P_{urea} (mM)	6.85 \pm 0.29 (32)	5.37 \pm 0.21 (33)	<0.001
P_{cr} (mg/dl)	0.63 \pm 0.04 (5)	0.62 \pm 0.03 (6)	NS
U_{osm}^{\parallel} (mosmol/kg H_2O)	896.00 \pm 85.00 (20)	340.00 \pm 37.00 (19)	<0.001
Urine concentration of Li^{\parallel} (mM)	—	12.70 \pm 1.7 (29)	—

* Fourth week.

§ Denotes mean \pm SE; number of rats in parentheses.

‡ For significance of differences (t test).

\parallel From bladder urine, taken under anesthesia (see Methods).

Table IV. Content of Water and Solutes in Kidney Tissue of Control Rats and Li-treated Rats*

	Control	Li-treated	P value‡		Control	Li-treated	P value‡
Water content (% of tissue wet weight)				K⁺ content (meq/kg H ₂ O)			
Cortex	75.9±0.1§	76.0±0.4	NS	Cortex	112.6±2.9	110.4±2.9	NS
Medulla	80.3±0.4	80.4±0.4	NS	Medulla	89.3±3.2	101.6±3.1	<0.01
Papilla	84.5±0.5	85.0±0.5	NS	Papilla	76.1±5.0	71.1±4.1	NS
Na⁺ content (meq/kg H ₂ O)				Urea content (mmol/kg H ₂ O)			
Cortex	76.4±2.7	76.9±2.6	NS	Cortex	27.7±1.4	15.9±1.5	<0.001
Medulla	96.8±3.2	99.0±5.6	NS	Medulla	68.0±5.4	24.3±2.2	<0.001
Δ	+27±4%	+27±7%	NS	Δ	+151±21%	+59±10%	<0.001
Papilla	177.0±6.5	150.4±9.8	<0.05	Papilla	256.0±36.7	65.5±10.1	<0.001
Δ	+132±8%	+94±11%	<0.02	Δ	+833±145%	+302±36%	<0.005
Total solutes¶ (mosmol/kg H ₂ O)							
Cortex	407.3±10.4	392.6±7.5	NS				
Medulla	441.7±13.9	427.1±15.3	NS				
Δ	+9±3%	+8±3%	NS				
Papilla	764.2±45.6	515.2±25.7	<0.001				
Δ	+88±12%	+31±5%	<0.001				

* For experimental details, see the Methods section. ‡ For significance of difference between values from control and Li-treated rats (*t* test). § Each value denotes mean±SE of 11 paired experiments. || Δ denotes relative (percentage) increase of solute content compared with cortex of the same kidney. Each percentage value is a statistically significant increase (*P* < 0.025 or higher degree of significance; paired *t* test). ¶ Total solutes is a calculated value according to formula 2(Na⁺ + K⁺) + urea.

presence of LiCl in the incubation medium may further influence the extent of cAMP accumulation in PCD. Incubation of PCD from Li-rats with added AVP, AVP plus MIX, or with forskolin was not further diminished in the presence of 10 mM LiCl added in the incubation mixture (data not shown).

In the next series of experiments, we examined several major components of cAMP metabolism in MCT from control and from Li-treated animals (Table VII). The basal activity of AdC, and AdC activity stimulated by 10⁻⁶ M AVP, by 10⁻⁴ M forskolin, or by 10⁻² M NaF, were all significantly lower in MCT from kidneys of Li-rats (Table VII A). No significant

Table V. Accumulation of cAMP in MCT Microdissected from Control Rats and Li-treated Rats*

Condition	N	Control	Li-treated	P value‡	Δ%§
Basal (no additions)	6	13.4±1.2 (20)	17.2±2.1 (19)	NS	+38.0±38.0
AVP (10 ⁻⁶ M)	6	542.0±60.0 (37)	141.0±15.0 (42)	<0.001	-68.1±8.2¶
Forskolin (10 ⁻⁴ M)	3	4,796.0±465.0 (16)	1,834.0±253 (17)	<0.001	-63.3±1.4¶
AVP (10 ⁻⁶ M) + forskolin (10 ⁻⁴ M)	5	6,190.0±494.0 (26)	2,573.0±252 (27)	<0.001	-50.6±12.7¶
AVP (10 ⁻⁶ M) + MIX (5 × 10 ⁻⁴ M)	9	1,259.0±74.0 (53)	780.0±64.0 (52)	<0.001	-37.8±7.5¶
AVP (10 ⁻⁶ M) + MIX (5 × 10 ⁻⁴ M) + forskolin (10 ⁻⁴ M)	5	9,077.0±633.0 (24)	5,132.0±300.0 (24)	<0.01	-35.2±13.9

* Content of cAMP is expressed in femtomoles per micrograms protein. N denotes number of paired experiments. Incubation time was 10 min at 30°C. For further details, see Methods. ‡ For significance of difference from corresponding controls (*t* test). § Relative difference (percentage, mean±SEM) from controls evaluated on paired basis. || Denotes mean±SEM; total number of samples from all experiments in parentheses. ¶ Statistically significant percentage difference (*P* < 0.02 or high degree of significance, paired *t* test).

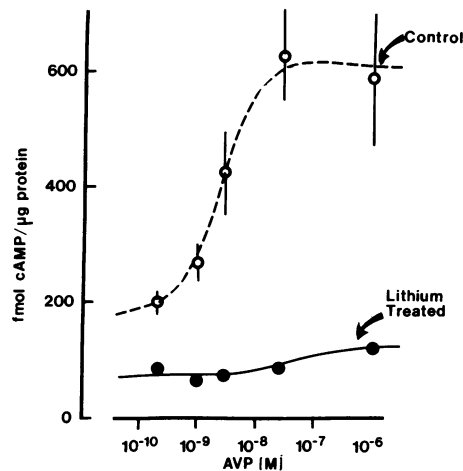


Figure 1. AVP-dependent accumulation of cAMP in MCT microdissected from control rats (○) and rats treated chronically with Li (●). Samples of MCT were incubated for 10 min at 30°C as detailed in the Methods section. Abscissa: concentration of AVP [M]. Ordinate: content of cAMP (femtomoles per micrograms protein). Each point denotes mean±SEM of 4–6 samples.

difference was observed between activity of cAMP-PDIE in MCT from the control and from Li-rats (Table VII B). Likewise, the content of ATP in MCT, incubated in vitro under the identical conditions, was not significantly different between tubules from Li-rats and control rats (Table VII C). The addition of 15 mM LiCl into the incubation mixture had little inhibitory effect (~10%) on AVP-stimulated AdC activity either in MCT from the control rats or in MCT from Li-rats (data not shown). The activities of AdC and of cAMP-PDIE were concisely examined also in MAL from the control and Li-rats. Both the basal and AVP-stimulated activities of AdC were decreased in MAL of Li-rats, while the activity of cAMP-PDIE did not differ from controls (Table VIII).

The rate of $^{14}\text{CO}_2$ production from 1,4- ^{14}C succinate was not significantly different between MAL from Li-rats and MAL from controls (Fig. 2). The addition of 10^{-3} M furosemide inhibited the $^{14}\text{CO}_2$ production to a similar degree in MAL from the control rats (–83%) and in MAL from Li-rats (–81%) (data not shown). In control rats, measured under the same incubation conditions, the rate of $^{14}\text{CO}_2$ production from 1,4-

^{14}C succinate by MCT was ~4 times lower than in MAL (Fig. 2). In MCT from Li-rats, the rate of 1,4- ^{14}C succinate oxidation to $^{14}\text{CO}_2$ was significantly ($\Delta +43\pm 6\%$, mean±SEM; $P < 0.025$, paired *t* test) higher than in MCT of the control rats (Fig. 2).

Cyclic AMP accumulation in various segments of collecting tubule system, MCT, PCD, and CCT microdissected from chronically polyuric HHDI rats were compared with their corresponding controls (Table IX). In contrast to Li-polyuric rats, the AVP-stimulated cAMP accumulation was not diminished in collecting tubules of polyuric HHDI Brattleboro rats (Table IX).

Discussion

A chronic administration of Li by oral route and with adequate supply of NaCl prevents intermittent increases in plasma Li, the Li-induced Na depletion, and keeps plasma Li in a concentration range similar to therapeutic levels in man (18–22). This polyuric syndrome with NDI in rats has characteristics functionally similar to an analogous renal syndrome caused by therapy with Li salts in human subjects (1, 2, 5), including chronicity and P_{Li} less than or equal to 1 mM (1, 5). Since the past studies investigated the pathogenic role of Li mainly by studying the acute Li infusion in vivo, addition of Li in vitro, or the effects of Li were examined usually only after a short (circa 1 wk) and/or parenteral treatment with Li (2, 10, 39), analysis of the chronic model in rat (18–20) reflects more closely the NDI syndrome in clinical treatment with Li salts.

Our present study points to several key abnormalities of AVP-regulated water excretion in this model of iatrogenic NDI. In contrast to results of our previous study (39), which analyzed effects of subacute (1 wk) exposure to parenterally administered Li, the MCT from chronically Li-rats had wider diameter, and higher kidney weight, consistent with the results of recent morphologic studies of the same model (19, 22).

Evidently, the AVP-dependent cAMP metabolism is severely blunted in MCT and in PCD of Li-rats (Tables V–VIII, Fig. 1). Lower accumulation of cAMP also in the presence of the nonhormonal stimulant forskolin (40) suggests that the observed abnormality in cAMP metabolism is not limited to the AVP-sensitive cAMP system of MCT. Analogous findings of blunted cAMP metabolism in PCD indicates that this defect is not confined to the medullary portion of collecting tubules (Table

Table VI. Accumulation of cAMP in PCD from Control Rats and Rats Chronically Treated with Li*

Condition	N	Controls	Li-treated	P value‡	Δ%§
AVP (10^{-6} M)	3	722±77 (18)	250±23 (18)	<0.001	63±7 [¶]
AVP (10^{-6} M) + MIX (5×10^{-4} M)	3	1,406±134 (18)	946±71 (18)	<0.01	23±7
Forskolin (10^{-4} M)	3	2,595±276 (18)	1,877±293 (16)	NS	32±13

* Content of cAMP is expressed in femtomoles per micrograms of protein. PCD were incubated at 30°C for 10 min. N denotes number of paired experiments. For further details, see Methods. ‡ For significance of difference from corresponding controls (*t* test). § Relative difference (percentage, mean±SEM) from control evaluated on paired basis. ^{||} Denotes mean±SEM; total number of samples in parentheses. [¶] Percentage change statistically significant (paired *t* test).

Table VII. Activity of cAMP-PDIE, and Content of ATP, in MCT from Control and Li-treated Rats*

	N	Controls	Li-treated	P value‡	Δ%§
AdC activity (pmol/30 min per μg protein)					
Basal activity	6	0.91±0.054 (30)	0.53±0.024 (34)	<0.001	-39.3±5.5 [¶]
AVP (10 ⁻⁶ M)	6	13.49±0.65 (34)	7.79±0.37 (33)	<0.001	-39.8±4.2 [¶]
NaF (10 ⁻² M)	4	13.61±0.89 (20)	9.58±0.62 (19)	<0.001	-28.8±6.9 [¶]
Forskolin (10 ⁻⁴ M)	3	21.01±1.22 (15)	14.89±0.55 (14)	<0.001	-28.0±3.4 [¶]
cAMP PDIE (fmol/min/μg protein)	7	635.00±27.00 (40)	607.00±32.00 (39)	NS	-3.7±7.6
Content of ATP (pmol/μg protein)	5	12.03±0.70 (43)	10.74±0.48 (41)	NS	-2.66±17.3

* AdC activity is expressed in picomoles cAMP/30 min per μg protein; cAMP-PDIE in femtomoles of cAMP hydrolyzed per min, per microgram protein; and ATP in picomoles per microgram protein. N denotes number of paired experiments. For further details, see Methods. ‡ For significance of difference between Li-treated and controls (*t* test). § Relative (percentage) difference from paired controls. ^{||} Denotes mean±SEM; total number of samples in parentheses. [¶] Difference significant (*P* < 0.05 or higher degree of significance; paired *t* test).

VI). Analysis of major components of cAMP metabolism (Table VII) indicates that the impairment of AdC activity is a key factor that accounts for low capacity to generate and accumulate cAMP in MCT from Li-rats. The limited supply of substrate ATP or an excessive cAMP breakdown apparently does not play a role. A finding of decreased AVP-dependent cAMP accumulation in MCT or PCD with MIX present (Tables V and VI) also argues against anomalously high cAMP catabolism.

The finding that AdC activity was decreased with or

without stimulation by both hormonal and nonhormonal agents suggests that chronic exposure to Li impairs the AdC complex in MCT at the catalytic subunit and/or guanyl nucleotide coupling protein (40, 41). The decrease in the activity of catalytic subunit alone may explain the pattern of changes in AdC activity observed in MCT from Li-rats (Table VII). While forskolin acts primarily to stimulate the catalytic subunit of AdC (40), the other components are apparently required for its action (41). Therefore, more than one component of the AdC complex might have been affected by chronic exposure to Li. The observation that cAMP accumu-

Table VIII. Activities of AdC and cAMP-PDIE in MAL from the Control and Li-treated Rats*

	Controls	Li-treated	P value‡
AdC activity (pmol/30 min/μg protein)			
Basal	0.15±0.012 [§] (12)	0.096±0.011 (12)	<0.005
AVP (10 ⁻⁶ M)	6.77±0.380 (10)	2.450±0.30 (12)	<0.001
cAMP-PDIE activity (fmol/min/μg protein)	569.00±68.00 (24)	523.00±65.00 (24)	NS

* For details, see Methods.

‡ For significance of difference from controls (*t* test).

§ Denotes mean±SEM; total number of samples in parentheses (for AdC from two experiments, for cAMP-PDIE from three experiments).

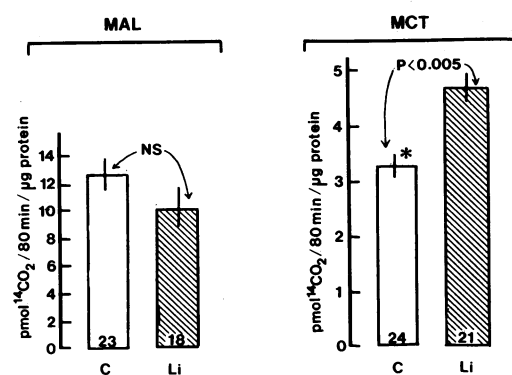


Figure 2. The rate of ¹⁴CO₂ production from 1,4-[¹⁴C]succinate in MAL (left) and in MCT (right) microdissected from control rats (□) and rats chronically treated with Li (▨). Each bar denotes mean±SEM of total number of samples, denoted at the bottom of the bars, from three experiments. P value is for difference from corresponding control (*t* test); * denotes value significantly (*P* < 0.005) different from controls of MAL (*t* test). For further details, see text of the Methods and Results sections.

Table IX. Accumulation of cAMP and Outer Tubular Diameter in CCT, MCT, and in PCD from Homozygote Brattleboro Rats with HHDI and from Control Rats (Normal Rats, Long-Evans strain)*

Tubular segment	cAMP content		Outer tubular diameter	
	Control rats	HHDI rats	Control rats	HHDI rats
			μm	μm
CCT	33.5 \pm 3.5‡ (12)	32.5 \pm 1.5 (12)	32.8 \pm 1.3 (13)	34.6 \pm 1.4 (11)
MCT	30.9 \pm 2.4 (12)	38.6 \pm 2.4§ (12)	36.7 \pm 1.2 (9)	39.8 \pm 1.4 (12)
PCD	41.2 \pm 4.7 (12)	56.6 \pm 9.6 (11)	46.8 \pm 3.1 (12)	46.8 \pm 1.9 (9)

* Samples of tubules were incubated either in isotonic (300 mM) medium (CCT), or hypertonic (600 mM) medium (MCT, PCD) for 10 min at 30°C with 10^{-6} M AVP and 5×10^{-6} M MIX. Since tubular diameter between control and HHDI did not differ, the cAMP content was expressed per millimeter of tubular length (23, 28, 29). Content of cAMP is expressed in femtomoles cAMP per millimeters per 10 min; tubular diameter is expressed in micrometers. For further details, see Methods.

‡ Denotes mean \pm SEM; total number of samples (from two paired experiments) in parentheses.

§ Significantly different ($P < 0.05$; t test) from controls.

lation in MCT is blunted when stimulated both by submaximal and maximal doses of AVP (Fig. 1) is also consistent with impairment of AdC components distal to the V_2 receptor; V_2 receptor is typically associated with AdC in cells of collecting tubules and ascending Henle's loop (15). While it cannot be excluded that AVP binding on the V_2 receptor is also impaired, taken together, the present results suggest that observed chronic Li effects are not due simply to decreased affinity of V_2 receptors to AVP. Future detailed analysis of V_2 receptor-AdC complex ought to clarify the exact molecular mechanism of impairment caused by chronic exposure to Li. The question may arise whether decreased capacity of MCT and PCD to generate cAMP in response to AVP detected in vitro can account for the functional resistance of collecting tubules to AVP in vivo (6, 13). Although conclusions should be guarded, analogy from other models of NDI suggests that indeed the deficient cAMP accumulation, as detected in vitro, corresponds to functional refractoriness to AVP in vivo (3, 6, 13, 24, 25).

Our previous study suggested that with shorter exposure to Li, the AVP-sensitive AdC is decreased only in MCT (39). However, after a more chronic course of Li administration, the impairment of AdC activity in MAL (Table VIII) was analogous to that in MCT (Table VII), which indicated that length of Li treatment and perhaps also the route of Li administration (17, 22) can determine the pattern of impairment. Lowering the AVP-sensitive AdC in both medullary segments, MCT and MAL can explain that the decreased AVP-sensitive AdC has been observed after chronic Li treatment in the past studies even in a mixed membrane preparation from the whole undivided medullary tissue (10, 11).

The observed defects in collecting tubules (Tables I, V–VII) are most likely the result from the chronic exposure of cells to Li, rather than due to the immediate presence of Li ions in body fluids. At $P_{Li} < 1$ mM the tissue content of Li in bladder urine (Table III) or in papilla (8, 36), even when

increased by AVP (37), did not exceed <13 mM. In the previous studies (7, 39), as well as in the present study, Li added in vitro at concentrations $\cong 10$ mM did not influence the cAMP accumulation. Therefore, while the presence of Li ions might contribute to decreased tubular response to AVP in vivo, as suggested by microperfusion studies of Cogan and Abramow (6, 13), this factor is expected to be a quantitatively minor one.

Decreased AVP-dependent cAMP accumulation and AdC activity seen in Li-rats is not likely the consequence of chronic polyuria per se. First, the AVP-sensitive AdC activity in MCT from HHDI Brattleboro rats with chronic polyuria does not differ from the controls (42). Second, results of our own control experiments on polyuric HHDI Brattleboro rats, not treated by Li, strongly indicate that polyuric state per se does not lead to blunting of AVP-dependent cAMP accumulation MCT, PCD, or CCT (Table IX), as does the polyuria in Li rats (Tables V and VI). Moreover, the previous studies examining the renal concentrating ability have shown that while Li-polyuric rats failed to concentrate urine after standard dehydration (until 7% drop in b wt), the control rats with polyuria induced by drinking of glucose water showed a normal concentration in response to the same stimulus (43).

Cortico-papillary gradient of solutes, another essential prerequisite for the antidiuretic effect of AVP, has not yet been, to our knowledge, completely analyzed in NDI caused by chronic oral administration of Li. Our finding of decreased tonicity of papillary tissue (Table IV) suggests that the decreased tubule-to-interstitium osmotic gradient in Li-rats can contribute to diminished water reabsorption in the papilla, even if the AVP-controlled water permeability of tubular epithelium would be partially preserved. The lower papillary osmolality may also influence the AVP-dependent cAMP accumulation (3, 15, 29). In PCD, the decreased osmolality in the medium diminishes markedly the AVP-dependent cAMP accumulation (29). Consequently, in Li-rats with much lower papillary osmolality (Table IV), the AVP-dependent cAMP accumulation in PCD may be further decreased in situ and the differences from the controls may be even more pronounced than suggested by comparison in vitro under the same test conditions (Tables V and VI).

The diminished papillary tonicity is due almost completely to severely decreased papillary content and low cortico-papillary gradient of urea in the kidneys of Li-rats (Table IV). The mechanism by which this renal depletion of urea develops in Li-rats should be briefly considered. Water diuresis in conscious rats was shown to lower P_{urea} and the cortico-papillary gradient of urea (44, 45); urea content in the renal cortex is also lower in HHDI rats with chronic water diuresis (37). Based on other reports (19, 20) and our survey of P_{cr} , chronic treatment with oral Li apparently does not diminish GFR. Consequently, because of low P_{urea} in Li-rats (Table IV), the filtered load of urea entering renal countercurrent concentrating system (46) is low in the Li-rats, and this factor alone may account for the decreased cortico-papillary gradient of this solute. Possibly, increased renal clearance of urea in Li-rats may result in depletion of urea from plasma and other body fluids.

The lumen-to-interstitium transport of NaCl in thick ascending limb of Henle's loop, namely in MAL, is a major factor in build-up and maintenance of medullary and papillary hypertonicity (46). The finding that the rate of 1,4- $[^{14}\text{C}]$ succinate oxidation, linked to NaCl cotransport, was not altered in MAL

of Li-rats (Fig. 2) suggests, in an indirect way (23, 34), that NaCl reabsorption in MAL is not impaired. This interpretation is compatible with unchanged Na⁺ content in the medullary zone of Li-rats (Table IV). The relatively small drop of Na⁺ content in papilla of Li-rats (Table IV) could be due to increased tubular fluid flow and lesser reabsorption of Na⁺ in MCT and PCD. The markedly lower rate of 1,4-[¹⁴C]succinate oxidation in MCT compared with MAL (Fig. 2) most likely reflects lower activity of SDH in MCT than in MAL (21). Unlike in MAL, the ¹⁴CO₂ production in MCT from Li-rats was increased (Fig. 2). While the functional significance of this finding is not yet apparent, the observation is consistent with the histochemical finding of increased SDH in MCT in Li-rats by Jacobsen et al. (21).

In conclusion, the results of the present study point to two major factors that may be the underlying cause of the NDI syndrome consequent to chronic oral administration of Li. As far as the *in vitro* studies could be extrapolated to *in vivo* situations, our present results suggest that diminished hydro-osmotic response of collecting tubules to AVP is due to a decreased AVP-dependent accumulation of cAMP in MCT and PCD, which is similar to some other nephrogenic concentrating defects (3), e.g., hereditary NDI in mice (24) and concentrating defect in adrenal insufficiency (25). However, in contrast to these two other syndromes (24, 25), the decreased AVP-stimulated cAMP accumulation in Li-induced NDI is primarily due to impairment of AVP-dependent cAMP generation rather than to anomalously high cAMP catabolism (24, 25). The defect in AVP-sensitive AdC in MCT and PCD seems to be a consequence of chronic exposure to Li (10, 11), rather than due to chronic polyuria or direct interaction with Li ions (7, 9). It is not excluded that additional defects exist also in the cellular steps that are distal to the cAMP generation, as has been surmised on some past studies (2, 8, 7, 14). The decrease in papillary tonicity, the urea depletion and low cortico-papillary gradient in urea in the kidneys of rats treated chronically with Li, is another substantive factor that can contribute to a blunted antidiuretic response to AVP (1, 2, 19, 20, 43). Also, possible role of prostaglandins and/or AVP-inhibiting putative circulating factor (47) in this disorder remains to be explored.

Acknowledgments

Mrs. Julie L. Werness and Ms. Martha J. Keller provided expert technical assistance, and Ms. Jacquelyn Trefz and Mrs. Carolyn Blankenship provided excellent secretarial assistance.

This study was supported by National Institutes of Health grant AM-16105 and by the Mayo Foundation.

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