A Micropuncture Study of the Renal Handling of Lithium

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Abstract. Although clearance studies in man and experimental animals indicate that filtered lithium is reabsorbed primarily in the proximal tubule, it is unclear whether lithium is also reabsorbed in distal portions of the nephron. Micropuncture studies were, therefore, performed to determine the nephron sites involved in lithium transport during free flow. A method was established to estimate the concentration of lithium in nanoliter samples, using the Helium Glow photometer, which permitted the accurate measurement of lithium in tubular fluid samples over a range from 0.5 – 30.0 mM.

Approximately 56 % of filtered lithium and tubular fluid was reabsorbed at the end of the proximal convolution, while at the early distal tubule 75 % of filtered lithium and water was reabsorbed. There was no change in net transepithelial movement of lithium beyond the loop of Henle.

These data suggest that lithium transport is localized to the proximal tubule, including the pars recta. Lithium reabsorption does not occur in distal tubule or collecting duct. Beyond the early distal tubule net movement of lithium and sodium is dissociated.

Key words: Lithium transport — Micropuncture analysis.

Introduction

Although lithium is not a major endogenous substance, recent interest in the renal handling of lithium and its effect on transport processes of the renal tubule has been generated by its use in clinical medicine to treat neuropsychiatric disorders. Since lithium has a low therapeutic index and serum lithium levels are largely influenced by the rate of renal lithium clearance, an understanding of the tubular sites of reabsorption of this substance is important in the prevention and treatment of lithium toxicity.

Clearance studies in man and experimental animals have shown that 70 – 80 % of the filtered load of lithium is reabsorbed predominantly in the proximal tubule [18, 19]. It is unclear, however, whether a small fraction of the filtered load is also reabsorbed in more distal portions of the nephron. For example, although clearance experiments have attempted to evaluate the handling of lithium in the distal nephron by using pharmacologic agents and physiological perturbations which are thought to influence sodium transport in specific nephron segments, the results, as recently reviewed by Thomsen, have been contradictory [17]. Analysis of this question, therefore, necessitates a direct study of fractional transport rates in tubular segments at the level of the individual nephrons.

To evaluate the transport sites for lithium reabsorption along the nephron, a method was developed to estimate its concentration in samples of tubular fluid and micropuncture studies were performed in normal, non-diuretic rats. The results of this study directly demonstrate that approximately 75 % of filtered lithium is reabsorbed in the proximal tubule and loop of Henle and that there is no evidence for further reabsorption at more distal nephron sites.

Methods

Male Sprague-Dawley rats, weighing 200 – 300 g, were used in all experiments and were fed regular Purina Chow and allowed to drink tap water ad lib until the time of study. Following induction of anesthesia with Inactin (Promonta, Hamburg, Germany) in a dose of 80 – 100 mg per kg body weight, a tracheostomy was performed, two polyethylene catheters (PE 50) were placed into jugular veins for the administration of fluid and a catheter was placed into the urinary bladder for collection of urine. Following surgery 0.15 M NaCl, equal in volume to 1 % of body weight, was infused intravenously to replace surgical losses of body fluid. Animals were prepared for micropuncture as previously described in our laboratory [3].
After administering a priming dose of 100 μg of methoxy-inulin-H₂ in 0.5 ml of 0.3 M LiCl, a sustaining infusion was administered to deliver 100 μg inulin-H₂ per hour in a volume of 1.2 ml of 0.3 M LiCl. Previous studies in our laboratory have shown that that priming and sustaining dose of LiCl resulted in relatively constant plasma levels of lithium of 2 – 4 mEq per liter [2]. Following a 45 min equilibration period, three 30 min urine collection periods were performed to determine the clearance of H₂-inulin and the urinary excretion of lithium and sodium. A blood sample was obtained from the tail at the mid-point of each urine collection to determine plasma H₂-inulin activity.

During clearance periods timed collections of tubular fluid were obtained from end proximal and early distal tubular sites, using sharpened glass capillary micropipettes. The selection of puncture sites was made on the basis of the transit of 2% FD & C green dye (Keystone Afinline and Chemical Co.). The terminal portion of the last accessible portion of the proximal tubule was determined during the rapid transit of dye. Early sites in the distal tubule were identified by the ratio of dye transit to the tubular segment selected for study to the dye transit to the earliest distal segment to fill with dye, after intravenous injection. A ratio of less than 1.3 was taken to identify the early portion of the distal tubule [21]. Dye was allowed to completely clear from tubular fluid before punctures were performed. Tubular fluid collections were analyzed for the concentration of lithium and sodium. A blood sample was obtained from the tail at the end of each urine collection period. Three 30 min urine collection periods were performed to determine the plasma concentration of lithium and H₂-inulin activity.

Determination of lithium in samples of nanoliter size was performed on a Helium Glow Photometer (American Instrument Co., Inc.). Lithium emission at 6708 Å was obtained with an interference filter (Baird-Atomic Inc., Bedford, Mass.). Characteristics of the filter provided by manufacturer included the following: 90 Å band width at 1/8 Å peak transmission; peak transmission 54% at 1/8 Å band width. Emission was recorded by photomultiplier tube R446, supplied by American Instrument Co. for measurement of potassium. Standard solutions were made from reagent grade LiCl dissolved in demineralized water or 0.15 M NaCl. The concentration range of lithium tested was 0.5 to 30 mM. With this system the sensitivity was found to be approximately 5 x 10⁻¹² Moles and the precision was reflected by the determination of a standard containing 2.50 mEq/l of lithium. The mean ± SD of 18 samples was 2.51 ± 0.07. Samples were diluted 1:4 in 5 mM CsNO₃ and 5 mM NH₄PO₄ and the sample size measured by the photometer was 4 – 5 nl. The emission profile is shown in Fig. 1.

To determine the accuracy of the micro method in the measurement of lithium recovery studies were performed in which lithium concentration was estimated in solutions containing varying concentrations of LiCl dissolved in Ringer's solution before and after the addition of a sample, to the wire of the photometer, containing LiCl dissolved in water. The results of that study are shown in Fig. 2 and indicate that the recovery of the added lithium was 103 ± 1%. Similar experiments were performed in which LiCl, dissolved in water, was added to samples of distal tubule fluid obtained from animals infused with LiCl. Recovery was 100 ± 1%. These data indicate that the constituents of tubular fluid do not interfere with the accurate estimation of lithium by the microanalytical method. Since changes in the concentration of other cations within tubular fluid may have influenced the emission of lithium, the effect of variations in the concentration of NaCl and KCl on the estimation of lithium concentration was examined. In this experiment solutions with the same concentration of LiCl were prepared in which the concentration of NaCl varied between 90 – 150 mM and KCl varied between 5 – 50 mM, to simulate variations in Na and K that could be expected to occur in proximal and distal fluid. Neither the presence nor changes in concentration of NaCl influenced the emission readings of lithium, compared to values obtained from a solution containing the same concentration of LiCl dissolved in water.

Fig. 1. Lithium emission profile. LiCl, 6 mMoles, dissolved in 0.15 M NaCl. The period of integration is shown by the time square well.

Data relating to lithium standards to demonstrate quantitative recovery.

The concentration of lithium in tubular fluid samples were determined by the Helium Glow photometer from the average value of quadruplicate determinations. Since the photometer was more sensitive for sodium [2] than for lithium, sodium concentration was not estimated in tubular fluid samples. Estimation of sodium would have required a higher dilution than used for measurement of lithium. Lithium concentration in plasma and urine was determined by a Perkin-Elmer Mass Spectrometer and sodium in plasma and urine by a flame photometer with an internal standard. The activity of H₂-inulin (New England Nuclear) was determined with a liquid scintillation counter (Packard Instruments).

Results are presented as mean ± SE and the Student's t-test was used for statistical comparison.

Results

Studies were performed in 14 animals during the infusion of lithium, under non-diuretic conditions. Plasma lithium levels averaged 3.90 ± 0.27 mEq per liter. The glomerular filtration rate averaged 442.3 ± 58.8 μl/min/100 g BW per kidney. Data relating to