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A Study on Na^+ and Water Reabsorption in the Nephron Segment Beyond Proximal Tubule Measured by Lithium Clearance

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= ABSTRACT =

During the past few years it has been proposed that lithium clearance can be used as a reliable measure for the outflow of tubular fluid from the proximal tubule. This study was aimed to characterize the inflow dependent reabsorption of Na in renal tubule beyond the proximal tubule. For this purpose, lithium clearance was used as a measure for the inflow from the proximal tubule and the changes in reabsorption fraction of Na and water were determined in rabbits.

Rabbits were pretreated with hypotonic saline solutions for an hour (50 mM/L NaCl, 20 ml/hr/kg). And then a hypertonic solution of 500 mM/L NaCl (20 ml/kg) was administered intraperitoneally in conjunction with a bolus of LiCl solution (2 mM/kg, i.v.) for conditioning the C_{Li} and urine flow rate. To rule out the effect of Li^+ on tubular functions, a bolus of NaCl solution (2 mM/kg, i.v.) was administered.

Fifteen, thirty, and sixty minutes after injection of hypertonic saline arterial blood and urine samples were taken. Urinary and plasma concentrations as well as urinary output of Li^+, Na^+ and K^+ were measured. From these C_{Li}, C_{Na} and the reabsorption fraction of Na and water (Fr_{Na} & FrH_2O) were calculated. These results were compared with those from control groups in which the same amount of isotonic saline (145 mM/L NaCl) and of 15% dextran solution were administered in the same way as that in experimental group.

Followings are the results obtained.

1) The plasma concentration of Na^+ in rabbits injected with hypertonic saline reached the peak value after 15 min and thereafter no significant change was observed. Hematocrit values did not show any change, while urinary excretion of Na^+ increased markedly during the first 15 min and decreased thereafter. These results were not affected by an injection of a small amount of LiCl.

2) The clearances of Li^+, Na^+ and K^+ in rabbits injected with hypertonic saline and LiCl solution decreased.

3) In spite of the variation in C_{Li}, Fr_{Na} did not show any significant change while FrH_2O increased gradually.

4) C_{Li} decreased also in rabbits received isotonic saline. Fr_{Na} tended to be higher than that in hypertonic saline group, while FrH_2O and Fr_{Na} did not associated with the decrease in C_{Li}.

5) C_{Li} of the rabbits received dextran solution fluctuated persistently and Fr_{Na} and FrH_2O did not change in along with C_{Li}, although Fr_{Na} had a tendency to be higher than that in hypertonic saline group.

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6) From the above results it was concluded that:

(a) In rabbits with normal body store of Na^+, the Fr_{Na} of renal tubule beyond proximal tubule, calculated from C_{Li} as a measure of inflow from proximal tubule is constant in spite of variations in C_{Li}.

(b) The FrH_2O calculated from C_{Li} is dependent largely upon ADH rather than inflow from proximal tubule.

(c) When there is a decrease in plasma Na^+ concentration or ineffective body fluid, Li^+ reabsorption may occur in the thick segment of Henle's loop and hence the determination of Fr_{Na} and FrH_2O will not be easy one, but Fr_{Na} is constant under the same experimental conditions.

Key Words: Lithium clearance, Sodium and water reabsorption fraction, Nephron segment beyond proximal tubule.

INTRODUCTION

Approximately 50-60% of filtered lithium is reabsorbed in proximal convolution and at the end of pars recta 70-80% of filtered lithium is reabsored. Urinary excretion rate of Li^+ is about 20-30%, so total reabsorption of Li^+ is similar with that of Na^+ in proximal tubule (Hayslett & Karshgarian, 1979).

FE_{Li} (*fractional excretion of lithium*) was increased when proximal reabsorption of Na^+ was blocked by osmotic diuresis, acetazolamide, sodium bicarbonate. But FE_{Li} was not changed when the reabsorption of Na^+ in ascending limb of Henle or in distal tubule was blocked by furosemide, ethacrynic acid, and spironolactone (Steel et al, 1975).

The rate of fractional reabsorption of Li^+ (Fr_{Li}) was similar to the rate of fractional reabsorption of Na^+ in proximal tubule but beyond the ascending limb of the loop of Henle lithium reabsorption does not occur (Hayslett & Kashgarian, 1979).

Thomson (1981) carried out a direct comparison among the three methods for quantitative determination of the fractional delivery of fluid from the proximal tubules to the loop of Henle: the lithium excretion method, the transit time/occlusion time method, and the micropuncture method. It was reported that lithium clearance (C_{Li}) can be used as a quantitative measure of the delivery of tubular fluid to the loop of Henle (V_{prox}).

Navar & Schafer (1987) argued that Li^+ cannot be substituted for Na^+ in furosemide sensitive coupled electroneutral (1 Na^+, 1 K^+, 2 Cl^-) transport mechanism in thick ascending limb of Henle (TALH) cell but Li^+ is transported about 70% of Na^+ in cation movement mechanism through cleft between epithelial cell (Herbert & Andreoli, 1984).

C_{Li} is not a quantitative measure of the delivery of tubular fluid to Henle's loop because under these circumstances Li^+ is also reabsorbed to some in TALH with low salt diet (Thomsen & Leyssac, 1986).

But Li^+ reabsorption beyond the proximal tubule was reported with in vitro study in which 50% of tubular perfusate of Na^+ was replaced by Li^+ (Greger, 1981), and in vivo study, with low Na^+diet or tubular Li^+ concentration is above 5 mEq/L (Webb et al, 1975; Martinez-Maldonado & Opava-Stitzer, 1977; Thomsen & Leyssac, 1986).

But Li^+ reabsorption beyond the proximal tubule does not occur in therapeutic range of Li^+ concentration or with normal Na^+ diet (Steel et al, 1975; Thomsen et al, 1981).

So C_{Li} can be used as a valid measure of V_{prox} as C_{in} reflects the GFR (Thomsen & Leyssac, 1986; Thomsen & Schon, 1986).

Proximal tubular reabsorption of filtered water occurs according to the Na^+ concentration gradient produced by Na^+ reabsorption due to glomerulo-tubular balance. But

beyond the loop of Henle, reabsorption of Na and water is dissociated.

Body fluid volume is controlled by entire tubule system. But osmolar concentration is controlled by distal nephron beyond the loop of Henle.

Na^+ reabsorption in TALH and distal tubule is increased by ADH, PTH, CT, glucagon and aldosterone, but it is decreased by PG. Water reabsorption is influenced greatly by ADH & PG (Culpepper & Andreoli, 1983).

The study of the reabsorption of Na^+ and water has been done with in vitro model using single nephron or medullary fragment. But studies conducted in vivo, it is very difficult to separate proximal and distal tubular function, so we can only approximate the function of proximal and distal tubule in consideration of several factors. High concentration of Li^+ suppresses tubular reabsorption of Na^+ and water, but in low concentration of Li^+ which do not affect renal function, C_{Li} can be used as a valid measure of Vprox (Thomsen et al, 1981; Thomsen & Leyssac, 1986).

As proximal tubular water reabsorption rate is determined by C_{Li} and C_{in} (Thomsen et al, 1981; Thomsen & Leyssac, 1986), the reabsorption of Na^+ and water beyond proximal tubule is determined by in vivo study in narrow range using C_{Li}, urine excretion rate, and Na^+ excretion rate.

To evaluate the change of Fr_{Na} and FrH_2O beyond proximal tubule according to C_{Li} change, we have measured Fr_{Na} and FrH_2O after infusion of large amounts of hypertonic saline intraperitoneally in rabbits pretreated with hypotonic saline which restricts ADH action.

We also have measured Fr_{Na}, FrH_2O, C_{Li} in other rabbits after infusion of isotonic saline or dextran solution intraperitoneally, which affect osmotic concentration or effective circulating blood volume.

From these results we interpreted the change of Fr_{Na} and FrH_2O according to C_{Li} change and we discussed the application of C_{Li}.

METHODS

Animal preparation

Adult rabbits were used in all experiments. The rabbits were restrained and then anesthetized with nembutal by ear vein in a dose of 30 mg per kg body weight. After anesthesia, heparin (100 IU/kg) was injected intravenously for anticoagulation treatment. Polyethylene catheters were placed into the jugular vein for the administration of fluid and the carotid artery for blood pressure monitoring via a pressure transducer and for arterial blood sampling. Catheter was placed into the uninary bladder for collection of urine samples.

Animals were pretreated with hypotonic saline (50 mM/L) for 1 hr at 20 ml/hr/kg. Urine samples were collected after 40-50 min equilibration period.

Experimental design

Rabbits with control and experimental groups. After one hour infusion of hypotonic saline, we immediately infused hypertonic saline (500 mM/L) intraperitoneally in the proportion of 20 ml/kg. In the experimental group, we infused 2 mmol/kg of LiCl solution intravenously, whereas in the control group, we infused 2 mM/kg of NaCl solution intravenously.

Sampling of arterial blood and urine were made at 15, 30 and 60 min after the infusion. Arterial blood was centrifuged immediately to separate the plasma.

For comparison of the results, we infused isotonic saline or dextran instead of hypertonic saline in other group. After administration of isotonic saline (145 mM/L) or 15 % dextran solution (M.W.=175,000) intraperitoneally at 20 ml/kg, we infused 2 mmol/kg of LiCl solution, and then arterial blood and urine samples were obtained at 15, 30, and 60 min.

In dextran treated rabbits, urine sample was not adequate to collect at 15 min so we obtained blood and urine samples at 90 and 180 min.

Measurement and calculation

Li^+ concentration in plasma and urine was determined by atomic absorption spectrophotometry (Shimazu AA-670/G V-5).

Na^+ and K^+ concentrations in plasma and urine were determined by flame photometry (Corning 430). C_{Li} and C_{Na} were calculated according to the conventional expression as $Cx = U\ V/Px$.

Fr_{Na} and FrH_2O can be derived from following equations.

$$C_{Li} = \frac{U_{Li}V}{P_{Li}} \quad \cdots\cdots ①$$

where U_{Li} is urine concentration of Li^+, P_{Li} is plasma concentration of Li^+, V is urine flow rate (μl/min/kg).

Equation ① can be replaced to equation ②

$$\frac{V}{C_{Li}} = \frac{1}{(U/P)_{Li}} \quad \cdots\cdots ②$$

in which V/C_{Li} denotes urine excretion rate from V_{TDNA}, so FrH_2O of distal tubule can be calculated $1\text{-}V/C_{Li}$.

If we assume the Na^+ concentration to inflow of nephron segment beyond pars recta equals to plasma Na^+ concentration, reabsorption rate of Na^+ (T_{Na}) may be expressed to equation③

$$T_{Na} = C_{Li} \cdot P_{Na} - U_{Na}V \quad \cdots\cdots ③$$

in which $C_{Li} \cdot P_{Na}$ denotes Na^+ inflow and $U_{Na} \cdot V$ is Na^+ excretion rate.

So, Fr_{Na} can be expressed as follows ;

$$Fr_{Na} = \frac{C_{Li} \cdot P_{Na} - U_{Na}V}{C_{Li} \cdot P_{Na}} \quad \cdots\cdots ④$$

where $U_{Na}V/P_{Na}$ is C_{Na}

So equation ④ can be changed to equation ⑤

$$Fr_{Na} = 1 - \frac{C_{Na}}{C_{Li}} \quad \cdots\cdots ⑤$$

Statistics

We compared the results of hypertonic saline-LiCl group with that of control group. We considered time related changes of C_{Li}, Fr_{Na}, and FrH_2O in hypertonic saline infusion group.

In isotonic saline or dextran group, the results were compared with that of hypertonic saline group and the data within group. Results are expressed as the mean± SE. Statistical analysis was performed by Students t-test and a probability of 5% was used as the criterion of significance.

RESULTS

Plasma and urine Na^+ concentration and urine excretion rate in hypertonic saline infused rabbits

Data on plasma and urine Na^+ concentration, urine excretion rate obtained at 15, 30, 60 min after infusion of hypertonic saline in rabbits pretreated with hypotonic saline are given in Table 1.

After infusion of hypertonic saline, plasma Na^+ concentration gradually increased but there was no significant statistical difference.

Urine excretion rate increased greatly 15 min after hypertonic saline infusion and then decreased gradually so that at 60 min it has significantly low value.

Urine Na^+ concentration gradually increased so that it has significantly high value at 60 min compared to the values of 15 min. After infusion of 2 mM/kg of LiCl, there was no diuresis or natriuresis due to Li^+ treatment.

So we performed 2 mmol/kg of LiCl treatment in all our experiments.

C_{Li} in hypertonic saline infused rabbits

C_{Li} at 15 min after infusion of hypertonic saline was 5140.9±840.27 μl/min/kg and it

Table 1. Effect of LiCl injection (2 mM/kg) on changes of plasma Na^+ concentration after intraperitoneal injection of hypertonic saline in rabbits pretreated with intravenous hypotonic saline

Group / Time	Control (N=10)				LiCl (N=15)			
	Hct (%)	P_{Na} (mEq/L)	UV (ul/min/kg)	U_{Na} (mEq/L)	Hct (%)	P_{Na} (mEq/L)	UV (ul/min/kg)	U_{Na} (mEq/L)
before	32.3±0.98	132.5±2.08	70.0±20.00	110.8±23.36	34.5±1.01	135.1±1.03	49.9±10.12	119.0±22.59
15 min	31.6±0.92	142.5±3.12	432.9±94.90	123.6±13.56	34.1±0.97	139.6±1.63	615.1±80.39	109.0±4.78
30 min	30.8±0.95	140.6±4.14	153.5±37.60	167.2±15.76	34.0±0.98	140.8±1.74	166.1±41.65	136.5±4.51
60 min	33.1±0.89	142.1±3.01	63.7±19.91	203.6±19.22*	34.6±1.08	141.0±2.32	42.5±10.22	163.7±13.57*

* $P<0.05$ compared with the value of 15 min

Table 2. Changes in clearances of Li^+, and K^+ after intraperitoneal injection of hypertonic saline (20 ml/kg) in rabbits pretreated with hypotonic saline (20 ml/hr/kg) for an hour (μl/min/kg) (N=15)

Time \ Clearance	C_{Li}	C_{Na}	C_K
15 min	5140.9±840.27	454.0±71.48	4014.7±505.03
30 min	2236.8±634.76*	175.9±48.95**	1394.1±387.55**
60 min	668.3±127.67***	49.0±13.88	443.9±108.52***

$P<0.01$; **$P<0.005$; compared with the value of 15 min
*** $P<0.05$; compared with the value of 30 min

Table 3. Fractions of Na^+ and water reabsorption in nephron segment beyond proximal tubule after intraperitoneal injection of hypertonic saline (20 ml/kg) in the rabbits pretreated with hypotonic saline (20 ml/hr/kg) for an hour (N=15)

Time \ Fraction	$1-\frac{UV}{C_{Li}}$	$1-\frac{C_{Na}}{C_{Li}}$	$\frac{1-UV/C_{Li}}{1-C_{Na}/C_{Li}}$
15 min	0.883±0.0158	0.905±0.0118	0.975±0.0056
30 min	0.912±0.0171	0.914±0.0174	0.998±0.023 **
60 min	0.932±0.0129*	0.912±0.0242	1.024±0.0164**

* $P<0.0025$, ** $P<0.005$ compared with the value of 15 and 30 min

decreased to 2236.8±634.76 μl/min/kg ($P<0.01$) at 30 min and 668.3±127.67 ($P<0.05$) at 60 min. C_{Na} and C_K also decreased significantly.

Comparison of the value at 30 min with that at 15 min, it decreases significantly ($P<0.05$). The value at 30 min decreased significantly ($P<0.05$) compared with that at 15 min.

Also the value at 60 min decreased significantly ($P<0.05$) compared with that at 30 min (Table 2).

Fr_{Na} and FrH_2O in hypertonic saline treated animal

Data on Fr_{Na} and FrH_2O in nephron seg-

ment beyond proximal tubule with hypertonic saline treated animals are given in Table 3. Measured Fr_{Na} ($1-C_{Na}/C_{Li}$) was constant in spite of the change of C_{Li} (Fig. 1).

Measured FrH_2O ($1-UV/C_{Li}$) increased as time went by so the value at 60 min was significantly higher than that at 15 min ($P<0.0025$).

The ratio of FrH_2O/Fr_{Na} was 0.975 ± 0.0056 at 15 min, and increased significantly to 0.0998 ± 0.0023 at 30 min, and then increased more to 1.024 ± 0.0165 at 60 min (each $P<0.005$).

Isotonic saline treated rabbits

Data on Hematocrit, P_{Na}, UV, U_{Na} after intraperitoneal infusion of isotonic saline in rabbits pretreated with hypotonic saline are given in Table 4, and Table 5.

Same as in hypertonic saline treated group, there was no change of hematocrit value in isotonic saline treated group.

Urine excretion (UV) tended to decline when compared with hypertonic saline treat-

Table 4. Changes of plasma Na^+ concentration, hematocrit, urine excretion and urine Na^+ concentration after intraperitoneal injection of normal saline (20 ml/kg) in the rabbits pretreated with hypotonic saline (20 ml/hr/kg) for an hour (N=6)

Time	Hct (%)	P_{Na} (mEq/L)	UV (ul/min/kg)	U_{Na} (mEq/L)
15 min	34.2±1.40	127.5±0.50*	442.6±152.70	89.0±16.00
30 min	34.3±1.90	134.0±6.00	50.3± 19.30	119.0±21.00
60 min	35.6	129.5±8.50**	17.1± 10.40	93.0± 0.00***

$P<0.005$, ** $P<0.05$ and *** $P<0.025$ compared with the value of Table 1

Table 5. Changes of Li^+ clearance and fraction of Na^+ and water reabsorption in the nephron segment beyond proximal tubule after intraperi-toneal injection of normal saline (20 ml/kg) in the rabbits pretreated with hypotonic saline (20 ml/hr/kg) for an hour (N=6)

Time \	C_{Li}	C_{Na}	$1-\frac{C_{Na}}{C_{Li}}$	$1-\frac{UV}{C_{Li}}$	$\frac{1-UV/C_{Li}}{1-C_{Na}/C_{Li}}$
15 min	7695.5±3439.50	328.8±163.45	0.959±0.0025*	0.940±0.0075	0.980±0.0104
30 min	1128.0± 548.05	42.2± 11.15	0.958±0.0105	0.953±0.055	0.995±0.0051
60 min	500.2± 223.20	13.7± 9.55	0.977±0.0085	0.969±0.0070	0.992±0.0014**

* $P<0.05$, ** $P<0.005$ compared with the values of Table 2 and Table 3.

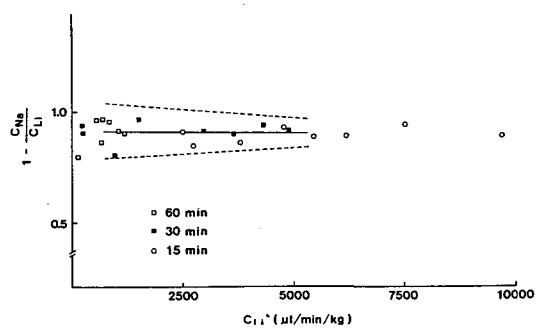

Fig. 1. The range (mean±2 S.D.) of sodium reabsorption fraction as a function of lithium clearance after i.p. injection of hypertonic saline (20 ml/kg) in rabbits pretreated with hypotonic saline (20 ml/kg).

Table 6. Changes of plasma Na^+ concentration, urine excretion rate and urine Na^+ concentration after intra-peritoneal injection of 15% dextran solution (20 ml/kg) in the rabbits pretreated with hypotonic saline (20 ml/hr/kg) for an hour (N=5)

Time	P_{Na} (mEq/L)	UV (ul/min/kg)	U_{Na} (mEq/L)
30 min	133.5±0.50	120.1±54.90	88.0±29.00**
60 min	130 ±2.89*	72.1±53.27	86.3±41.91***
120 min	134.3±2.03	80.8±44.01	93.3±29.33
180 min	131.6±0.88	9.3±4.00	79.9±19.30

P<0.05, ** P<0.005 and *** P<0.025 compared with the value of Table 1

Table 7. Changes of Li^+ clearance and the fraction of Na^+ and water reabsorption in the nephron segment beyond proximal tubule after intraperi-toneal injection of 15% dextran solution (20 ml/kg) in the rabbits pretreated with hypotonic saline (20 ml/hr/kg) for an hour (N=5)

Time	C_{Li}	C_{Na}	$1-\frac{C_{Na}}{C_{Li}}$	$1-\frac{UV}{C_{Li}}$	$\frac{1-UV/C_{Li}}{1-C_{Na}/C_{Li}}$
30 min	668.0	88.2±65.75	0.966	0.902	0.934
60 min	1572.1± 965.17	38.5±19.29	0.977±0.0083	0.969±0.0119	0.002±0.0133*
90 min	2214.8±1027.20	37.5±11.19	0.985±0.0015	0.969±0.0195	0.984±0.0183
120 min	263.2± 147.55	7.2± 3.70	0.972±0.0015	0.961±0.0070	0.989±0.0057

* P<0.05 compared with the value of Table 2 and Table 3.

ed group, but we couldn't recognize significant difference because of large individual variance. Compared with hypertonic saline treated group, plasma Na^+ concentraton showed significant low value at 15 min and at 60 min (P<0.005 at 15 min, P<0.05 at 60 min) and also urine Na^+ concentration showed significant low values at 60 min (P <0.005).

Dextran treated rabbits

In rabbits treated with 15% dextran solution intraperitoneally after treatment of hypotonic saline, there was no measurable urine excretion at 15 min but 120 μl/min/kg of urine was excreted at 30 min.

In Table 6, plasma Na^+ concentration at 60 min was significantly low value (P< 0.025) and also U_{Na} was significantly low value (P<0.05 at 30 min P<0.025 at 60 min) compared with hypertonic saline treated group.

C_{Li} fluctuated greatly with time (Table 7). Fr_{Na} seemed to be increased but we couldn't recognize statistically significant difference.

Fr_{Na}/FrH_2O had an almost constant value and it was significantly low value at 60 min compared with hypertonic saline treated group (P<0.005).

DISCUSSION

Li^+ is reabsorbed with Na^+ in proximal tubular epithelial cell and Li^+ uses Na^+ channel when it is excreted in basolateral membrane. But Li^+ can not be substituted for Na^+ in electroneutral transport mechanism (1 Na^+, 1 K^+, 2 Cl^-) in TALH cell (Herbert & Andreoli, 1984; Navar & Schafer, 1987) and low concentration of Li^+ does not participate in Na^+ reabsorption

mechanism throughout distal tubule (Steel et al, 1975).

So the reabsorption of Na^+ and Li^+ is similar at the end of proximal tubule, but it is quite different thereafter. Li^+ reabsorption in distal tubule can be observed only when large amount of Li^+ is present in tubular fluid or with low salt diet. In therapeutic range of Li^+ concentration, $U_{Li}V$ (Li^+ excretrion rate) means Li^+ inflow to TALH and C_{Li} can be considered as a measure of outflow of tubular fluid from vasa recta (Thomsen & Leyssac, 1986).

By using C_{Li}, we can assume the reabsorption of Na^+ and water beyond TALH.

Because qualitative and quantitative control of body fluid by urine excretion is occurred beyond TALH, the application of C_{Li} has advantage of that it can give clear explanation of reabsorption beyond proximal nephron when compared with the application of C_{in}.

So we attempted to measure Na^+ and water reabsorption rate (Fr_{Na} & FrH_2O) beyond pars recta by using the equations of $Fr_{Na}=1-C_{Na}/C_{Li}$ and $FrH_2O=1-UV/C_{Li}$(Methods 3)

Such equations have been made on the basis of two assumptions;

1. Li^+ reabsorption rate and Na^+ reabsorption rate are same in proximal tubule.

2. Li^+ is not reabsorbed beyond Henle's loop. It is reported that Fr_{Na} and Fr_{Li} is similar in proximal tubule (Hayslett & Kashgarian, 1979), so first assumption has no problem. And the Li^+ concentration used in this experiment is not reabsorbed (Thomsen & Leyssac, 1986).

Even though it has been suggested that Li^+ is reabsorbed in the same proportion of Na^+ in thin limb of Henle's loop (Navar & Schafer, 1987), the amount of Li^+ reached to this segment is 22% of filtered Li^+ and urine excretion of Li^+ is 21% (Hayslett & Kashgarian, 1979) and then Li^+ amount in this experiment is low, so we can ignore the reabsorption of Li^+ in this segment.

We infused 50 mmol/L of hypotonic saline as a pretreatment to reduce Li^+ reabsorption in this segment as much as possible.

Hypotonic saline can induce measurable urine excretion and can rule out ADH action and increase medullary blood flow, so the osmotic concentration in medulla will be decreased. As a result, the reabsorption of Na^+ and water in Henle's loop will be reduced.

We can consider C_{Li} measured in this situation as an inflow of tubular fluid to TALH in which the ADH action about Na^+ and water reabsorption is ruled out.

FrH_2O at 15 min after infusion of hypertonic saline C_{Li} had the value much higher than that of Brattleboro white rat (Thomsen & Leyssac, 1986).

So we can attribute this difference to species difference, or to inherent ADH deficient animal, the most probable cause is remained ADH action since after 30 min FrH_2O was increased.

However Fr_{Na} fluctuated a little at constant level in spite of great decrease of C_{Li}.

Na^+ reabsorption at TALH is about 15-20 % of filtration and it is increased by several hormones (Hebert & Andreoli, 1984). Na^+ reabsorption at TALH and distal tubule is influenced greatly by hormones; ADH, glucagon, PTH, PG, CT, aldosterone (Greger, 1981; Doucet, 1987; Culpepper & Andreoli, 1983) and also influenced by medullary osmotic concentration and flow rate (Herbert & Andreoli; 1984).

When tubular perfusion rate is greatly increased during water diuresis, delivery of fluid to distal tubule is greatly increased.

As a result reabsorption rate will be decreased.

As reabsorption rate decrease, urine concentration of substrate will be near to plasma concentration.

The more the reabsorption rate decrease, the nearer urine concentration of substrate will be to inflow fluid concentration and at

last to plasma concentration. In this experiment C_{Na} and urine excretion rate followed the change of C_{Li} and urine Na^+ concentration was the lowest value at 15 min when C_{Li} was the highest value. Urine Na^+ concentration increased as C_{Li} decreased.

As a whole, we can conclude that there is no reabsorption disturbance of Na^+ and water secondary to flow increase in this experimental range of C_{Li}.

If the outflow from proximal tubule is large, increase of medullary blood flow will reduce medullary osmotic concentration. As a result, reabsorption of Na^+ and water in thin limb of Henle's loop will be decreased and then inflow to TALH will be increased and the increase of Na^+ reabsorption in TALH will be followed. In this experiment, C_{Na} changed secondary to C_{Li} change but Fr_{Na} was nearly constant despite C_{Li} change.

We found that Fr_{Na} was constant beyond Henle's loop in this experimental range of C_{Li} so the increase of excretion rate followed the increase of inflow rate. But true reabsoption will increase as inflow rate increases.

1 Na, 1 K, 2 Cl reabsorption in TALH by predescribed hormones and ADH did not play a major role (Sasaki & Imai, 1980). When we consider FrH_2O value at 60 min after hypertonic saline treatment, we can't expect ADH action in TALH.

With other possibility, we can consider medullary osmotic concentration. Only if medullary osmotic concentration is high, there happens back flow through paracellular shunt (Morgan & Berliner, 1969). Since the medullary osmotic concentration is thought to be low in this experiment, we can rule out concentration effect. So we can explain the Fr_{Na} constancy irrespective of inflow.

The Fr_{Na} constancy beyond Henle's loop may therefore be explained that reabsorption rate as well as urine excretion rate increases with the increase of inflow rate.

However, we can't rule out the effect of aldosterone on Na^+ reabsorption in distal tubule.

With aldosterone effect, Fr_{Na} should be decreased according to secretion difference as time goes by. But we can rule out aldosterone effect due to following reasons; aldosterone effect appears late and maintains for a long time and in the early phase of experiment-when the aldosterone secretion is large-ANP will be produced due to blood volume increase by infused solution.

Though ADH has no apparent action on the reabsorption of Na^+ in TALH, if increase of urea reabsorption accompanied with FrH_2O increase cause medullary osmotic concentratin increment, it is possible backflow in TALH to occur. But we can rule out ADH action since ADH effect on the water reabsorption is not large enough.

Unlike Fr_{Na}, FrH_2O increased as C_{Li} decreased. When we consider water reabsorption change in distal tubule, we should think three different points; simple flow change, the effect of ADH secretion change, the effect of medullary osmotic concentration.

Generally, 1% increment of plasma osmotic concentration make urine osmotic concentration two times via increase of ADH secretion (Schrier et al, 1979). In this experiment, plasma Na^+ concentration only tend to be increased as time goes by. But we can't rule out Na^+ concentration effect on water reabsorption.

If ADH secretion increases, water reabsorption will be followed, and then increase of urea reabsorption will contribute to the increase of medullary osmotic concentration.

Since plasma Na^+ concentration tends to be increased with the decrease of C_{Li} in this experiment, we can conclude that FrH_2O increase may be attributed to the increase of ADH action. ADH also accelerates Na^+ reabsorption in TALH. But water and Na^+ reabsorption mechanism by ADH is quite different (Herbert & Andreoli, 1984).

Fr_{Na} did not change in this experiment, it is in accordance with the reports of in

vitro study; in rabbit ADH does not influence the Na^+ reabsorption in TALH (Sasaki & Imai, 1980) and Na^+ reabsorption in TALH is constant when the flow rate is the same (Herbert & Andreoli, 1984; Greger, 1981).

If we rule out the effects of aldosterone and ADH, we can confirm the report of in vitro study by in vivo study conducted with the application of C_{Li} that Fr_{Na} is constant despite the change of inflow.

Since we tried to find the relation of Fr_{Na} and FrH_2O with C_{Li} in regard with ADH, we infused isotonic saline intraperitoneally to block ADH secretion completely (Dunn et al, 1973).

At first C_{Li} was nearly same value with that of hypertonic saline treatment and after 30 min it tended to decrease.

Fr_{Na} tended to increase compared with that of hypertonic saline treatment but Fr_{Na} was constant as well despite C_{Li} change.

Therefore, we can confirm that Fr_{Na} is constant without regard to inflow and ADH has little effect on Fr_{Na}.

Even if plasma Na^+ concentration is low, Na^+ and water reabsorption in proximal tubule is taken place isotonically. So the Li^+ and Na^+ concentration in TALH may be decreased but since decrement of two ions is the same proportion, Fr_{Na} calculated as $1-C_{Na}/C_{Li}$ shows the same value.

Therefore we can attribute the increasing tendency of Fr_{Na} with isotonic saline treatment to the action of peptide hormones via cAMP or to the increase of Na^+ reabsorption in distal tubule by aldosterone. But we can't evaluate that with this experiment.

FrH_2O in isotonic saline treatment showed increasing tendency with C_{Li} decrease but the change was not so significant and therefore we can't expect ADH effect. But in fact FrH_2O was generally not lower value than that of hypertonic saline treatment.

Thomsen & Leyssac (1986) reported that with low sodium diet Li^+ was also reabsorbed to some extent in the distal nephron segment, so the C_{Li} was reduced.

Therefore C_{Li} measured in this experiment has the possibility to show lower value than true outflow rate of proximal tubule and in that circumstances, FrH_2O should be lowered, so C_{Li} measured in hypotonic saline treatment must be corrected.

From the results of isotonic saline treatment, we can confirm that Fr_{Na} is constant despite C_{Li} change and therefore, both the reabsorption rate and excretion rate of Na^+ increase according to the increase of inflow increases.

We infused dextran solution intraperitoneally to reconfirm the inflow rate dependency of Fr_{Na} and found out great decrease of C_{Li}.

If dextran makes effective fluid volume decrease, the GFR decreases, and then reabsorption of Na^+ and water in proximal tubule increases, so the decrease of C_{Li} is quite natural.

By studies conducted in vitro it was reported that the range of Na^+ reabsorption change is very large; and if the Na^+ concentration and the velocity of inflow fluid is low, the Na^+ reabsorption in TALH stops; the highest reabsorption rate may be reached to value of 10×10^{-9} mol/S/cm^2.

But in vivo study it is very difficult to confirm the records due to various factors (Schlatter & Greger, 1984).

Especially if body fluid volume is decreased, the reabsorption in proximal tubule is increased via the control mechanism of body fluid volume and therefore inflow rate to TALH will be decreased. But treatment of loop blocker induce natriuresis (Greger, 1981).

With dextran infusion, C_{Li} decreased but Fr_{Na} did not show C_{Li} dependent change, and FrH_2O had similar value to Fr_{Na}. That is, we confirmed the result of in vitro study that Na^+ reabsorption in TALH is proportional to inflow amount. The slight higher value than that when body fluid volume is

increased may be attributed to ADH effect due to decrease of body fluid.

It is known that in rabbit ADH effect in TALH is not large, but it is also reported that up to 150 mM/L of Na^+ concentration, cAMP increase by ADH can be observed (Sasaki & Imai, 1980). So we can expect ADH increase due to volume decrease FrH_2O showed the tendency to be increased compared with that when body fluid volume is increased, we can also expect ADH action.

But we can not elucidate whether Li^+ is reabsorbed in TALH or not with this experiment.

From the above results, we can confirm that; Fr_{Na} calculated from C_{Li} shows constant value despite inflow rate—it is in accordance with the report of in vitro study.

Even if the absolute value of Fr_{Na} is changed by aldosterone or ADH, Fr_{Na} is constant in certain flow range despite flow velocity. Since FrH_2O calculated from C_{Li} was dependent upon ADH rather than upon flow velocity, FrH_2O measured in hyponatremic condition was not evaluated only by C_{Li}. Modification about expected Li^+ reabsorption in TALH may be needed.

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