Kawasaki Med. J. Vol. 1: 145-151, 1975

MOVEMENT OF ^{14}C-UREA IN MICROPERFUSED HENLE'S LOOP OF ADRENALECTOMIZED RAT KIDNEY

Satoru WATANABE, Yasuko HAYASHI, Tsutomu NOHNO and Yoshimichi MURAYAMA

Depatment of Pharmacology, Kawasaki Medical School, Kurashiki, 701-01, Japan

Accepted for publication on Sep. 8, 1975

Abstract

Henle's loops of normal and adrenalectomized rats under nondiuretic condition were perfused with isotonic saline containing ^{3}H-methoxyinulin and ^{14}C-urea, and distal fluid was subsequently collected by micropuncture. Osmolality and radioactivities of tubular fluid, and concentrations of sodium, potassium, urea and inulin and osmolalities of urine and plasma were determined. In the adrex rats, the distal tubular fluid to plasma ratio of osmolality was elevated and the distal fractional recovery of ^{14}C-urea was significantly increased, but the water reabsorption did not change. The distal recovery of ^{14}C-urea in the control rats was not correlative to urinary osmolality. These results suggest that the increase in distal fractional recovery of the tracer in adrex rats is possibly produced by the lowered permeability of Henle's loop to urea but not by the decreased solvent drag.

INTRODUCTION

Sodium and urea are main solutes which are concentrated in the renal medulla, and it is generally accepted that sodium is accumulated in the medulla by active reabsorption in the ascending limb of Henle's loop and urea is also passively accumulated in the same portion by recyclage from the collecting duct to the loop and distal tubule. In addition, it is well known that the urine concentrating ability in adrenalectomized (adrex) animals is lower than that in intact[1,2], suggesting that sodium transport out of the ascending limb of the loop is depressed by adrenalectomy[3,4,5]. On the other hand, renal handling of urea in adrex animals, particularly permeability of tubular wall to urea, have not yet been studied. In the present experiments, therefore, urea movement in Henle's loop, which is a barrier against the recyclage of the molecule in the renal medulla, was studied by microperfusion of ^{14}C-urea into the loop of adrex rat kidney.

METHODS

Female Wistar rats weighing 200 to 300 g were used and the animals were given free access to food (CE-2). Control rats were kept on tap water, which was replaced by 1 % saline for 7 to 9 days after adrenalectomy. Animals were anesthetized by intraperitoneal injection of Nembutal (50 mg/kg for control and 30 mg/kg for adrex rats) and placed on temperature-controlled operating board. The left kidney was exposed for micropuncture. The temperature of superficial oil over the exposed kidney was controlled between 35 to 36°C. During the surgical procedures for micropuncture and clearance experiments, 2 ml of 0.9 % saline was intravenously infused to replace surgical losses. Subsequently, the animals were primed with 0.4 ml of 5 % inulin (2.5 % inulin for adrex) in 1 % saline and then 2 % inulin (1.0 % inulin for adrex) in 2 % saline was infused at 33.4 μl/min. After a period of 1 hr allowed for equilibration of the inulin, urine samples were collected from the left ureter at about 30 min intervals and blood samples were also obtained from the femoral artery at the midpoint of clearance periods.

The loop of Henle was perfused according to the method of Cortney et al.[6] as previously described[4]. The proximal tubular segment was filled with colored oil to stop the flowing of glomerular filtrate, and the perfusion pipette was inserted into the late proximal tubule in order to perfuse the single nephron. One percent saline colored with 0.05 % lissamine green was used as perfusate and in some experiments the colored solution with ^{3}H-methoxyinulin (N. E. N., 0.5 mCi/ml) and ^{14}C-urea (Amerciam, 0.125 mCi/ml) was applied. The osmolality and urea concentration in the solution prepared were 310 mOsM and 7.8 mM respectively. The perfusion rate was 29 nl/min. Micropunctures were performed during clearance periods.

Osmolalities of the tubular sample, ureteral urine and plasma were determined with a microosmometer (Iiodenki). Radioactivities of ^{3}H and ^{14}C in tubular fluids were measured with a Tricarb liquid scintillation counter (Model 3385). Sodium and potassium concentrations in plasma and urine were determined with a flame photometer (Hitachi Model 205), urea concentration with urea N-Test Kits (Wako) and inulin concentrations in plasma and urine by the method of Vurek and Pegram[7] after the deproteinization by 10 % trichloracetic acid.

RESULTS

1. *Clearance experiments*

The summarized results are shown in Table 1.

TABLE 1.
Summarized result of clearance experiments in normal and adrenalectomized rats

	No. of rats	Concentration in plasma (mM) Na	K	Urea	Excretion (per min per kidney) V (μl)	Na (μmol)	Urea (μmol)
Control	5	148 ±9	4.8 ±0.4	7.5 ±1.1	4.8 ±1.8 (28)	0.93 ±0.90 (28)	3.74 ±0.96 (21)
Adrex	21	149 ±9	6.8 ±0.7	12.2 ±3.7	2.8 ±1.3 (40)	0.37 ±0.46 (35)	0.87 ±0.69 (33)
p			<0.001	<0.025	<0.001	<0.001	<0.001

	No. of rats	Uosm (OsM)	$\left(\frac{U}{P}\right)_{in}$	Cin (ml/min.kidney)	Fractional excretion of urea
Control	5	2.38 ±0.39 (28)	281 ±147 (27)	1.20 ±0.51 (27)	0.455 ±0.158 (19)
Adrex	21	1.11 ±0.44 (40)	173 ±157 (29)	0.38 ±0.19 (29)	0.248 ±0.156 (19)
p		<0.001		<0.001	<0.001

Standard deviations were calculated
Numbers of samples were given in parentheses.

The characteristic signs of adrenal insufficiency were detected in potassium and urea concentrations in plasma[8], and the significant decreases in urine volume, sodium excretion and urinary osmolality (U_{osm}) were observed as previous report[5].

With adrenalectomy urea excretion was decreased from a level of 3.74 μmol/min in control rats to a level of 0.87 μmol/min. Inulin clearance (C_{in}) in adrex rats was lowered to about one third of control value and fractional excretion of urea to about a half. Urea excretion theoretically depends on either C_{in}, urea concentration in plasma, or

fractional excretion of urea. The Urea excretion as a function of C_{in} is illustrated in Fig. 1, showing that the data obtained from adrex rats were located inside the reach of normal distribution obtained from the data in non-adrenalectomized rats[9]. Therefore, the decreased urea excretion in adrex rats appears to depend in large part on the lowered C_{in}, which resulted in slower tubular flow.

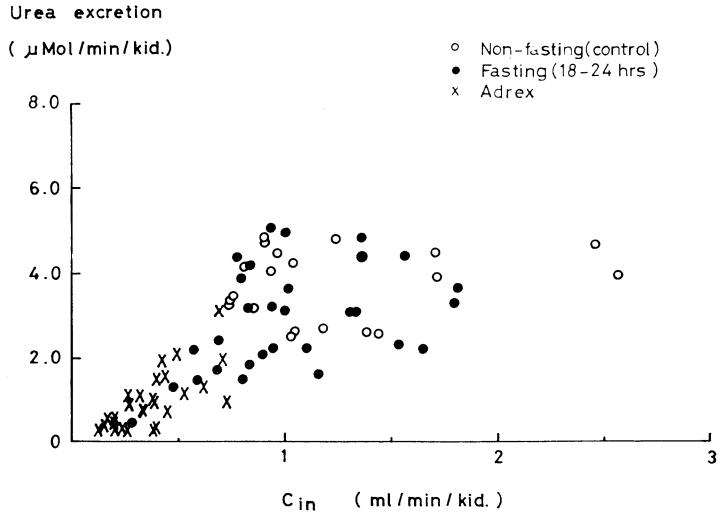

Fig. 1. The urea excretion in control (non-fasted), fasted (18-24 hrs) and adrex rats, in relation to the glomerular filtration rate (C_{in}).

2. *Microperfusion experiments*

As shown in Table 2, the distal tubular fluid (TF) to plasma (P) ratio of osmolality, $(TF/P)_{osm}$, in adrex rats (0.82 ± 0.15) was significantly different from that in control animals (0.60 ± 0.09). The difference was similar to the data previously reported[4]. The fractional water reabsorption through the loop of adrex rats (calculated from the inulin concentration ratio of perfusate (Per) to distal tubular fluid) was not different from that of normals. The distal fractional recovery of ^{14}C-urea microperfused into Henle's loop of adrex rats (0.45 ± 0.18) was significantly higher than a control level of 0.30 ± 0.09, which approximated to the value (0.32) obtained by Wilczewski et al.[11] using the similar microperfusion technique (at 25 nl/min).

DISCUSSION

The elevation of distal $(TF/P)_{osm}$ in constant microperfusion experi-

ment is theoretically due to either increased water reabsorption, decreased sodium reabsorption, or change of urea movement in Henle's loop. From the present result that water reabsorption was not changed by adrenalectomy, the elevation of the osmotic ratio should be resulted from the latter two factors. Although sodium and urea concentrations in collected distal tubular fluid was not determined unfortunately and, therefore, accurate information could not be taken, the decreased recovery of ^{14}C-urea at distal tubule in adrex rats may dissolve the several problems which will be discussed as follows.

The absorption of urea through biological membrane generally depends on solvent drag, permeability to urea, and transtubular concentration gradient of the molecule. The decrease of solvent drag in Henle's loop of adrex rats was excluded because of no change of water reabsorption (Table 2). The influence of transtubular concentration

TABLE 2.

Effects of adrenalectomy on the distal tubular fluid to plasma ratio of osmolality, the fractional reabsorption of water in Henle's loop and the distal fractional recovery of ^{14}C-urea in microperfusion experiments

	No. of rats	$\left(\frac{TF}{P}\right)_{osm}$	$1-\left(\frac{Per}{TF}\right)_{in}$	$\left(\frac{TF}{Per}\right)_{^{14}C\text{-urea}} / \left(\frac{TF}{Per}\right)_{in}$
Control	6	0.60 ±0.09 (23)	0.37 ±0.15 (19)	0.30 ±0.09 (19)
Adrex	4	0.82 ±0.15 (16)	0.37 ±0.22 (15)	0.45 ±0.18 (15)
p		<0.001		<0.005

Numbers of punctured samples were given in parentheses.
Standard deviations were calculated.
P=plasma, Per=perfusate, TF=distal tubular fluid,
osm=osmolality, in=inulin, ^{14}C-urea=radioactivity of the tracer.

gradient of urea on the distal recovery of ^{14}C-urea would be considered because the urea concentration in plasma of adrex rats (12.2 mM) was significantly higher than a control level of 7.5 mM which was similar to the concentration of perfused solution (7.8 mM) and because the urea concentration in outer medulla of adrex rats (118 mM) was lower than that in normal animals (238 mM)[5]. However, the effect of the concentra-

tion difference may be excluded from the previous results[10] that change of the fractional recovery did not occure even when higher concentration of urea (100 mM) was perfused into the Henle's loop of desert rodent (Merion) and that the recovery did not depend on the change of U_{osm} (0.5 to 3.5 OsM). In the present experiments, the distal recovery of ^{14}C-urea in adrex rats appears to be correlative to U_{osm} ($r=-0.660$, $p<0.01$, Fig. 2), but in control rats it was almost independent and the

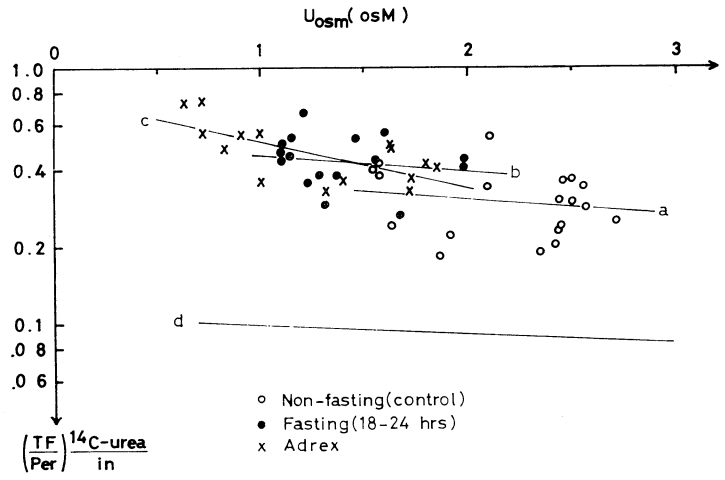

Fig. 2. The distal fractional recovery of the perfused ^{14}C-urea as a function of the urinary osmolality in control, fasted and adrex rats.
Line a (control): $Y=-0.064\ X-0.390$ ($r=-0.204$)
Line b (fasted): $Y=-0.067\ X-0.272$ ($r=-0.189$)
Line c (adrex): $Y=-0.187\ X-0.108$ ($r=-0.660$)
Line d was cited from the data obtained by Murayama et al.[10], using the same microperfusion technique applied to the Henle's loop of desert rodents.

regression line of control was parallel with that of merions and fasted (18-24 hrs) rats with adrenal glands. Accordingly, the increase in distal fractional recovery of ^{14}C-urea may indicate the lowered permeability of the loop to urea, which may in part lead to the reduction of recyclage[12] of urea from the collecting duct to the loop and distal tubule in adrex rats. However, it is necessary to study further for supporting the possible mechanism above described.

In the present experiments, the increase in distal fractional recovery of ^{14}C-urea in adrex rats was not reflected to the overall fractional and

absolute excretion. This may result from slower tubular flow because of remarkable decrease of GFR in adrex animals.

According to the results of free flow micropuncture experiments under mannitol saline diuresis[13], in which the sodium concentration in the distal tubule and fractional sodium reabsorption in Henle's loop were not changed by adrenalectomy while the osmolality in the distal tubule was elevated, it has been assumed that the elevated osmolality might result from the increased urea concentration produced by increased addition of urea into the loop or increased osmotic equilibration in ascending limb because the free flow rate through the loop of adrex rat kidney was slower. From the present results, the lowered permeability of Henle's loop to urea may be also considered as another factor which produces an increase of osmolality in the distal tubule of adrex rat kidney.

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