Decreased Renal Excretion of Uric Acid Following Diuretic Administration in Rats

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Abstract—In order to evaluate the cause of diuretic-induced hyperuricemia which has been well documented in clinical studies, clearance experiments were performed in rats using furosemide and trichlormethiazide. The net flux in the tubular transport of uric acid was reabsorptive, and the fractional excretion of uric acid responded sensitively to the transtubular transport inhibitors, sodium probenecid and pyrazinoic acid. When the hemoconcentration was induced by highly potent doses of test diuretics, the inulin clearance and the fractional excretion of uric acid clearly decreased. The contraction of body fluid produced by intraperitoneal administration of polyethylene glycol resulted in marked decrease of inulin clearance and fractional excretion of uric acid. The decrease of uric acid excretory capacity under the treatment with trichlormethiazide was completely corrected by saline loading. Moreover, no significant change was found in the pyrazinoic acid-suppressible fractional excretion of uric acid between the diuretic-treated rats and the control animals. These studies suggest that furosemide and trichlormethiazide-induced changes in the renal handling of uric acid are mediated by the fluid volume contraction and that the decrease in fractional excretion of uric acid by test diuretics is the result of reabsorptive enhancement of uric acid.

Hyperuricemia incident to the treatment with diuretics is well recognized (1–6), and obstruction in the renal transport system of uric acid has been discussed as the cause (2–6).

The transport of uric acid in the mammalian kidney involves glomerular filtration, tubular reabsorption and tubular secretion, though the relative importance of uric acid movement in each direction differs among species (7, 8). The rat is a useful animal for evaluating drug effects on uric acid excretion because the net flux in the tubular excretory transport of uric acid is reabsorptive, although the fractional excretion of uric acid is obviously higher than that in man (9–13). Thus, we utilized rats as the animal for evaluating the action of diuretics on the uric acid transport system.

The animals treated with furosemide and trichlormethiazide clearly decreased the fractional excretion of uric acid, together with the decrease of inulin clearance and the increase of plasma uric acid level. These results corresponded well to the reports on clinically observed hyperuricemia induced by the diuretics (2–6). Accordingly, the present communication contributes to the understanding of the hyperuricemia-inducing effect of diuretics in pharmacological studies with experimental animals.

Materials and Methods

Animals and drug treatments: The drugs used were sodium probenecid, pyrazinoic acid (Nakarai Chem.), furosemide, trichlormethiazide (Shionogi) and polyethylene glycol 4000 (average molecular weight 3000, Wako Chem.). Sodium probenecid and furosemide were prepared in our laboratory from commercially available probenecid (Sigma) and furosemide solution for injection (Lasix, Hoechst). Sodium probenecid was dissolved in saline. Pyrazinoic acid was dissolved in
0.2 N sodium hydroxide solution, then neutralized with hydrochloric acid. Furosemide and trichlormethiazide were suspended in 1% gum arabic solution. These agents were administered at 2 ml/kg of body weight. Polyethylene glycol was dissolved at a concentration of 0.2 g/ml in water and was administered at 3 ml/rat. The animals of the control group were given saline or 1% gum arabic solution instead of the test agent.

Experiments were performed on male Wistar strain rats weighing 270–300 g. They were permitted free access to food and water until the time of the experiment. The animal room was maintained at 22–23°C and illuminated at intervals of 12 hr from 8:00 a.m. to 8:00 p.m.

In experiments on the effects of diuretics, the animals were housed in metabolism cages to measure the change in urine volume and deprived of food but not water. Furosemide (30 mg/kg, i.p.) and trichlormethiazide (10 mg/kg, p.o.) were administered twice at 10:00 a.m. and 6:00 p.m. A 24-hr urine was collected after the first administration, and then the rats were sacrificed by decapitation to determine blood components or used for clearance studies. The procedures for the treatments with sodium probenecid, pyrazinoic acid and polyethylene glycol are described in the legend of each figure or table.

Clearance studies: The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Polyethylene catheters were inserted into the left femoral vein for infusion of fluid, into the right femoral artery for collection of blood, and into the urinary bladder for collection of urine. Next, the animals were placed on a thermostatically controlled heated table to maintain the body temperature at 37–38°C and were infused with 0.25% sodium chloride solution containing 4% mannitol, 1.5% inulin and 0.26% sodium pentobarbital at a flow rate of 1.8 ml/hr. After a 60-min equilibration period, continuous 20-min urine collections were taken. A 0.2 ml arterial blood sample was obtained at the midpoint of each urine collection period.

Analysis: The sampled blood was immediately cooled in ice water, then centrifuged as soon as possible. To determine the plasma concentrations of inulin and uric acid, the resultant plasma was mixed with 10 volumes of 0.007 N acetic acid, heated for 2 min in boiling water and then centrifuged. Inulin concentrations in deproteinized plasma and diluted urine were measured by a fluorometric method adapted from the procedure described by Vurek and Pegram (14). Uric acid concentrations in the above specimens were measured by the fluorometric method described previously (15).

Hematocrits were measured in heparinized microhematocrit tubes. Sodium and potassium in plasma were determined by atomic absorption spectrophotometry, and other components in plasma were determined by a standard autoanalyzer technique (Technicon Autoanalyzer, SMA-MICRO system) in which chloride, creatinine, urea-nitrogen and protein were estimated by the method of Zall et al. (16), Chasson et al. (17), Marsh et al. (18) and Skeggs and Hochstrasser (19), respectively.

The abbreviations used in this paper are \( C_{\text{in}} \), inulin clearance; \( P_{\text{ur}} \), plasma uric acid; \( U_{\text{ur}} \), urine-excreted amount of uric acid; \( C_{\text{ur}} \), uric acid clearance; and \( FE_{\text{ur}} \), fractional excretion of uric acid (\( C_{\text{ur}}/C_{\text{in}} \)). All data are given as means±S.E. and Student’s t-test was used to assess statistical significance.

Results

Effects of transtubular transport inhibitors on renal handling of uric acid: First, studies were undertaken to confirm whether the inhibitor of renal tubular reabsorption and renal tubular secretion of uric acid in man are also effective in rats used in the present clearance experiments. Sodium probenecid (100 mg/kg, i.v.) and pyrazinoic acid (50 mg/kg, i.v.) were administered just after the first urine collection. Sodium probenecid produced marked elevations in the urine-excreted amount, the clearance and the fractional excretion of uric acid compared to the pre-treatment period. The inulin clearance and the plasma uric acid level did not change in the treatment with sodium probenecid (Fig. 1A). On the other hand, pyrazinoic acid produced decreases in the urine-excreted amount, the clearance and the fractional excretion of uric acid compared to the pre-
treatment period. The plasma uric acid level slightly elevated in the pyrazinoic acid-treated rats, but the inulin clearance did not change in the treatment with pyrazinoic acid (Fig. 1B). Thus, both transtubular transport inhibitors showed reasonable effects, but all parameters did not change in the treatment with saline.

Effects of furosemide and trichlormethiazide on blood components and renal handling of uric acid: Furosemide (30 mg/kg, i.p.) and trichlormethiazide (10 mg/kg, p.o.) given twice daily, sufficient to produce marked diuresis, increased the hematocrit and produced significant changes in blood components as detailed in Table 1. Plasma uric acid level increased in both diuretic-treated groups. Moreover, creatinine, urea-nitrogen and protein levels in plasma were significantly higher in the drug-treated group compared to the control. With respect to the plasma electrolytes, both drugs did not change the potassium level, but decreased the chloride level. Sodium level decreased only with furosemide treatment.

Next, clearance studies were performed in order to understand changes in the renal transport of uric acid due to the treatments
with both diuretics. As indicated in Fig. 2, the two drugs gave similar results in the clearance experiment. The inulin clearance was lower and the plasma uric acid level was higher in the diuretic-treated animals compared to the control. The urine-excreted amount, the clearance and the fractional excretion of uric acid were significantly lower in the drug-treated animals.

Effect of polyethylene glycol-induced volume contraction on renal handling of uric acid: The acute effect of contraction of the body fluid on renal transport of uric acid was studied using rats which had received an intraperitoneal administration of polyethylene glycol. Fluid loss into the peritoneal cavity produced by polyethylene glycol resulted in a marked rise of the hematocrit at 3 hr after the administration, from 47.8±0.5% in the nontreated group to 55.4±0.7% (P<0.01). In the clearance experiment, the inulin clearance and the urine-excreted amount, the clearance and the fractional excretion of uric acid were lower, and the plasma uric acid...
level was higher in volume-contracted animals compared to the nontreated group (Fig. 3).

**Effect of saline loading on the decrease of uric acid excretion induced by trichlormethiazide:** The correction of body fluid volume by saline loading was performed using rats with marked diuresis produced by treatment with trichlormethiazide. Volume restoration to the control level at 2 hr after saline loading was indirectly evidenced by the decrease in the hematocrit from 50.5±0.7% in the trichlormethiazide-treated group to 47.6±0.5% (P<0.01) in the group that received saline loading. The hematocrit of the control group was 47.2±0.4%. In the clearance experiment, the decreases of the inulin clearance, the urine-excreted amount of uric acid, the uric acid clearance and the fractional excretion of uric acid by trichlormethiazide treatment returned to the control levels in the animals loaded with saline. The increase of the plasma uric acid level by trichlormethiazide treatment was also prevented by saline loading (Fig. 4).

**Pyrazinoic acid-suppressible fractional excretion of uric acid under treatment with diuretics:** The dose-response relationship between the decrease in fractional excretion of uric acid induced by pyrazinoic acid and its dose was determined in the control rats (Fig. 5). Pyrazinoic acid was administered intravenously just after the first urine collection. The fractional excretion of uric acid decreased temporarily at the 10 mg/kg dose and rapidly returned to the pre-treatment level. Doses over 25 mg/kg resulted in a lasting decrease in the fractional excretion of uric acid. The maximum response was
observed at 50 mg/kg. The decrease in the fractional excretion of uric acid in rats treated with 100 mg/kg was similar to that in the animals treated with 50 mg/kg.

Figure 6 shows the result of the study to assess the pyrazinoic acid-suppressible and -nonsuppressible components in the fractional excretion of uric acid in the diuretic-treated animals. As previously stated, the treatments with furosemide and trichlormethiazide significantly decreased the fractional excretion of uric acid. This excretion in diuretic-treated rats responded well to pyrazinoic acid, and the decrease in the excretion was almost parallel to that in control animals. Pyrazinoic acid-suppressible fractional excretion of uric acid was 0.25±0.02, 0.21±0.03 (P>0.05) and 0.19±0.02 (P>0.05) in the control, furosemide-treated and trichlormethiazide-treated rats, respectively.

Discussion

While net reabsorption of uric acid in the rat nephron has been described by many investigators, the value of fractional excretion of uric acid varies from 0.1 to 0.9 (8–11, 20–22). The value in the present study was approximately 0.4, and it responded reasonably to sodium probenecid and pyrazinoic acid, known to be uricosuric and antiuricosuric in man, respectively. Thus, we concluded that the rat kidneys used in the present clearance study have considerably greater bidirectional fluxes in regard to the transtubular uric acid transport. On the basis of the effectiveness of this experimental design, we clarified the main subject of diuretic-induced decrease of uric acid excretion.

Uric acid retention accompanied by diuretic therapy is presumed to result either from diminished secretion or accelerated reabsorption of uric acid in the renal tubules (2–6). This change in renal handling of uric acid is known to be closely associated with diuretic-induced extracellular fluid volume contraction (4–6). A close relationship between the state of hydration in the extracellular fluid volume and the renal transport of uric acid in rats was also noted by Weinman et al. (20). Thus, highly potent doses of furosemide and trichlormethiazide were given to the rat to rapidly change the body fluid volume. As seen in Table 1, the increase of plasma protein level together with the elevated hematocrit value obviously reflects the hemoconcentration, and the changes of creatinine, urea-nitrogen and chloride levels in plasma suggest obstructions in renal excretory capacity and electrolyte balance. In the clearance study, inulin clearance, urine-excreted amount of uric acid and uric acid clearance decreased, while the level of plasma uric acid increased in diuretic-treated rats compared to the control group. Moreover, the fractional excretion of uric acid fell in the experimental animals due to secretory inhibition and/or reabsorptive enhancement.

Further experiments were performed to investigate the mechanism of diuretic-induced changes in renal handling of uric acid. By employing polyethylene glycol, often used to produce volume-depleted animals (23, 24), in the clearance study, we were able to confirm the importance of the extracellular fluid volume contraction on both the reduction in the filtration rate and the transtubular transport for uric acid. Moreover, the restoration of uric acid excretory capacity in the animals loaded with saline after trichlormethiazide treatment suggests that the
diuretic-induced uric acid retention might be triggered by the volume contraction. The mechanism underlying this process is not entirely clear but several hypotheses have been clinically proposed (3, 5, 6).

Pyrazinoic acid is the active metabolite of pyrazinamide that inhibits the renal tubular secretion of uric acid (12, 25). We utilized pyrazinoic acid to dissect out the contribution of secreted uric acid to urinary excretion of uric acid. The treatment with pyrazinoic acid decreased the fractional excretion of uric acid according to its dose, and the maximum effect was attained at the dose of 50 mg/kg. Moreover, the response to pyrazinoic acid was almost intact in rats in which the fractional excretion of uric acid had already been diminished by treatment with furosemide and trichlormethiazide. Therefore, the animals treated with both diuretics seem to have an accelerated reabsorptive capacity of uric acid.

In most mammalian species, except man and certain nonhuman primates, hepatic urate oxidase degrades most of the uric acid formed into allantoin which is easily excreted from the kidney in the normal state. However, in spite of this species difference in uric acid metabolism, we were able to elucidate the characteristics of test diuretics acting on uric acid retention even in the experiments with rats. Moreover, the findings of the present study have a similar sequence of events to that proposed for clinically detectable uric acid retention induced by diuretics (4, 6).

Further investigation is required on the influence of chronically administered diuretics on the handling of uric acid in the rat kidney because hyperuricemia is a frequent side effect of prolonged treatment with conventional diuretic antihypertensives.

References
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