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Research Article

Urinary Excretion and Diuretic Action of Furosemide in Rats: Increased Response to the Urinary Excretion Rate of Furosemide in Rats with Acute Renal Failure

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A urinary excretion–response curve representing the urinary excretion rate of furosemide versus the urinary excretion rate of ($Na^+ + K^+$) was used to analyze furosemide action in rats with uranyl nitrate-induced acute renal failure (ARF) with and without dopamine coadministration. Urinary excretion of furosemide, but not its serum concentration, was the determinant for the diuretic action of furosemide. Increased diuretic response was observed in ARF rats, although the total diuretic response and urinary recovery of furosemide within 2 hr decreased. Dopamine enhanced furosemide-induced diuresis in ARF rats in terms of the total urine output and urinary electrolyte excretion, although the urinary excretion–response curves were not different. This enhancement by dopamine was found to be caused by the augmented urinary excretion of furosemide and the increased response to this drug in ARF rats. These findings suggest the contribution of decreased concentrating ability along the nephron and/or increased sensitivity of cells at the site of action to this drug.

KEY WORDS: furosemide; acute renal failure; increased response; urinary excretion of furosemide; dopamine.

INTRODUCTION

Furosemide, one of the most potent loop diuretics, has been used clinically for patients with increased extracellular fluid, congestive heart failure, liver cirrhosis, and renal failure. However, the diuretic action of furosemide in such patients is frequently decreased. For example, patients with congestive heart failure or liver cirrhosis showed reduced maximal responses to furosemide (1–3), and those with renal failure do not fully respond to the drug (4,5). Among these diseases, the prognosis of acute renal failure (ARF)[4] is influenced directly by the response to diuretics. Therefore, large doses of furosemide or coadministration of furosemide and other diuretics has been tried, but many patients have responded poorly. Thus, some patients have required dialysis.

It is thought that the pharmacological action of loop diuretics involves inhibition of the reabsorption process of $Na^+/K^+/2Cl^-$ at the ascending limb of Henle's loop (6–9). However, details of this action are not yet clear. Individual differences of diuretic action vary widely and the extent of effect differs among reports (5,10–12), because the response is attenuated by physiological conditions in patients or experimental animals.

In the preceding paper, we proposed a method for analyzing the diuretic action of furosemide using the urinary excretion rate of ($Na^+ + K^+$) in rats (13). In this paper, urinary excretion and diuretic activity of furosemide were investigated in normal and uranyl nitrate-induced ARF rats. The effects of dopamine on furosemide-induced diuresis were also evaluated in these animals using a furosemide excretion–response curve.

MATERIALS AND METHODS

Animal Experiment for Diuretics

Male Wistar rats weighing 220–260 g were treated as described previously (13). Briefly, under urethane anesthesia (1 g/kg), the urinary bladder and right jugular vein were cannulated for sample collection and drug administration. After surgery, infusion (2.3 ml/hr) of inulin in 5% (w/v) glucose solution via the jugular vein cannula was started and continued throughout the experiment. After control periods, furosemide was injected via the jugular vein cannula at doses of 5, 10, or 20 mg/kg. Urine samples were collected periodically for 2 or 2.5 hr after furosemide injection. At the end of the experiment, a blood sample was collected via the abdominal aorta. In some animals, the carotid artery was

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[4] Abbreviations used: ARF, acute renal failure; AUC, area under the concentration–time curve; GFR, glomerular filtration rate; HPLC, high-performance liquid chromatography; MRT, mean residence time; RPF, renal plasma flow; UFR, urine flow rate; UV_{FM}, urinary excretion rate on furosemide; UV_{Na+K}, urinary excretion rate of ($Na^+ + K^+$).

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also cannulated by polyethylene tubing to take blood samples periodically. The serum was separated from blood. The volume of the urine sample was determined by weight. In the case of dopamine coadministration, dopamine was dissolved in infusate and administered at the rate of 3 μg/min/kg. Urine and serum samples were shielded and stored at 4°C until assay.

The glomerular filtration rate (GFR) was calculated using a serum sample and the last urine sample. Data are expressed as the mean ± SE and a statistical test was performed using the one-tailed or two-tailed t test with a significance level of $P < 0.05$.

Analytical Methods

For the assay of furosemide concentrations in urine and serum, samples were diluted with 2 vol of pH 5.0 phosphate buffer and 6 vol of methanol. The mixture was centrifuged and the supernatant was assayed by high-performance liquid chromatography (HPLC) (Trirotar III, JASCO, Tokyo). A C_{18} reverse-phase column (Chemcosorb 5-ODS-H, 150 × 4.6 mm, Chemco Co., Osaka, Japan) was used for the separation of furosemide, with a mobile phase of 0.01 M sodium acetate:methanol = 60:40, 1 ml/min. Furosemide was detected by UV absorption at 280 nm (Uvidec-100-III UV spectrophotometer, JASCO, Tokyo). The concentration of furosemide was calculated by the peak height using a calibration curve. For pharmacokinetic analysis, the area under the serum concentration–time curve (AUC) was calculated by the trapezoidal integration. The mean residence time (MRT) of furosemide was also calculated by the trapezoidal integration accoring to the following equation: $MRT = \int_0^T t * C_s dt / \int_0^T C_s dt$, where C_s, t, and T are the serum concentration of furosemide, time, and duration of the experiment, respectively. Total-body clearance (CL_{TB}) was estimated by dose/AUC and renal clearance (CL_R) was calculated from the urinary recovery of furosemide multiplied by CL_{TB}. Then nonrenal clearance (CL_{NR}) was obtained from $CL_{TB} - CL_R$.

Urinary and serum concentrations of Na^+, K^+, and Cl^- were determined using an ion meter (F-8AT, Horiba Ltd., Kyoto, Japan) with ion-specific electrodes (Na^+-, K^+-, or Cl^--specific electrodes for Sera-100, Horiba Ltd., Kyoto, Japan). Inulin concentrations in urine and serum were measured by the modified method of Dische and Borenfreund (14).

Materials

Furosemide was supplied by Hoechst Japan Ltd. (Kawagoe, Japan) and was used as the standard for the HPLC assay. For animal experiments, furosemide injection (Lasix, Hoechst Japan Ltd., Kawagoe) and dopamine hydrochloride injection (Inovan, Kyowa Hakko Co., Tokyo) were used. Inulin was purchased from American Hoechst Co. (La Jolla, Calif.). All other chemicals were of reagent grade.

RESULTS

Urinary Excretion of Furosemide and Urine Flow Rate

Time courses of the serum concentration of furosemide, urinary excretion rate of furosemide (UV_{FM}), and urine flow rate (UFR) after a 10-mg/kg injection of furosemide were investigated in both normal and uranyl nitrate-induced ARF rats. Serum concentrations were changed slightly in ARF rats compared with normal rats but were not significantly different at any time point ($P > 0.05$) (Fig. 1). AUC, CL_{TB}, CL_{NR}, and MRT in both groups also did not differ significantly (Table I). However, UV_{FM} at each time point and CL_R were decreased markedly in ARF rats ($P < 0.01$) (Fig. 1 and Table I). In the same rats, the urine output was also reduced significantly ($P < 0.01$) (Table I). In the case of different doses of furosemide (5, 10, and 20 mg/kg) in normal rats, the time course of the logarithmic plot of the UV_{FM} showed a pattern similar to that of the normal scale plot of the diuretic response expressed by the urinary excretion rate of ($Na^+ + K^+$) (UV_{Na+K}) in each dose (Fig. 2). The urinary recoveries of furosemide did not differ significantly at all three doses (38.0 ± 3.2, 30.7 ± 5.4, and 44.7 ± 5.5% of the dose for 5, 10, and 20 mg/kg, respectively; $P > 0.05$).

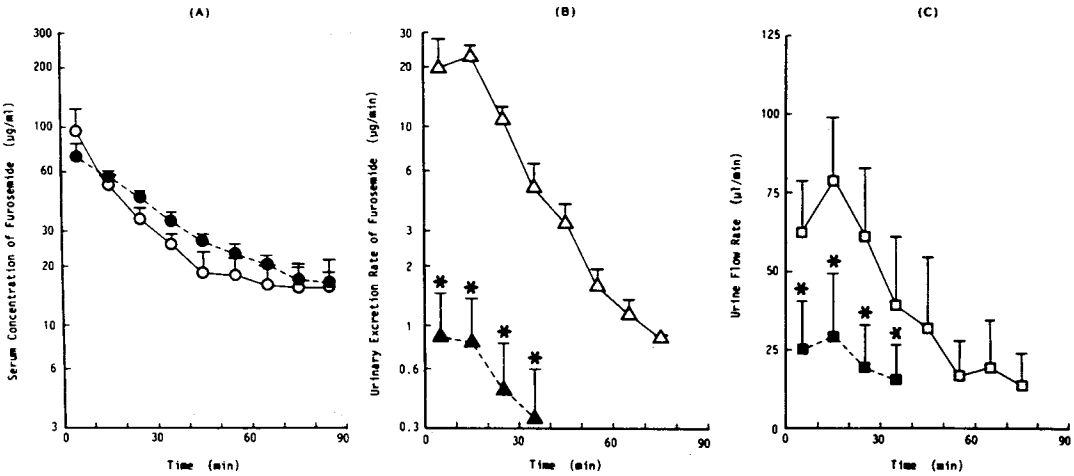

Fig. 1. Time courses of (A) serum concentration of furosemide, (B) urinary excretion rates of furosemide, and (C) urine flow rates in normal and acute renal failure (ARF) rats. Furosemide was injected at a dose of 10 mg/kg. Points and bars represent the mean values ± SE of six animals. Open and filled symbols denote the normal and ARF rat values, respectively. (*) Significantly different from normal rats ($P < 0.01$).

Table I. Pharmacokinetics and Diuretic Action of Furosemide After a 10-mg/kg Injection in Normal and Acute Renal Failure Rats

	Normal rats	ARF[a] rats
Body weight (g)	265 ± 1	236 ± 5**
AUC[b] (mg * min/ml)	2.57 ± 0.57	2.80 ± 0.13
CL_{TB}[c] (ml/min/kg)	4.34 ± 1.04	3.61 ± 0.38
CL_R[d] (ml/min/kg)	1.08 ± 0.46	0.03 ± 0.04**
CL_{NR}[e] (ml/min/kg)	3.25 ± 0.64	3.58 ± 0.18
MRT[f] (min)	28.8 ± 1.5	31.5 ± 2.3
Recovery[g] (%)	23.6 ± 5.1	0.8 ± 0.6**
Urine output (ml)	3.23 ± 0.87	0.79 ± 0.61*

[a] Acute renal failure.
[b] Area under the concentration curve.
[c] Total-body clearance.
[d] Renal clearance.
[e] Nonrenal clearance.
[f] Mean residence time.
[g] Urinary recovery of furosemide (% of dose).
* Significantly different from normal rats ($P < 0.05$).
** Significantly different from normal rats ($P < 0.01$).

Effect of Acute Renal Failure and Dopamine on Furosemide-Induced Diuresis

The urinary recoveries of furosemide, UFR, and UV_{Na+K} for 2 hr after a 10-mg/kg injection of furosemide were compared in normal and ARF rats with or without dopamine infusion. Data shown in Fig. 3 are expressed as a percentage of the values in normal rats without dopamine. Urinary recovery of furosemide decreased markedly in ARF rats without dopamine infusion ($P < 0.001$). Although UFR and UV_{Na+K} also decreased in ARF rats ($P < 0.01$), the reductions were not proportional to the furosemide recovery (Fig. 3). This finding suggests that the response to furosemide in ARF rats is different from that in normal rats.

With dopamine infusion, the urinary recovery of furosemide increased in normal rats (30.7 ± 5.4% of the dose without dopamine and 43.8 ± 2.1% of the dose with dopamine; $P < 0.05$). The increase in urinary recovery of furosemide was small in ARF rats (1.2 ± 0.4% of the dose without dompanine and 1.9 ± 0.1% of the dose with dopamine; $P < 0.05$), but those of UFR and UV_{Na+K} were marked ($P < 0.01$) (Fig. 3). In this experiment, the discrepancy between urinary recovery and diuretic response was again evident in ARF rats. On the other hand, these increases were essentially proportional in normal rats. Figure 3 also shows the changes of GFR in these experiments. The GFR in normal rats with dopamine infusion was not changed. However, the GFR in ARF rats without dopamine was reduced markedly ($P < 0.01$) and it recovered significantly with dopamine infusion in those animals.

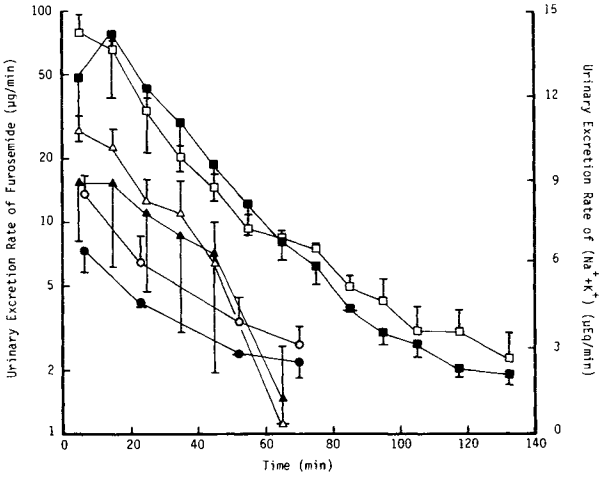

Fig. 2. Time courses of urinary excretion rates of furosemide and ($Na^+ + K^+$) in normal rats. Furosemide was injected at a dose of 5 (○, ●), 10 (△, ▲), and 20 (□, ■) mg/kg. Points and bars represent the mean values ± SE of three to five animals. Open symbols denote furosemide excretion rates. Filled symbols denote ($Na^+ + K^+$) excretion rates.

Urinary Excretion–Response Curve in Normal and Acute Renal Failure Rats

Diuretic response, UV_{Na+K}, was plotted against UV_{FM} to evaluate the properties of the response in normal and ARF rats. In normal rats, response curves for three different doses of furosemide were almost the same (Fig. 4). No saturation was observed in these dosing ranges. In uranyl nitrate-induced ARF rats, the plots shifted markedly to the left (Fig. 4). That is, the diuretic response to UV_{FM} was increased in ARF rats compared with that in normal rats.

On the other hand, the coadministration of dopamine with furosemide did not change the apparent sensitivity of the diuretic response in either normal or ARF rats (Fig. 4). Only corresponding increases in UV_{FM} and UV_{Na+K} were observed, even in ARF rats.

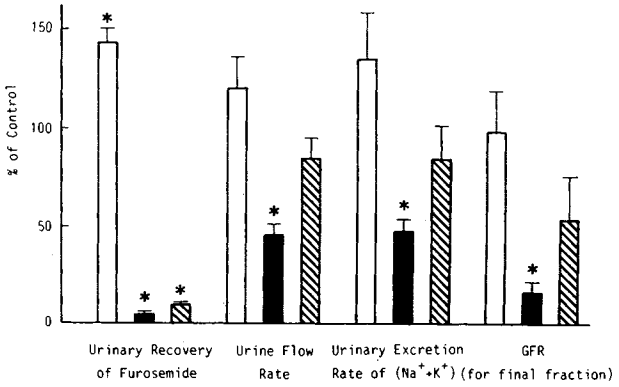

Fig. 3. Effects of dopamine infusion on diuresis for furosemide in normal and acute renal failure (ARF) rats. Urine flow rate and urinary excretion rate of ($Na^+ + K^+$) were calculated as the mean value during the experiment. Glomerular filtration rate (GFR) was estimated from the final urine sample. Furosemide was injected at a dose of 10 mg/kg with or without dopamine infusion. Data are shown as a percentage of the values in normal rats without dopamine infusion. The values in those rats ($N = 5$; mean ± SE) are as follows: urinary recovery of furosemide, 30.7 ± 5.4% of the dose; urine flow rate, 2.24 ± 0.75 ml/hr; urinary excretion rate of ($Na^+ + K^+$), 232 ± 93 μEq/hr; and GFR, 1.00 ± 0.18 ml/min. Columns and bars denote the mean values ± SE in normal rats with dopamine ($N = 6$; □), ARF rats without dopamine ($N = 5$; ■), and ARF rats with dopamine ($N = 5$; ▨). (*) Significantly different from control rats by one-tailed t test ($P < 0.05$).

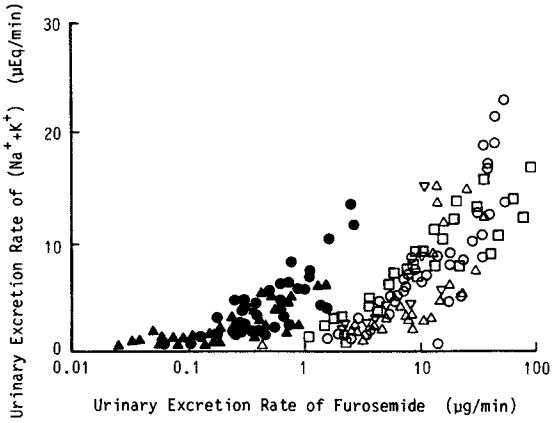

Fig. 4. Furosemide excretion–response curve in normal and acute renal failure (ARF) rats. Open symbols denote normal rat data and filled symbols denote ARF rat data. Furosemide was injected at doses of 5 mg/kg (N = 3, ▽), 10 mg/kg (N = 5, △; N = 5, ▲), 20 mg/kg (N = 3, □), or 10 mg/kg with dopamine (N = 6, ○; N = 5, ●).

DISCUSSION

Most urinary furosemide is delivered by renal tubular secretion, because this drug has high protein binding (91–99% bound) and its glomerular filtration rate is very low for this reason (15–17). The secreted furosemide acts on tubular cells from the luminal side at the ascending limb of Henle's loop, inhibits the reabsorption of $Na^+/K^+/2Cl^-$, and then induces diuresis (6–9). In the present experiment, both the pharmacological activity of furosemide and the urinary excretion of this drug in ARF rats decreased markedly, while the serum concentration of furosemide was not altered significantly (Fig. 1 and Table I). Therefore, the diuretic response to furosemide in ARF rats seems to be explained well with the same urinary excretion rate of this drug as in normal rats (18,19), rather than with its concentration in serum in rats. This is reasonable when the site of action of this agent is considered (6–9). Thus, the urinary excretion rate of furosemide was considered to be the better reflector for the concentration at the action site of this drug than the serum furosemide concentration, and a blood sample was withdrawn at the end of the experiment in all other experiments. Although the AUC increased only 9% in ARF rats compared with normal rats, a 17% decrease in the mean CL_{TB} was observed in ARF rats (Table I). This reduction corresponds to that of the mean urinary recovery of furosemide from 23.6% of the dose in normal rats to 0.8% of the dose in ARF rats. The discrepancy between the AUC and the CL_{TB} may be explained by the change of other factors such as the plasma protein binding, which was not determined in the present study.

Dose-dependent pharmacokinetics and pharmacodynamics of furosemide were observed in rats at relatively high doses of the drug (20). If the renal tubular secretion is saturated or if the maximal response is achieved, then dose dependency occurs. In the present study, however, urinary recoveries of furosemide were proportional to doses up to 20 mg/kg within 2 hr after the injection. When the diuretic response was plotted against the logarithmic scale of UV_{FM}, response curves for three different doses of the drug did not show the maximal response (Fig. 4). Although interindividual variations in response curves were observed, the plots were almost identical for the three doses in normal rats.

It also has been reported that furosemide-induced diuresis is decreased in patients with renal failure (4). In particular, patients with ARF sometimes do not respond to this agent. However, we observed an increased response in ARF rats when the urinary excretion–response curve was used for the analysis (Fig. 4). Although the total urine output decreased in ARF rats, this reduction was less marked than the diminished urinary recovery of furosemide (Fig. 3). This is an interesting observation, because a reduced response is expected in ARF rats.

It was reported that the diuretic treatment of furosemide with a low-dose infusion of dopamine was effective in patients with ARF (21). Thus, the mechanism by which dopamine enhances the effects of furosemide has been extensively investigated (22–25). Furthermore, for dopamine direct inhibition of sodium reabsorption and increased GFR mediated by increased renal plasma flow (RPF) were reported (26,27). In our previous paper (13), dopamine had no effect on the relationships between the urinary excretion of electrolytes and that of water. In the present experiment, coadministered dopamine slightly increased the urinary recovery of furosemide, but it markedly augmented the urine output in ARF rats (Fig. 3). This increased urine output coincides with that observed in patients with ARF (21–25). As for the urinary excretion–response curve, both UV_{FM} and UV_{Na+K} were increased with dopamine infusion (Fig. 4). Therefore, the synergic effect of coadministrated dopamine could be explained as follows: dopamine increases the RPF, which augments GFR (Fig. 3). The increased RPF mediates the increase of renal excretion of furosemide, and then the diuretic action of excreted furosemide is amplified by the increased response. Thus, the increase in response is greater than the furosemide recovery in ARF rats.

The increased response in ARF rats after furosemide injection with or without dopamine may be due to a decreased concentrating ability in the latter part of nephron. Decreased concentrating ability and increased fractional excretion of Na^+ have been observed in uranyl nitrate-treated ARF rats (28,29). Therefore, if the concentrating ability is decreased from the distal tubule to the collecting duct in the ARF rat, the electrolytes and water would be excreted in larger amounts than in normal rats. Another possibility is that this agent has a greater effect on functionally impaired tubules, since this agent inhibits the normal physiological function.

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