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Research Article

A Quantitative Method of Evaluating the Diuretic Response to Furosemide in Rats

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Furosemide effects are usually evaluated by measuring the urinary excretion rate of Na^+ (UV_{Na}) in humans. In the present study, however, UV_{Na} showed a nonlinear relationship with urine flow rate after intravenous injection of furosemide in rats. In contrast, when the urinary excretion rate of ($Na^+ + K^+$) (UV_{Na+K}) was plotted against the urine flow rate, a linear regression line was observed, with small interindividual variations in normal rats and in rats with uranyl nitrate-induced acute renal failure (ARF). Piretanide, a loop diuretic, also showed a similar relationship, while other types of diuretics revealed different slope values for the relationship. Although the urinary excretion rate of Cl^- (UV_{Cl}) vs UV_{Na+K} is expected to show a linear relationship in normal rats, the correlation coefficient of the linear regression line was smaller than that of the urine flow rate vs UV_{Na+K}. Further, the slope of UV_{Cl} vs UV_{Na+K} was slightly different in ARF rats. Therefore, UV_{Na+K} provides a better quantitative measure of diuretic response to loop diuretics than UV_{Na} or UV_{Cl}.

KEY WORDS: furosemide; diuresis; sodium excretion; potassium excretion; chloride excretion; urinary excretion rate; urinary electrolytes in rats.

INTRODUCTION

Loop diuretics possess the highest potency of various diuretics. Furosemide is used frequently as a typical loop diuretic in the clinical management of edematous states, particularly for patients with impaired renal function (1,2). However, a diuretic response to furosemide is sometimes decreased in those patients, accompanied by large interindividual variations (3–10). Factors involved in these clinical problems have not yet been elucidated.

The potency of furosemide action in humans is usually measured by the urinary excretion of Na^+ instead of by the urine volume to distinguish the response from water diuresis. This index is usually applied to experimental animals including rats (11–13). However, larger amounts of K^+ are excreted by rats than by humans. Urinary excretion of K^+ is also increased by furosemide. Therefore, it is questionable to omit K^+ excretion from consideration.

To analyze the diuretic response to furosemide, we systematically investigated the relationships between urinary excretion rates of electrolytes and water in normal and acute renal failure (ARF)[4] rats. We propose a method of evaluating the diuretic action of furosemide using the urinary excretion rate of ($Na^+ + K^+$).

MATERIALS AND METHODS

Animal Experiment for Diuretics

Male Wistar rats weighing 220–260 g were anesthetized by intraperitoneal injection of urethane [50% (w/v) in saline, 2 ml/kg] to avoid fluctuation of blood pressure during the experiment. The urinary bladder was exposed via abdominal midline incision and cannulated by polyethylene tubing (Intermedic PE-50, Becton Dickson and Co., Parsippany, N.J.) to collect urine samples. The right jugular vein was also cannulated with the same size tubing to infuse or inject solutions. After the wounds were closed by surgical suture, 20 mg/ml of inulin in 5% (w/v) glucose solution was injected via the jugular vein cannula at a volume of 3 ml/kg, followed by 0.2 ml of the glucose solution. Eight milligrams per milliliter of inulin in the glucose solution was infused at the rate of 2.3 ml/hr during the course of the experiment to maintain the water balance. After two consecutive 30-min urine samplings for control periods, furosemide was injected via the jugular vein cannula at a dose of 10 mg/kg. Additional injections of small volumes of glucose solution followed. In some experiments, furosemide was replaced by piretanide (10 mg/kg), mannitol (400 mg/kg), or acetazolamide (20 mg/kg). In the case of dopamine coadministration, dompanine was dissolved in the infusate and administered at the rate of 3 μg/min/kg. After the injection of diuretics, urine samples were collected periodically for 2 or 2.5 hr. At the end of the

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[4] Abbreviations used: ARF, acute renal failure; GFR, glomerular filtration rate; UFR, urine flow rate; UV_{Cl}, urinary excretion rate of Cl^-; UV_K, urinary excretion rate of K^+; UV_{Na}, urinary excretion rate of Na^+; UV_{Na+K}, urinary excretion rate of ($Na^+ + K^+$).

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experiment, a blood sample was withdrawn via the abdominal aorta, and serum was separated. The volume of the urine sample was measured by weight, as the specific gravity of urine equaled 1.0. 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 two-tailed t test.

Acute Renal Failure Animals

Experimental acute renal failure was induced by uranyl nitrate (10 mg/kg, subcutaneous injection). Standard purine chow with free tap water was provided. The urine collection was started 48 hr after the injection. Conditions of the animal were monitored by the measurement of blood urea nitrogen and serum creatinine concentrations.

Analytical Methods

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). Blood urea nitrogen and serum creatinine were assayed using an automatic assay system, Clinalyzer (JCA-SIM6R, Nihon Denshi Co. Ltd., Tokyo).

Materials

Furosemide injection (Lasix, Hoechst Japan Ltd., Kawagoe), piretanide injection (Arelix, Hoechst Japan Ltd., Kawagoe), dopamine hydrochloride injection (Inovan, Kyowa Hakko Co., Tokyo), and sodium acetazolamide injection (Diamox, Lederle Japan Ltd., Tokyo) were used for animal experiments. Inulin was purchased from American Hoechst Co. (La Jolla, Calif.). All other chemicals were of reagent grade.

RESULTS

Urine Flow Rate and Urinary Excretion Rate of Electrolytes After Furosemide Injection in Normal Rats

The relationship between urine flow rate (UFR) and urinary excretion rate of Na^+ (UV_{Na}) after a 10-mg/kg dose of furosemide showed a nonlinear pattern (Fig. 1A). In the same animals, the urinary excretion rate of K^+ (UV_K) vs UV_{Na} also exhibited a saturable nonlinear pattern (Fig. 1B). During these experiments, urinary excretion rates of inulin were almost constant except for the first period after furosemide injection. In preliminary experiments under the same conditions, serum concentrations of Na^+, K^+, and Cl^- were not altered before and after a furosemide injection. When the urinary excretion rate of ($Na^+ + K^+$) (UV_{Na+K}) instead of UV_{Na} was used, a good linear relationship was obtained (Fig. 2A). A regression line of these plots produced a very high correlation coefficient (Table I). The urinary excretion rate of Cl^- (UV_{Cl}) vs UV_{Na+K} would be expected to show a good linear relationship in terms of the action mechanism of furosemide (15–19), but a rather large scattering of data points was observed (Fig. 2B). The linear regression line of this relationship had a poor correlation compared with that of UFR vs UV_{Na+K} (Table I). UFR vs UV_{Cl} plots also represented larger variations and a poor correlation (data not shown).

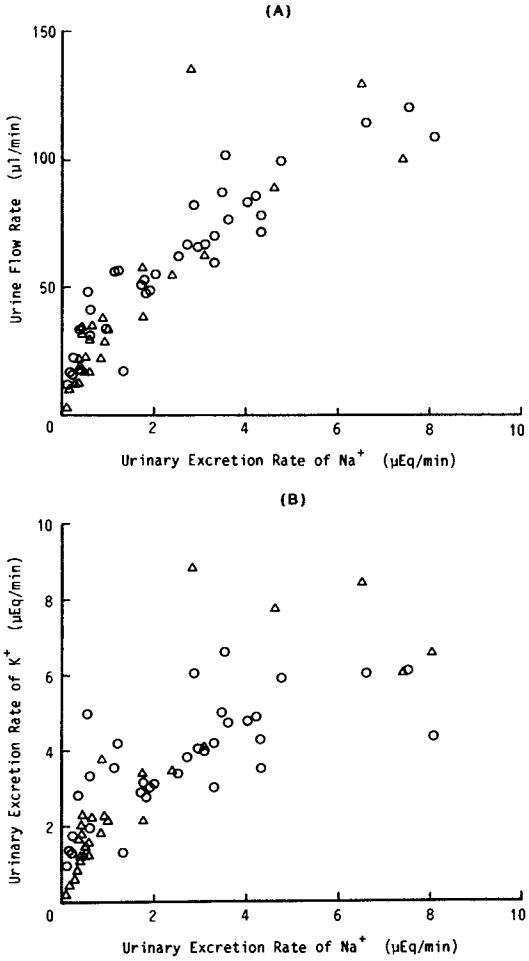

Fig. 1. Urinary excretion rate of Na^+ versus (A) urine flow rate and (B) urinary excretion rate of K^+ after furosemide injection in normal rats. Circles represent furosemide injection alone. Triangles represent furosemide injection with dopamine infusion. Each point represents the data obtained from five rats for furosemide alone and six rats for furosemide with dopamine, with 5–10 sampling periods for each animal.

Urine Flow Rate and Urinary Excretion Rate of the Sum of Sodium and Potassium After the Injection of Various Diuretics in Normal Rats

The relationships between UFR and UV_{Na+K} after the injection of other diuretics were compared with furosemide. Piretanide, a loop diuretic, showed a similar pattern and essentially the same regression slope to furosemide (Fig. 3 and Table I); the difference in the slope value was not significant ($P > 0.05$). However, mannitol, an osmotic diuretic, showed a very steep slope, while acetazolamide, a carbonic anhydrase inhibitor, yielded a gentler slope than that of furosemide (Fig. 3). The slope values of the linear regression line of the latter two drugs were significantly different from those of the loop diuretics ($P < 0.01$).

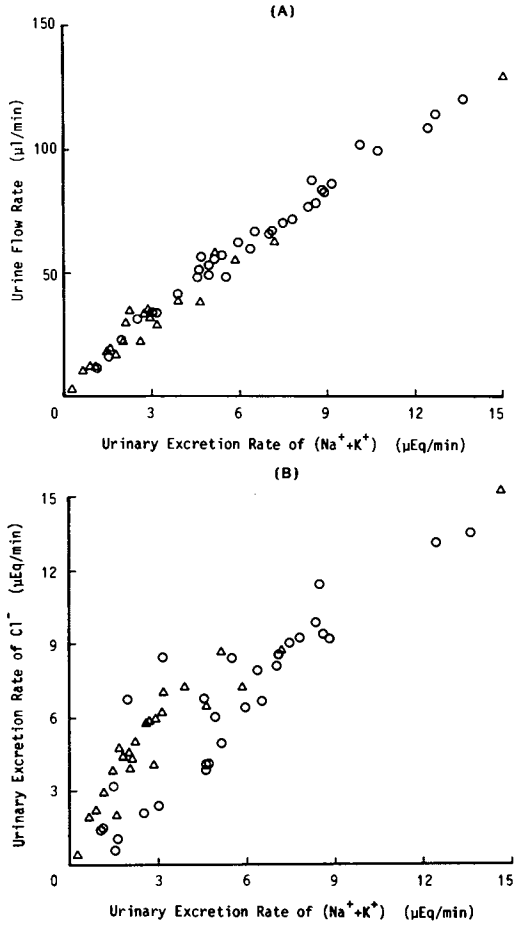

Fig. 2. Urinary excretion rate of $(Na^+ + K^+)$ versus (A) urine flow rate and (B) urinary excretion rate of Cl^- after furosemide injection in normal rats. Circles represent furosemide injection alone. Triangles represent furosemide injection with dopamine infusion. Each point represents the data obtained from five rats for furosemide alone and six rats for furosemide with dopamine, with 5–10 sampling periods for each animal.

Effect of Acute Renal Failure on Urinary Excretion of Water and Electrolytes After Furosemide Injection

The relationships between UV_{Na+K} and either UFR, UV_{Cl}, or UV_K are shown in Fig. 4 in uranyl nitrate-induced ARF rats and normal rats. The relationship between UV_{Na+K} and UFR was similar to that in normal rats (Fig. 4A), although ARF rats showed a significantly smaller range of responses than normal rats [total urinary excretion of $(Na^+ + K^+)$, 204 ± 34 μEq ($N = 5$) in normal rats and 111 ± 13 μEq ($N = 6$) in ARF rats; $P < 0.05$]. Interestingly, the regression lines were almost identical between normal and ARF rats (Table I). However, the relationship between UV_{Na+K} and UV_{Cl} differed slightly between ARF rats and normal rats, but not significantly (Fig. 4B and Table I). When UV_{Na+K} vs UV_K was plotted for normal and ARF rats, a decrease in UV_K in ARF rats was observed (Fig. 4C). The ratio of total urinary K^+ excretion vs $(Na^+ + K^+)$ excretion in normal rats (0.70 ± 0.04; $N = 5$) was significantly different from that in ARF rats (0.51 ± 0.05; $N = 6$) ($P < 0.05$).

Effect of Dopamine Coadminstration on Urinary Excretion of Water and Electrolytes After Furosemide Injection in Normal and Acute Renal Failure Rats

During the infusion of dopamine, no changes were detected in the relationships between URF and UV_{Na+K} in normal and uranyl nitrate-induced ARF rats (Figs. 2 and 4 and Table I), although total urine output and electrolyte excretions were increased by dopamine infusion in both normal and ARF rats, which was observed from the shift of plots in Fig. 4. Relationships between urinary excretion rates of water and electrolytes also were not affected by dopamine infusion in any groups.

DISCUSSION

Furosemide is known as a potent natriuretic agent (1,2). UV_{Na} is usually employed as an index of furosemide-induced diuresis (3–10). This is a good index in humans, because UFR shows an essentially linear relationship with UV_{Na}. In rats, many studies have also used UV_{Na} as an index of furosemide action (11–13). However, as shown in Fig. 1, UFR did not show a linear relationship with UV_{Na} in rats. This finding indicates that UV_{Na} does not quantitatively reflect a diuretic response to furosemide in rats. Some researchers have used the ratio of urinary concentrations or urinary excretion rates of Na^+ to K^+ (20,21). Judging from the present result (Fig. 1), however, this ratio would also show a nonlinear relationship. Therefore, we investigated other indices for the analysis of furosemide diuresis in rats. To provide the precise analysis, the experiment was performed with the standard diet, appropriate water supplement, and optimum furosemide dose. Under this condition, physiological factors such as serum electrolyte concentrations and GFR were not changed before and after a furosemide injection.

Table I. Linear Regression for the Relationships Between Urinary Excretion Rate of $(Na^+ + K^+)$ (X) and (A) Urine Flow Rate (Y) or (B) Urinary Excretion Rate of Cl^- (Y) in Normal and Acute Renal Failure (ARF) Rats After Furosemide Injection Alone or Furosemide Injection with Dopamine Infusion

	N^a	Regression line[b]	r^c
		A	
Normal rats			
Furosemide	5	$Y = 7.55X + 8.79$	0.977
+ Dopamine	6	$Y = 8.14X + 11.08$	0.973
ARF rats			
Furosemide	5	$Y = 8.32X + 5.95$	0.931
+ Dopamine	5	$Y = 7.34X + 13.41$	0.962
		B	
Normal rats			
Furosemide	5	$Y = 1.37X + 1.14$	0.969
+ Dopamine	6	$Y = 1.30X + 1.06$	0.719
ARF rats			
Furosemide	5	$Y = 1.13X - 0.64$	0.865
+ Dopamine	5	$Y = 1.00X - 0.81$	0.953

[a] Number of animals.
[b] All slope values of linear regression lines were not significantly different between the corresponding two groups.
[c] Correlation coefficient.

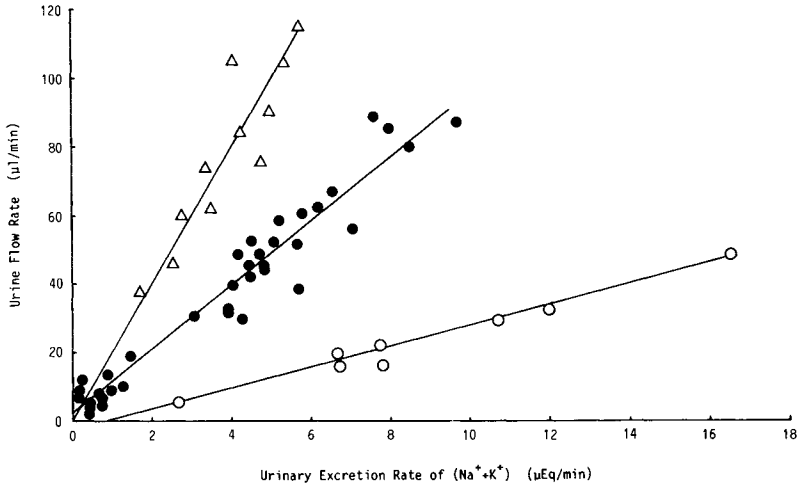

Fig. 3. Effects of diuretics on the relationship between urinary excretion rate of $(Na^+ + K^+)$ and urine flow rate in normal rats. The regression lines are as follows: piretanide ($N = 5$) (●)—$Y = 8.97X + 2.95$, $r = 0.908$; acetazolamide ($N = 3$) (○)—$Y = 2.01X - 3.09$, $r = 0.778$; and mannitol ($N = 3$) (△)—$Y = 19.88X + 0.02$, $r = 0.731$.

Loop diuretics inhibit the reabsorption of $Na^+/K^+/2Cl^-$ at the ascending limb of Henle's loop (15–19). In humans, K^+ excretion into urine is very low compared with Na^+ excretion during both nondiuretic and diuretic conditions (4,7,22). The contribution of K^+ can be ignored in evaluating the diuretic action, even if the loop diuretic is used. In rats, however, K^+ is excreted at a higher rate than Na^+ during nondiuretic conditions and at higher or similar rates during furosemide-induced diuresis. Consequently, K^+ excretion into urine cannot be ignored even in diuretic conditions in rats. From these considerations, the urinary excretion rate of $(Na^+ + K^+)$ was selected as an index of diuresis. This index, UV_{Na+K}, was related linearly to UFR, with a high correlation coefficient and very small interindividual variation. As shown in Fig. 1, nonlinear relationships of UFR vs UV_{Na} and UV_{Na} vs UV_K may be explained by an extensive and saturable exchange of Na^+ and K^+ from the late distal tubule to the collecting duct in the rat.

The relationship between UV_{Na+K} and UV_{Cl} was also investigated, because the Cl^- excretion has been used occasionally for the index of diuresis to furosemide (6). The pharmacological action of furosemide at the site of action is the inhibition of reabsorption of $Na^+:K^+:Cl^- = 1:1:2$ (15–19). Therefore, this slope value of the linear regression line would be equal to 1.0. The result in normal rats showed a slightly higher slope value than 1.0 but was not significantly different (Table I). However, scattering of the plots was noted and a smaller correlation coefficient was obtained compared with that of UFR vs UV_{Na+K}. The electrolyte composition would be attenuated along the tubule after the site of action. Thus, UV_{Na+K} and UV_{Cl} were also considered to be indices of diuretic response to furosemide in normal

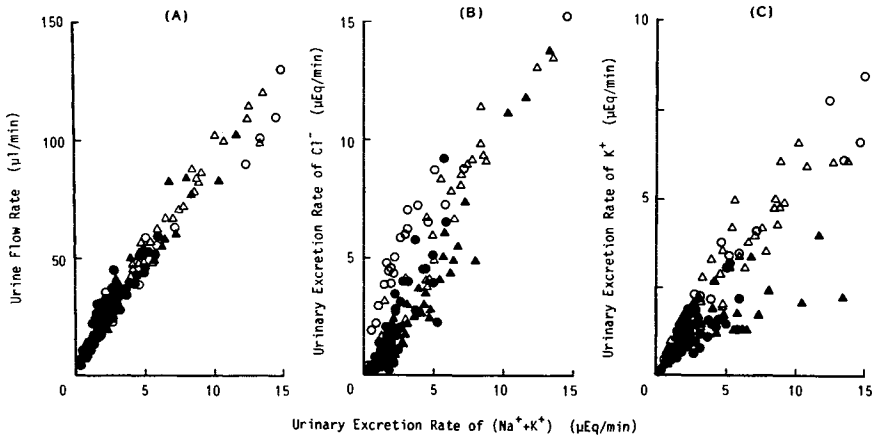

Fig. 4. Urinary excretion rates of electrolytes and water after furosemide injection in normal and acute renal failure (ARF) rats. Urinary excretion rates of $(Na^+ + K^+)$ are plotted against (A) urine flow rate, (B) urinary excretion rate of Cl^-, and (C) urinary excretion rate of K^+ in normal (open symbols) and ARF (filled symbols) rats after furosemide injection alone (circles) or furosemide injection with dopamine infusion (triangles). Each point represents the data obtained from five or six rats for each group, with 5–10 sampling periods for each animal.

rats, although UV_{Cl} showed a poor correlation to UFR compared with that of UV_{Na+K}.

The responses to other diuretics were also investigated using this index. Piretanide, a loop diuretic, showed a pattern of urinary excretions of water and electrolytes similar to that of furosemide. On the other hand, different types of diuretics showed a different response pattern (Fig. 3). An osmotic agent, mannitol, showed a very high slope value for the relationship between UFR and UV_{Na+K}. A carbonic anhydrase inhibitor, acetazolamide, showed a smaller slope value than that of furosemide. These differences of slopes reflect the different action mechanisms of these drugs (23).

The relationship between UFR and UV_{Na+K} in uranyl nitrate-induced ARF rats was similar to that in normal rats (Fig. 4), although the magnitude of the response was smaller in ARF rats. On the other hand, UV_K against UV_{Na} was decreased in ARF rats. The decreased UV_K in disease states against UV_{Na} suggests reduced saturable Na^+-K^+ exchange from the late distal tubule to the collecting duct. The slight change of UV_{Cl} may also be caused by the difference of reabsorption of water and electrolytes after the site of action of furosemide. For these reasons, UV_{Na+K} is thought to be the best index for quantitative evaluation of the diuretic action of furosemide in rats.

Dopamine is known to cause vasodilation of the renal vasculature, resulting in increased renal plasma flow and GFR (24–26). Graziani *et al.* (27) and Lindner (28) found that furosemide combined with a low-dose infusion of dopamine was an effective diuretic treatment in furosemide-resistant AFR patients. However, the mechanism involved in the increased diuretic action of furosemide with dopamine is not yet clear. In the present experiments, although the total urine output and electrolyte excretions were increased by dopamine infusion, we could not detect any differences in the relationships between the excretions of water and electrolytes in both normal and ARF rats. Consequently, the intrinsic natriuretic activity of dopamine (29,30), if any under our condition, may be negligible in comparison with that of furosemide.

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