

## INPUT RATE AS A MAJOR DETERMINANT OF FUROSEMIDE PHARMACODYNAMICS: INFLUENCE OF FLUID REPLACEMENT AND HYPOALBUMINEMIA

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### ABSTRACT:

To investigate how the response to a bolus and an infusion of furosemide is modulated by the rate of fluid replacement and by hypoalbuminemia, rabbits received 5 mg/kg of furosemide as a bolus or infused over 60 min, whereas diuresis was replaced with 13, 121, or 238 ml/h NaCl 0.9%/glucose 5% (50:50). Natriuretic and diuretic efficiencies were greater with the infusion than with the bolus of furosemide. Fluid replacement increased natriuretic and diuretic efficiency of furosemide bolus but only diuretic efficiency of furosemide infusion. Furosemide net fluid depletion reached a plateau when fluid replacement increased beyond 121 ml/h. Repeated plasmapheresis decreased plasma albumin by 30% ( $P < .05$ ) and increased furosemide unbound fraction ( $P < .05$ ). Compared with control rabbits, hypoalbuminemia decreased the natri-

uresis of the bolus ( $22.7 \pm 1.5$ – $16.6 \pm 1.3$  mmol,  $P < .05$ ) but not that elicited by furosemide infusion ( $26.2 \pm 1.8$  mmol). Given as a bolus, furosemide natriuretic and diuretic response as a function of its urinary rate of excretion exhibited an hyperbolic relationship, and after its infusion a clockwise hysteresis, denoting tolerance. Plasma renin activity was increased by the bolus and the infusion of furosemide, even in the presence of 121 ml/h of fluid replacement. It is concluded that: 1) the increase in natriuretic/diuretic efficiency of the bolus induced by fluid replacement is greater than when furosemide is infused, 2) furosemide net effect does not increase proportionally to fluid replacement, and 3) the infusion of furosemide prevents the hypoalbuminemia-induced decrease in response of furosemide given as a bolus.

Conventional pharmacodynamic models assume that the pharmacological response is determined by the concentration of the drug at its site of action; accordingly, the effect-concentration relationship should be independent of drug input rate and route of administration, and of the distribution and elimination of the drug (Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988). Once secreted into the tubular fluid, furosemide exerts its diuretic and natriuretic effects on the luminal side of the loop of Henle (Branch et al., 1977; Odland and Beermann, 1980), i.e., furosemide excretion rate reflects the drug concentration at its site of action (Lant, 1985; Hammarlund-Udenaes and Benet, 1989). However, several studies have shown that furosemide natriuretic and diuretic effects are greater than expected when the input rate of the drug is slowed, either by an i.v. infusion (Lee et al., 1986; van Meyel et al., 1992; Wakelkamp et al., 1997) or by the use of oral modified release formulations (Beermann, 1982; Ebihara et al., 1983; Alván et al., 1992). Slow furosemide input appears to enhance its response secondary to the time course of drug delivery to its site of action (Kaojarern et al., 1982; Alván et al., 1990; van Meyel et al., 1992; Wakelkamp et al., 1997).

In vivo, the response to selected drugs is the net result between direct drug action and the physiological responses triggered by homeostatic mechanisms, including the sympathetic, arginine-vasopres-

sin, and renin-angiotensin-aldosterone systems, which limit the pharmacological effect (Brater, 1985; du Souich et al., 1989; Loon et al., 1989). Under such conditions, slow drug input may limit/retard the activation of homeostatic mechanisms and increase the net or measured response. This is the case for most antihypertensive agents (Castañeda-Hernandez et al., 1994) and for furosemide, where the replacement of urinary volume and electrolyte losses enhances its effect, likely by limiting the extent of homeostatic responses (Li et al., 1986).

The concentration of drug at the receptor biophase is directly related to the concentration of free drug in plasma. Therefore, it is assumed that drug effect is inversely associated to the extent of drug binding to plasma proteins (du Souich et al., 1993). It is noteworthy that the resistance to loop diuretics, especially to furosemide, occurs more frequently in patients with severe hypoalbuminemia (Inoue et al., 1987), secondary to the increase in its renal metabolic clearance (Pichette et al., 1996). That is, in the case of furosemide, an increase in its free fraction reduces its natriuretic and diuretic response. The objective of this study was to determine how fluid replacement and hypoalbuminemia modulate the changes in effect generated by the rate of input of furosemide. To this purpose, the pharmacodynamics of furosemide were studied in conscious rabbits receiving the diuretic as either an i.v. bolus or an infusion without or with fluid loss replacement and hypoalbuminemia.

### Materials and Methods

**Animals.** Male New Zealand rabbits (3.0–3.5 kg) purchased from Ferme Cunicole (Ste-Hyacinthe, PQ, Canada) were used throughout this study. Ani-

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mals were maintained on Purina pellets and water ad libitum in individual, well ventilated cages for at least 10 days before undertaking any experimental work. Before each experiment, a sterile Bardex Foley catheter (8–10) was introduced into the bladder of the rabbits, which were then placed in restraining cages (Plaslabs, Lansing, MI). A catheter (Butterfly-21; Abbott Ireland Ltd., Sligo, Ireland) was introduced in the central artery of the left ear to allow for blood sampling, and a polyethylene catheter (PE 50; Becton Dickinson and Co., Parsippany, NJ) was placed into a lateral vein of the left ear to infuse the replacement solution. Furosemide, as either a bolus or an infusion, was administered through a lateral vein of the right ear. All of the experiments were conducted in agreement with the Canadian Council on Animal Care guidelines for care and use of laboratory animals.

**Experimental Protocol.** *Effect of fluid replacement on the dynamics of furosemide given at different rates.* Six groups of six rabbits each were included in this experiment. All animals received a total dose of furosemide of 5 mg/kg, and a basal hydration with a solution of 0.9% NaCl and 5% glucose (50:50, v/v) infused at a rate of 0.217 ml/min for 60 min (total 13 ml) to replace losses due to ventilation and blood sampling (Babini and du Souich, 1986). Rabbits of groups 1, 2, and 3 were given furosemide (solution 10 mg/ml, Sabex, Montreal, PQ) as an i.v. bolus of 0.5 ml/kg in 30 s, and rabbits of groups 4, 5, and 6 received the same amount of furosemide but diluted in the basal fluid replacement solution and infused over 60 min (Syringe pump, model 341; Sage Instruments, Orion Research Incorporated, Cambridge, MA). The dose of 5 mg/kg of furosemide was selected because in the rabbit: 1) furosemide kinetics is first order, 2) it elicits a response close to the ED<sub>50</sub> (Homsy et al., 1995), and 3) it elicits a diuresis comparable to that observed in humans after an i.v. dose of 40 mg (Lambert et al., 1983). Because in the rabbit furosemide half-life is slightly less than 15 min, the response of furosemide was assessed for 60 min because at this time only around 5% of the dose administered as a bolus remains in the body (Babini and du Souich, 1986; Babini et al., 1991).

Rabbits in groups 1 and 4 received only basal fluid replacement, i.e. 13 ml/h infused over 60 min (Syringe pump, model 355; Sage Instruments). To determine the rate of fluid replacement of groups 2 and 5, it was assumed that the diuresis (mean 108.2 ml/h) observed in four rabbits from group 4 receiving the infusion of furosemide reflected the optimal response. Therefore, rabbits of groups 2 and 5 received, in addition to basal fluid replacement, 108 ml/h of a solution of 0.9% NaCl and 5% glucose (50:50) infused at various rates, simulating the rates of diuresis. When furosemide was administered as a bolus, of the 108 ml, 92.2 ml were infused over the first 30 min, and 15.8 ml were infused over the 30- to 60-min period. When furosemide was given by infusion, the volume was distributed over six periods of 10 min, that is 22.5, 46.3, 16.3, 7.1, 7.8, and 8.0 ml. In both groups 2 and 5, basal fluid replacement of 13 ml was infused over 60 min.

In rabbits of groups 1, 2, 4, and 5, to assess the effect of fluid replacement on furosemide-induced homeostatic reactions, blood samples were drawn at 0 and 30 min, and plasma renin activity (PRA)<sup>1</sup> was estimated using a commercial radioimmunoassay kit (RIANEN 026; New England Nuclear, Billerica, MA). Thirty minutes was selected because preliminary results showed that at that time, furosemide response was significantly reduced. To estimate furosemide pharmacodynamics, urine was collected at the following intervals: 0- to 10-, 10- to 20-, 20- to 30-, 30- to 40-, 40- to 50-, and 50- to 60-min.

To investigate further the effect of fluid replacement on the response to furosemide, rabbits in groups 3 and 6 received the basal fluid replacement of 13 ml/h of a solution of NaCl 0.9%/glucose 5% (50:50, v/v), as well as an additional 225 ml of the same solution, of which 191.75 ml was infused over a period of 30 min, and the remaining 33.25 ml over the second 30-min period. The volume of 225 ml was selected according to the maximal diuresis elicited by the infusion of 5 mg/h to rabbits in group 5 receiving a fluid replacement of 121 ml/h. Rabbits in group 3 received 5 mg/kg of furosemide as an i.v. bolus, and rabbits in group 6 received the same dosage of furosemide, but infused over the period of 60 min. Urine was collected every 10 min for 60 min.

To assess the net effect of furosemide, three additional groups of four rabbits each were used to assess the effect of the infusion of 13, 121, and 238 ml/h of a solution of NaCl 0.9%/glucose 5% (50:50, v/v), respectively, on the

baseline natriuresis and diuresis, in absence of furosemide. The net effect of furosemide was estimated by subtracting from the diuresis induced by furosemide the diuresis provoked by fluid replacement.

*Effect of hypoalbuminemia on the dynamics of furosemide given at different rates.* To assess the effect of hypoalbuminemia on the pharmacodynamics of various rates of furosemide input, hypoalbuminemia was produced by repeated plasmapheresis as described elsewhere (Pichette et al., 1996). Briefly, blood (10 ml/kg) was withdrawn from a central ear artery and centrifuged at 2500 rpm. Plasma was discarded and was replaced volume per volume by sterile Lactate Ringer (Abbott Laboratories, Montréal, Québec, Canada) and both the red cells and Lactate Ringer were reinfused. Five exchanges daily for 2 days were done. Pharmacodynamic studies were performed on the third day. Three groups of six rabbits each were included in this experiment, one control receiving a bolus of 5 mg/kg of furosemide, and two groups of rabbits with hypoalbuminemia receiving the bolus or the infusion of furosemide. All rabbits received the basal fluid replacement of 13 ml/h and in addition, 108 ml/h of the NaCl 0.9%/glucose 5% (50:50, v/v) solution. Urine was collected every 10 min for 60 min.

In the samples of blood withdrawn at 3 min, plasma protein binding of furosemide was assessed using the ultrafiltration method. The 3-min blood sample was selected on the assumption that furosemide was homogeneously distributed in the vascular compartment and, therefore, furosemide plasma concentration values were the higher (Pichette and du Souich, 1996). Plasma (1.0 ml) was centrifuged at 3500 rpm in Centrifree System devices (Amicon; W.R. Grace & Co., Beverly, MA) for 30 min at -25°C. The concentration of unbound furosemide was assayed in 250 µl of the resulting ultrafiltrate.

**Data Analysis.** Urinary volume was measured, and sodium and potassium concentrations were determined using a IL943 automatic flame photometer (Instrumentation Laboratory, Lexington, MA). Plasma albumin was determined with a Hitachi 717 analyzer (Boehringer Mannheim Canada, Laval, Québec, Canada). Furosemide concentration in urine samples was determined by HPLC as described previously (Lambert et al., 1982). Furosemide efficiency was estimated as the ratio of the measured effect divided by furosemide excretion during a given period of time.

The influence of the various experimental conditions on furosemide response and efficiency was evaluated with a one-way ANOVA followed by Tukey's test. PRA values within each group at 0 and 30 min were compared by Student's *t* test for paired data. The significance threshold was fixed a priori at *P* < .05.

## Results

Independently of the volume of fluid replacement, when furosemide was given as a bolus, its urinary excretion rate peaked during the 0- to 10-min collection period, to decrease thereafter. By contrast, when furosemide was infused, its urinary excretion rate increased gradually to remain rather constant after 10 to 20 min of infusion (Fig. 1). The cumulative amount of furosemide excreted in the 60-min urine collection was always lower after the infusion than after the bolus (Table 1). Fluid replacement did not alter the extent or the time course of furosemide excretion.

In the absence of furosemide, fluid replacement at the rates of 13, 121, and 238 ml/h induced a diuresis of  $6.0 \pm 3.2$ ,  $28.6 \pm 6.9$ , and  $90.4 \pm 17.5$  ml/h. Furosemide-induced natriuresis increased with the rate of fluid replacement but was not affected significantly by the mode of administration, i.e., bolus versus infusion. The diuresis also increased with the rate of fluid replacement, and tended (*P* > .05) to increase with the mode of administration (Table 1). When furosemide was given as a bolus, fluid replacement increased its efficiency, i.e., the number of mmol of Na<sup>+</sup> or the volume (ml) excreted in urine per milligram of furosemide recovered in urine, but after the infusion of furosemide, fluid replacement increased only its diuretic efficiency (Table 1). On the other hand, after the infusion of furosemide, the natriuretic and diuretic efficiency was increased at least 2-fold compared with the efficiency of furosemide given as a bolus. After furosemide bolus and infusion, the net diuresis, i.e., the diuresis of

<sup>1</sup> Abbreviation used is: PRA, plasma renin activity.

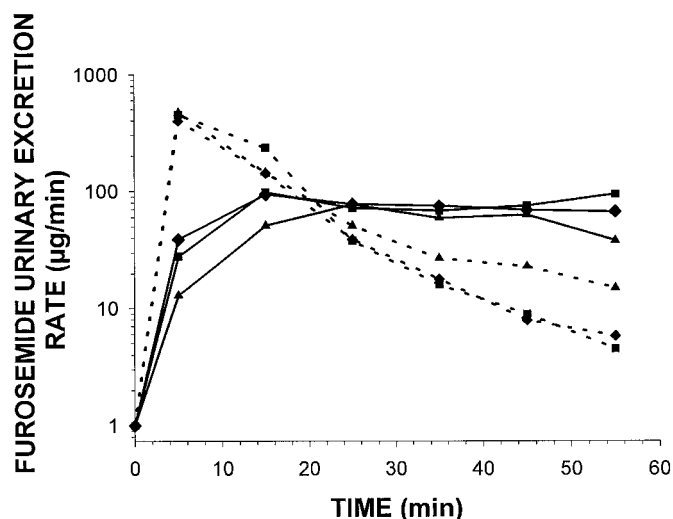


FIG. 1. Furosemide urinary excretion rate in rabbits receiving 13 (■), 121 (◆), or 238 ml/h (▲) of fluid replacement after the administration of 5 mg/kg furosemide as either an i.v. bolus (.....) or as an infusion over 60 min (—).

Each point represents the mean of six rabbits.

rabbits treated with furosemide and receiving fluid replacement minus the diuresis of rabbits with only fluid replacement, was 73.9, 130.1, 129.6 ml/h, and 98.3, 154.1, 153.9 at the fluid replacement rate of 13, 121, and 238 ml/h, respectively.

The changes in efficiency as a function of the rate of fluid replacement and of the mode of administration are clearly depicted when furosemide response is plotted against furosemide urinary excretion rate (Fig. 2). After the bolus of furosemide to rabbits receiving 13 ml/h of fluid replacement, maximal natriuresis and diuresis were achieved at furosemide urinary excretion rates of around 200  $\mu\text{g}/\text{min}$ . The maximal response to furosemide increased when animals received a fluid replacement of 121 ml/h, and peaked at furosemide urinary excretions of around 150  $\mu\text{g}/\text{min}$ . A further increase in fluid replacement to 238 ml/h did not augment the response to furosemide, but diminished the furosemide urinary excretion rate eliciting the maximal effect to around 50  $\mu\text{g}/\text{min}$ . That is, the extent of fluid replacement shifted the dose-response curve upwards and to the left, i.e., it apparently increased furosemide efficiency (Fig. 2).

Compared with rabbits receiving 13 ml/h and the bolus of furosemide, the infusion of furosemide elicited a greater maximal natriuretic and diuretic response, and that at furosemide excretion rates of around 95  $\mu\text{g}/\text{min}$  (Fig. 2). As a matter of fact, the maximal effect elicited by the infusion of furosemide to rabbits receiving 13 ml/h of fluid was similar to that obtained with the bolus of furosemide to rabbits receiving a replacement of 238 ml/h of fluid. Increasing the replacement of fluid to 121 and 238 ml/h shifted slightly the dose-response curve to the left and upwards. After the infusion of furosemide, the natriuresis/diuresis as a function of furosemide urinary excretion rate curves did not depict a linear relationship, but rather a clockwise hysteresis, suggesting the presence of tolerance to the natriuretic and diuretic effect to furosemide and that in presence of all three rates of fluid replacement.

Repeated plasmapheresis decreased plasma albumin concentrations by around 30% ( $P < .05$ ) (Table 2). As a consequence, furosemide unbound fraction increased from 1.3 to almost 8%. In rabbits with hypoalbuminemia and receiving the bolus of furosemide, the total amount of furosemide recovered in urine in 60 min was significantly reduced by comparison with control rabbits receiving the bolus ( $P < .05$ ). However, by comparison with control rabbits receiving the bolus

of furosemide, in rabbits with hypoalbuminemia and receiving the infusion of furosemide, the excretion of furosemide was not decreased. Hypoalbuminemia reduced ( $P < .05$ ) the furosemide-induced natriuresis in rabbits receiving the bolus of the diuretic, but did not prevent the increase ( $P < .05$ ) in natriuresis elicited by the infusion of furosemide. A similar trend was observed with the diuresis. The natriuretic efficiency of furosemide was not affected by hypoalbuminemia, independently of the mode of administration; on the other hand, the diuretic efficiency of furosemide was increased in hypoalbuminemic rabbits receiving the infusion of furosemide (Table 2). Plotting furosemide natriuresis and diuresis as a function of furosemide urinary excretion rate demonstrates that the maximal effect of the bolus of furosemide was decreased by hypoalbuminemia (Fig. 3). The infusion of furosemide to hypoalbuminemic rabbits elicited its maximal effect at furosemide excretion rates of 75  $\mu\text{g}/\text{min}$ , an effect that was greater than that observed after the bolus of furosemide to control rabbits. After the bolus of furosemide to control and hypoalbuminemic rabbits, furosemide response as a function of its excretion rate depicted an hyperbolic relationship, and after the infusion of furosemide to hypoalbuminemic rabbits, the dose-response curve depicted a clockwise hysteresis relationship.

In rabbits receiving 13 ml/h, PRA was almost doubled by the bolus of furosemide (Fig. 4). Compared with rabbits receiving 13 ml/h, in rabbits receiving a fluid replacement of 121 ml/h, baseline PRA was 50% lower, but fluid replacement did not prevent the furosemide-induced increase in PRA. In rabbits receiving 13 ml/h, the infusion of furosemide increased the PRA by more than 600%, and fluid replacement of 121 ml/h was unable to prevent the increase in PRA.

## Discussion

The present results show that the diuresis and the natriuresis elicited by the infusion of furosemide tends to be greater ( $P > .05$ ) than the effect elicited by the bolus of furosemide. On the other hand, after the infusion of furosemide, the amount of diuretic recovered in urine is around 50% of that recovered after the bolus. Therefore, the natriuretic and diuretic efficiencies of infused furosemide are greater than those estimated after the bolus of furosemide. Similar differences between bolus and infusion of furosemide have been reported in healthy volunteers (Wakelkamp et al., 1997), in patients with heart failure (Dormans et al., 1996; Aaser et al., 1997), and in patients with chronic renal failure (Rudy et al., 1991). The diuretic and natriuretic efficiencies of furosemide given as a bolus increase proportionally to the rate of fluid replacement; however, when furosemide is infused, fluid replacement has a small repercussion on its diuretic efficiency and does not affect the natriuretic efficiency. The difference in net loss or depletion of fluid (mean furosemide-induced diuresis minus mean fluid replacement-induced diuresis) between the bolus (73.90 ml) and the infusion of furosemide (98.30 ml) is 24.4 ml for a replacement of 13 ml/h, a difference that is not modified by the rates of replacement of 121 and 238 ml/h, i.e., 24.0 and 24.3 ml, respectively. When fluid replacement increases to 121 ml/h, the net loss of fluid induced by the bolus or the infusion of furosemide increase by 76%; however, with a fluid replacement of 238 ml/h, the net loss of fluid does not increase further. These observations suggest that the increase in effectiveness of both the bolus and the infusion of furosemide as a function of fluid replacement is not linear, reaching a plateau when the rate of fluid replacement increases beyond 121 ml/h.

The differences in response between the bolus and the infusion of furosemide have been ascribed to the development of acute tolerance secondary to the appearance of homeostatic reactions due to the volume depletion induced by the bolus of furosemide (Sjöström et al., 1988a,b; Wakelkamp et al., 1997). However, the plot of furosemide

TABLE 1

Cumulative urinary excretion of furosemide, natriuresis, and diuresis over a 60-min period, and furosemide natriuretic and diuretic efficiency in rabbits receiving fluid replacement of 13, 121, or 238 ml/h, and 5 mg/kg furosemide as either an i.v. bolus or as an infusion over 60 min

Data are presented as mean  $\pm$  S.E.

Group	Furosemide Excretion	Natriuresis	Natriuresis Efficiency	Diuresis	Diuresis Efficiency
	mg	mmol	mmol/mg	ml	ml/mg
Bolus 13 ml/h	7.85 $\pm$ 0.71	10.2 $\pm$ 0.7	1.6 $\pm$ 0.2	79.9 $\pm$ 3.8	5.1 $\pm$ 0.1
Bolus 121 ml/h	6.06 $\pm$ 0.79	18.5 $\pm$ 1.7 <sup>a</sup>	3.6 $\pm$ 0.3 <sup>a</sup>	158.7 $\pm$ 14.4 <sup>a</sup>	14 $\pm$ 1 <sup>a</sup>
Bolus 238 ml/h	7.30 $\pm$ 0.33	27.5 $\pm$ 1.7 <sup>a</sup>	3.9 $\pm$ 0.3 <sup>a</sup>	219.0 $\pm$ 11.7 <sup>a</sup>	30 $\pm$ 2 <sup>a</sup>
Infusion 13 ml/h	3.66 $\pm$ 0.57 <sup>a</sup>	12.9 $\pm$ 0.7	8.9 $\pm$ 1.4 <sup>a</sup>	104.3 $\pm$ 4.8	33 $\pm$ 6 <sup>a</sup>
Infusion 121 ml/h	3.85 $\pm$ 0.81 <sup>c</sup>	22.3 $\pm$ 1.0 <sup>b</sup>	7.0 $\pm$ 1.2	182.7 $\pm$ 10.3 <sup>b</sup>	53 $\pm$ 11
Infusion 238 ml/h	3.52 $\pm$ 0.27 <sup>d</sup>	27.4 $\pm$ 3.2 <sup>b</sup>	7.6 $\pm$ 0.6 <sup>d</sup>	244.3 $\pm$ 11.0 <sup>b</sup>	66 $\pm$ 2 <sup>b,d</sup>

<sup>a</sup>  $P < .05$  compared with bolus 13 ml/h.

<sup>b</sup>  $P < .05$  compared with infusion 13 ml/h.

<sup>c</sup>  $P < .05$  compared with bolus 121 ml/h.

<sup>d</sup>  $P < .05$  compared with bolus 238 ml/h.

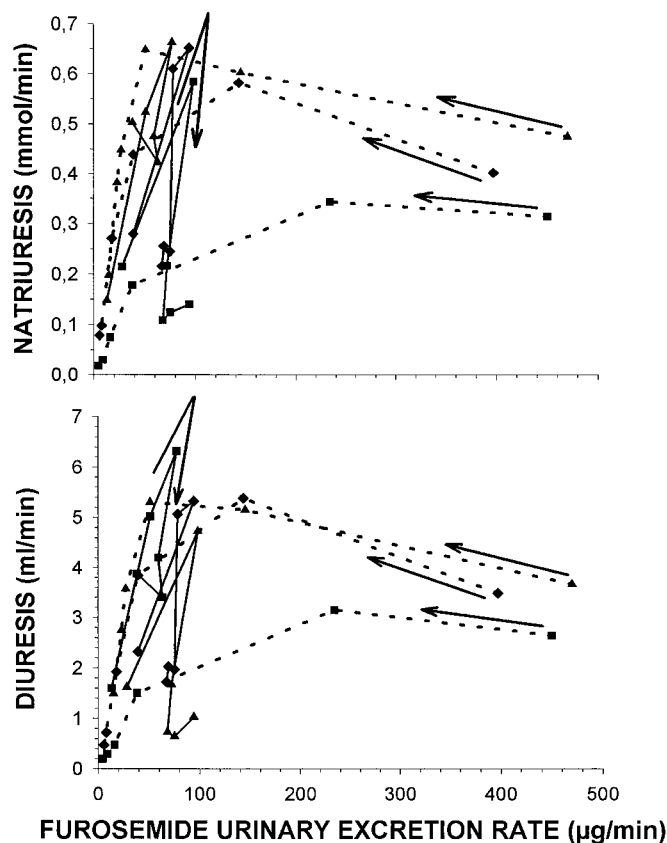


FIG. 2. Furosemide-induced natriuresis and diuresis as a function of furosemide urinary excretion rate in rabbits receiving 13 (■), 121 (◆), or 238 ml/h (▲) of fluid replacement after the administration of 5 mg/kg furosemide as either an i.v. bolus (.....) or as an infusion over 60 min (—).

Arrows indicate the time sequence the data was recorded. Each point represents the mean of six rabbits.

response as a function of furosemide urinary excretion rate after the bolus injection (Fig. 2) depicts an hyperbola, i.e., the diuretic/natriuretic response to furosemide is directly associated to the rate of furosemide urinary excretion (Holford and Sheiner, 1981). Fluid replacement does not change the pattern of the response of furosemide as a function of its urinary excretion rate curve, although it displaced the curve upwards and to the left.

When furosemide was infused, the plot of the response as a function of furosemide urinary excretion rate depicts a clockwise hysteresis,

TABLE 2

Effect of hypoalbuminemia on furosemide plasma protein binding and on its natriuretic and diuretic response to 5 mg/kg furosemide as a bolus or infused over 60 min given to conscious rabbits receiving a fluid replacement of 121 ml/h

Data are presented as mean  $\pm$  S.E.

Xu, amount of furosemide recovered in urine in 60 min.

	Control Bolus	Hypoalbuminemia Bolus	Hypoalbuminemia Infusion
Albumin (g/l)	33.0 $\pm$ 0.3	23.8 $\pm$ 1.0 <sup>a</sup>	24.0 $\pm$ 0.5 <sup>a</sup>
Binding (%)	98.7 $\pm$ 0.1	92.9 $\pm$ 1.9 <sup>a</sup>	92.1 $\pm$ 1.6 <sup>a</sup>
Xu (mg)	5.39 $\pm$ 0.38	3.80 $\pm$ 4.67 <sup>a</sup>	4.67 $\pm$ 0.57
Natriuresis (mmol)	22.7 $\pm$ 1.5	16.6 $\pm$ 1.3 <sup>a</sup>	26.2 $\pm$ 1.8 <sup>b</sup>
Efficiency (mmol/mg)	4.23 $\pm$ 0.15	4.83 $\pm$ 0.64	5.86 $\pm$ 0.64
Diuresis (ml)	183 $\pm$ 12	142 $\pm$ 17	229 $\pm$ 14 <sup>a,b</sup>
Efficiency (ml/mg)	34.0 $\pm$ 0.7	40.7 $\pm$ 6.3	51.6 $\pm$ 5.7 <sup>a</sup>

<sup>a</sup>  $P < .05$  compared with control rabbits.

<sup>b</sup>  $P < .05$  compared with hypoalbuminemic rabbits receiving the bolus of furosemide.

suggesting the presence of tolerance. This clockwise hysteresis is not abolished by the increase in fluid replacement, even when the replacement was adapted to the pattern of the response, i.e., spread over the period of 60 min. We may speculate that it is improbable that the development of tolerance after the infusion of furosemide is associated with a homeostatic reaction secondary to fluid depletion, because at the greater rate of fluid replacement (238 ml/h), the absolute depletion (diuresis minus fluid replacement) was of the order of 6 ml/h. Furthermore, after the bolus of furosemide, even in presence of large absolute fluid depletion, i.e., 67 and 37 ml/h in the groups receiving 13 and 121 ml/h, no tolerance was apparent. Supporting our hypothesis, Sjöström et al. (1988a) reported that in healthy male volunteers, tolerance to furosemide was not related to dehydration, but rather to the activation of the sympathetic nervous system and/or the renin-angiotensin-aldosterone system (Sjöström et al., 1988b).

The administration of furosemide as a bolus to rabbits receiving a fluid replacement of 13 ml/h nearly doubled the PRA. In the presence of a fluid replacement of 121 ml/h, the bolus of furosemide also doubled the PRA. During the infusion of furosemide to rabbits receiving a fluid replacement of 13 ml/h, PRA increased 7-fold, and fluid replacement of 121 ml/h reduced the increase of PRA, i.e., it increased only 200%. These results suggest that the infusion of furosemide, due to its great efficiency, promotes an important secretion of PRA and the appearance of tolerance. On the other hand, because the increase in PRA does not prevent the greater response to furosemide during the infusion, we may speculate that the relevancy of the renin-angiotensin system as a limiting factor to the response to furosemide in healthy animals is minimal. Supporting such a hypoth-

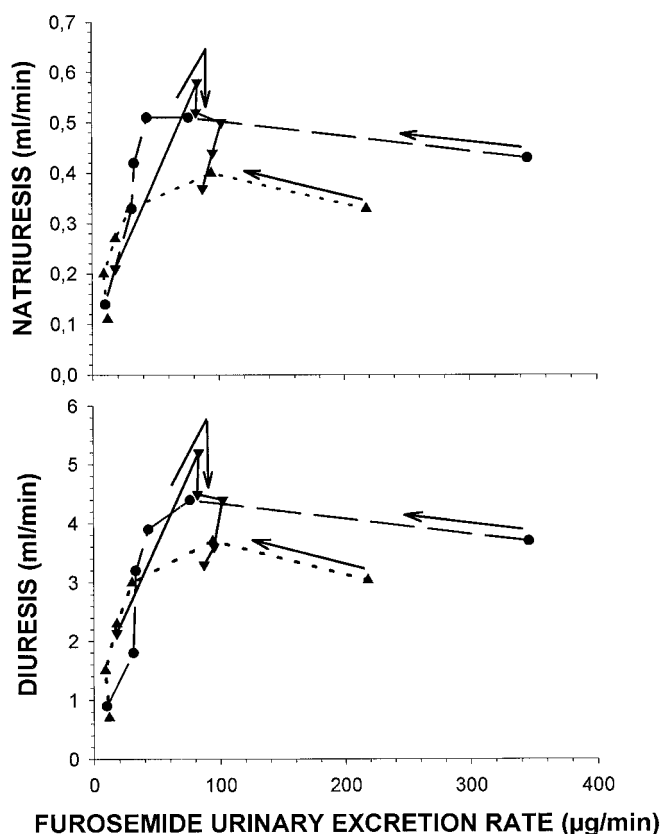


FIG. 3. Furosemide-induced natriuresis and diuresis as a function of furosemide urinary excretion rate in three groups of rabbits receiving 121 ml/h of fluid replacement.

Control (—●—) and one group of rabbits with hypoalbuminemia (---▲---) received a bolus of 5 mg of furosemide, whereas the other group of rabbits with hypoalbuminemia (—▼—) received 5 mg of furosemide by infusion. Arrows indicate the time sequence the data was recorded.

esis, it has been shown in healthy subjects that angiotensin-converting enzyme inhibition does not prevent the acute tolerance to an infusion of furosemide (Kron et al., 1994). Furthermore, in patients with congestive heart failure, the reduced efficiency of a bolus of furosemide does not appear to be associated with the activation of the renin-angiotensin-aldosterone system (Reed et al., 1995; Aaser et al., 1997), and the use of converting enzyme inhibitors may even reduce the efficiency of furosemide (Flapan et al., 1991), reduction partially associated to the dose of converting enzyme inhibitor used (Motwani et al., 1992). There are conflicting reports concerning the role of the sympathetic neuronal system in the appearance of tolerance to furosemide in animal models (Petersen et al., 1991), healthy volunteers (Kron et al., 1994), or in patients with congestive heart failure (Lang et al., 1993).

The modifications in the pharmacokinetics of furosemide induced by hypoalbuminemia or analbuminemia are associated with significant alterations in the pharmacodynamics of furosemide, i.e., a reduction in the excretion of sodium and in the urine volume (Inoue et al., 1987; Pichette et al., 1996, 1999). In analbuminemic animals, the decrease in the renal secretion of furosemide and in its natriuretic and diuretic effects is prevented when furosemide is administered mixed with albumin volume (Inoue et al., 1987; Pichette et al., 1999). In the current study, hypoalbuminemia reduced ( $P < .05$ ) the natriuresis and tended ( $P > .05$ ) to diminish the diuresis elicited by the bolus of furosemide, secondary to a decrease in the urinary excretion rate of furosemide, confirming the results published previously in the rat and

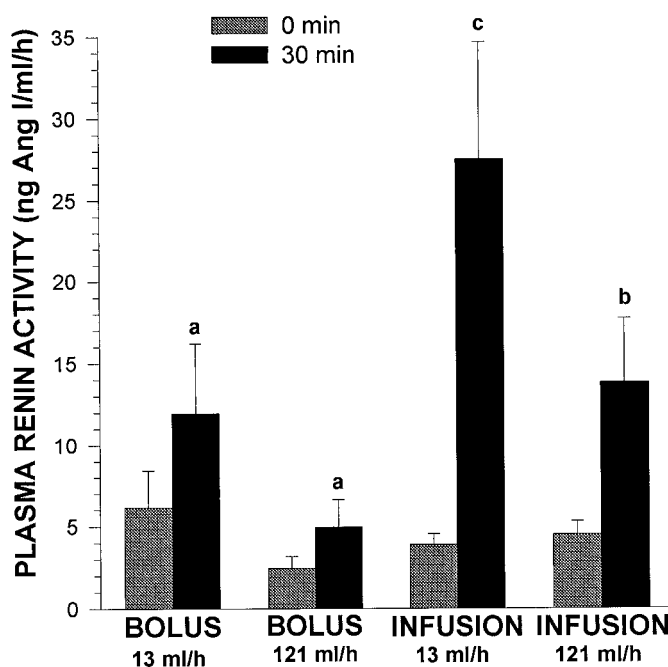


FIG. 4. PRA measured at 0 and 30 min after the administration of 5 mg/kg furosemide as either a bolus or an infusion over 60 min to rabbits receiving 13 or 121 ml/h of fluid replacement.

Data are presented as the mean  $\pm$  S.E. of six rabbits. <sup>a</sup> $P < .05$  compared with baseline values (0 min), <sup>b</sup> $P < .05$  compared with baseline values (0 min) and infusion 13 ml/h at 30 min, and <sup>c</sup> $P < .05$  compared with baseline values (0 min) and bolus 13 ml/h at 30 min.

humans (Inoue et al., 1987), and in rabbits (Pichette et al., 1996, 1999). Interestingly, a reduction in the rate of input of furosemide into the body not only prevents the hypoalbuminemia-induced decrease in response, but increases furosemide response above the effect elicited by the bolus in control rabbits, despite the presence of tolerance and smaller amounts of diuretic in urine. The present results show that the infusion of furosemide may be an alternative approach to overcome the hypoalbuminemia-induced decrease in furosemide response.

In summary, compared with the administration of furosemide as a bolus, the diuretic and natriuretic efficiency of furosemide are increased whenever the rate of input of the diuretic into the body is slowed, an increase that is not limited by the presence of tolerance or by the activation of the renin-angiotensin system. In addition, the infusion of furosemide reverses the hypoalbuminemia-induced decrease in furosemide response. These results may be helpful in understanding why an infusion of a loop diuretic is more effective than multiple boluses of the diuretic in patients with congestive heart failure (Dormans et al., 1996), in patients with chronic renal failure (Rudy et al., 1991), or in patients with hepatic cirrhosis (Uchino et al., 1983).

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