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Effects of furosemide on renal haemodynamics and proximal tubular sodium reabsorption in conscious rats

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1 The effects of furosemide given as constant i.v. infusion (7.5 mg kg^{-1} h^{-1}) or bolus injections (0.5, 7.5 and 120 mg kg^{-1}) on renal haemodynamics and proximal tubular Na reabsorption were studied in conscious water diuretic rats. The clearance of Li (C_{Li}) was used as marker for Na delivery from the proximal tubules, and clearance of [^{14}C]-tetraethylammonium (C_{TEA}) and [^{3}H]-inulin (C_{In}) as markers for renal plasma flow (RPF) and glomerular filtration rate (GFR), respectively.

2 Furosemide caused a transient increase of RPF and GFR followed by a secondary decrease below baseline levels; the latter could in part be counteracted by volume replacement. The filtration fraction (FF = GFR/RPF) was not significantly changed by furosemide. Fractional proximal Na excretion (C_{Li}/C_{In}) was significantly increased by all doses of furosemide independent of changes in RPF, GFR and FF.

3 The peak diuretic/natriuretic effect of furosemide was markedly potentiated by volume replacement, probably due to prevention of antinatriuretic mechanisms triggered by volume depletion.

4 It is concluded that following i.v. furosemide administration there is a biphasic change in renal haemodynamics in conscious, restrained rats, and that the inhibition of proximal Na reabsorption, as manifested by changes in fractional Li excretion, is not likely to be due to changes in total renal haemodynamics.

Introduction

Furosemide is the most popular loop diuretic. Its major natriuretic and diuretic action is due to inhibition of Na reabsorption in the thick ascending limb of Henle (Goldberg, 1973). However, changes in renal haemodynamics and inhibition of proximal tubular Na reabsorption are believed to be integral components of the natriuretic response, at least after i.v. administration (Brater, 1983). In recent studies, using the renal Li clearance to assess changes in segmental Na reabsorption in conscious rats, we found that maximal inhibition of proximal Na reabsorption occurs at relatively low doses of furosemide and that the effect of the diuretic on proximal reabsorption is rapidly compensated for when volume depletion is allowed to develop (Christensen *et al.*, 1986; 1987).

The mechanisms underlying the inhibition of proximal tubular Na reabsorption after i.v. furosemide remain unknown. It has been suggested that furosemide, due to its acute renal vasodilator action, increases the renal plasma flow (RPF) relative to the glomerular filtration rate (GFR), i.e. decreases filtration fraction (FF = GFR/RPF), and that the resulting decrease in peritubular oncotic pressure would reduce proximal Na reabsorption by increasing back-leak of tubular fluid in the proximal convolution (Brater, 1983; Weinman *et al.*, 1971). However, this hypothesis has never been confirmed experimentally, and experiments on isolated perfused tubules have indicated that removal of peritubular proteins may directly inhibit active NaCl transport in the proximal tubule (Baum & Berry, 1985).

The aim of the present study was to elucidate specifically, whether inhibition of proximal tubular Na reabsorption by furosemide is correlated to changes in total renal haemodynamics and FF. For this reason we determined simultaneous changes in GFR, RPF, and $FE_{Na\ prox}$ (FE = fractional excretion) in conscious rats given furosemide i.v. as constant infusion or bolus injections. The results indicate that inhibition of proximal Na reabsorption by furosemide may occur in the absence of measurable changes in renal haemodynamics and FF.

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Methods

Female Wistar specific pathogen-free rats, weighing 190–210 g, were used. Before experiments they were acclimatized in a temperature (22°C) and moisture (60%) controlled room with a 12 h light-dark cycle (light on from 06 h 00 min to 18 h 00 min). They were offered commercial rat pellets with a Na content of about 100 mmol kg^{-1} and tap water *ad libitum*.

Clearance technique

Clearance experiments were performed between 08 h 00 min and 13 h 00 min. Just before experiments the rats were given an oral test dose of 0.5 mmol kg^{-1} LiCl (10 ml kg^{-1} of 0.05 M LiCl), which maintained a relatively constant plasma Li concentration between 0.15 and 0.30 mM throughout the experiments. In experiments where the renal Li clearance was enhanced, adequate amounts of LiCl were added to the infusate in order to maintain plasma Li levels above 0.10 mM. The rats were anaesthetized with 1–3% Halothane in N_2O/O_2 (2 + 1) for surgical implantation of Silastic catheters into one jugular vein and the urinary bladder via the urethra. Postoperative stress was minimized by careful suturation and spraying with a local anaesthetic (lignocaine) and liquid plaster. After operation, lasting 15–20 min, the rats were restrained in plastic cylinders mounted on electronic balances and allowed to wake up. Infusion of isotonic or hypotonic saline with clearance markers was started when the animals had regained consciousness.

Experiments with constant infusion of furosemide

In this series, 145 mM NaCl, to which was added tracer amounts of [^{3}H]-inulin (Amersham; specific activity 3.9 Ci $mmol^{-1}$; 4 μCi h^{-1}) and [^{14}C]-tetraethylammonium bromide (TEA) (New England Nuclear, Boston, MA, U.S.A.; specific activity 4.8 mCi $mmol^{-1}$; 2 μCi h^{-1}) was infused i.v. 6 ml h^{-1} for 15 min, followed by 1.5 ml h^{-1} throughout the experiments. The infused dose of TEA was 0.44 mg kg^{-1} h^{-1} resulting in plasma levels of about 0.2 μg ml^{-1}. This concentration is supposed to be without any significant effects on the cardiovascular system. After a 1 h equilibration period, 30 min clearance determinations were started with automatic urine collection and midpoint blood sampling (300 μl from the tail tip) every 60 min. After 3 control periods with constant urine flow, furosemide (Furix, Alfred Benzon A/S, Copenhagen, Denmark), 7.5 mg kg^{-1} h^{-1}, was added to the saline infusion and clearance determinations were continued for 3 h (4 × 15 min + 4 × 30 min). During furosemide diuresis, volume losses were replaced by increasing the rate of infusion of 145 mM NaCl + 0.5 mM LiCl in the subsequent clearance period (delayed replacement).

Experiments with bolus injection of furosemide

In this series water diuresis was produced by constant infusion (6 ml h^{-1}) of 35 mM NaCl + 80 mM glucose, to which was added [^{3}H]-inulin (4 μCi h^{-1}) and [^{14}C]-tetraethylammonium (2 μCi h^{-1}).

After a 1.5 h equilibration period, clearance determinations were started with automatic urine collection and midpoint blood sampling (300 μl from the tail tip) every 40 min. After three 20 min control periods with constant urine flow, furosemide was injected as an i.v. bolus over 1 min in doses of 0.5, 7.5 and 120 mg kg^{-1} ($n = 5$ for each dose level). In one series of experiments no replacement fluid was given. In another series urinary volume losses were instantly replaced by 100 mM NaCl + 10 mM KCl + 0.5 mM LiCl infused at a rate which kept the body weight constant (±0.1 g). After furosemide administration, urine was collected for 6 × 5 min followed by 3 × 10 min.

Analyses

Urine volume was determined by weighing to the nearest mg. Lithium and Na were determined in plasma and urine by atomic absorption spectrophotometry as previously described (Christensen *et al.*, 1987). [^{3}H]-inulin and [^{14}C]-tetraethyl-ammonium bromide were determined in plasma and urine by double label scintillation counting on a LKB-Wallac liquid scintillation counter, model 1217. Thirty μl of sample and 270 μl of water were added to 2.5 ml of Optifluor; a constant degree of quenching was checked by the external standard method. The difference in plasma levels of clearance markers between two consecutive samples never exceeded 30%.

Calculations and statistics

Renal clearances and fractional excretions were calculated by the standard formula:

$$C = \frac{U \times V}{P}; \quad FE = \frac{C}{C_{In}},$$

where C = renal clearance, U = urine concentration, V = urine volume and P = plasma concentration.

The renal Li clearance was used as an estimate for the delivery of Na from the proximal tubules

(Thomsen, 1984):

$$C_{Li} = C_{Na\ prox}$$

The fractional proximal Na excretion was accordingly calculated:

$$FE_{Na\ prox} = C_{Li}/C_{In}$$

The filtration fraction was determined as FF = GFR/RPF, where GFR = C_{In} and RPF = C_{TEA}/0.90. In the latter formula 0.90 is the mean extraction fraction of tetraethylammonium (TEA) (Petersen & Christensen, 1987). TEA was used as clearance marker for RPF because a previous study indicated that the renal secretion of *p*-aminohippurate, as opposed to that of TEA, may be inhibited by high doses of furosemide (Petersen & Christensen, 1987).

All results are mean values ± s.e.mean. One-way analysis of variance followed by Duncan's test was used to evaluate differences between means in Figure 6.

Results

As also shown in a previous paper (Christensen *et al.*, 1986), control rats given basal infusion without drugs exhibited constant clearance parameters for several hours (data not shown).

Constant infusion of furosemide with delayed volume replacement

Constant infusion of furosemide in submaximal doses caused a sustained diuretic-natriuretic response (Figure 1). FE_{Na}, an indicator of whole nephron Na rejection, increased from 0.5% in the control state to about 20% during steady state natriuresis. Proximal tubular Na reabsorption was markedly inhibited, as indicated by the increase of $FE_{Na\ prox}$, from 36% in the last control period to more than 60%. This natriuretic response was not associated with significant changes in renal haemodynamics, as judged from measurements of C_{In} and C_{TEA}. Calculated values for FF remained constant, except for a tendency to decrease in the first 15 min period of furosemide infusion.

Furosemide bolus injections without volume replacement

The time course and magnitude of the diuretic-natriuretic response to bolus injections of furosemide

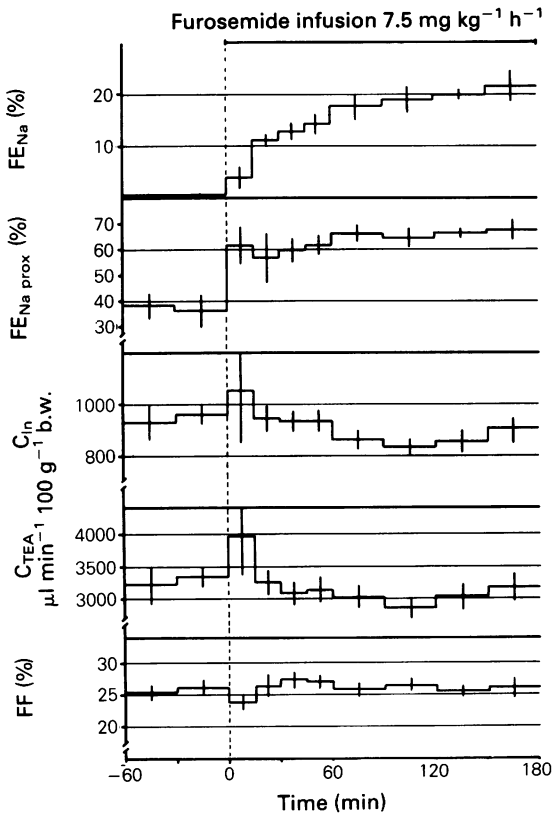

Figure 1 Effects of constant submaximal furosemide infusion (7.5 mg kg^{-1} h^{-1}) on fractional Na excretion (FE_{Na}), fractional proximal Na excretion ($FE_{Na\ prox}$ = C_{Li}/C_{In}) (C = renal clearance) and renal haemodynamics in conscious rats (n = 6). During furosemide infusion, urinary volume losses were replaced by infusion of 145 mM NaCl in the subsequent period (delayed replacement). Mean values with s.e.mean shown by vertical lines are indicated.

in water diuretic rats were similar to those previously observed in non-diuretic rats (Christensen *et al.*, 1987) (Figure 2). Maximal inhibition of whole nephron Na reabsorption occurred 5–10 min after furosemide injection; FE_{Na} increased from 0.7% in the control state to 22.6% after the highest (near-maximal) dose of furosemide. Proximal tubular Na reabsorption was markedly inhibited at all dose levels; $Fe_{Na\ prox}$ increased from less than 30% in the control state to 50–60% independent of the dose of furosemide (Figure 3). The natriuretic response to bolus injections was associated with parallel changes in the clearance markers for GFR and RPF: a small initial increase followed by a sustained dose-dependent decrease. Calculated values for FF

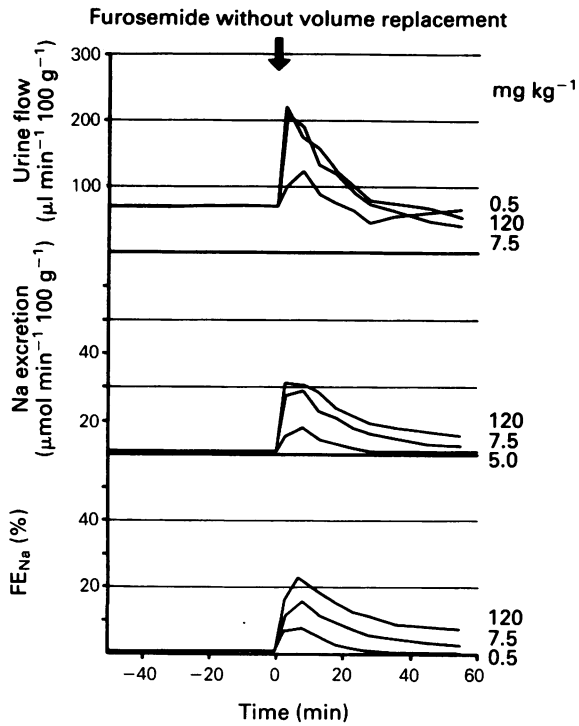

Figure 2 Effects of furosemide bolus injections (0.5, 7.5 and 120 mg kg^{-1}) on urine flow, total and fractional Na excretion in conscious rats. Urinary volume losses were not replaced. Curves indicate mean values of 5 animals.

showed only small and insignificant changes (Figure 3). There was no significant correlation between either individual or mean values for FF and $FE_{Na\ prox}$ determined before and at different times after furosemide administration (not shown).

Furosemide bolus injections with volume replacement

Because volume depletion caused by furosemide has marked effects on renal haemodynamics (Figure 3) and is known to activate antinatriuretic mechanisms, which oppose the effects of the diuretic on proximal Na reabsorption (Christensen *et al.*, 1986), the above experiments were repeated under conditions where volume depletion was prevented by instant infusion of an adequately composed replacement solution. As seen from Figure 4, the diuretic-natriuretic responses were magnified at all dose levels when volume depletion was prevented (cf. Figure 2). The peak natriuretic response after 120 mg kg^{-1} furosemide was doubled both in terms of absolute and fractional Na excretion.

The changes in renal haemodyamics (Figure 5) were similar to the changes observed without volume

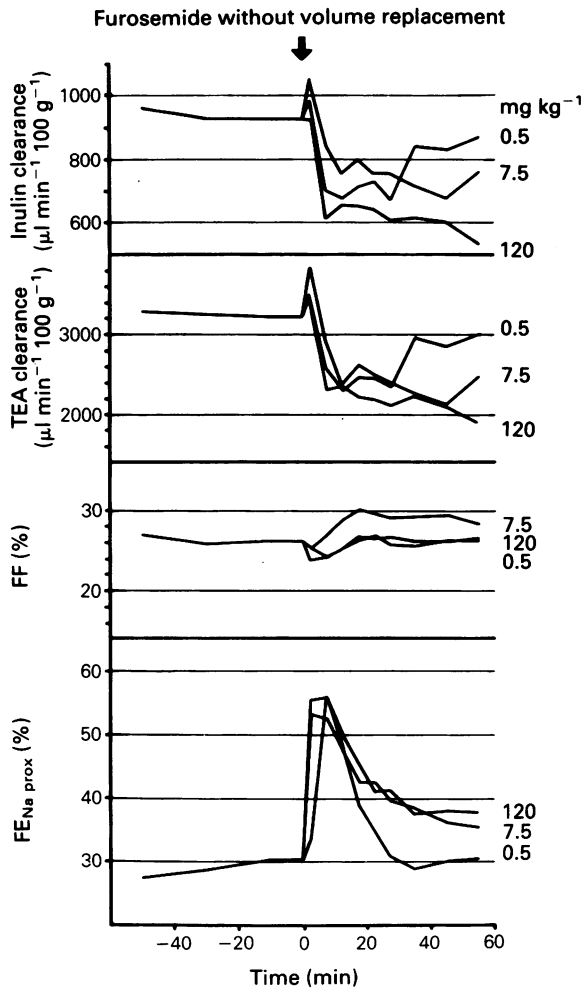

Figure 3 Effects of furosemide bolus injections (0.5, 7.5 and 120 mg kg^{-1}) on inulin clearance, tetraethylammonium (TEA) clearance, filtration fraction (FF) and fractional proximal Na excretion ($FE_{Na\ prox} = C_{Li}/C_{In}$) in conscious rats. (C = renal clearance) Urinary volume losses were not replaced. Curves indicate mean values of 5 animals.

replacement (Figure 3), except that the secondary decreases of C_{In} and C_{TEA} were less pronounced. Again, furosemide diuresis was not accompanied by any changes in the calculated values of FF. The inhibition of proximal Na reabsorption was potentiated by volume replacement. Thus $FE_{Na\ prox}$ increased to 74% after 7.5 and 120 mg kg^{-1} furosemide, compared with 56% without replacement. The secondary increases of all clearance parameters observed after the low dose of furosemide may represent extracellular over-replacement.

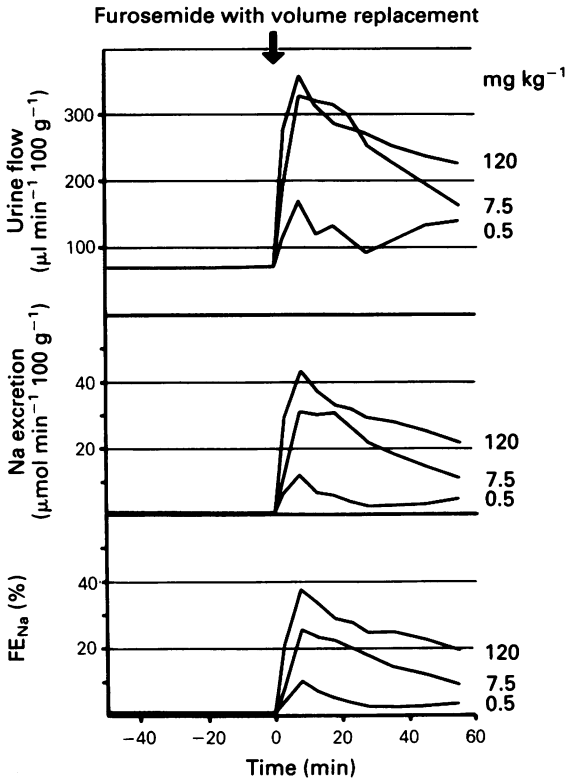

Figure 4 Effects of furosemide bolus injections (0.5, 7.5 and 120 mg kg^{-1}) on urine flow, total and fractional Na excretion in conscious rats. Urinary volume losses were replaced by i.v. infusion of 100 mM NaCl + 10 mM KCl + 0.5 mM LiCl to keep the body weight constant. Curves indicate mean values of 5 animals.

As in the series without volume replacement, there was no significant correlation between individual or mean values for FF and FE_{Li} measured before and at different times after administration of furosemide (not shown).

Dose-response curves

The present experiments were designed to elucidate the relation between haemodynamic and tubular effects of furosemide, but also provided some information about the role of changes in extracellular volume for the response to furosemide. Figure 6 indicates dose-response curves for selected parameters with and without volume replacement. In these curves, mean values for peak responses are indicated, whenever they occurred. It is seen that the peak natriuretic response was markedly enhanced by volume replacement, particularly after the higher doses of furosemide. Maximal $U_{Na}V$ was thus enhanced from 22 to 44 µmol min^{-1} 100 g^{-1} b.w. and maximal FE_{Na} from 23 to 38%. Both proximal and distal nephron segments contributed to the enhanced natriuretic response to furosemide induced by volume replacement (Figure 6).

Discussion

Although the effects of furosemide on renal haemodynamics have been studied extensively, the numerous reports on this subject have not been concordant. Thus, parameters estimating RBF or RPF have been shown to increase (Vorburger, 1964; Hook *et al.*, 1966; Ludens *et al.*, 1968; Stowe & Hook, 1970), remain unchanged (Tucker & Blantz, 1984; Gerber & Nies, 1980; Nies *et al.*, 1983) or even decrease (present results) after furosemide administration. Undoubtedly, differences in species, Na intake, urine flow, anaesthesia, dosage, and mode of administration have contributed to the different results.

In the present study we examined renal haemodynamic changes caused by furosemide in conscious rats under different protocols with respect to dosage, mode of administration, and state of hydration. In order to avoid bladder dead space artifacts, causing an erroneous increase of clearance parameters due to the abrupt rise in urine flow, we used water-diuretic rats in the experiments where furosemide was given as bolus injections.

The general finding was that, in conscious rats on normal Na intake, furosemide has a biphasic effect on total renal haemodynamics, as judged from changes in established clearance markers for RPF and GFR. Both parameters showed small short-term increases within 0–5 min after furosemide injection (Figures 3 and 5) and calculations indicated that these increases could not be due to dead space error (total space in the bladder and urine collecting catheter approx. 70 µl). The transient increases were, however, followed by a marked and sustained decrease between 5 and 10 min after furosemide injection. This secondary fall in renal haemodynamics may be related to diuretic-induced extracellular volume depletion, since it could be partly counteracted by volume replacement (Figure 6). It thus appears that in the unanaesthetized, sodium-repleted rat, furosemide does not cause any persistent increase in total renal blood flow, as has been demonstrated in other mammalian species (Vorburger, 1964; Hook *et al.*, 1966; Ludens *et al.*, 1968; Stowe & Hook, 1970). However, this apparent discrepancy among studies may to some extent be artifactual, since most studies reporting an increase of RBF or RPF were performed in anaesthetized animals having a low baseline RBF; also many studies were not controlled for bladder dead space

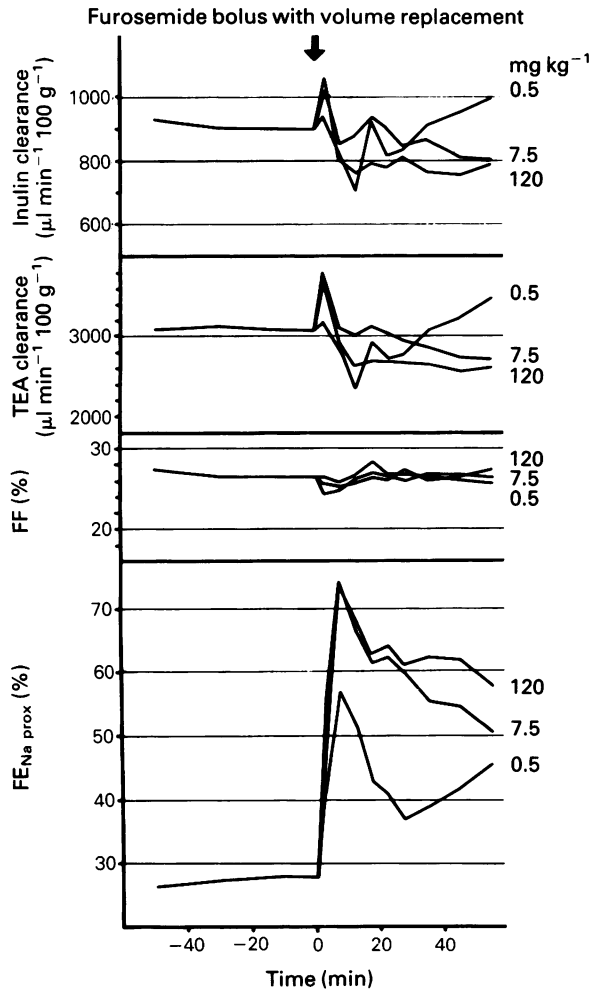

Figure 5 Effects of furosemide bolus injections (0.5, 7.5 and 120 mg kg^{-1}) on inulin clearance, tetraethylammonium (TEA) clearance, filtration fraction (FF) and fractional proximal Na excretion ($FE_{Na\ prox}$ = C_{Li}/C_{In}) in conscious rats. Urinary volume losses were replaced by i.v. infusion of 100 mM NaCl + 10 mM KCl + 0.5 mM LiCl to keep the body weight constant. Curves indicate mean values of 5 animals.

artifacts, tending to give an erroneous rise in clearances.

Furosemide may influence the intrarenal blood flow distribution as well as the total renal blood flow. Thus, Stein *et al.* (1972) found in anaesthetized dogs that 10 mg kg^{-1} bolus + 10 mg kg^{-1} h^{-1} furosemide i.v. increased total renal blood flow by 26% and this increase was due to a selective increase in the midcortical zone without changes in the outer cortical or juxtamedullary zones. With the clearance technique used in the present study we were unable to detect possible changes in intrarenal blood flow distribution. However, there is only little evidence that changes in intrarenal blood flow distribution may be of significance for the natriuretic effect of furosemide (Stein *et al.*, 1972).

The main objective of the present study was to analyze the relation between effects of furosemide on renal haemodynamic factors and proximal Na-reabsorption, as assessed by Li clearance. We have previously published arguments supporting the intrepretation that the rise in C_{Li} observed after furosemide administration reflects an increased output of Na from the proximal tubules rather than an inhibition of Li reabsorption in the distal nephron (Christensen *et al.*, 1986; 1987), and this interpretation has been strengthened by recent experiments indicating that furosemide does not increase C_{Li} in rats during maximal saline diuresis (Christensen *et al.*, 1988). In the present study the high values of FE_{Na} (mean 38%) determined after furosemide administration with volume replacement (Figure 4) is another indication of the inhibition of proximal tubular Na reabsorption.

According to the classical view, an effect of furosemide on proximal tubular Na reabsorption could be mediated by a decrease in FF, which would decrease the oncotic pressure or protein concentration in the peritubular fluid (Weinman *et al.*, 1971; Brater, 1983). Our results were clear in that furosemide only caused minor and inconsistent changes in FF, which were not related to the much more consistent inhibition of proximal tubular Na reabsorption. Therefore, the effects of furosemide on proximal tubular Na reabsorption are not likely to be mediated by factors influencing peritubular oncotic pressure or protein concentration, as previously suggested. The mechanism by which furosemide changes Na and volume reabsorption in the proximal tubule remains unknown. Our present and previous studies (Christensen *et al.*, 1987) show that contrary to the general view (Goldberg, 1973), the effect of furosemide on proximal Na reabsorption is observed after relatively low doses of the diuretic and becomes maximal at half-maximal diuretic doses (Figure 6).

The present study also provided important information about the importance of extracellular volume status for the diuretic response to furosemide. In a previous study (Christensen *et al.*, 1987) we described the dose-response relationship for bolus injections of furosemide in rats without volume replacement and suggested that the natriuretic response would be enhanced by volume replacement. This was actually confirmed in the experiments where the body weight was kept constant by replacement infusions (Figure 6). As would be expected, the synergistic effect of

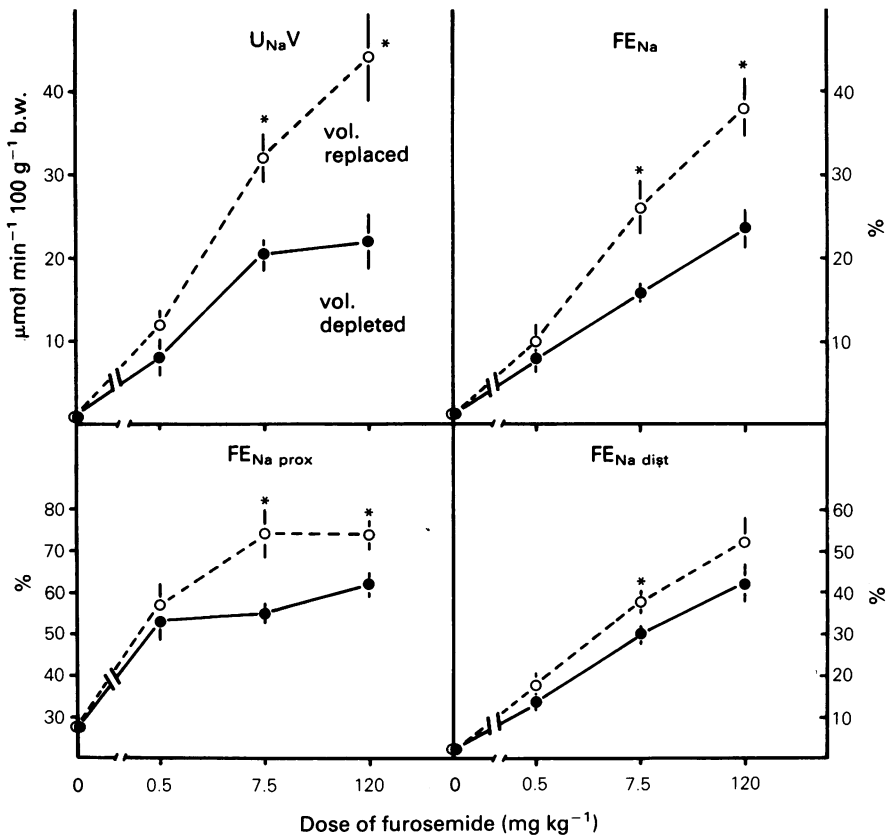

Figure 6 Dose-response curves indicating the effects of furosemide bolus injections on total Na excretion ($U_{Na}V$), fractional Na excretion (FE_{Na}), fractional proximal Na excretion ($FE_{Na\,prox} = C_{Li}/C_{In}$) and fractional distal Na excretion ($FE_{Na\,dist}C_{Na}/C_{Li}$) in conscious rats. Points indicate mean values ± of 5 animals with s.e.mean shown by vertical lines. Urinary volume losses were replaced (○) or not replaced (●). Asterisks indicate significant differences between volume replaced and volume depleted groups, evaluated by analysis of variance followed by Duncan's test.

volume replacement was more pronounced at high doses of furosemide; thus, at 120 mg kg^{-1} the peak natriuretic response of furosemide was doubled by volume replacement. These results suggest that diuretic-induced volume depletion triggers some very fast-operating Na-retaining mechanisms which, within a few minutes, counteract the tubular effects of furosemide. Both proximal and distal nephron segments participate in this 'diuretic-induced antinatriuresis' (Figure 6).

In conclusion, intravenous furosemide administration to unanaesthetized rats produces a biphasic response in renal haemodynamic parameters. An initial small increase of RPF and GFR is followed by a more sustained decrease, which may be related to volume depletion. The inhibition of proximal tubular Na reabsorption by furosemide is not likely to be mediated by haemodynamic changes influencing peritubular oncotic pressure or protein concentration.

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