Factors Modifying the Early Nondiuretic Vascular Effects of Furosemide in Man

The Possible Role of Renal Prostaglandins

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SUMMARY. Animal experiments have suggested that salt-balance, prostaglandin synthesis, and renal function are important determinants of the nondiuretic vascular effects of furosemide. To investigate the influence of these factors in humans, we studied 10 normal volunteers and five anephric patients. The volunteers were studied on three occasions: when on a 10 mEq/day sodium diet, on a 250 mEq/day sodium diet, and on a 10 mEq/day sodium diet with indomethacin, 200 mg/day. The anephric patients were studied immediately after dialysis. Plethysmographic methods were used to measure venous capacitance and blood flow in the calf before, and at 5, 10, and 15 minutes after furosemide, 80 mg, iv. Blood was obtained before and 15 minutes after furosemide for determination of plasma renin activity by radioimmunoassay and of plasma 6-keto-prostaglandin F1α by chromatography-mass spectrometry. We found that furosemide significantly increased venous capacitance in the calf of the normal volunteers on a low salt diet. Indomethacin, high salt intake, or lack of renal function was sufficient to inhibit this effect. Plasma renin activity increased only in the group that had the increase in venous capacitance. Limb blood flow decreased gradually in the 15 minutes following administration of furosemide in the normal volunteers, regardless of salt balance or indomethacin, but remained unchanged in the anephric patients. Plasma 6-keto-prostaglandin F1α was less than 30 pg/ml in all samples. Indomethacin concentration averaged 1.3 μg/ml in volunteers on the drug. To determine whether indomethacin, salt intake or renal function affected another venodilator, we studied an additional group of normal and uremic volunteers who received 0.6 mg nitroglycerin sublingually. An increase in venous capacitance was produced by the nitroglycerin in all subjects regardless of salt balance, indomethacin treatment, or renal function, in contrast to the findings with furosemide. We conclude that the early effect of furosemide to increase venous capacitance in man requires functional kidneys, a salt-retaining state, and prostaglandin synthesis, but that venodilation produced by nitroglycerin is independent of these variables. Individuals without renal function, or who are receiving indomethacin, do not show increased venous capacitance in the lower extremity which is the early effect of furosemide. (Circ Res 53: 630–635, 1983)

FUROSEMIDE is a potent diuretic that is frequently used for the treatment of acute left ventricular failure and pulmonary edema. Although furosemide decreases cardiac preload by producing a natriuresis with a resultant reduction in extracellular fluid volume, an acute fall in left ventricular filling pressure has been observed within a few minutes of intravenous drug administration, before a diuretic response is apparent (Dikshit et al., 1973). Plethysmographic studies in humans have demonstrated that furosemide increases the venous pooling that can account for the early decrease in venous return to the heart. In addition to effects on peripheral veins, furosemide is known to increase renal blood flow acutely (Ludens et al., 1968), an effect that has been postulated to be of benefit in the early management of acute tubular necrosis (Cantarovich et al., 1971).

There is increasing evidence in animals that both the renal and extrarenal vascular effects of furosemide require prostaglandin synthesis (Williamson et al., 1975; Bourland et al., 1977; Data et al., 1978). Furthermore, we have demonstrated that salt-loaded animals do not have a renovascular response to furosemide, whereas the vascular response is maximal in animals in a salt-retaining state (Gerber and Nies, 1980). Finally, Bourland et al. (1977) have found that nephrectomy in the dog abolishes the ability of furosemide to produce an acute fall in left ventricular filling pressure.

Although data are accumulating which concern the factors that modify the acute nondiuretic effects of furosemide in animals, the area is largely unexplored in man. Therefore, we undertook the present clinical study to determine whether the early extrarenal vascular effect of furosemide, characterized by changes in venous capacitance, was influenced by the subject's salt balance or prior administration of...
the prostaglandin synthetase inhibitor, indomethacin. In addition, we studied a group of uremic patients to determine whether a functional kidney was essential for the extrarenal vascular response to occur. Since prostacyclin (PGI₂) is the only known vasodilator prostaglandin that could circulate to produce systemic effects, evidence was also sought for increases in circulating levels of this substance.

Methods

The furosemide study was divided into two parts. Ten healthy female volunteers (age 23–32 years) participated in the first part of the experiment and five uremic patients (3 males, 2 females, age 30–49 years) in the second part. The group of 10 volunteers was studied on three separate occasions with a period of at least 1 month between each study. Subjects received a low salt diet (10 mEq/day) on the first occasion, a high salt diet (250 mEq/day) on the second, and a 10 mEq/day salt diet followed by oral indomethacin on the last occasion. Subjects received the diets for a period of 5 days and oral indomethacin 50 mg four times a day for 2 days before the third part of the experiment. The last dose of indomethacin was taken 1 hour before the experiment started and 2 hours before furosemide was administered. In the second part, five uremic patients—two surgically anephric and three functionally anephric—were studied. These patients were on hemodialysis three times weekly at a dialysis center. The study was performed in these patients following dialysis when they were considered to be at "dry weight." Serum electrolytes were within the normal range in both the volunteers and uremic subjects at the time of the study.

All normal subjects rested in the supine position for a period of 1 hour before the experiment, at the end of which a timed urine collection was obtained for estimation of urinary sodium. Three baseline measurements of venous capacitance, calf blood flow, blood pressure, and heart rate were obtained at 5-minute intervals over the next 15 minutes. Blood then was withdrawn for determination of plasma renin activity (PRA) and 6-keto PGF₁α concentration. For the 6-keto PGF₁α determination, 60 ml of blood were drawn into syringes containing 6 ml of 3.8% sodium citrate and 1 ml indomethacin; for the PRA, 5 ml were immediately placed in tubes at 4°C containing 0.15 ml 10% ethylenediamine tetraacetate (EDTA). In the indomethacin-treated group, an additional 5 ml of blood were obtained for determination of plasma indomethacin concentration. Furosemide (80 mg) then was administered as an intravenous bolus, and the hemodynamic measurements were repeated at 5-minute intervals for 15 minutes. Ten minutes after furosemide administration, 65 ml of blood were obtained for repeat PRA and 6-keto PGF₁α concentration; 15 minutes after furosemide administration, a second urine sample was collected for sodium estimation. Intravenous saline then was administered to replace salt and water losses. Two of the 10 volunteers did not participate in the experiment in which indomethacin was administered—one was unable to tolerate indomethacin and the other became pregnant after the first two parts of the study. The study design in the five uremic patients was similar, with the exception that no urine was produced and intravenous saline was not required.

To determine whether salt intake, indomethacin, or renal function affected the response to another venodilator, we performed additional studies in healthy females maintained for 5 days on a 250 mEq/day sodium intake (n = 5), a 20 mEq/day sodium intake (n = 4), or a 20 mEq/day sodium intake plus indomethacin (50 mg) four times a day for 2 days prior to the study (n = 4). Also, three additional uremic patients maintained on chronic hemodialysis were studied following dialysis. These volunteers rested supine with their right leg elevated for 30 minutes before their venous capacitance was measured. They then received 0.6 mg nitroglycerin sublingually, and the change in venous capacitance 4–6 minutes later was measured.

Calf blood flow and venous capacitance were measured by venous occlusion plethysmography with a mercury-in-rubber strain gauge to detect changes in calf circumference (Whitney, 1952). A pneumatic ankle cuff was inflated to suprasystolic pressures to eliminate blood flow to the foot, and the leg was suspended by the ankle with the knee slightly flexed so that the calf was above the level of the heart. Venous occlusion pressure was applied with a thigh cuff. Calf blood flow was calculated from the initial rate of change in calf circumference following inflation of the thigh cuff. The venous capacitance was determined by the equilibrium technique at a venous occlusion pressure of 30 mm Hg (Mason and Braunwald, 1965). For the furosemide studies, intermittent venous capacitance measurements were made by inflating the thigh cuff suddenly to 30 mm Hg. Calf volume became stable within 3 minutes, and measurements were made consistently at 3½ minutes after the cuff was deflated. For the nitroglycerin studies, the technique was changed slightly, in order to detect the early peak effects of the drug. After baseline measurements of calf volume, the cuff pressure was increased to 30 mm Hg. Predrug measurements were made during a stable plateau 10 minutes after cuff inflation. Nitroglycerin was then given sublingually with the venous occluding cuff pressure maintained at 30 mm Hg. Within 2 minutes of drug administration, the calf volume began to increase rapidly to a new plateau. Measurements were made 4–6 minutes after nitroglycerin administration and compared with measurements taken just prior to the drug. Measurements made over the 4-minute time period before administration of nitroglycerin showed no significant change in venous volume, indicating the stability of the plateau values. In four of the volunteers receiving nitroglycerin while in balance on a 20 mEq/day sodium diet, the two methods of measuring venous capacitance changes were compared. Two of the volunteers had capacitance determined initially by intermittent inflation of the thigh cuff to 30 mm Hg with measurement of calf volume 3½ minutes after inflation prior to and following 0.6 mg nitroglycerin sublingually. Capacitance changes in the other two volunteers were measured during the stable plateau produced by continued inflation of the thigh cuff to 30 mm Hg. After determination of the effects of nitroglycerin, the four volunteers rested for 45 minutes, after which the effects of nitroglycerin on venous capacitance were determined, again by the alternate method of measurement.

Blood pressure was measured with a sphygmomanometer. Plasma 6-keto-PGF₁α was estimated by gas chromatography-mass spectrometry following extraction, purification, and derivatization as described previously (Gerb et al., 1981). Plasma renin activity was determined by angiotensin I (AI) radioimmunoassay, and urinary sodium was measured by flame photometry. Indomethacin levels in the plasma were measured by high performance liquid
The change in venous capacitance (ml/100 ml of tissue) is plotted against time (min) after furosemide administration (80 mg, iv, at time 0). In panel A are the means ± SEM in the normal volunteers on 10 mEq/day sodium balance (○), on 150 mEq/day sodium balance (■), and on 10 mEq/day sodium balance plus indomethacin 200 mg/day for 2 days (□). In panel B, the means ± SEM in the five anephric subjects studied immediately following hemodialysis (●).

Mean comparisons of paired data were made by two-way analysis of variance with Dunnett's procedure for multiple comparisons or Student's paired t-test for single comparisons. Unpaired f-test was used to compare the means of unpaired data, and regression analysis was used for slope comparison. The level of significance was chosen as P < 0.05, and results were expressed as the mean ± SEM.

Results

In the salt-depleted group, venous capacitance following furosemide increased 15% ± 2.6% (P < 0.05) over the first 5 minutes and remained significantly elevated during the entire 15-minute period (Fig. 1A; Table 1). Indomethacin abolished the increase in venous capacitance produced by furosemide in the salt-depleted subjects. In the salt-loaded group, there was no increase in venous capacitance, and, in fact, a significant decrease in venous capacitance was apparent at 10 and 15 minutes following furosemide administration. The uremic individuals had no change in venous capacitance with furosemide (Fig. 1B; Table 1), whether or not the kidneys had been surgically removed. Except for the uremic patients, a progressive fall in calf blood flow was observed after furosemide, which became significant at 10 and 15 minutes (Table 1). No differences in the slope of the decline were apparent. The small but significant increases in blood pressure and heart rate observed in the salt-depleted and salt-loaded volunteers following furosemide were abolished by indomethacin and were not seen in the anephric patients (Table 1). Twenty-four-hour urinary sodium excretion extrapolated from the timed urinary collection prior to furosemide administration was 13.8 ± 2.6 mEq/day, 249 ± 53 mEq/day, and 4.8 ± 2.2 mEq/day for the salt-depleted, salt-loaded, and salt-depleted/indomethacin-treated groups, respectively, confirming that the diets had been followed. Urinary sodium output was significantly increased after furosemide (Table 1). The sodium excretion rate for the 15-min period after furosemide for the salt-loaded group was significantly higher than for the salt-depleted groups, but the response

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* P < 0.05 compared with 0 time (Dunnett's test).
of two salt-depleted groups did not differ from each other. Furosemide-induced increases in supine plasma renin activity (from 2.2 ± 0.3 to 4.80 ± 0.7 ng AL/ml per hour; P < 0.05) were observed in the low salt group but not in any of the other groups (Table 1). The baseline supine plasma renin activities were significantly (P < 0.05) higher when the volunteers were salt depleted (2.2 ± 0.3 ng AL/ml per hour) than when they were salt loaded (1.1 ± 0.4 ng AL/ml per hour). Indomethacin treatment did not significantly alter the baseline plasma renin activity. The high plasma renin activity in the uremic group is accounted for by one patient with a value of 16 and 18.9 ng AL/ml per hour before and after furosemide, respectively.

In seven volunteers in whom technically satisfactory analysis of plasma for 6-keto-PGF_{1alpha} concentration was performed, all samples had <30 pg/mL 6-keto-PGF_{1alpha} (our detection limit is 30 pg/mL) before and after furosemide. The plasma indomethacin concentration drawn 2 hours after the last dose in the eight healthy volunteers receiving the drug was 1.3 ± 0.3 µg/mL, and in all the subjects, indomethacin levels were greater than 0.3 µg/mL, a concentration that has been shown to produce almost complete inhibition of PGE_{2} synthesis in man (Rane et al., 1978).

The effects of nitroglycerin were not affected by salt balance, indomethacin, or renal function. In five volunteers on a 250 mEq sodium diet, nitroglycerin produced a peak increase of venous capacitance of 10.0 ± 0.5% (P < 0.05). In four volunteers on a 20 mEq sodium intake, the nitroglycerin-induced increase in venous capacitance was 9.8 ± 1.0% (P < 0.05), and after indomethacin, nitroglycerin produced a 10.8 ± 1.5% increase (P < 0.05). In three uremic volunteers, nitroglycerin produced a 6.7 ± 0.9% increase in venous capacitance. In the four volunteers where the two methods of measuring venous capacitance response to nitroglycerin were compared, the two methods were found to be equivalent. With the intermittent thigh cuff inflation, the response to nitroglycerin was 9.3 ± 1.8% compared to 9.8 ± 1.0% for the continuous thigh cuff inflation.

**Discussion**

It is generally accepted that the nondiuretic vascular effect of furosemide to reduce venous return is therapeutically important in bringing about rapid symptomatic relief for patients with left ventricular failure. This study clearly demonstrates that this effect is not due to the direct action of furosemide on blood vessels, as was proposed by Dikshit et al. (1973), but depends on prostaglandin synthesis, the salt balance of the individual, and the presence of functional kidneys. In contrast, the effects of nitroglycerin were evident regardless of salt intake, indomethacin treatment, or the presence of renal function.

These observations agree with previously published animal data. Bourland et al. (1977) demonstrated in the dog that the early effect of furosemide to reduce mean pulmonary artery wedge pressure was abolished by pretreatment with indomethacin or nephrectomy, and they postulated that a prostaglandin released from the kidney by furosemide was responsible for the extrarenal vascular effects. Bayne and Williamson (1979) reported that nephrectomy, but not acute ureteral ligation, prevented the furosemide-induced increase in venous capacitance in the hindlimb of the dog. In a previous study, we found that the increase in renal blood flow produced by furosemide in the dog was dependent on prostaglandin synthesis, as well as the salt balance of the animal (Gerber and Nies, 1980). The renal vascular response was seen only in those animals that had been sodium deprived and was absent in animals that had been salt loaded or treated with a prostaglandin synthesis inhibitor.

Our current results that the extrarenal vascular effects of furosemide in man are also dependent on salt balance and the presence of renal function indicate the key role of the kidney in the production of all of furosemide's vascular effects. Although the effect of furosemide on venous capacitance in normal volunteers was abolished by salt loading, a paradoxical situation occurs in patients with congestive heart failure, who, despite salt overloading and volume expansion, show the effect of furosemide on venous capacitance (Dikshit et al., 1973). However, when one considers that salt overloading in heart failure occurs secondary to reduced renal blood flow, reduced glomerular filtration, and increased sodium and chloride reabsorption, the picture clearly resembles that seen in healthy individuals who are salt depleted, i.e., the kidney is in a salt-retaining state. On the other hand, although the kidney behaves as though the subject is salt depleted in congestive heart failure, the effect of changes in dietary sodium intake in this condition as compared with normal individuals has still to be assessed.

In considering the effect of sodium in the diet, it is interesting that nitroglycerin was able to produce venodilation in volunteers on a 250 mEq sodium intake in contrast to the venoconstriction produced by furosemide. This finding indicates that the effect of sodium is not related to an inability of the veins to respond. In addition, our data indicate that some kidney function is required for furosemide to produce venodilation. We found no response in two surgically anephric and three functionally anephric patients who were considered to be at dry weight immediately following dialysis. These data confirm the findings of Mukherjee et al. (1981) in 11 functionally anephric individuals. In contrast, Dikshit et al. (1973) found that a venodilator response occurred in three patients in heart failure who were anuric but did not have any prior evidence of renal failure. It is possible that, although kidneys may be poorly filtering, their ability to produce vasoactive sub-
stances, such as renin, prostaglandins, and probably kinins, may be preserved (Bayne and Williamson, 1979). How much renal function is required has yet to be assessed.

Perhaps of greater clinical importance is our observation that indomethacin completely abolished the increase in venous capacitance produced by furosemide in the volunteers on a low salt diet. Plasma indomethacin concentrations sufficient to inhibit prostaglandin synthesis (Rane et al., 1978) were achieved in all subjects, implying that the responses were prostaglandin mediated. It has been shown in animals and man that furosemide releases arachidonic acid from renal lipid stores and increases the urinary output of several prostaglandins (Cibattoni et al., 1979; Gerber and Nies, 1981). Although PGE$_2$ is a potent dilator of vascular smooth muscle, 95% of intravenously infused PGE$_2$ is oxidized to inactive metabolites during a single passage through the lung (Ferreira and Vane, 1967). Since prostacyclin (PGI$_2$) has been shown to escape pulmonary metabolism (Gerkens et al., 1978), we postulated that prostacyclin might be released by the kidney, recirculate, and affect the systemic vasculature. Our present study fails to confirm this hypothesis. Our assay for plasma 6-keto-PGF$_{1α}$ should detect circulating prostacyclin as well as any circulating 6-keto-PGF$_{1α}$. Although a concentration of PGI$_2$ less than 30 pg/ml may have been present, current literature implies that such concentrations are too low to produce physiological effects (Steer et al., 1980). Likewise, Patrono et al. (1982) recently reported venous plasma 6-keto-PGF$_{1α}$ concentration (measured by radioimmunoassay) to be less than 7.5 pg/ml following furosemide administration to normal females.

Another possibility for the extrarenal vascular effects would be that furosemide causes the release of a substance from the kidney that is dependent on prostaglandins for its release, or that circulates and induces release of prostaglandins in or near capacitance vessels. Vascular tissue is capable of producing the vasodilators, PGE$_2$ and PGI$_2$, and it has been shown that angiotensin II can increase prostaglandin production by human vascular endothelium (Moncada et al., 1977; Gimbrone and Alexander, 1978). It is conceivable that release of renin from the kidney by furosemide with subsequent angiotensin II production could have caused generation of a vasodilator substance in the vasculature to produce the effects we observed. In contrast to its profound effects on arterioles, angiotensin II produces little or no direct constriction of capacitance vessels (DePasquale and Burch, 1963; Somlyo and Somlyo, 1966). It has been observed in several vascular beds that angiotensin II stimulates production of a vasodilator prostaglandin that modulates the arteriolar constriction produced by angiotensin (Messina et al., 1976; Gryglewski et al., 1980). A similar production of a venodilator prostaglandin could result in a net increase in venous capacitance, since angiotensin II has no direct venoconstrictor effect. If this hypothesis were correct, the ability of indomethacin to inhibit the venodilation produced by furosemide could be due to inhibition of the local vasodilator production and/or inhibition of renin release from the kidney. Whatever the mechanism of the venodilation produced by furosemide, indomethacin at normal therapeutic doses abolishes it. In contrast, the effects of nitroglycerin were not affected by salt balance, indomethacin treatment, or renal function. Nitroglycerin has been shown to cause prostacyclin production by venous endothelial cells in vitro (Levin et al., 1981). One preliminary report has suggested that the effects of nitroglycerin on venous compliance could be inhibited by indomethacin, 150 mg/day (Van Dusen and Fischl, 1981). However, our data obtained with a higher dose of indomethacin indicate that the venodilation produced by nitroglycerin is not dependent on cyclooxygenase products.

Apart from changes in venous capacitance, furosemide produced a substantial progressive fall in calf blood flow over the 15-minute observation period in all groups except the anephric patients. Presumably, these changes were secondary to a reduction in plasma volume and depended on a diuretic response, hence the absence of an effect in the anephric subjects. These changes in limb blood flow are identical to those found by direct measurement in conscious normal and anephric dogs (Ludens et al., 1970), but contrast with reports showing an increase in limb blood flow in patients with heart failure or hypertension (Dikshit et al., 1973; Mukherjee et al., 1981). Increases in heart rate and blood pressure in the normal volunteers may reflect increased sympathetic activity occurring in response to the diuretic. Why indomethacin prevented these responses is unclear, but the recent observation that indomethacin decreases plasma norepinephrine concentration in normal man may be relevant (Gullner et al., 1979).

In conclusion, this study demonstrates that the early nondiuretic vascular effect of furosemide on venous capacitance in humans is dependent on salt balance, prostaglandin synthesis, and renal function. A high salt diet, indomethacin, or lack of renal function will abolish the ability of furosemide to produce effects on venous capacitance in man, but these factors do not influence the venodilatory response to nitroglycerin. The pharmacodynamic drug-drug interaction between furosemide and indomethacin (and, probably, other commonly used non-steroidal anti-inflammatory drugs) may be clinically significant in the treatment of patients with acute left ventricular failure where an early increase in peripheral venous capacitance following furosemide is desired. However, the relative importance of the venodilator and diuretic effects of furosemide in this setting is unknown.
Vascular Effects of Furosemide

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INDEX TERMS: Furosemide • Prostaglandins • Venodilation

Indomethacin
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Circ Res. 1983;53:630-635
doi: 10.1161/01.RES.53.5.630

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