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The dose-response characteristics of the acute non-diuretic peripheral vascular effects of frusemide in normal subjects

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1 The peripheral venous and arterial effects of frusemide, plasma renin activity and plasma frusemide concentrations were examined during the 15 min period following the i.v. administration of 0, 5, 10, 20, 40 and 80 mg of frusemide in a group of nine salt depleted volunteers. The responses to 80 mg frusemide given orally during the 1 h period after administration were also examined.

2 Increases in venous capacitance 5 min after administration of 5 and 10 mg frusemide were observed ($P < 0.05$) but no significant increases were apparent after the higher doses. At 10 min, the increases in venous capacitance showed a flat dose response effect with significant increases throughout the dose range (5–80 mg).

3 Decreases in forearm blood flow occurred at 5 and 10 min after frusemide administration at all the doses studied ($P < 0.05$) while blood pressure responses at 10 min showed increasing effect with increasing dose.

4 An oral dose of 80 mg frusemide produced a rise in venous capacitance 15 min after administration and a decrease in forearm blood flow during the period 15–60 min without any alteration in heart rate or blood pressure.

Keywords frusemide dose-response characteristics non-diuretic effects

Introduction

The diuresis produced by frusemide increases with increasing dosage and a relationship between natriuresis and the urinary excretion of frusemide has been described (Branch *et al.*, 1977). In addition to the drug's diuretic properties, frusemide causes an increase in venous capacitance and a decrease in the left ventricular filling pressure in patients with congestive heart failure (Dikshit *et al.*, 1974) and an increase in venous capacitance, a reduction in peripheral blood flow and an elevation in blood pressure in salt depleted volunteers (Johnston *et al.*, 1983a). These peripheral vascular effects occur within minutes of administration and before a diuresis is observed.

There is accumulating evidence in man that the acute vascular effects of frusemide are dependent on renin release from the kidney and do not occur when frusemide induced renin release is suppressed (Johnston *et al.*, 1983a,1984) or angiotensin II formation is inhibited (Johnston *et al.*, 1983b).

The present study was undertaken to determine the effects of increasing doses of frusemide on the acute peripheral responses, the relationship between effect and plasma renin activity, and the dose which would produce an optimum venodilator response.

Methods

Nine healthy volunteers, seven males and two

females aged 18–22 years, were studied after full clinical examination and having given informed consent. The subjects had normal renal function as assessed by serum creatinine (90.4 ± 2.8, range 83–108 μmol/100 ml). The protocol of the study had been approved by the Ethical Committee of The Queen's University, Belfast. The study for the intravenous frusemide dose characteristics was carried out on six separate occasions at the same time of day with a period of at least 1 week between studies. The 80 mg oral study was performed 1 week after the intravenous study had been completed.

For 3 days before each part of the experiment subjects received 60 mmol of sodium in their diet and 80 mg of oral frusemide daily. On the day of each experiment, timed urine samples were obtained from 08.00 to 13.00 h to estimate urinary sodium and thus determine crudely whether subjects had adhered to the protocol. A 19 gauge butterfly needle was then inserted into a hand vein of the right arm for drug administration and another into the left antecubital vein for blood sampling. Blood pressure was measured with a Hawksley random zero sphygmomanometer (Wright & Dore, 1970) and heart rate from a direct writing electrocardiograph as the mean of ten consecutive R-R intervals. Mean blood pressure was calculated as diastolic + ⅓ (systolic–diastolic). Forearm blood flow and venous capacitance were measured in the right arm by venous occlusion plethysmography (Whitney, 1952). Venous capacitance was determined by the equilibrium technique at a venous occlusion pressure of 30 mm Hg (4 kPa) and forearm blood flow determined from the initial rate of change in forearm circumference at an occlusion pressure of 60 mm Hg (8 kPa). Changes in forearm circumference were measured with a mercury in rubber strain gauge. Room temperature throughout was maintained at 24 ± 0.5°C.

After rest for 1 h in the supine position, three baseline measurements of venous capacitance, forearm blood flow, blood pressure and heart rate were made at 5 min intervals and a blood sample taken for measurement of plasma renin activity and plasma frusemide concentration. Subjects then received 0, 5, 10, 20, 40 or 80 mg frusemide according to a randomized double-blind design. Frusemide was administered as an 8 ml volume on each occasion and delivered over a 10 s period.

Venous capacitance, forearm blood flow, blood pressure and heart rate were then recorded at 5, 10 and 15 min after intravenous frusemide administration. Blood samples were taken at each observation time for plasma frusemide concentrations and at 10 min for plasma renin activity.

In addition, on one study day 80 mg of frusemide was administered orally and measurements of venous capacitance, forearm blood flow, blood pressure and heart rate made at 0. 5, 10,15, 30 and 60 min after drug administration.

For estimation of plasma renin activity, 10 ml of blood was immediately placed in tubes at 0°C containing 0.3 ml of 10% sodium ethylenediamine tetra-acetate (EDTA), centrifuged at 4°C and the plasma stored at −40°C. Plasma renin activity was expressed as ng of angiotensin I (ANG I) generated h^{-1} ml^{-1} of plasma at pH 7.4 and at 37°C. ANG I was measured by radioimmunoassay with a Gammacoat Kit (Clinical Assays, Travenol Laboratories Inc., Haber *et al.*, 1969). Plasma frusemide concentrations were measured by high performance liquid chromatography (Lin *et al.*, 1979).

Comparisons of multiple data were made using analysis of variance and Duncan's multiple range test. Comparisons were made with the corresponding placebo values. Student's paired *t*-test was used for single paired data. and unpaired *t* for unpaired data. Comparisons between data were also made using product-moment correlations. Results are expressed as the mean ± s.e. mean and the level of significance chosen as $P < 0.05$.

Results

The increases in venous capacitance 5 min after 5 and 10 mg of intravenous frusemide were significantly greater than the corresponding placebo values (0.58 ± 0.12 ml/100 ml and 0.47 ± 0.11 ml/100 ml respectively (Figure 1) (Table 1) ($P < 0.05$). With increasing doses, a progressive decline in the venous responses at 5 min was observed so that over the dose range 20–80 mg, no significant increases were observed. An inverse correlation between the increases in venous capacitance and log plasma frusemide concentration was apparent at 5 min ($n = 45$, $r = 0.43$, $P < 0.01$) (Figure 2). On the other hand, 10 min venous responses showed a flat dose response effect (Figure 1), the increases being statistically greater than placebo at all the doses studied ($P < 0.05$). No relationship between the degree of venodilatation and plasma renin activity was apparent.

Decreases in forearm blood flow at 5 and 10 min after frusemide were observed throughout the dose range (Figure 1). Although decreases were greater at 10 min than 5 min, the differences were not statistically significant. No correlations between the decrease in forearm blood flow, log plasma concentration or plasma renin activity were observed. The acute decreases in

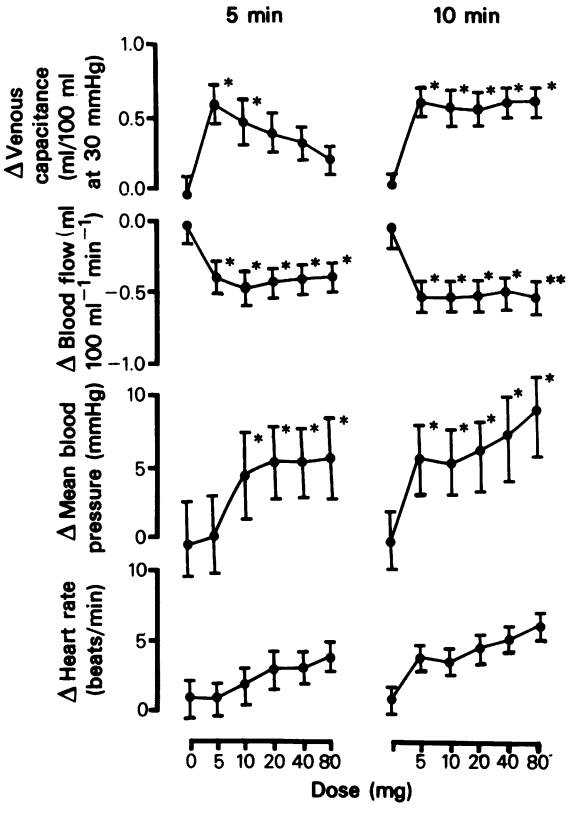

Figure 1 The effects of intravenous frusemide (5–80 mg) and placebo (0 mg) on venous capacitance, forearm blood flow, mean blood pressure and heart rate 5 and 10 min after administration. The changes are measured from pre-treatment baselines and expressed as mean ± s.e. mean. $*P < 0.05$; $**P < 0.01$.

forearm blood flow during the 10 min period after administration were therefore independent of dose. The blood pressure responses at 5 min showed significant increases over baseline in the dose range 10–80 mg frusemide ($P < 0.05$, Figure 1). At 5 min, no correlation between the increases in blood pressure and the log plasma concentration was apparent. At 10 min, a significant increase in blood pressure was observed at all the doses studied and positive correlations between the increases in blood pressure, log plasma frusemide concentrations ($n = 45$, $r = 0.34$, $P < 0.05$) (Figure 3) and plasma renin activity were apparent ($n = 45$, $r = 0.36$, $P < 0.05$) (Figure 4). Small increments in heart rate were seen over the dose range 20–80 mg at 5 and 10 min but changes were not statistically significant.

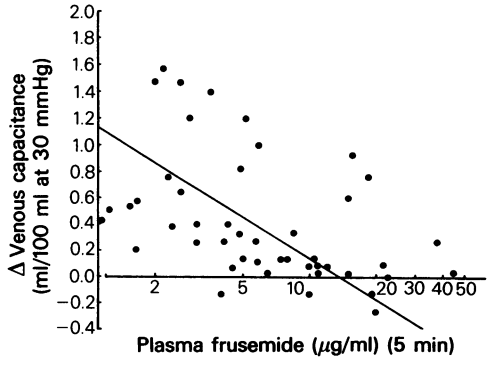

Figure 2 Relationship between the change in venous capacitance and the plasma concentration of frusemide, 5 min after intravenous administration ($n = 45$, $r = 0.43$, $P < 0.01$).

Oral frusemide (80 mg) produced an increase in venous capacitance 15 min after administration ($P < 0.05$) (Figure 5) (Table 2) but not at the other times studied. This compared with a maximum increase in venous capacitance at 10 min after the same dose given intravenously (Figure 5). Decreases in blood flow were also seen after oral frusemide but occurred later, so that changes seen after 5 min ($P < 0.05$) following intravenous frusemide were not seen until 15 min after oral administration (Figure 5).

Table 1 The vascular effects of frusemide, plasma renin activity, and plasma frusemide concentrations before and at 5 and 10 min after drug administration

Dose (mg)	*Time (min)*	*Venous capacitance (ml/100 ml)*	*Forearm blood flow (ml 100 ml^{-1} min^{-1})*	*Blood pressure (mm Hg)*	*Heart rate (beats/min)*	*Plasma renin activity (ng Al ml^{-1} h^{-1})*	*Plasma frusemide concentration (μg/ml)*
	0*	2.86 ± 0.31	2.07 ± 0.33	82.7 ± 3.5	70.4 ± 5.9	2.52 ± 0.61	—
0	5	2.69 ± 0.32	2.04 ± 0.32	80.4 ± 1.6	71.3 ± 5.7	—	—
	10	2.87 ± 0.29	1.95 ± 0.34	79.4 ± 2.0	71.6 ± 5.9	2.42 ± 0.45	—
	0*	2.83 ± 0.31	1.98 ± 0.32	79.9 ± 3.5	71.0 ± 5.8	2.29 ± 0.52	—
5	5	3.41 ± 0.41†	1.59 ± 0.39†	79.8 ± 3.7	72.1 ± 5.9	—	2.67 ± 0.67
	10	3.54 ± 0.35†	1.39 ± 0.31†	86.6 ± 3.7†	74.9 ± 5.6	4.21 ± 1.03†	1.45 ± 0.23
	0*	2.94 ± 0.28	2.09 ± 0.35	81.3 ± 3.7	70.3 ± 5.5	2.15 ± 0.31	—
10	5	3.41 ± 0.41†	1.61 ± 0.35†	85.4 ± 3.4†	72.1 ± 5.3	—	3.86 ± 0.88
	10	3.51 ± 0.35†	1.45 ± 0.30†	85.6 ± 2.2†	73.7 ± 5.8	3.68 ± 0.57†	2.63 ± 0.68
	0*	2.89 ± 0.30	2.22 ± 0.40	80.2 ± 2.9	69.9 ± 5.6	2.35 ± 0.52	—
20	5	3.28 ± 0.55	1.88 ± 0.31†	85.6 ± 3.2†	72.7 ± 5.9	—	5.52 ± 0.97
	10	3.33 ± 0.47†	1.61 ± 0.27†	87.6 ± 3.1†	74.7 ± 5.7	5.20 ± 1.13†	4.01 ± 0.79
	0*	2.95 ± 0.39	2.15 ± 0.36	81.1 ± 2.7	72.3 ± 6.1	3.07 ± 0.63	—
40	5	3.27 ± 0.37	1.86 ± 0.33†	82.6 ± 2.5†	74.8 ± 5.8	—	12.40 ± 1.99
	10	3.57 ± 0.46†	1.72 ± 0.31†	88.2 ± 2.8†	80.0 ± 6.2	6.02 ± 1.25†	7.74 ± 1.40
	0*	2.96 ± 0.34	2.07 ± 0.37	80.7 ± 3.6	70.4 ± 5.8	3.25 ± .72	—
80	5	3.18 ± 0.34	1.75 ± 0.32†	85.6 ± 3.2†	74.2 ± 5.9	—	21.10 ± 3.97
	10	3.61 ± 0.34†	1.54 ± 0.29††	89.9 ± 3.5†	77.6 ± 6.3	7.18 ± 0.91††	15.05 ± 3.55

* Mean of three readings
† Change compared to baseline < 0.05
†† Change compared to baseline < 0.01

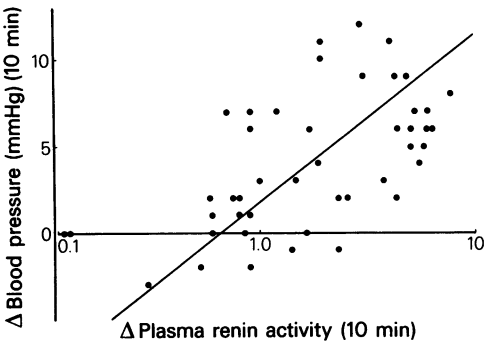

Figure 3 Relationship between the increase in blood pressure and the plasma concentration of frusemide 10 min after intravenous administration ($n = 45$, $r = 0.43$, $P < 0.05$).

In addition comparable changes in blood flow were observed 15 min after intravenous and 60 min after oral frusemide (Figure 5). Unlike the blood pressure increases seen 10 and 15 min after 80 mg frusemide intravenously, no changes in heart rate or blood pressure were observed over the one hour period after oral administration (Figure 2). Estimated 24 h sodium excretion did not exceed 35 mmol in any individual during the study.

Discussion

Unlike its diuretic responses, the acute peripheral vascular effects of frusemide do not show increasing effects with increasing dose. In fact, if one examines the venous responses at 5 min, a negative relationship with log plasma concentration is apparent.

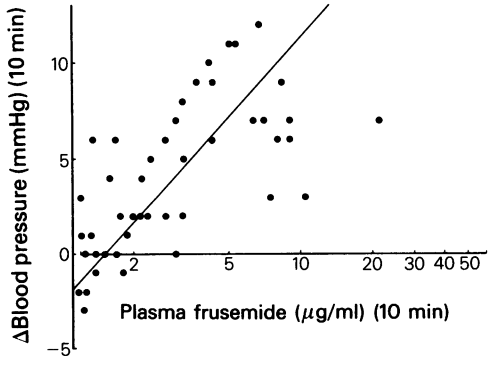

Figure 4 Relationship between the increase in blood pressure and the increase in plasma renin activity 10 min after intravenous administration ($n = 45$, $r = 0.36$, $P < 0.05$).

In a previous study we demonstrated that captopril, an angiotensin converting enzyme inhibitor, abolished the acute effects of frusemide on venous capacitance, forearm blood flow and blood pressure in salt depleted volunteers (Johnston *et al.*, 1983b). In addition, if frusemide stimulated renin-release was suppressed by indomethacin, salt overloading (Johnston *et al.*, 1983a) or propranolol (Johnston *et al.*, 1984) venodilatation failed to occur. These observations suggest that frusemide stimulated renin release and subsequent angiotensin II formation is important in producing the acute peripheral vascular effects of frusemide. Could acute renin release and angiotensin II formation be responsible for the peripheral vascular dose-response characteristics seen in this study?

Angiotensin II is a powerful constrictor of the arterioles and the changes in forearm blood flow and the increases in blood pressure could be due to angiotensin formation. In addition, angiotensin II causes release of vasodilator substances from the vessel wall, particularly the prostaglandins PGE_2 and PGI_2 (Messina *et al.*, 1976; Gryglewski *et al.*, 1980). The release of these vasodilator substances could explain why increasing doses of frusemide (with increasing renin release) failed to produce increasing arterial constriction. The increasing blood pressure seen with increasing dose could then be due to increases in myocardial contractility associated with angiotensin II (Koch-Weser, 1965).

Although angiotensin II has a marked arteriolar constrictor effect, the hormone appears to have little venoconstrictor effect *in vivo* (Haddy *et al.*, 1962; De Pasquale & Burch, 1963), and the response appears to depend on the degree of pre-existing venoconstriction (Laing *et al.*, 1978). Release of venodilator prostaglandins and kinins from the vein wall in response to angiotensin II could produce a net venodilator effect and the variation with dose observed 5 min after intravenous administration could represent a balance between a dose dependent constrictor effect and secondary venodilatation.

Oral administration of 80 mg frusemide produced venodilator and arterial constrictor effects at 15 min, showing that these effects do not depend on acute intravenous drug administration. Although it is likely that little diuresis had occurred by this stage one could not be absolutely sure that these peripheral vascular responses were 'non-diuretic'. In conclusion, this study shows that unlike the diuretic responses, the peripheral vascular effects of frusemide are independent of dose throughout the range 5–80 mg intravenously. Venodilatation and arterial construction do not depend on intravenous drug administration and effects are seen after oral

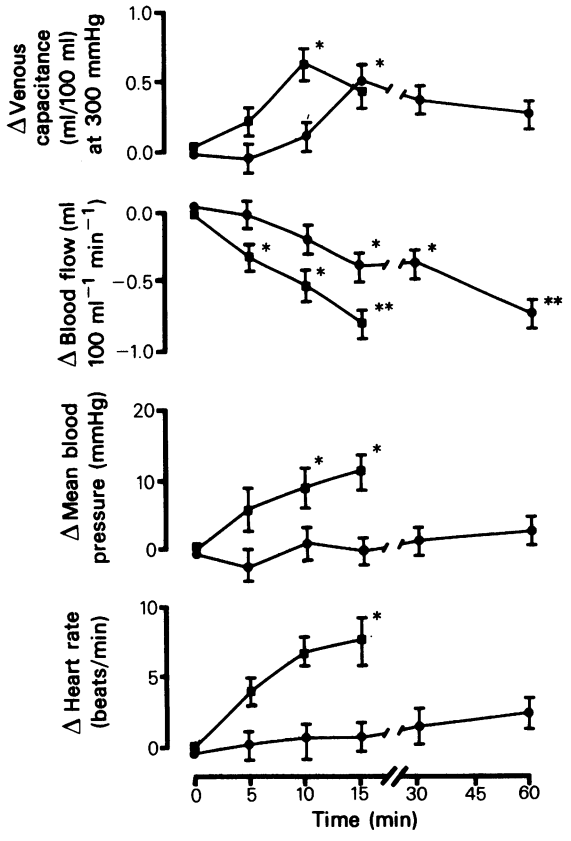

Figure 5 The effects of 80 mg frusemide, given intravenously, over a 15 min period (—) and orally, over a 1 h period (—), on venous capacitance, forearm blood flow, mean blood pressure and heart rate. The changes are measured from pre-treatment baselines and expressed as mean ± s.e. mean. $^{*}P < 0.05$; $^{**}P < 0.01$.

therapy. From this and other studies it is likely that the vascular effects are related to acute frusemide induced renin release with angiotensin formation and secondary release of vasodilator substances from the kidney or vessel wall.

Table 2 The vascular effects of 80 mg frusemide before and for the 1 h period following oral administration

	Time (min)	*Venous capacitance (ml/100 ml)*	*Forearm blood flow (ml 100 ml^{-1} min^{-1})*	*Blood pressure (mm Hg)*	*Heart rate (beats/min)*
Pre	−10	2.86 ± 0.32	2.12 ± 0.30	88.0 ± 3.3	70.0 ± 6.2
treatment	− 5	2.80 ± 0.32	2.00 ± 0.26	86.6 ± 2.8	70.5 ± 6.3
	0	2.93 ± 0.32	2.20 ± 0.31	87.1 ± 2.9	70.1 ± 6.9
	5	2.82 ± 0.35	2.08 ± 0.33	84.9 ± 2.2	70.8 ± 6.6
	10	2.87 ± 0.38	1.92 ± 0.27	88.3 ± 3.3	71.8 ± 6.3
	15	3.39 ± 0.41†	1.71 ± 0.26†	86.9 ± 3.2	72.8 ± 6.6
	30	3.31 ± 0.46	1.74 ± 0.30†	88.2 ± 2.7	73.8 ± 6.4
	60	3.16 ± 0.54	1.36 ± 0.21††	89.1 ± 3.4	75.2 ± 6.4

† Change compared to baseline < 0.05
†† Change compared to baseline < 0.01

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