The effects of propranolol and digoxin on the acute vascular responses to frusemide in normal man

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1 To examine the importance of acute frusemide-induced renin release in the production of the acute peripheral venous and arterial responses to frusemide in man, the effects of two drugs, previously described as inhibitors of acute frusemide-induced renin release, propranolol and digoxin, were examined.

2 Propranolol abolished the acute increases in venous capacitance and blood pressure and attenuated the increases in forearm vascular resistance produced by frusemide. The acute increases in plasma renin activity and plasma aldosterone concentrations were also abolished.

3 Pre-treatment with digoxin had no effect on the acute peripheral vascular responses to frusemide and failed to inhibit the acute increases in plasma renin activity and plasma aldosterone produced by frusemide.

4 The study provides further evidence of a relationship between acute frusemide-induced renin release and the acute peripheral vascular effects of frusemide in man.

Keywords digoxin frusemide propranolol non-diuretic effects

Introduction

The loop diuretic frusemide causes a decrease in left ventricular filling pressure accompanied by venodilatation and peripheral venous pooling within minutes of administration and before a diuresis is apparent (Dikshit *et al.*, 1973). We have previously shown that this only occurs when the renin-angiotensin system is activated and when frusemide gives rise to acute renin release. If frusemide-induced renin release is inhibited by salt overloading or indomethacin these acute venodilator effects do not occur (Johnston *et al.*, 1983a). Further evidence that renin release and angiotensin formation after frusemide administration are related to the acute vascular effects is the observation that pre-treatment with captopril abolishes the venodilator and acute pressor responses to frusemide (Johnston *et al.*, 1983b). Digoxin and

β -adrenoceptor blocking agents have been previously described as inhibiting acute frusemide stimulated renin release (Ferrari, 1973; Bravo *et al.*, 1974). Since digoxin and β -adrenoceptor antagonists are frequently used with frusemide in the treatment of cardiovascular disease, and since these drugs appear to reduce angiotensin II by different mechanisms the following experiment was undertaken to determine the effects of pre-treatment with digoxin and propranolol on the acute vascular effects of frusemide in man.

Methods

Twelve healthy volunteers, six males and six 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 (93.4 ± 2.9 , range 84–110 $\mu\text{mol}/100\text{ ml}$). The protocol of the study had been approved by the Ethics Committee of The Queen's University, Belfast. The study was carried out at the same time of day on three separate occasions with a period of at least 1 week between studies. For the 3 days before each part of the experiment, subjects received 60 mmol of sodium daily in their diet and 80 mg of oral frusemide daily (Johnston *et al.*, 1983b). On the day of each experiment timed urine samples were obtained to calculate urinary sodium concentration and determine whether subjects had adhered to the protocol. A 19 gauge butterfly needle was then inserted into an antecubital vein of the left arm for blood sampling and drug administration. 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 + $\frac{1}{3}$ (systolic-diastolic). Forearm blood flow and venous capacitance were measured in the right arm by venous occlusion plethysmography (Whitney, 1953). Venous capacitance was determined by the equilibration technique at a venous occlusion pressure of 30 mm Hg (4 kPa) and forearm 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. Forearm vascular resistance was determined by dividing the mean blood pressure by the blood flow. Room temperature throughout was maintained at $24 \pm 0.5^\circ\text{C}$.

After a 1 h period of rest 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 aldosterone concentrations. Subjects then received 750 μg digoxin, 10 mg propranolol (dissolved in 20 ml normal saline) or 20 ml normal saline delivered at a rate of 1 ml min^{-1} according to a double-blind, randomised cross-over design. Forty five minutes after the end of each infusion, a further three sets of readings were taken and 5 mg frusemide administered as an intravenous bolus over 10 s. Venous capacitance, forearm blood flow, blood pressure and heart rate were again measured at 5 min intervals over the next 15 min. Plasma renin activity and plasma aldosterone concentrations were measured immediately before digoxin, propranolol or

placebo, before frusemide and 10 min after frusemide administration.

For estimation of plasma renin activity 10 ml of blood was immediately placed in glass 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 (A I) generated $\text{h}^{-1}\text{ ml}^{-1}$ of plasma at pH 7.4 and at 37°C . A I was measured by radioimmunoassay with a Gamma Coat Kit (Clinical Assays, Travenol Laboratories Inc.) (Haber *et al.*, 1969). Plasma aldosterone concentrations were measured with a radioimmunoassay kit supplied by C.I.S. (UK) Ltd, North Finchley, London, and urinary sodium concentrations by flame photometry.

Comparisons of multiple data were made by using analysis of variance and Duncan's multiple range test. Values after each treatment were compared with their immediate pre-treatment values at -45 min (before placebo, propranolol or digoxin) or 0 min (before frusemide) (Figure 1). For single paired data, the Wilcoxon matched pairs signed rank test was used and a Mann-Whitney U test was used for unpaired data. Results are expressed as the mean $\pm$ s.e. mean, and the level of significance chosen as $P < 0.05$.

Results

In the placebo treated group, venous capacitance 10 min after 5 mg frusemide intravenously increased from 2.15 ± 0.16 to $2.46 \pm 0.18\text{ ml } 100\text{ ml}^{-1}$ ($P < 0.05$). In the digoxin pre-treated group an increase was also observed from 1.74 ± 0.15 to $2.05 \pm 0.16\text{ ml } 100\text{ ml}^{-1}$ ($P < 0.05$). No venodilator response was observed following frusemide in the group pre-treated with propranolol (Figure 1). Venous capacitance measured under the same conditions in a group of 40 volunteers receiving placebo showed a within day coefficient of variation of 18% and a between day coefficient of 26%.

Forearm vascular resistance increased in the digoxin and propranolol treated groups 45 min after drug administration, but these changes were not statistically significant. At 10 and 15 min after frusemide, significant increases in forearm vascular resistance were observed in the placebo and digoxin pre-treated groups (Figure 1). Although small increases were observed in the propranolol treated group, there were no significant increases in forearm vascular resistance following frusemide administration (Figure 1). The increases in vascular resistance at 10 and 15 min after frusemide,

Figure 1 Changes in venous capacitance (ml 100 ml⁻¹ at 30 mm Hg, forearm vascular resistance (mm Hg ml⁻¹ 100 ml⁻¹ min⁻¹) and blood pressure (mm Hg) following 5 mg frusemide i.v. in the presence of placebo, propranolol and digoxin. Changes are compared with the immediate pre-treatment baseline measurements (-45 min or 0 min). * $P < 0.05$; ** $P < 0.01$.

however, were not different in the three groups when compared to the original baseline values (-45 min). Coefficients of variation for forearm vascular resistance in 40 volunteers under the same conditions were 32% (within day) and 42% (between day). No changes in mean blood pressure were observed 45 min after the administration of placebo, propranolol or digoxin (Figure 1). Mean blood pressure increased in the placebo and digoxin pre-treated groups following frusemide but no increase was observed after propranolol (Figure 1). No changes in heart rate were observed in the three groups.

Plasma renin activities after a 1 h period of rest were 1.76 ± 0.16 (placebo group), 1.89 ± 0.3 (propranolol group) and 1.83 ± 0.4 ng AI h⁻¹ ml⁻¹ (digoxin group). These levels were significantly greater than the mean plasma

renin activity obtained in this laboratory in a group of 66 salt replete healthy volunteers obtained in the standing position (1.07 ± 0.07). Plasma renin activity and plasma aldosterone concentrations were reduced 45 min after placebo ($P < 0.05$), digoxin ($P < 0.05$) and propranolol ($P < 0.01$) (Figure 2). An increase in plasma renin activity following frusemide was observed in the presence of placebo (1.41 ± 0.16 to 2.27 ± 0.17 ng AI h⁻¹ ml⁻¹; $P < 0.01$) and digoxin (1.41 ± 0.20 to 1.86 ± 0.40 ; $P < 0.01$) but not in the group pre-treated with propranolol (1.19 ± 0.18 to 1.23 ± 0.18 ; NS). Similarly, plasma aldosterone increased following frusemide in the placebo (109 ± 16 to 143 ± 21 pg ml⁻¹; $P < 0.01$) and digoxin (85 ± 13 to 122 ± 10 pg ml⁻¹; $P < 0.01$) treated groups but remained unchanged in the propranolol-

Figure 2 Changes in plasma renin activity (ng AI h⁻¹ ml⁻¹) and plasma aldosterone concentrations (pg ml⁻¹) before placebo, propranolol or digoxin (-45 min), before frusemide (0 mins) and 10 min after frusemide administration. Increases and decreases are compared with the immediate pre-treatment baseline values (-45 or 0 min). ♦♦ $P < 0.05$ (increase); ♦ $P < 0.05$ (decrease); ♦♦♦ $P < 0.01$ (increase); ◇◇ $P < 0.01$ (decrease).

treated group (105 ± 16 to 100 ± 14 , NS). Urinary sodium concentrations in the three groups at the beginning of each study day were 11.8 ± 2.3 mmol/l (placebo), 8.6 ± 1.5 mmol/l (propranolol) and 9.6 ± 2.4 (digoxin).

Discussion

We have previously shown that the early non-diuretic vascular effects of frusemide on venous capacitance in man are dependent on salt balance, prostaglandin synthesis and renal function (Johnston *et al.*, 1983a). A high salt diet, pre-treatment with indomethacin and severely impaired renal function will prevent the acute venodilator effects of frusemide (Johnston *et al.*, 1983a). In these situations acute frusemide-induced renin release and prostaglandin formation (PGE₂, PGI₂) are reduced. In addition we have shown that captopril, an angiotensin converting enzyme inhibitor prevents both the acute venodilator and peripheral arterial con-

strictor effects of frusemide in man suggesting that angiotensin II formation, occurring secondary to acute renin release from the kidney, is probably involved in producing these effects (Johnston *et al.*, 1983b). Since frusemide does not produce significant elevations of epoprostenol (PGI₂) in venous blood to account for the venodilator effects (Steer *et al.*, 1980; Patroni *et al.*, 1982; Johnston *et al.*, 1983a), and since the venous and arterial effects are abolished by captopril, we postulated that angiotensin II might stimulate the release of vasodilatory substances—PGE₂, PGI₂ or kinins in or near the venous capacitance vessels (Moncada *et al.*, 1977; Gimborne & Alexander, 1978) while in the arterial system the overall effect would be of vasoconstriction. If this were the case, then drugs which prevent frusemide induced renin release from the kidney by mechanisms which do not involve inhibition of prostaglandin formation, e.g. β -adrenoceptor blocking drugs (Gerber *et al.*, 1981) would be expected to prevent the drug's peripheral vascular effects.

The observation that propranolol but not digoxin abolished frusemide-induced renin release, prevented the acute venodilatation and attenuated the peripheral arterial constrictor effects would be in keeping with this theory. The reasons why digoxin failed to inhibit frusemide-induced renin release are unclear but the effects of digoxin on the renin-angiotensin system in salt depleted volunteers could be different from those occurring in patients with congestive heart failure (Covit *et al.*, 1983) or hypertension (Montanaro *et al.*, 1980).

One other possible explanation of the peripheral vascular effects of frusemide in the presence of β -adrenoceptor blockade is that propranolol alters the vascular responsiveness to circulating vasoactive materials and the arterial responsiveness to vasoconstrictor sub-

stances can depend on the degree of pre-existing vascular tone (Laing *et al.*, 1978). The observation that frusemide had less effect on vascular resistance in the presence of propranolol could have been due to the fact that propranolol had already increased vascular tone due to β_2 -adrenoceptor blockade. Since propranolol had no effect on venous tone, it is difficult to explain the observed venous responses on this basis.

In conclusion, pre-treatment with propranolol prevents the acute venodilator effects of frusemide and attenuates the arteriolar constrictor responses. This study provides further evidence of a relationship between acute renin release and the acute peripheral vascular effects of frusemide in man.

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