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INTRARENAL STIMULATION OF RENIN SECRETION BY FRUSEMIDE IN THE ISOLATED KIDNEY OF THE RAT

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1 Intrarenal infusion of frusemide markedly stimulated renin secretion in the isolated perfused kidney of the rat.

2 Renin values increased from 24 ± 6 to 195 ± 34 units of secretion rate (renin concentration (nmol angiotensin I/h per litre) $\times$ flow rate (ml/min)) following administration of frusemide for 8 min, compared with corresponding control values of 13 ± 2 ($P > 0.05$) and 47 ± 18 ($P < 0.001$). This stimulatory effect was also observed when urine flow was interrupted by ligation of the ureters.

3 The changes in renal perfusion pressure and perfusate flow rate were not significantly different from the control values.

4 These findings indicate the existence of an intrarenal site of action for frusemide on renin secretion.

5 Since frusemide did not appear to alter perfusion pressure or flow rate, and was effective when urine flow was abolished, a dominant role for a vascular or macula densa receptor mechanism seems unlikely. A direct effect of frusemide on the renin secreting cell is therefore suggested.

Introduction

The administration of potent natriuretic agents such as frusemide leads to marked stimulation of renin release (Fraser, James, Brown, Isaac, Lever & Robertson, 1965; Rosenthal, Boucher, Nowaczynski & Genest, 1967; Vander & Luciano, 1967). The simplest explanation is that renin secretion is stimulated by the reduction in renal perfusion pressure (Skinner, McCubbin & Page, 1964) or the secondary release of catecholamines (Gordon, Küchel, Liddle & Island, 1967; Winer, Chokshi, Yoon & Freedman, 1969) subsequent to an acute fall in plasma volume. However, studies in dogs (Vander & Carlson, 1969) and rabbits (Meyer, Menard, Papanicolaou, Alexandre, Devaux & Milliez, 1968) have demonstrated that the stimulation of renin secretion by frusemide persists even when changes in body sodium and water balance are prevented. This is supported by a recent study in man showing that the rapid increase in plasma renin activity following intravenous frusemide did not correlate with plasma volume changes (Hesse, Nielsen & Lund-Jacobson, 1975).

Although these observations suggest an intrarenal site of action of frusemide on renin release, extrarenal factors cannot be excluded by the experimental conditions employed. In the study described here the isolated perfused kidney of the rat (Vandongen, Peart & Boyd, 1973) has been used to investigate a possible intrarenal site of action of frusemide on renin release and to relate this to changes in renal perfusion pressure and flow rate.

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Methods

Kidney perfusion

Male wistar rats (150–250 g) maintained on similar diets were anaesthetized with sodium pentobarbitone (0.1 mg/g by intraperitoneal injection) and given heparin (50–100 units intravenously). The left kidney was cannulated and perfused with Krebs–Ringer saline by means of a pulsatile flow inducer pump (Watson Marlow MHRE 1000) as previously described (Vandongen *et al.*, 1973) except that in most experiments the ureter was occluded by ligature before starting the perfusion. Perfusion pressure was monitored continuously by a pressure transducer (Bell and Howell) and recorder (Devices MX2). Approximately 5 min after starting the perfusion, which was designated 0 min, a timed collection of effluent perfusate was obtained for determination of renin concentration. At 1 min the infusion of frusemide (Lasix, supplied by Hoechst Australia, Pty. Ltd.) diluted in distilled water to the required concentration, or distilled water only (control study), was started at the rate of 0.04 ml/min and this was continued for the duration of the experiment. Further collections of perfusate were obtained at 5 min and 9 min (4 and 8

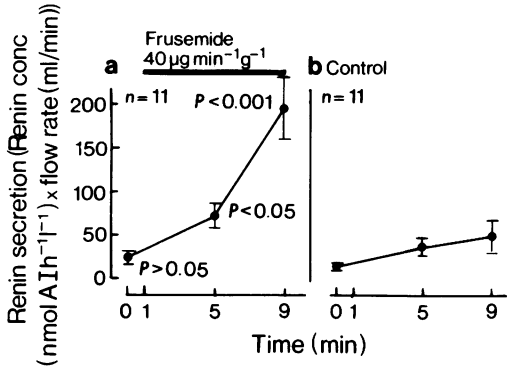

Figure 1 The effect of frusemide, infused from 1 to 9 min, on renin secretion rate (a). The control study without frusemide infusion is shown in (b). All values are means of 11 experiments. Vertical lines show s.e. means. *P* values refer to significance of difference between frusemide and corresponding control values (Student's nonpaired *t* test). AI = angiotensin I.

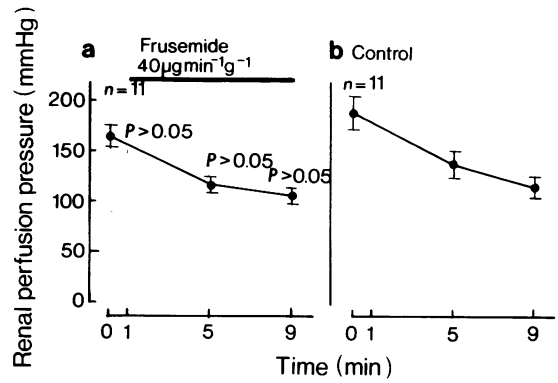

Figure 2 The effect of frusemide, infused from 1 to 9 min, on renal perfusion pressure (a). The control study without frusemide infusion is shown in (b). All values are means of 11 experiments. *P* values refer to significance of difference between frusemide and corresponding control values (Student's nonpaired *t* test).

min respectively after the start of frusemide or distilled water infusion). The doses of frusemide are expressed per g wet kidney weight (non-perfused kidney). Control and frusemide experiments were performed alternately.

Renin assay

Timed collections of perfusate were treated according to the method of Skinner (1967) for plasma renin activity. After incubation for 6 h with nephrectomized rat plasma as renin substrate source (treated as sheep substrate by the method of Skinner) further reaction was terminated by heating at 85°C for 5 min, and the samples assayed without extraction for angiotensin I by radioimmunoassay (Boyd, Adamson, Fitz & Peart, 1969). Renin concentration is expressed in nmol equivalents of 1-Asp-5-Ile-angiotensin I generated per h per litre of perfusate and converted into secretion rate by multiplying by the flow rate (ml/min). All values shown are mean values ± s.e. mean. Statistical analysis was carried out using non-paired Student's *t* test and 2-tailed probability tables. All samples from any one experiment were processed and assayed at the same time.

Results

Figure 1 compares the response in renin secretion rate to frusemide (40 μg min^{-1} g^{-1}), after infusion for 4 and 8 min in 11 experiments, with that observed in 11 control experiments conducted over the same period of time. Following the introduction of frusemide at 1 min, renin secretion (as defined in the Methods section) increased from 24 ± 6 at 0 min to 72 ± 13 at 5 min and to 195 ± 34 at 9 minutes. In 2 experiments where the ureter was not occluded, renin secretion increased from 24 and 15 at 0 min to 355 and 246, respectively, at 9 minutes. In the control studies the rise in renin secretion observed, 13 ± 2 at 0 min to 35 ± 10 at 5 min and 47 ± 18 at 9 min, was similar to that reported in previous studies with this preparation (Vandongen & Greenwood, 1975b) but was significantly lower than during the infusion of frusemide. When the individual changes are expressed as a percentage of the 0 min value, the mean increase at 9 min is 283% in the control and 927% in the frusemide group ($P < 0.05$).

The response to frusemide is at least partly determined by the basal secretion rate at 0 min since a significant inverse relationship was found between this and the percentage increase at 9 min ($r = -0.66$, $P < 0.05$).

The changes in renal perfusion pressure during the infusion of frusemide and in the corresponding control study are shown in Figure 2. Although the initial and subsequent pressures were somewhat higher in the control group, these values and the actual percentage fall from 0 min to 9 min (36 ± 3% for the frusemide and 35 ± 4% for the control group) were not significantly different. During this progressive fall in renal perfusion pressure flow rates increased marginally, 7.4 ± 0.4 at 0 min to 8.3 ± 0.4 ml/min at 9 min in the frusemide and 8.4 ± 0.7 to 9.5 ± 0.5 ml/min in the control group, there being no significant differences between the two groups ($P > 0.05$).

This finding indicates that renal autoregulatory function was preserved.

Discussion

Previous observations that intravenous frusemide increased plasma renin levels in animals even when urinary losses were replaced (Bailie, Davis & Loutzenhiser, 1973) have been cited in support of an intrarenal action of frusemide on renin secretion. However the experimental conditions in these studies were such that extrarenal factors cannot be completely ruled out.

Conclusive evidence for an intrarenal effect of frusemide on renin secretion is provided by the present study in which the isolated kidney of the rat was used. As seen with isoprenaline (Vandongen *et al.*, 1973) and diazoxide (Vandongen & Greenwood, 1975a), the response to a given dose of frusemide in this model varied considerably between experiments. One important determinant of this response appears to be the basal secretion rate, but other less apparent factors may also be involved. Although occlusion of the ureters alone will increase renin secretion (Kaloyanides, Bastron & Dibona, 1973) the basal renin values in this study are similar to values obtained with patent ureters recently reported from this laboratory (Vandongen & Greenwood, 1975b). It is apparent that the response to frusemide is not dependent on occlusion of the ureters as it is also observed when the ureter is patent. The similarity of the changes in renal perfusion pressure and flow rate in the frusemide and control experiments makes it unlikely that these are important in mediating the response in renin secretion. This is in contrast with other studies where the increase in renin secretion following intravenous frusemide was associated with a fall in renal blood flow and variable changes in mean arterial blood pressure (Meyer *et al.*, 1968; Vander & Carlson, 1969; Bailie *et al.*, 1973). It is conceivable however that frusemide produced localized changes in pressure and flow distribution (Birtch, Zakheim, Jones & Barger, 1967) in the isolated kidney which would not be detected by the methods used. The effect of frusemide on renin secretion was observed after ligation of the ureters and the presumed abolition of urine flow (Kaloyanides *et al.*, 1973) suggesting that the macula densa, which may influence renin secretion by detecting changes in tubular sodium movement (Vander, 1967), is not involved. Further evidence against an important role for this mechanism is the observation that renin secretion is stimulated by intravenous frusemide in dogs with a single non-filtering kidney (Corsini, Hook & Bailie, 1975) where the macula densa is presumed to be destroyed (Blaine, Davis & Witty, 1970).

It has been postulated that frusemide inhibits sodium transport at the macula densa site and that the resulting lowering of intracellular sodium concentration initiates renin release (Vander & Carlson, 1969). Whilst this proposal remains speculative, a direct effect of frusemide on the renin-producing cell should also be considered. Such an effect has been suggested for isoprenaline (Vandongen *et al.*, 1973), adrenaline and noradrenaline (Vandongen & Greenwood, 1975b), which in small doses activate only the renal β-adrenoceptors mediating renin secretion, and diazoxide a sodium-retaining thiazide derivative (Vandongen & Greenwood, 1975a). These agents have been shown to stimulate renin secretion in the isolated kidney independently of changes in renal perfusion pressure and flow rate. However, like frusemide (Hook, Blatt, Brody & Williamson, 1966; Ludens, Hook, Brody & Williamson, 1968; Ludens, Heitz, Brody & Williamson, 1970), these agents are potential renal vasodilators. It is conceivable therefore that the cellular mechanism involved in renin secretion is functionally related in some way to the contractor process and evidence has been previously given that calcium may occupy an important intermediary role in this relationship (Vandongen, Peart & Boyd, 1974; Vandongen & Peart, 1974).

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