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Frusemide releases renin in the rat kidney when prostacyclin synthesis is suppressed

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1 The effect of inhibiting prostaglandin (PG) synthesis on basal and frusemide-stimulated renin secretion was examined in the rat isolated perfused kidney. The stable PGI_2 derivative, 6-keto $PGF_{1\alpha}$, was measured by radioimmunoassay in urine collected from the kidney.

2 Treatment of rats with indomethacin ($3.0\,mg\,kg^{-1}$) reduced 6-keto $PGF_{1\alpha}$ excretion from 121.3 ± 39.1 ($n=9$) to 15.5 ± 6.6 ($n=9$) $pg\,min^{-1}$ ($P<0.02$) but had no effect on basal renin secretion. Renal perfusion pressure, flow rate and vascular resistance were similar in treated and control rats. Mean urine flow was lower after treatment.

3 Infusion of frusemide ($250\,\mu g\,min^{-1}$) did not alter 6-keto $PGF_{1\alpha}$ excretion in control or indomethacin-treated ($P>0.05$) rats. Although renin secretion was increased during frusemide infusion, there was no significant difference between control ($1{,}806 \pm 384$ ng angiotensin I (AI) min^{-1}) and treated ($2{,}310 \pm 554$ ng AI min^{-1}) rats ($P>0.05$). Propranolol, at a dose ($8\,\mu g\,min^{-1}$) which suppressed renin secretion after isoprenaline stimulation, had no effect on the response to frusemide in indomethacin-treated rats.

4 These results demonstrate that frusemide-stimulated renin secretion in the rat kidney does not require intact renal PGI_2 synthesis and is independent of β-adrenergic mechanisms.

Introduction

There is evidence suggesting that prostaglandins are important mediators of renin secretion in response to reduced renal perfusion pressure (Berl *et al.*, 1978; Data *et al.*, 1978; Blackshear *et al.*, 1979); haemorrhage (Romero *et al.*, 1976) and sodium depletion (Frölich *et al.*, 1979; DeForrest *et al.*, 1980).

The prostaglandin precursor arachidonic acid stimulates renin release (Larsson *et al.*, 1974; Gerber *et al.*, 1979), probably after conversion to endogenous prostaglandins as the effect is blocked by the prostaglandin cyclo-oxygenase inhibitor indomethacin (Larsson *et al.*, 1974; Data *et al.*, 1978). Further studies with individual prostaglandins have shown that PGE_2, PGD_2 and PGI_2 are each capable of releasing renin in the denervated, non-filtering kidney (Gerber *et al.*, 1979; Seymour *et al.*, 1979). PGI_2 which is a product of vascular endothelium, is found in substantial amounts in the renal cortex where the renin containing glomeruli are also located. Studies on rabbit renal cortical slices (Whorton *et al.*, 1977) and perfused rat and rabbit kidney (Schwertschlag *et al.*, 1982) clearly indicate a direct stimulatory effect of PGI_2 on renin release. PGI_2 appears to be considerably more potent than PGE_2 (Whorton *et al.*, 1977; Güllner *et al.*, 1980) although which of these is the major prostaglandin involved in the control of renin secretion has not been resolved (Franco-Saenz *et al.*, 1980).

Frusemide stimulates renin release in the dog (Corsini *et al.*, 1975) and rat kidney (Lyons & Churchill, 1975; Vandongen, 1977) by a direct intrarenal action. Since increased urinary PGE_2 excretion has been shown to be associated with frusemide administration in man (Scherer & Weber, 1979; Brater *et al.*, 1980) and in dogs (Patak *et al.*, 1979; Sreenivasan *et al.*, 1981) it has been suggested that prostaglandins are involved in mediating the renal haemodynamic and renin response to frusemide. Furthermore, frusemide also appears to enhance production of PGI_2-like material from the rat aorta (Sullivan & Patrick, 1981) and increased urinary excretion of 6-keto $PGF_{1\alpha}$, the stable metabolite of PGI_2, was found in man after intravenous administration of frusemide (Patrono *et al.*, 1982).

The rat isolated perfused kidney was used in this study to examine the dependence of frusemide-

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induced renin release on renal prostaglandin production thereby avoiding potentially confounding systemic influences. Because PGI_2 is the most likely prostaglandin involved in the control of renin release, 6-keto $PGF_{1\alpha}$ was measured in urine during frusemide administration both before and after cyclo-oxygenase inhibition with indomethacin.

Methods

Male Wistar rats fed a standard commercial diet (0.13 mM sodium g^{-1}) were anaesthetized with sodium pentobarbitone (0.1 mg g^{-1}), given by intraperitoneal (i.p.) injection. Approximately 30 min later the left carotid artery was cannulated, heparin (50–100 u) was administered and an endotracheal tube inserted. Through a midline incision the abdomen was opened and the left ureter cannulated. Urine was collected over a 10 min period in a preweighed tube. The left kidney was then isolated and perfused at constant pressure with modified Krebs-Ringer buffer containing Hemaccel (35 g l^{-1} colloidal solution, Hoechst, W. Germany), oxygenated with 95% O_2 plus 5% CO_2 and maintained at 37°C as previously described (Vandongen *et al.*, 1973). Urine was collected from the perfused kidney over 10 min and effluent perfusate over 1 min for determination of 6-keto $PGF_{1\alpha}$ and renin concentration, respectively. Perfusion pressure (mmHg) was continuously recorded from the renal arterial cannula and maintained between 80–90 mmHg by adjusting the flow rate. Flow rate (ml min^{-1}) of the effluent perfusate was measured by timed collection in a graduated cylinder. Renal vascular resistance was calculated as the ratio of perfusion pressure and flow rate and expressed in arbitrary units. All drugs were diluted in 0.9% w/v NaCl solution (saline) and infused at 0.04 ml min^{-1}.

Effect of indomethacin on frusemide-stimulated renin secretion and urinary 6-keto prostaglandin $F_{1\alpha}$ excretion

Two groups of rats were used. One group ($n = 9$), weight 322 ± 15.6 g(mean ± s.e.mean), were given indomethacin (Merck, Sharp and Dohme, Australia) 3.0 mg kg^{-1} by i.p. injection at the same time as the anaesthetic was administered. Indomethacin was dissolved in 0.2 M K_2HPO_4 buffer and freshly prepared on the day of administration. The other group ($n = 8$, weight 309 ± 15 g) were given 0.2 M K_2HPO_4 buffer alone and were controls. The animals were ready for urine collection and kidney perfusion, as described above, about 1 h after the administration of indomethacin or buffer.

When perfusion pressure had stabilized, usually within 30 min of starting perfusion (time 0), 2 consecutive 10 min collections of urine were obtained for determination of 6-keto $PGF_{1\alpha}$ excretion. Halfway through each collection period (i.e. at 5 and 15 min) perfusate was sampled over 1 min for determination of basal renin concentration. Perfusion pressure and flow rate were also recorded at these times. Frusemide, diluted in saline, was then infused into the arterial cannula, 250 μg min^{-1} for 10 min. Urine was collected over this 10 min period, perfusate sampled over 1 min, 5 and 10 min after commencing frusemide infusion (i.e. at 25 and 30 min), and flow rate and perfusion pressure recorded.

Effect of indomethacin and propranolol on frusemide-stimulated renin secretion and urinary 6-keto prostaglandin $F_{1\alpha}$ excretion

In the first instance the effect of propranolol on a known β-adrenergic stimulus, isoprenaline, was determined. A group of rats ($n = 9$, weight 314 ± 8.5 g) was prepared for perfusion and after stabilization of perfusion pressure (time 0), 1 min collections of perfusate were obtained at 5 and 15 min for determination of basal renin concentration. (±)-Propranolol HCl (ICI, Australia) was then infused at 8 μg min^{-1} and continued for the duration of the experiment. Perfusate collections were obtained at 20 and 30 min. The infusion of isoprenaline (0.01 μg min^{-1}) was then commenced and further perfusate collected at 35 and 45 min. Perfusion pressure and flow rate were noted at all the times that perfusate was sampled. A control group ($n = 12$, weight 272 ± 13.6 g), which received isoprenaline infused alone and saline substituted for propranolol, was also included.

In a further group of rats ($n = 9$, weight 352 ± 11.7 g) indomethacin (3.0 mg kg^{-1}) was given by i.p. injection approximately 1 h before urine collection and kidney perfusion. The procedure outlined above was then followed except that frusemide (250 μg min^{-1}) was infused instead of isoprenaline.

Measurement of renin secretion rate and urinary 6-keto prostaglandin $F_{1\alpha}$ excretion

Renin concentration was measured by radioimmunoassay of angiotensin I (AI) generated by incubating renal perfusate with renin substrate (prepared from nephrectomized rats) at 37°C and pH 7.4, in the presence of disodium EDTA (0.003 M), 8-hydroxyquinoline (0.0035 M) and dimercaprol (0.0014 mM). Renin secretion rate was obtained by multiplying renin concentration by flow rate and therefore represents the quantity of angiotensin generated by the amount of renin produced by the kidney in 1 min.

The stable metabolite of PGI_2, 6-keto $PGF_{1\alpha}$, was measured directly in urine collected from the kidney

by radioimmunoassay using a specific antiserum (Ono Pharmaceutical Co., Japan) and ^{125}I-labelled histamine coupled to 6-keto $PGF_{1\alpha}$ (Upjohn Co., USA) by the technique of Maclouf *et al.*, (1976). Free iodinated prostaglandin was separated from bound by dextran coated charcoal. In this assay PGD_2 and 6,15-diketo $PGF_{1\alpha}$ cross-react less than 1%, and PGE_2 and $PGF_{2\alpha}$ less than 5%. The lower limit of sensitivity was 15 pg ml^{-1} and the interassay coefficient of variation was 12.3%. Direct assay of 6-keto $PGF_{1\alpha}$ in urine from the perfused kidney gives similar levels and is closely correlated with values obtained after organic separation and chromatographic separation, as previously described (Vandongen *et al.*, 1982).

In order to validate the direct measurement of 6-keto $PGF_{1\alpha}$ in urine collected before isolating and perfusing the kidney, we compared these values with those obtained after extraction and chromatography in urine samples collected from rats in metabolic cages before and after twice weekly i.p. indomethacin (3.0 mg kg^{-1}) ($n=9$) or an equivalent volume of 0.2 M K_2HPO_4 buffer (control, $n=9$) for 2 weeks.

All values are means ± s.e.mean. Paired data were analysed by the Wilcoxon matched pairs of signed-ranks test and unpaired data by the Mann-Whitney U test. Comparison of more than 2 values was by one-way analysis of variance.

Results

Indomethacin and frusemide-stimulated renin secretion and urinary 6-keto prostaglandin $F_{1\alpha}$

Basal urinary 6-keto $PGF_{1\alpha}$ excretion, before isolation and perfusion of the kidney, was 197 ± 81.2 pg min^{-1} ($n=7$) in the control group given phosphate buffer. Administration of indomethacin (3.0 mg kg^{-1}) 1 h before reduced this to 26 ± 7.4 pg min^{-1} ($n=9$, $P<0.006$). In one of the control groups basal urine collections were not obtained.

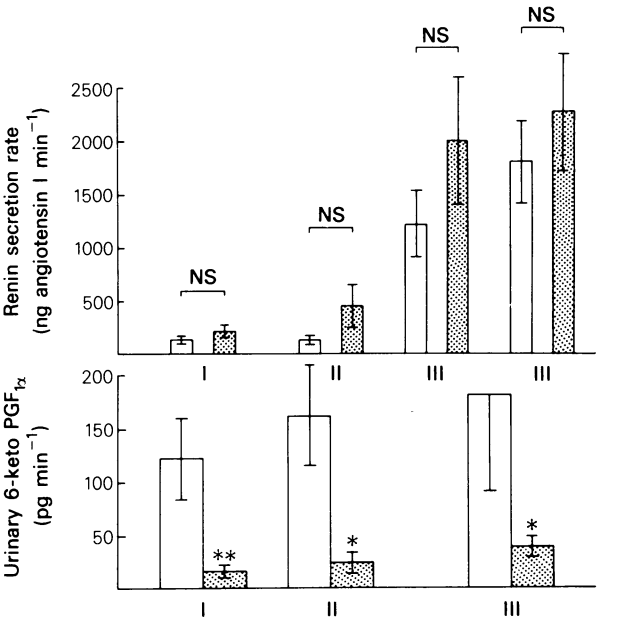

Figure 1 Renin secretion rate and urinary 6-keto prostaglandin (PG) $F_{1\alpha}$ excretion in the perfused kidney of control (open columns) and indomethacin-treated (3.0 mg kg^{-1}) (stippled columns) rats under basal conditions (I & II) and during infusion of frusemide (250 μg mm^{-1}) (III). $^{NS}P>0.05$, Columns represent means ± s.e.mean (vertical bars). $^{*}P<0.05$, $^{**}P<0.02$, compared to control excretion (Mann-Whitney U test).

As shown in Figure 1, 6-keto $PGF_{1\alpha}$ in the urine of the perfused kidney was significantly reduced by prior administration of indomethacin and remained suppressed during infusion of frusemide.

Table 1 Renal perfusion pressure, flow rate and resistance (arbitrary units) measured before (5 and 15 min) and during (25 and 30 min) infusion of frusemide (250 μg min^{-1})

	Time (min)			
	5	*15*	*25*	*30*
			Frusemide	
Control ($n=8$)				
Perfusion pressure (mm Hg)	83.8 ± 1.2	82 ± 1.7	80 ± 2.5	78.3 ± 2.3
Flow rate (ml min^{-1})	5.1 ± 0.8	6.0 ± 0.8	6.5 ± 1.0	6.8 ± 1.1
Resistance	19 ± 2.6	16 ± 3.0	15.4 ± 3.5	14.4 ± 3.3
Indomethacin ($n=9$)^{NS}				
Perfusion pressure (mm Hg)	83 ± 1.7	81.8 ± 1.6	82.8 ± 1.2	83.4 ± 1.4
Flow rate (ml min^{-1})	5.4 ± 0.6	7.1 ± 0.8	7.6 ± 0.9	7.9 ± 1.0
Resistance	16.5 ± 1.7	12.7 ± 1.5	12.3 ± 1.6	12.0 ± 1.7

^{NS}Values are means ± s.e.; no values were significantly different from control ($P>0.05$) (Mann-Whitney U test).

Table 2 Urinary volumes ($\mu l\ min^{-1}$), collected over 10 min before (10 and 20 min) and during (30 min) infusion of frusemide ($250\ \mu g\ min^{-1}$) in control and indomethacin-treated groups

	Time (min)		
	0–10	*10–20*	*20–30*
			Frusemide
Control ($n=8$)	15.8 ± 4.6	29.3 ± 10.2	53.5 ± 17.8
Indomethacin ($3.0\ mg\ kg^{-1}$) ($n=9$)	7.2 ± 3.6*	13.7 ± 7.5^{NS}	32.1 ± 15.1^{NS}

For significance of difference from control values ^{NS} $P > 0.05$; * $P < 0.05$ (Mann-Whitney U test).
Values are means ± s.e.

Renin secretion increased considerably during administration of frusemide ($F = 12.41$, $P < 0.005$) without a concomitant increase in urinary 6-keto $PGF_{1\alpha}$ ($F = 0.27$, $P > 0.05$). Pretreatment with indomethacin, which substantially reduced 6-keto $PGF_{1\alpha}$ levels, did not alter resting renin secretion or the response to frusemide when compared with the control values (Figure 1) ($F = 7.22$, $P < 0.005$).

Renal perfusion pressure, flow rate and renal vascular resistance (Table 1) were not altered in indomethacin-treated rats, but urine flow rates were lower although this was significant only for the first 10 min collection (Table 2). The apparent increase in urine flow during the experiment was not significant in either control ($F = 2.83$, $P > 0.05$) or indomethacin-treated ($F = 1.90$, $P > 0.05$) groups.

Effect of indomethacin and propranolol on frusemide-stimulated renin secretion and urinary 6-keto prostaglandin $F_{1\alpha}$

Figure 2 illustrates that stimulation of renin secretion by isoprenaline ($0.01\ \mu g\ min^{-1}$) to levels comparable with those achieved during frusemide administration is suppressed by propranolol ($8\ \mu g\ min^{-1}$). This confirms previous findings in this preparation demonstrating that the renin response to isoprenaline is mediated by a β-adrenoceptor mechanism (Vandongen *et al.*, 1973).

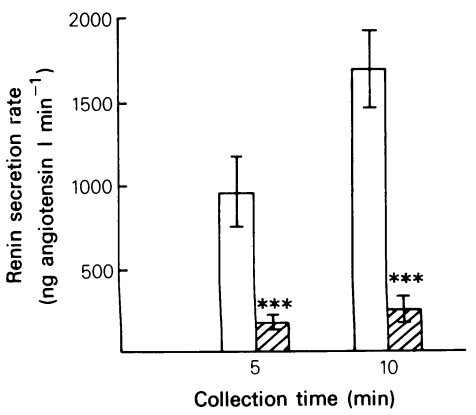

Figure 2 Suppression of isoprenaline ($0.01\ \mu g\ min^{-1}$)-stimulated renin secretion (open columns) by propranolol ($8\ \mu g\ min^{-1}$) (hatched columns). Columns represent means ± s.e.mean (vertical bars). ***$P < 0.002$, significance of differences determined by Mann-Whitney U test.

As shown in Figure 3, pretreatment with indomethacin ($3.0\ mg\ kg^{-1}$) and administration of propranolol at a dose demonstrated to suppress isoprenaline-stimulated renin release, failed to affect the response to frusemide. The excretion of 6-keto $PGF_{1\alpha}$ in urine collected before ($40.4 \pm 16.3\ pg\ min^{-1}$) and during perfusion (Figure 3) were comparable to values previously obtained after indomethacin treatment.

Renal perfusion pressure, flow rate and resistance did not significantly change during propranolol and

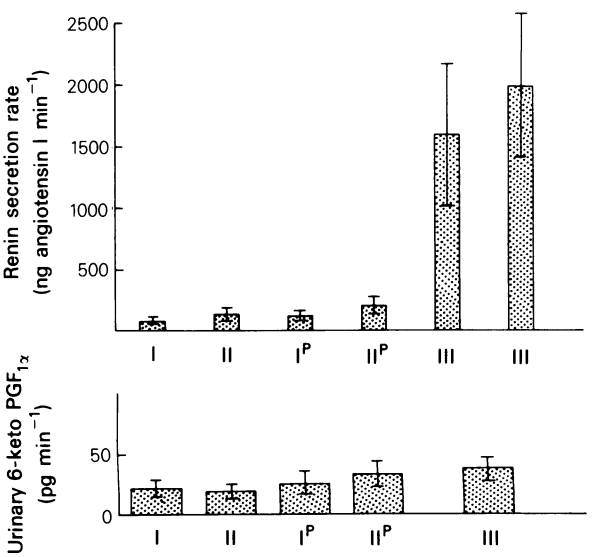

Figure 3 Renin secretion rate and urinary 6-keto prostaglandin (PG) $F_{1\alpha}$ excretion in the perfused kidney in indomethacin-treated ($3.0\ mg\ kg^{-1}$) rats under basal conditions (I & II), during propranolol infusion ($8\ \mu g\ min^{-1}$) (I^P & II^P) and during combined propranolol and frusemide infusion ($250\ \mu g\ min^{-1}$) (III). Columns represent mean values ± s.e.mean (vertical bars).

Table 3 Twenty four hour urinary concentration of 6-keto prostaglanding $F_{1\alpha}$ measured by radioimmunoassay directly (unextracted) and after organic extraction and chromatographic separation (extracted), before and after treatment with indomethacin (3.0 mg kg^{-1})

	6-keto $PGF_{1\alpha}$ (ng $24\,h^{-1}$)	
	Before	*After buffer/ indomethacin*
Buffer ($n=9$)		
Unextracted	97.2 ± 14.5	112.4 ± 17.5^{NS}
Extracted	20.4 ± 2.1	17.8 ± 3.2^{NS}
Indomethacin ($n=9$)		
Unextracted	126 ± 21	27.6 ± 5.9**
Extracted	22.1 ± 2.7	8.4 ± 2.2**

For significance of differences before and after treatment ^{NS} $P>0.05$; **$P<0.01$ (Wilcoxon matched pairs signed-ranks tests).

frusemide administration and were similar to the values shown in Table 1.

Comparison of 6-keto prostaglandin $F_{1\alpha}$ measured in urine before and after extraction and chromatography

The values in Table 3 indicate that although immunoreactive 6-keto $PGF_{1\alpha}$ is considerably higher in urine before extraction and chromatographic separation, levels are reduced to a similar degree by indomethacin.

Discussion

These studies have confirmed previous observations in the rat isolated kidney demonstrating a stimulating effect of frusemide on renin secretion without changes in renal vascular resistance (Vandongen, 1977). Since prostaglandins, particularly PGI_2, have been shown to release renin (Whorton *et al.*, 1981; Lin *et al.*, 1981; McGiff *et al.*, 1982) and intravenous administration of frusemide increases urinary excretion of prostaglandins in man (Scherer *et al.*, 1978), dog (Sreenivasan *et al.*, 1981) and rat (Sullivan & Patrick, 1981), a causal relationship is suggested with prostaglandins as potential mediators in the sequence of events linking stimulation and release of renin.

However, in the isolated kidney preparation, devoid of neurovascular connections and perfused at near constant pressure and flow rate, frusemide had no effect on the urinary excretion of 6-keto $PGF_{1\alpha}$, the stable metabolite of PGI_2. In contrast, renin secretion was markedly stimulated by frusemide. Pretreatment with indomethacin, which considerably reduced 6-keto $PGF_{1\alpha}$ excretion in urine collected from the kidney before and after isolation, did not alter basal renin secretion or the response to frusemide. These results are similar to those found in dogs (Sreenivasan *et al.*, 1981) where constant infusion of frusemide increased renal vein renin activity during inhibition of urinary PGE_2 excretion by meclofenamate. Similarly, indomethacin given daily in comparable intraperitoneal doses for 2 weeks, did not alter basal levels of plasma renin or the response to frusemide (Cangiano, *et al.*, 1981). PGE_2 was not measured in the present study but it is likely that the dose of indomethacin used suppressed the synthesis of other prostaglandins in addition to PGI_2.

An assumption is made that measurement of the stable but less active degradation product 6-keto $PGF_{1\alpha}$ accurately reflects continuing PGI_2 synthesis in the kidney. Although a proportion of the 6-keto $PGF_{1\alpha}$ in urine before isolation of the kidney may originate from extra-renal vascular tissue, once isolated it must represent renal origin. Also since complete inhibition of PGI_2 synthesis was not achieved, it is conceivable that a critical concentration persisted to mediate the renin response to frusemide, particularly as it is not certain as to how closely urinary 6-keto $PGF_{1\alpha}$ levels reflect intracellular concentrations of PGI_2.

Assays performed on urine directly give levels of 6-keto $PGF_{1\alpha}$ that are higher than after chromatography, although indomethacin produced a comparable degree of suppression. As we have shown earlier (Vandongen *et al.*, 1982) this discrepancy is considerably smaller in urine collected from the perfused kidney, presumably because less cross-reacting substance is filtered.

These findings in the rat isolated kidney disagree with those obtained in intact animals and in man where the renin response to frusemide was blocked by inhibitors of prostaglandin synthesis (Romero *et al.*, 1976; Goldiner *et al.*, 1981). However, systemic haemodynamic changes resulting from administration of frusemide may have released renin by the renal baroceptor mechanism in which a role for prostaglandins had been demonstrated (Data *et al.*, 1978; Blackshear *et al.*, 1979). A potential direct effect of frusemide on renin secretion may be obscured under these conditions by the marked sodium retaining action of prostaglandin inhibitors, perhaps not seen in the *in vitro* perfused kidney. It is conceivable that the apparent relationship between renin release and prostaglandin production is based on a common response to changes in sodium balance. The increase in prostaglandin production observed in some studies following frusemide administration may also be the result rather than the cause of increased circulating renin and angiotensin II levels. Stimulation of prostaglandin production under these circumstances helps to counteract the vasoconstrictor action of angiotensin II on local vascular beds.

Since vasoconstriction was not observed in the isolated kidney, it is unlikely that significant amounts of angiotensin II were generated during active secretion of renin. This may account for the lack of effect of frusemide on 6-keto $PGF_{1\alpha}$ excretion in these experiments.

Species differences may also be responsible for some of these inconsistencies, as in the dog denervated kidney intrarenal infusion of indomethacin markedly reduced prostaglandin secretion and renin activity in the venous effluent and attenuated the renin response to intravenous frusemide (Seymour & Zehr, 1979). Also in the intact animal a fall in plasma potassium concentration following frusemide administration could stimulate renin release and there is some evidence that this response is mediated by prostaglandins (Lazar & Whorton, 1980).

Dissociation of renal PGI_2 synthesis and β-adrenergic stimulation of renin has been found in several species (Beierwaltes *et al.*, 1980; Vandongen *et al.*, 1981), and is analogous to the findings described here with frusemide in the rat.

The precise mechanism whereby frusemide stimulates renin secretion remains to be elucidated. A β-adrenoceptor mechanism appears to be excluded as renin release was not altered by doses of propranolol sufficient to block a comparable renin response to isoprenaline, and a previous report indicates that the stimulation of renin release occurs independently of urine flow (Vandongen, 1977) and intact macula densa function (Corsini *et al.*, 1975).

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