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A COMPARATIVE STUDY OF THE ACTION OF FRUSEMIDE AND METHYCLOTHIAZIDE ON RENIN RELEASE BY RAT KIDNEY SLICES AND THE INTERACTION WITH INDOMETHACIN

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1 The effects of frusemide (a diuretic acting on the loop of Henle) and methyclothiazide (a thiazide diuretic) on renin release were studied on rat kidney slices.

2 Frusemide at concentrations of 1.5 and 7.5 mmol/l produced significant increases in renin release but had no effect at 0.15 mmol/l.

3 Methyclothiazide in a similar concentration range did not increase renin release; instead, at the highest concentration used, methyclothiazide (3.5 mmol/l) inhibited renin release.

4 Indomethacin (25 μmol/l) did not inhibit the increase of renin induced by frusemide.

5 Our limited study *in vitro* is consistent with the findings of other workers who have shown *in vivo*, in the absence of systemic electrolyte depletion, that only 'loop diuretics' increase renin secretion. Under our experimental conditions, it is suggested that frusemide exerts a direct action either upon the epithelioid cells or upon the macula densa since the renal prostaglandin system does not intervene.

Introduction

The *in vivo* administration of 'loop diuretics', such as frusemide, leads to a stimulation of renin secretion even in the absence of systemic electrolyte depletion (Meyer, Menard, Papanicolaou, Alexandre, Devaux & Milliez, 1968; Vander & Carlson, 1969; Cooke, Brown, Zacherle & Walker, 1970; Imbs, Desaulles, Velly, Bloch & Schwartz, 1972). This intrarenal mechanism involves the afferent arteriolar baroreceptor, a sodium- (or chloride)-sensitive receptor at the level of the macula densa and possibly the renal prostaglandin system (Hook & Bailie, 1977). This mechanism is peculiar to loop diuretics, as the administration of thiazide diuretics does not lead to any increase in renin release under such conditions (Cooke *et al.*, 1970; Imbs *et al.*, 1972). Having previously shown that frusemide increases renin secretion by rat kidney slices (Desaulles & Schwartz, 1974), the present study was undertaken to compare frusemide with the thiazide diuretic, methyclothiazide (Ford, 1960) under *in vitro* conditions, in which changes in haemodynamics, and tubular and blood composition are eliminated.

Furthermore, although the *in vitro* results suggest that frusemide acts directly upon the juxtaglomerular apparatus, indomethacin was used to investigate whether or not the renal prostaglandin system is involved, as has been suggested by many authors (Oliw, Kövér, Larsson & Änggård, 1976; Frölich, Hollifield, Dormois, Frölich, Seyberth, Michelakis & Oates, 1976).

Methods

Incubation of kidney slices

Kidneys were removed from male Wistar rats (weighing 278 ± 1 g, s.e. mean) anaesthetized with sodium pentobarbitone (50 mg/kg). After decapsulation, the kidneys were cut along the sagittal plane, the poles were ablated and the half kidney was cut manually into transverse cortico-medullary slices less than 0.5 mm thick, with an average weight of 20 mg. In each tube a single slice was incubated at 37°C in 2 ml of gassed (95% O_2: 5% CO_2) medium. Only one kidney slice was taken from each animal.

Each slice was incubated for three successive 40 min periods (I, II, III) preceded by a 10 min and a 20 min preincubation to rid the slices of tissue debris. After each period the medium was changed. No drugs were added during period I (reference

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period). During period II, the medium contained frusemide or methyclothiazide dissolved in NaOH 0.2 N, the final concentration of 0.2 N NaOH in the medium being 5%; NaOH in the same concentration was added to the medium during periods I and III. The composition of the medium was as follows: medium 199 (Difco), 5% horse serum (Difco) and 5% NaOH 0.2 N; the pH was 7.2.

When indomethacin was used, it was dissolved in NaOH 1 N which was thereafter partially neutralized with HCl 0.2 N; 10 µl of this solution was injected into the medium at the beginning of periods I and II. At the end of period II, the slice was washed. Period III was to verify whether the phenomena observed during period II (i.e. the actions of drugs) were reversible. The amounts of renin released during periods II and III are expressed as percentages of renin released during reference period I (Desaulles, Miesch & Schwartz, 1978).

Measurement of renin

Physiological saline (0.3 ml) and nephrectomized rat plasma (0.5 ml) were incubated with a 0.2 ml aliquot of the medium for 2 h at 37°C, in the presence of disodium edetate (EDTA, 2.6 mM), 2,3-dimercaptopropanol (BAL, 1.6 mM) and 8-hydroxyquinoline (3.4 mM). We have observed that under these conditions there is a block of angiotensinases and of the converting enzyme (Ryan, McKenzie & Lee, 1968; Haber, Koerner, Page, Kliman & Purnode, 1969). The reaction was stopped by acidification (pH 5.5) followed by 10 min in a boiling water bath. After 10 min centrifugation at 3000 rev/min and filtration on a Millipore HAWPO 1300 filter, angiotensin I was measured by radioimmunoassay according to the method of Haber *et al.* (1969). The quantity of angiotensin I generated is proportional to the renin concentration, as we confirmed. The renin activity of the horse serum and nephrectomized rat plasma only accounted for an average of 1% of the total renin activity of the incubating medium. None of the drugs used interfered with this determination.

Drugs

The drugs used were: frusemide (Hoechst), methyclothiazide (Abbott), indomethacin (Merck, Sharpe & Dohme).

Statistical analysis

The differences between control and experimental groups were tested by Student's *t* test for unpaired data. The differences between doses were tested by variance analysis of linear regression. In Figure 2, statistical comparison between groups was made by the method of Newman-Keuls (Lellouch & Lazar, 1974). Results are always expressed as mean ±s.e. mean.

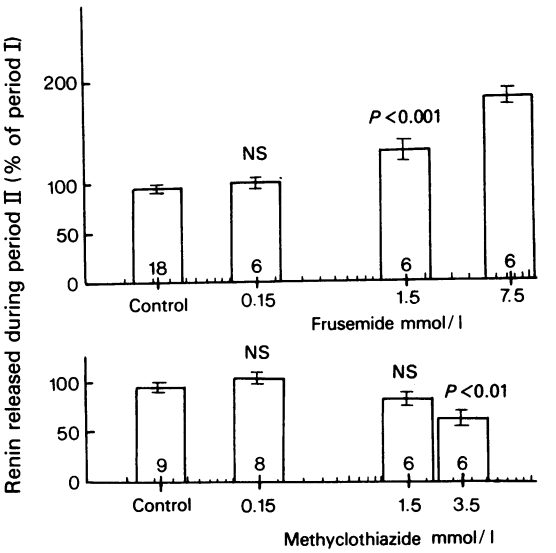

Figure 1 Effects of frusemide and methyclothiazide on renin release by rat kidney slices. Results were obtained during experimental period II when diuretics were added, and are expressed as percentages of the preceding reference period I. Mean values are plotted; vertical lines show s.e. mean. The numbers in the columns refer to number of experiments. *P* value refers to significance of differences between diuretics and control. NS = not significant ($P > 0.05$).

Results

Control

The amounts of angiotensin I generated by the control tissues in the radioimmunoassays were: 166 ± 12; 149 ± 11 and 105 ± 11 ng ml^{-1} h^{-1} during periods I, II and III respectively. The results expressed as a percentage of renin release during reference period I were: I = 100%; II = 93 ± 2%; III = 66 ± 2%.

Drugs

As shown in Figure 1, frusemide at a concentration of 1.5 mmol/l produced a significant increase in renin release which was even greater at a concentration of 7.5 mmol/l. When the data for 0.15 mmol/l were included, the variance analysis of linear regression on log dose gave the following results: deviation from linearity: NS; slope test: $P < 0.001$.

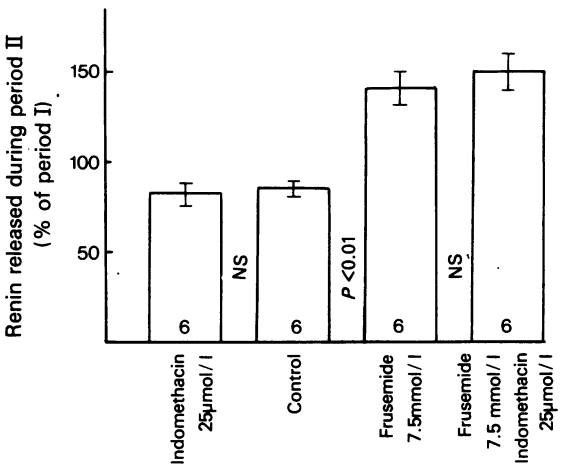

Figure 2 Effect of indomethacin on renin release induced by frusemide. Results were obtained during experimental period II when drugs were added and are expressed as percentage of the preceding reference period I. Mean values are plotted; vertical lines show s.e. mean. The numbers in the columns refer to number of experiments. *P* value refers to significance of difference between two adjacent columns; NS = not significant ($P > 0.05$).

No increase in renin release was observed with methyclothiazide at concentrations of 0.15 and 1.5 mmol/l. However, at a concentration of 3.5 mmol/l methyclothiazide inhibited renin release (Figure 1). Beyond this concentration, the product precipitated.

Indomethacin (25 μmol/l) by itself had no effect on renin release; furthermore, indomethacin did not inhibit the increase in renin release induced by 7.5 mmol/l frusemide (Figure 2).

Frusemide-induced renin release was reversed during period III (I = 100%; II = 144 ± 9%; III = 73 ± 6%). This was also true in the presence of indomethacin: I = 100%; II = 143 ± 13%; III = 73 ± 6%. However, the decrease in renin release observed with methyclothiazide at a concentration of 3.5 mmol/l was not reversed (I = 100%; II = 62 ± 7%; III = 32 ± 3%).

Discussion

The present study shows that, *in vitro*, as has been shown by others *in vivo*, in the absence of electrolyte depletion, the effects of a loop diuretic differ from those of a thiazide diuretic as far as renin release is concerned. However, contrary to our present findings, an inhibition of renin release has never been observed with thiazide diuretics *in vivo* (Imbs, Schmidt, Velly & Schwartz, 1977).

Under our experimental conditions, frusemide may have stimulated renin secretion by (a) a direct action on the epithelioid cells as has been suggested by Desaulles & Schwartz (1974) and Lyons & Churchill (1975) in their work on rat kidney slices and by Hofbauer, Zschiedrich, Hackenthal & Gross (1974) and Vandongen (1977) in their work on the isolated perfused kidney; (b) directly inhibiting sodium (or chloride) reabsorbtion by the macula densa cells as has been suggested by Vander & Carlson (1969) and Cooke *et al.* (1970); (c) liberation of medullary or cortical prostaglandins (Larsson & Änggård, 1973) which may play an intermediary role in this process.

Frusemide may exert its action either on the epithelioïd cells and/or the macula densa. As a renal vasodilator (see Hook & Bailie, 1977) and by its probable direct action upon the vascular smooth muscle (Larochelle, Mikulic & Ogilvie, 1973) it consequently may have a direct effect upon the epithelioïd cells which are located in the afferent arteriole and which are derived from smooth muscle cells (Barajas & Latta, 1967). Such an effect might account for the increase in renin release we observed, for Vandongen & Greenwood (1976) have shown that in the isolated perfused rat kidney, vasodilators increase renin release even in the absence of any haemodynamic modification, which also suggests a direct action of these drugs upon the juxtaglomerular cells. But frusemide, by inhibiting sodium (or chloride) transport in the ascending limb of Henle's loop (Meng, 1967) may also stimulate renin secretion by direct action upon the macula densa cells.

Certain differences between the modes of action of thiazide diuretics and frusemide could account for our results. The data obtained by Hook (see Hook & Bailie, 1977) suggests that thiazides, as opposed to 'loop diuretics', have a vasoconstrictor effect on the renal vascular bed. This may explain the decrease in renin release we observed at the higher concentration of methyclothiazide as, similarly, Vandongen & Peart (1974) and Desaulles, Forler, Velly & Schwartz (1975) suggested that vasoconstrictor agents may inhibit renin secretion by a direct effect on the renin secreting cells. However, the cause of this decrease could also be attributed to the possible toxicity of methyclothiazide at the highest concentration (3.5 mmol/l) since the inhibition we observed was not reversed by washing during the recovery period III. Also, thiazide diuretics differ from frusemide in their sites of action in the tubule (Meng, 1967), which explains why one and not the other may affect the macula densa.

Sirois & Gagnon (1974) have suggested that, *in vitro*, the release of prostaglandins is caused by a fresh synthesis rather than by the mobilization of intracellular pools. In our study, indomethacin was used at a concentration that would inhibit prostaglandin synthesis (Sirois & Gagnon, 1974). Therefore, our find-

ings that indomethacin did not have any action in this process suggest that prostaglandins play no intermediary role in frusemide-induced renin release *in vitro*. The renal prostaglandin system intervenes in the renin hypersecretion induced by frusemide *in vivo* (Oliw *et al.*, 1976; Frölich *et al.*, 1976) but under such conditions supplementary mechanisms are involved which might explain the difference between the *in vivo* and *in vitro* results.

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