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Calcium and Renin Release: Inhibition of Low Sodium-Induced Renin Secretion by High Calcium Concentration in Rat Kidney Perfusion

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The effects of changes in calcium on renin secretion have been studied in the isolated perfused rat kidney. Perfusion with free calcium buffer significantly decreases renin secretion as compared with control experiments (Ca^{++}: 2.5 mM/l). Other calcium concentrations (1.25 mM/l and 5 mM/l) do not affect basal renin secretion. When the renin release is previously increased by low sodium concentration (Na^{+}: 110 mM/l) however, perfusion with high calcium buffer (Ca^{++}: 5 mM/l) significantly inhibits this stimulation.

Available information about the role of calcium in the control of renin secretion is controversial. *In vivo* experiments show that hypercalcemia supresses stimulated renal renin release by sodium depletion (8, 9, 20). *In vitro* experiments show variable results: MICHELAKIS (13) and SARUTA and MASUKI (16) found that incubation of cortical renal slices in a medium free of calcium decreased renin secretion; similarly, other authors (3) report that calcium increases renin secretion from slices previously incubated in a calcium-free medium, while AOI *et al.* (1) and PARK and MALVIN (14) have not found alterations in renin secretion resulting from changes in calcium concentrations. LYONS (12), in cortical renal slices, and BAUMBACH and LEYSSAC (2), in isolated perfused glomeruli, however, found that the decrease in calcium concentration caused increased renin secretion.

Some authors (11, 15, 18) using the isolated perfused rat kidney to study the role of calcium on renin secretion, have found that renin secretion is stimulated not only when calcium concentration is decreased in the perfusate but also when

chelants such as EDTA of EGTA are added to the medium. Others have failed to confirm those reports (7, 10, 19). By contrast, LESTER and RUBIN (10) and HARADA *et al.* (6) have concluded that the presence of intracellular calcium is necessary for the synthesis and/or mobilisation of renin.

The present study was designed to test the possible role of intrarenal calcium in the control of renin secretion. The isolated rat kidney perfused *in situ* with a Hepes-dextrane solution was chosen to prevent the neural and humoral influences commonly present in *in vivo* experiments.

Materials and Methods

Materials. Male Wistar rats weighing 250-280 g, were anaesthetized with nembutal (0.1 mg/g). The left kidney was isolated and perfused as previously described (12). The perfusion fluid was a Hepes (Sigma) buffered electrolite dextran solution equilibrated with 100% oxygen. The perfusion fluid was delivered at a constant rate of 8 ml/min. The first sample was collected after 5-7 min. Samples were also collected at 10, 20 and 30 minutes after the first.

Types of infusion. The buffer solution in the control groups was of the following composition (mM/l): Na^+, 135; K^+, 5.5; Mg^{++}, 1.2; glucose, 10; and dextran, 36 g/l (m.w.: 70,000). The calcium concentration, mannitol added and the theorical osmolarity are summarized on table I. The osmolarity determined in the buffer solutions by an osmometer (Osmette, A, Precision System, Inc.) was 296 ± mOs/l in control and in low and free calcium fluids, while in the high calcium fluid the osmolarity measured was 302 ± 1.8 mOs/l.

Methods. Perfusate samples were dialysed successively to pH 4.5 (24 h) and pH 7.5 (24 h) at 4° C with phosphate buffer containing EDTA-Na_2 to remove angiotensinases (17). Samples were then incubated at 37° C for 3 h with nephrectomized rat plasma as substrate.

The enzymatic reaction was stopped by heating the samples to 85-90° C for 10 minutes. The angiotensin I was measured by radioimmunoassay.

Statistical analysis. All results are expressed as mean ± S.E.M. Analysis of signification was performed using the Student «t» test.

Results

There were no changes in perfusion flow in any of the experimental groups. A significant decrease ($p < 0.01$) in renin secretion compared with control group ($n = 17$) was observed when the kidney was infused with calcium free fluid ($n=8$) (fig. 1). By contrast, when calcium concentration in the perfusion fluid was increased to 5 mM/l ($n = 8$), or decreased

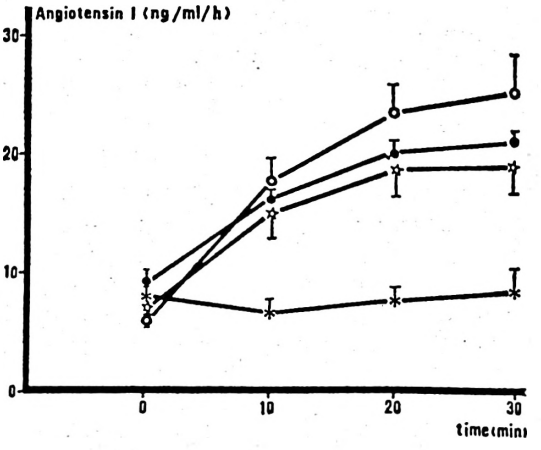

Fig. 1. *Effect of high, low and free calcium on renin secretion.*
Control: Ca^{++}: 2.5 mM/l (●—●); high calcium: 5 mM/l (○—○); low calcium: 1.25 mM/l (☆—☆). Free calcium (✱—✱).

Table I. *Calcium concentration, mannitol added, and theorical osmolarity of different experimental groups.*

Experimental group	Ca^{++} (mM/l)	Mannitol (g/l)	Osmolarity (mOs/l)
Control	2.499	—	306.40
High calcium	4.999	—	313.89
Low calcium	1.249	0.6826	306.40
Free calcium	—	1.3655	306.40

to 1.25 mM/l (n = 6), no changes in renin secretion were found.

Figure 2 shows the effects of low sodium and high calcium infusion on renin release. When renin secretion was increased by low sodium concentration ($p < 0.001$) (n = 11), the infusion with high calcium (n = 6) significantly inhibited the stimulation ($p < 0.01$). The inhibitory effect of high calcium was not able to reduce the renin secretion to control values, thus, the renin secretion was significantly higher compared with control (n = 17) ($p < 0.01$).

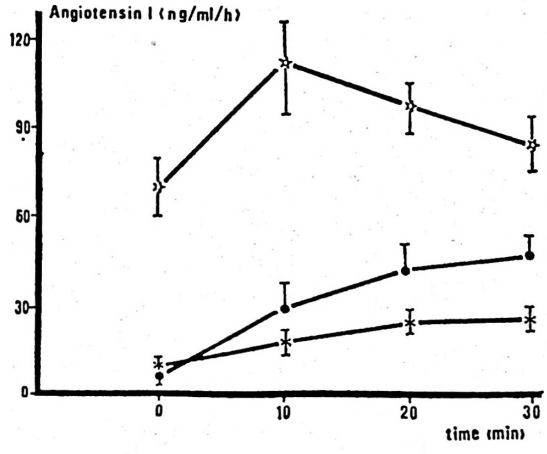

Fig. 2. *Effect of high calcium on renin release induced by low sodium.*
Control: Na^+ 135 mM/l, Ca^{++}: 2.5 mM/l (✳—✳); low sodium: 110 mM/l (☆—☆); low sodium and high calcium: Na^+: 110 mM/l, Ca^{++}: 5 mM/l (●—●).

Discussion

It has been shown that calcium plays a role in hormonal secretion. It is also believed to act as a coupler of stimulation and hormone secretion (4). The concentration of calcium in some intracellular sites is related to humoral release (12). LESTER and RUBIN (10) have reported that renin release from the juxtaglomerular cells agrees with this hypothesis (6). Other authors, however, have suggested that intracellular calcium concentrations may be inversely related to renin release (5, 15). On the other hand, others have found no changes in calcium dependent renin secretion (1, 14).

The present results (fig. 1) suggest that kidney perfusion with an isosmolar calcium-free buffer causes a significant decrease in basal renin secretion ($p < 0.01$). Perfusion with other calcium concentrations (Ca^{++}: 1.25 mM/l and 5 mM/l, respectively) does not affect renin release as compared with control (Ca^{++}: 2.5 mM/litre).

These results support the hypothesis that calcium does not play an essential role in the control of basal renin release, but the presence of calcium is needed for synthesis and/or mobilisation of the enzyme (13, 16, 19). Our results, nevertheless, do not confirm those obtained by others (11, 15, 18), suggesting an increase in renin secretion in response to a calcium-free kidney perfusion.

In vivo experiments in dogs (9, 20), and in humans (8) show that renin stimulation by sodium depletion is inhibited by administration of calcium chloride. Likewise, our results suggest that *in vitro*, high calcium concentrations inhibit the stimulatory effect of low sodium on renin secretion (fig. 2).

The suppressive effects of calcium on low sodium-increase in renin release seems to take place in the kidney, since our experiments were carried out in isolated

rat kidneys. Moreover, as the same results have been found in filtering and non filtering kidneys (20), it may be assumed that this inhibitory effect of calcium is directly mediated by juxtaglomerular cells.

We may conclude that, only when calcium is excluded from the medium, the renin release is affected (1, 6, 10, 14). In this way, we also support the view that, in basal renin secretion calcium has not an important role, suggesting that this ion can modulate the renin response to other stimuli.

Resumen

Se estudia en riñón aislado y perfundido de rata el efecto de distintas concentraciones de calcio en la secreción basal y previamente estimulada de renina. La perfusión con un tampón exento de calcio inhibe significativamente la secreción basal de renina en comparación con los experimentos controles (Ca^{++}: 2,5 mM/l). A otras concentraciones (Ca^{++}: 1,25 mM/l y 5 mM/l) no afecta a la secreción de renina. Sin embargo, cuando ésta se estimula previamente por la infusión de un medio de bajo contenido en sodio (Na^{+}: 110 mM/l) la adición a dicho medio de una elevada concentración de calcio (5 mM/l) disminuye significativamente la secreción de renina.

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