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Biphasic Alteration of Renin Release by Calcium (41820)

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Abstract. The effects of calcium reintroduction on renin release were examined. Calcium reintroduction to calcium-deprived rat renal cortical slices caused an initial stimulation of renin release (30–40 min) followed by a period of suppression of release (4.5 hr). With ouabain present the initial stimulation was enhanced but the subsequent fall in release was more pronounced. Our results suggest that although renin release from juxtaglomerular cells can be both stimulated and inhibited by raising intracellular calcium, it is the inhibition of release that is the more persistent effect.

Considerable evidence suggests that a rise in intracellular calcium in renal juxtaglomerular (JG) cells inhibits renin release (1–11). Paradoxically, the reintroduction of calcium after a period of calcium deprivation stimulates renin release (12–14). We investigated the possibility that if this stimulation is due to raising intracellular calcium, then the presence of ouabain during calcium reintroduction should enhance the stimulation. By its inhibition of Na^+-K^+ ATPase ouabain is believed to indirectly inhibit Na^+-Ca^{2+} exchange, a mechanism of calcium extrusion (2, 15). By suppressing the extrusion of calcium ouabain should potentiate the effects of any rise in intracellular calcium that may occur during the period of calcium reintroduction. The stimulation of renin release reported to occur during this period should then be enhanced if the stimulation is caused by raising intracellular calcium.

Materials and Methods. Female Sprague–Dawley rats (150–200 g) maintained on a normal sodium diet were sacrificed by decapitation. Kidneys were quickly excised, cleaned of surrounding connective tissues, and then sliced with a Stadie–Riggs microtome. The first cortical slices (20–40 mg) from the broad kidney surfaces were used for superfusion. During preparation intact kidneys and slices were bathed in cold calcium-free Krebs containing EGTA (Sigma). The Krebs composition (in m*M*) was as follows: NaCl, 119; $NaHCO_3$, 25; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; and either EGTA, 2.0, or $CaCl_2$, 2.7. The Krebs solution was gassed with 95% O_2–5% CO_2, adjusted to pH 7.4, and then gassed continuously during superfusions. The superfusion apparatus has been described previously (16).

Slices were washed three times in calcium-free Krebs + EGTA and then superfused with this buffer at 1 ml/min at 37°C. After 2 hr of superfusion for washout and baseline stabilization, 10-min fractions were collected for renin determinations. In our first study calcium was reintroduced after the first two collections (t = 20 min). EGTA was excluded from the buffer during calcium reintroduction. Control slices were maintained in calcium-free medium. In a second study, ouabain (100 μM, Sigma) was applied after 20 min of collection and then calcium was reintroduced after 80 min. Controls were not treated with ouabain prior to or during calcium addition.

Twenty five microliters of 2% bovine serum albumin (4× crystalline BSA, ICN) was added to 475 μl of each superfusate fraction for renin stabilization (0.1% BSA final concentration). The albumin-treated samples were kept at 0–4°C or frozen until incubation for angiotensin I generation. For substrate incubation 50 μl of each sample was combined under ice-cold conditions with 75 μl nephrectomized rat plasma, 90 μl Tris–acetate–lysozyme buffer (pH 7.4), 25 μl 4% EDTA, 5 μl dimercaprol, and 5 μl 8-hydroxyquinoline (16). This mixture was stoppered, quickly vortexed, and incubated for 1 or 2 hr at 37°C. The reaction was stopped by transferral of the incubates to an ice bath. Fifty microliters of each incubate was taken for radioimmunoassay of angiotensin I (Becton Dickinson PRA kit).

Because of high variability in the rates of absolute renin release, the results are expressed as means ± SE of percentages of the renin

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release rates measured during the 10 min that preceded calcium reintroduction. Statistical analysis was performed by unpaired t test (*$P < 0.05$, **$P < 0.01$).

Results and Discussion. When calcium was reintroduced after an initial period of calcium deprivation, renin release fluctuated in a biphasic manner (Fig. 1). Release rose 2.1-fold during the first 10 min and then declined to the control level. The stimulation period lasted 30–40 min. This was followed by suppression of release (4.5 hr). The renin release rate (mean ± SE) for the calcium-treated group during the first sample collection was 779 ± 119 pg AI/min superfusion/mg wet wt per 60 min of substrate incubation. The rate for the control group was 518 ± 74 (performed April–September 1981).

The initial rise in release may reflect triggering by calcium of release of prepackaged renin, while the fall and suppression may result from depletion of this renin store. Synthesis or intracellular transport of renin to the cell membrane may not be sufficient to maintain the high rate of release. Alternatively, the suppression may involve inhibition by calcium of renin synthesis or other steps which occur prior to the calcium-triggered step. A complicating factor is that calcium-deprived cell membranes have increased permeability to calcium (17). In our study as juxtaglomerular cell membranes regain calcium their permeability to calcium probably decreases, so that calcium influx is high at the start of reintroduction but then declines. The fall in renin release may therefore be related to decreasing calcium influx. Also, these results were obtained using a high concentration of calcium (2.7 mM), and perhaps different results would be obtained using a lower calcium concentration.

The results of the ouabain study are shown in Fig. 2. The release rate for the ouabain-treated group during the first sample collection was 176 ± 24 pg AI/min/mg, while the rate for the controls was 244 ± 57 (performed September–November, 1981). The reason for the disparity in the initial basal release values between the experiments of Figs. 1 and 2 is unclear. However, a previous report (18) suggests that renin release following calcium deprivation may be much higher during the spring and summer months than during the fall and winter. In that study renin release from isolated glomeruli of male Sprague–Dawley rats rose 700% following calcium deprivation between late April and midAugust, but the rise in release was only 125% during the rest of the year. The high values we observed under calcium-free conditions during April through early September relative to those between September and November are consistent with this earlier study.

As shown in Fig. 2, ouabain did not alter renin release during the calcium-free pretreatment period. The lack of a ouabain effect on

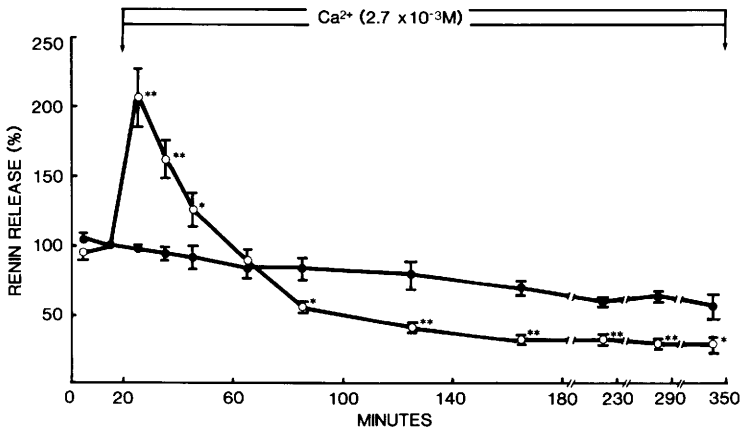

FIG. 1. Effects of calcium reintroduction (○) on renin release relative to calcium-free controls (●). For both groups $N \geq 7$ for each mean ± SE through 170 min. Between 170 and 350 min $N = 5$ for each mean for the calcium-reintroduced slices and $N = 3$ for the controls.

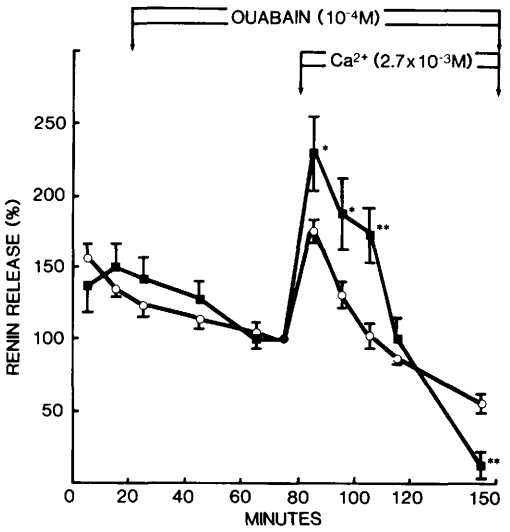

FIG. 2. Effects of ouabain (■) on renin release during calcium reintroduction. Ouabain was not added to the controls (○). The values are means ± SE of $N \geq 8$ experiments for the ouabain-treated group and $N \geq 7$ experiments for controls.

renin release under calcium-free conditions has already been reported (2, 8, 10). The magnitude of the stimulation caused by calcium in the ouabain-treated slices was significantly greater than the control during the first 30 min of reintroduction. Renin release then dropped precipitously in the ouabain-treated slices and was significantly lower than the controls after 70 min of calcium reintroduction. This inhibitory effect of ouabain on renin release with calcium present has also been shown previously (2, 8, 10), and is part of the body of evidence suggesting that a rise in intracellular calcium inhibits release.

Our results with ouabain indicate that the stimulatory and inhibitory effects of calcium are due to a rise in intracellular calcium. It has been suggested that by blocking Na^+-K^+ ATPase with ouabain, the sodium gradient across the JG cell membrane becomes reduced, and this inhibits the uptake of external sodium that occurs with the extrusion of intracellular calcium (2). By lowering calcium efflux in this manner ouabain should thus potentiate the effects of calcium influx. Since both stimulation and inhibition of renin release caused by calcium were enhanced by ouabain, it appears that both effects are mediated by raising intracellular calcium following the special condition of calcium deprivation.

Although the mechanism for these opposing effects is unknown, it is possible that an optimal level of intracellular calcium exists for renin release, and that severe calcium deprivation using EGTA lowers intracellular calcium below this level. When calcium is reintroduced the intracellular concentration may rise to this proposed optimum and cause an elevation in release, but further increases in the intracellular concentration suppress release. Since the more persistent effect of calcium reintroduction is suppression, calcium may "equilibrate" within the cell *in vitro* at an inhibitory level that is higher than the optimum for renin release.

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