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# Direct Stimulation of Renin Release by Calcium[1] (39441)

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Although it is well known that calcium plays a vital role in both endocrine and exocrine secretion (1), its role in the secretion of renin is less clear. Thus, some authors suggest that calcium retards renin release (2, 3), while others have provided evidence that calcium enhances renin release (4, 5). We have examined the direct effect of calcium on renin release *in vitro*, independent of neural or vascular control. Our results indicate that calcium can directly stimulate the release of renin.

*Methods.* The technique we have adopted has previously been utilized to demonstrate the direct stimulant action of calcium on adrenal medullary secretion, namely, reintroduction of calcium after a period of calcium depletion (6). Male Holtzman rats (250–300 g) maintained on regular diet were killed by a blow to the head and both kidneys were then removed. Renal cortex was separated and the capsule was removed by using a razor blade. The cortex, thus obtained, was cut into slices by hand, averaging 14 mg each. All of these procedures were performed at room temperature. Nine slices were skewered on a long needle and incubated at 37° in 2-ml volumes of oxygenated modified Krebs solution containing (m*M*): NaCl, 140; KCl, 4.0; $CaCl_2$, 1.0; dextrose, 10; Tris buffer (pH 7.4), 10. After a 30 min preincubation period the slices were transferred to successive tubes at 1 min intervals and aliquots of the medium were assayed for renin released from the slices. The standard method of stimulation adopted was to expose the tissues to calcium-free conditions for 30–35 min and then reintroduce calcium (0.5 m*M*) for 1 min, followed by the Ca-free medium again. Renin was assayed by radioimmunoassay of angiotensin I (7) generated from bovine serum substrate (8) at pH 7.0. The bovine serum substrate contained NaCl (150 m*M*), EDTA (10 m*M*), and phosphate buffer (pH 7.0, 50 m*M*). The final incubation mixture of renin and serum substrate contained 10 m*M* Cleland's reagent (DDT, dithiothreitol) and 3.3 m*M* EDTA. After incubation for 90 min at 37° the mixture was chilled on ice, diluted ninefold with 100 m*M* Tris buffer (pH 7.4) and assayed for angiotensin I (7). Angiotensin generation was proportional to renin concentration in the range utilized. Renin release is expressed as nanograms of angiotensin generated per hour of incubation with substrate per 100 mg tissue per minute of tissue incubation (ng/hr/100 mg/min).

*Results and discussion.* After 30 min incubation, the spontaneous release of renin reached a low and steady level, about 10–20 ng/hr/100 mg/min. If the kidney slices were pretreated with calcium-free medium, the reintroduction of calcium (0.5 or 6.0 m*M*) caused an immediate increase in renin release (Fig. 1). However, in the absence of calcium depletion (calcium maintained at 1.0 m*M*), addition of 0.5 or 6.0 m*M* calcium did not cause an increase in renin release (Fig. 1). To rule out secondary effects of calcium on $Na^+$ fluxes, experiments were done with Na-free medium (choline chloride replacing NaCl). Again, reintroduction of calcium caused a large increase in renin release (Fig. 2). Since the calcium-induced release of hormones from the posterior pituitary gland and from the adrenal medulla is dependent on a source of metabolic energy (9, 10), we have examined the effects of lowered temperature on the calcium-induced release of renin. When experiments were done at 20° instead of 37°, the release of renin in response to calcium was reduced (Fig. 3).

The present results parallel experiments on adrenal medullary secretion. It has been

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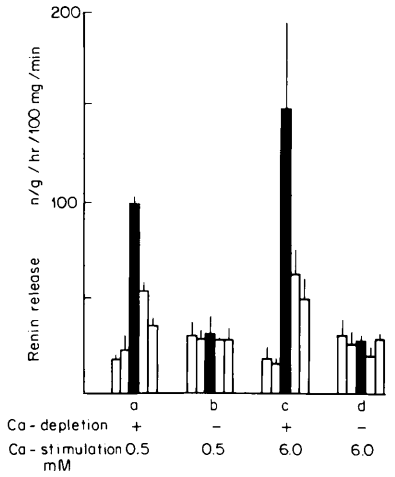


FIG. 1. Calcium-induced renin release. The vertical bars show renin released from four sets of rat kidney cortex slices (a–d). Slices were preincubated for three 10 min periods in Krebs solutions containing $CaCl_2$, 1.0 m*M* (b and d) or 0 (a and c). In the Ca-free experiments, EGTA, 1.0 m*M*, was present during the first 10 min period of preincubation. Renin released in five consecutive 1 min periods following the preincubation is shown in each experiment from left to right. In the third period (closed bars), calcium was added (0.5 m*M* in a and b; 6.0 m*M* in c and d). Renin release is expressed in ng angiotensin 1/hr substrate incubation/ 100 mg tissue/min tissue incubation. *N* = 3 for a–c and 5 for d. Each sample in each experiment was assayed in duplicates or triplicates. The thin line at the top represents the standard error.

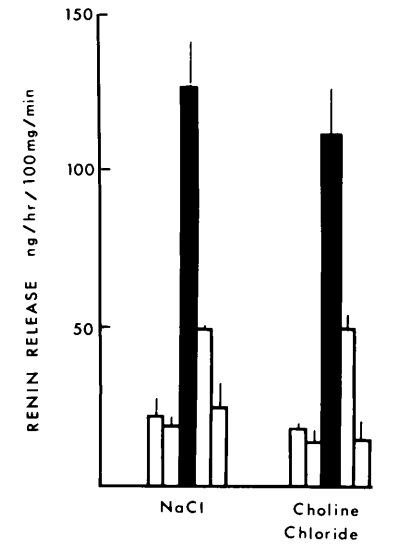


FIG. 2. Effect of NaCl replacement on calcium-induced renin release. The vertical bars show renin released from two sets of rat kidney cortex slices. Both sets of slices were preincubated for three 10 min periods in Ca-free Krebs solution, with 1.0 m*M* EGTA present during the first 10 min. In one set of slices, the NaCl in the Krebs solution was replaced by isotonic choline chloride throughout the experiment. Renin released in five consecutive 1 min periods following preincubation is shown from left to right in each experiment. In the third minute Ca (0.5 m*M*) was added. *N* = 3.

suggested that exposure of tissues to calcium-free medium increases their permeability to calcium so that subsequent treatment with calcium allows an enhanced calcium influx which then stimulates secretion (6). This may also be true for the juxtaglomerular cells and suggests that calcium can

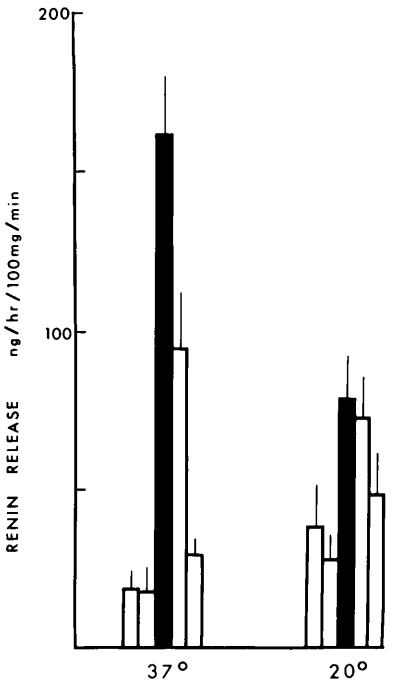


FIG. 3. Effect of temperature on calcium-induced renin release. The vertical bars show renin released from two sets of rat kidney cortex slices. Slices were preincubated for three 10 min periods in Ca-free Krebs solution, with 1.0 m*M* EGTA present during the first 10 min. Renin released in five consecutive 1 min period following preincubation is shown from left to right in each experiment. Calcium (0.5 m*M*) was added during the third min. One experiment was done at 37° and the other one at 20°. *N* = 4.

directly stimulate renin secretion. The present experiments, which were designed to eliminate the influence of neural, vascular, and sodium-dependent mechanisms on renin secretion, show that the cellular basis of renin release may be similar to that operating in catecholamine secretion. Further studies will be needed to determine if there are differences from the more established secretory systems.

*Summary.* Calcium directly stimulates renin release from rat kidney slices previously treated with calcium-free medium. The stimulant effect of calcium (0.5 or 6.0 m*M*) is not seen without the period of calcium depletion. The stimulant effect of calcium is still present in sodium-free medium but is reduced when the incubation is performed at 20° instead of 37°. The results suggest that the underlying mechanism of renin release may be comparable to that of catecholamine release, involving calcium-dependent and energy-dependent steps.

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