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Effect of Ca^{2+} Binding by EGTA on Renin Release in the Isolated Perfused Rabbit Kidney[1] (39409)

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Whether or not ionized calcium (Ca^{2+}) is a factor in renin release is still unclear. Michelakis (1) showed that renin release from kidney slices was depressed when calcium was omitted from the incubation fluid, and was maximal when the concentration of calcium in incubation media was increased to 2.5 m*M*/liter. Kotchen *et al.* (2), however, found that the acute injection of calcium into the renal artery of a dog suppressed renal venous PRA, and that PRA and renal renin content were decreased in rats during chronic administration of calcium. Previous studies from this laboratory failed to find any change in PRA during acute EDTA-induced hypocalcemia in normal subjects or subjects with mild renal failure (3), or during calcium infusions in subjects with varying degrees of renal failure (4).

In studies in the intact animal or human (2-4), where simultaneous changes in extrarenal and renal factors modulating renin release cannot be prevented, calcium could theoretically affect renin release by one or a combination of several mechanisms: (a) it might affect circulating catecholamines (5) which in turn could alter renin secretion (6); (b) it might have a direct action on intrarenal beta-adrenergic renin receptors via its known action on cyclic AMP (7); (c) it might affect urinary sodium excretion (8), thus changing sodium delivery to the macula densa as proposed by Kotchen *et al.* (2); and (d) calcium-induced changes in systemic and renal hemodynamics (9-10) might alter renin release.

The use of an isolated perfused kidney eliminates the possibility of changes in circulating catecholamines and enables maintenance of a constant perfusion pressure, permitting analysis of the intrarenal factors of renin release. Therefore, this model was utilized to study the effects of reduction of Ca^{2+} during stimulation with isoproterenol and theophylline.

Methods. Details of the isolated, perfused rabbit kidney model used in our laboratory has been described previously (11, 12). Each experiment consisted of two consecutive periods of 10 min each, a control period followed by an infusion period of known stimuli of renin release, isoproterenol (13, 14), or theophylline [theophylline ethylene diamine (1-3 dimethylxanthine)] (15). Both agents were administered into the renal artery during the first 8 min of the infusion period. The infusion rate of isoproterenol was 0.01 μg/kg/min, and that of theophylline was 7 mg/kg/8 min.

Two sets of experiments were performed. In the control group (Group A) the kidney was perfused with fresh rabbit blood; in Group B, EGTA [ethylene glycol bis(beta-aminoethyl ether) *N*,*N*,tetraacetic acid] was added to the blood perfusate in a concentration of 1 m*M*, calculated to bind essentially all of the circulating Ca^{2+} (7). The addition of EGTA to fresh rabbit blood *in vitro* in equimolar concentration to that of the experiments did not alter the velocity reaction of the hydrolyses of hog renin substrate by rabbit renin (Lineweaver-Burk

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plot). In six duplicate studies of unaltered rabbit serum compared to rabbit serum with EGTA added prior to incubation, radioimmunoassay of Angiotensin I yielded similar results.

In all experiments perfusion pressure was kept constant at 80 ± 3 mm Hg. Renal venous blood specimens were collected at the end of each 10-min period for measurement of serum renin concentration (SRC), and samples were drawn from the renal artery at each midperiod for determination of plasma creatinine and serum electrolytes. Urine collections were made during the theophylline experiments. Methods for determination of SRC, electrolytes, creatinine, glomerular filtration rate, and renal blood flow have been described previously (11, 12). Student's *t* test for paired data was used for statistical analysis.

Results. Effect of EGTA on isoproterenol-stimulated renin secretion (Fig. 1). In the control experiments, Group A, isoproterenol caused a 17% increase of renal vein serum renin concentration ($P < 0.001$). A similar increase was observed during perfusion with EGTA-containing blood, Group B. Renal vascular resistance and serum potassium did not change significantly during the control period, during isoproterenol administration, or following the addition of EGTA.

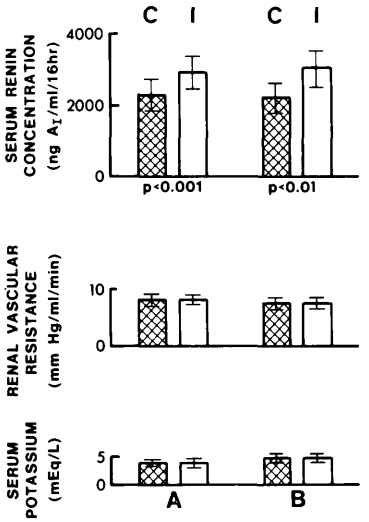

FIG. 1. The influence of isoproterenol (I) on renal vein serum renin concentration, renal vascular resistance, and serum potassium, as compared to a preceding 10-min control period (C). Perfusate in A is rabbit blood ($n = 15$); and in B is rabbit blood with 1 m*M* EGTA ($n = 6$).

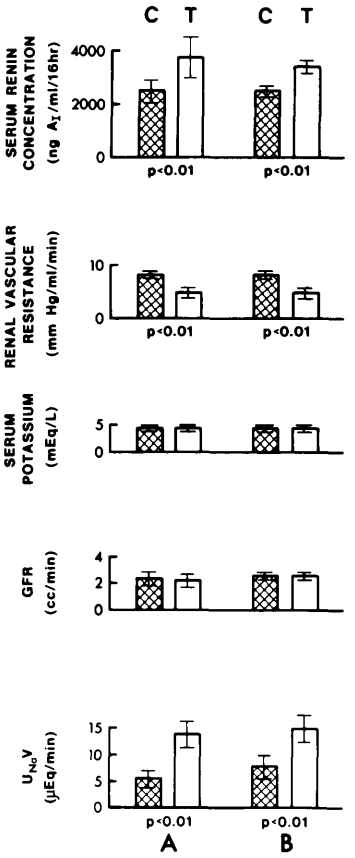

FIG. 2. The influence of theophylline (T) on renal vein serum renin concentration, renal vascular resistance, serum potassium, GFR, and $U_{Na}V$ as compared to a preceding 10-min control period. Perfusate in A is rabbit blood ($n = 6$); and in B is rabbit blood with 1 m*M* EGTA ($n = 6$).

Effect of EGTA on theophylline-stimulated renin secretion (Fig. 2). Renal vein serum renin concentration increased significantly ($P < 0.01$) during theophylline infusion in group A. The addition of EGTA (Group B) did not change the theophylline-induced renin release (N.S.). During infusion of theophylline, renal vascular resistance decreased from 8.1 to 5.0 mm Hg/ml/min ($P < 0.01$) without EGTA, and a similar decrease was observed when EGTA was added. GFR and serum potassium did not change during theophylline infusion or with

the addition of EGTA. Urinary sodium excretion increased from 5.5 ± 1.5 during the control period to 14 ± 2.5 μequiv/min during theophylline infusion ($P < 0.01$) without EGTA. A similar increase was observed when EGTA was added.

Discussion. Renin secretion by the kidney is regulated by an interrelated complex system, mediated by changes in renal perfusion pressure (16), renal resistance (17), urinary sodium excretion (16–19), serum potassium (20), sympathetic nerve stimulation (5, 21), and catecholamines (5, 6). The presence of abundant adrenergic nerve fibers in close proximity to the juxtaglomerular cells (22) and the fact that both catecholamines and cyclic AMP cause renin release when added to the kidney *in vitro* (16) has led to the assumption that catecholamines increase renin secretion by direct action on β-adrenergic receptors in the renin-secreting cells, mediated by cyclic AMP.

Cyclic AMP content is generally controlled by adenylcyclase, which increases cyclic AMP production, and phosphodiesterase activity, which decreases cyclic AMP content. In smooth muscle, reduction of external free Ca^{2+} increases phosphodiesterase activity with a resultant decreased cyclic AMP content (7). This might explain the decreased renin release by kidney slices *in vitro,* when calcium was omitted from the incubation fluid reported by Michelakis *et al.* (1). The influence of binding the free Ca^{2+} by EGTA was therefore examined when the perfused rabbit kidney was stimulated by isoproterenol, which activates adenyl cyclase, or by theophylline, which inhibits phosphodiesterase activity. Both of these stimuli should theoretically increase cyclic AMP.

The observations in the present study show that in the isolated kidney the increase of renal vein SRC during stimulation with isoproterenol and theophylline was unaffected by binding of free Ca^{2+} with EGTA. This does not exclude the possibility that in different circumstances changes in sodium excretion secondary to changes in filtered calcium may alter renin release (8). In the present experiments control $U_{Na}V$ was uneffected by the addition of EGTA, and the marked naturesis secondary to theophylline administration in both groups A and B could have masked any decrease in urinary sodium secondary to calcium-binding by EGTA.

Vandongen and Peart (23) studied renin secretion in the isolated, perfused rat kidney and observed that elimination of calcium from and the addition of EDTA to the perfusion fluid increased noradrenaline-induced renin release, as did the alpha-adrenergic blocking agent, phenoxybenzamine. They postulated that intrarenal vascular contractility and inhibition of renin secretion after alpha-adrenergic receptor activity may be related and may be calcium-dependent. In the present experiments, binding of the perfusate Ca^{2+} did not affect renin release secondary to direct beta-adrenergic stimulation (isoproterenol) or to changes in vascular reactivity (theophylline), two major mechanisms of renin release.

Summary. In the isolated, perfused rabbit kidney, binding of ionized calcium by EGTA did not affect the increased renin release secondary to two well-known stimuli, theophylline and isoproterenol. These results indicate that Ca^{2+} is not a major factor in the intrarenal regulation of renin secretion.

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