Control of Renin Release in Isolated Rat Glomeruli

WILLIAM H. BEIERWALTES, PH.D., SHARON SCHRYVER, B.A.,
PATRICIA S. OLSON, B.S.N., AND J. CARLOS ROMERO, M.D.

SUMMARY Glomeruli were isolated from rat kidneys using a passive sieving technique to study the mechanisms of basal and β-adrenergic stimulated renin release. Glomeruli were enclosed within glass chambers and continuously superfused with Krebs media, or modified Krebs as described below, at a rate of 0.3 ml/min. The chamber effluent was collected in 10-minute fractions and measured for renin concentration (ng angiotensin I (A-I) generated) by radioimmunoassay. Basal renin was approximately 3 ng A-I/ml/hr. Beta-adrenergic stimulation with isoproterenol (ISO), 178 μM, increased renin concentration threefold (11 ± 2 ng A-I). The β-blocker propranolol at 12 μM halved ISO-stimulated renin, and at 120 μM eliminated it. Doubling Krebs sodium concentration (280 mM) had no effect upon basal or ISO-stimulated renin release. Pretreating rats with DOCA and a high salt diet significantly reduced basal and abolished ISO-stimulated renin release. Increasing Krebs calcium (10 mM) did not affect basal but abolished ISO-stimulated renin release. Calcium-free Krebs had no significant effects. Increasing Krebs potassium (50 mM) increased basal renin fourfold (14 ng A-I) while the absolute increase from basal due to ISO remained the same (23 ng A-I). These results suggest that basal renin and ISO-stimulated renin are released via different mechanisms.

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KEY WORDS • renin • sodium • propranolol • isolated glomeruli • β-adrenergic • rat • isoproterenol • calcium

Renin release is controlled by an interplay between signals from the renal baroreceptors, macula densa, renal nerves, and different hormones. Normally there is a constant basal secretion of renin which can be increased or decreased by altering these signals. An in vitro preparation of isolated rat renal glomeruli provides a means by which to study the control of renin release in the absence of such modifying external stimuli seen in vivo. Also, isolated glomeruli provide a more homogeneous cell population than other more gross in vitro methods. Renin production has been localized primarily in the afferent arteriole, and remnant arteriolar attachments are thought to be the source of renin in this preparation.

We have previously reported that isolated glomeruli constantly release a basal amount of renin into our superfusion system, and that renin can be further stimulated by the β-adrenergic agonist isoproterenol. Various reports have suggested that basal renin release and renin evoked by specific stimuli are elicited through different mechanisms. This study, using isolated glomeruli, suggests that there may be differences in the means by which basal and β-adrenergic-stimulated renin are released.

Methods

Sprague-Dawley rats of either sex weighing 150–250 g were anesthetized with 5 mg/100 g body weight sodium pentobarbital i.p. (Fort Dodge Laboratories). Their abdomens were opened and the kidneys flushed of blood with Krebs Ringer solution by means of an aortic catheter. The kidneys were then excised, decapsulated, and the cortical tissue was removed. Renal glomeruli were isolated using a passive technique of progressively finer sieves, as previously described. Approximately 40–50 mg (wet weight) of glomeruli were placed into glass chambers enclosed by scintted glass filters, allowing for a constant superfusion with modified Krebs Ringer solution (140 mM NaCl, 5 mM KCl, 0.8 mM MgSO₄, 2 mM CaCl₂, 10 mM NaAc, 10 mM glucose, 20 mM Tris base, 2 MM...
NaPO₄, pH 7.4, or as modified below), which was continuously aerated with 95% O₂-5% CO₂. The chambers were superfused at a constant rate of 0.3 ml/min with a Buchler polystatic pump (Model 3-6100) and the chamber effluent collected in 10-minute fractions by a Gilson FC-800 microfractionator.

Glomeruli harvested from several rats were pooled such that different chambers could be run simultaneously using the same tissue with different experimental maneuvers. The n-values presented represent the number of chambers run using each experimental permutation. The renin concentration of chamber effluent was measured. Aliquots of 50 µl were mixed with 50 µl of sodium phosphate buffer (50 mM, pH 7.4) and 5 µl of 2.5% phenylmethylsulfonylfluoride (PMSF). Plasma obtained from nephrectomized rats (100 µl) was added as a substrate for a 1-hour incubation to produce angiotensin I (AI), measured by radioimmunoassay as previously described.

All values are presented as the mean renin concentration ± 1 standard error. Students t tests for paired and unpaired variates were performed for analysis of statistical significance. Analysis of changes in renin release was limited to those changes from basal concentration (40 minutes) to that concentration during stimulation (50 minutes). Results were considered significant when p values were less than 0.05.

**Experimental Protocol**

**Krebs Perfusion**

Chambers containing glomeruli were superfused with Krebs medium or modified Krebs medium, when appropriate, for 90 minutes, concurrently with each of the following experimental protocols.

**Isoproterenol**

Chambers containing glomeruli were superfused with Krebs medium over the initial four 10-minute periods. In the fifth period, Krebs solution containing the β-adrenergic agonist isoproterenol (Winthrop) at a concentration of 178 µM was perfused over 13 minutes, after which Krebs solution alone was resumed.

**Propranolol and Isoproterenol**

Chambers containing glomeruli were superfused with Krebs medium containing one of two concentrations of the β-adrenergic antagonist propranolol (12 µM, n = 7; or 120 µM, n = 4; propranolol HCl, Ayerst Laboratories). In the fifth period, Krebs solution containing propranolol and isoproterenol was perfused over 13 minutes, after which Krebs solution with propranolol was resumed.

**Increased Perfusate NaCl Concentration**

Chambers containing glomeruli were superfused with Krebs medium modified by doubling the NaCl concentration (280 mM). Isoproterenol was perfused during the fifth period, as previously described (n = 6).

**High Salt Diet**

A group of rats were maintained on a normal diet for 2 weeks, but were given 1% NaCl as a drinking solution and were injected with 5 mg of deoxycorticosterone acetate (DOCA) in oil (Ciba Pharmaceutical Company) twice a week for 2-3 weeks prior to isolation of glomeruli. Chambers containing glomeruli from DOCA and salt rats were perfused with Krebs, and with isoproterenol, during the fifth period as previously described.

**High Calcium**

Chambers containing glomeruli were superfused with Krebs solution containing increased calcium (10 mM) and with isoproterenol in the fifth period, as previously described (n = 7).

**Low Calcium**

Chambers containing glomeruli were superfused with Krebs solution containing no calcium, and with isoproterenol in the fifth period, as previously described (n = 8).

**High Potassium**

Chambers containing glomeruli were superfused with Krebs solution containing increased potassium (50 mM) and with isoproterenol in the fifth period, as previously described (n = 7). Four additional chambers were superfused with the high potassium, but received no isoproterenol.

**Results**

**Krebs Perfusion**

Renin concentration in the initial two 10-minute periods was inconsistent, but generally high, probably representing renin accumulated throughout the isolation procedure. This rapidly dissipated to approximately 3 ng AI ml⁻¹ min⁻¹ by the third period, after which it declined slowly to 1.5-2.0 ng AI ml⁻¹ min⁻¹ by the eighth period.

**Isoproterenol**

Isoproterenol superfusion consistently stimulated an approximate threefold increase in renin concentration. As illustrated in figure 1, basal renin in Period 4 was 3.1 ± 1.0 ng AI ml⁻¹ min⁻¹ by the third period, after which it declined slowly to 1.5-2.0 ng AI ml⁻¹ min⁻¹ by the eighth period.

**Propranolol and Isoproterenol**

Isoproterenol superfusion consistently stimulated an approximate threefold increase in renin concentration. As illustrated in figure 1, basal renin in Period 4 was 3.1 ± 1.0 ng AI ml⁻¹ min⁻¹ and increased significantly to 10.2 ± 1.9 (p < 0.01) in the fifth period in response to 178 µM isoproterenol.

**Increased Perfusate NaCl Concentration**

When the Krebs superfusate contained 120 µM propranolol (fig. 1), renin release in response to isoproterenol was completely abolished. When the concentration of propranolol was reduced to 12 µM, the renin response to isoproterenol was diminished when compared to results without propranolol (5.3 ±
FIGURE 1. Renin concentration in serial 10-minute superfusion effluent collections from isolated glomeruli in response to 178 μM isoproterenol (ISO), and with ISO during 12 or 120 μM propranolol (PROP). Asterisks represent a significant increase between 40 and 50 minutes.

FIGURE 2. Renin concentration in serial 10-minute superfusion effluent collections from isolated glomeruli in response to 178 μM isoproterenol (ISO), to ISO in Krebs medium containing 280 mM NaCl, and to ISO in Krebs superfusing glomeruli from rats pretreated with DOCA and salt. Asterisks represent a significant increase between 40 and 50 minutes.

1.1 vs 10.2 ± 1.9 ng AI ml⁻¹ min⁻¹, p < 0.05), but an increase in renin concentration was still elicited from basal values (from 1.9 ± 0.3, p < 0.05).

Increased Perfusate NaCl Concentration

Increasing the NaCl concentration twofold (fig. 2) had no effect upon basal renin concentration (3.8 ± 1.3 ng AI ml⁻¹ min⁻¹) or upon the renin response to isoproterenol (10.5 ± 2.7) when compared to the superfusion with normal Krebs solution.

High Potassium

Increasing the potassium concentration of the Krebs superfusate increased the basal renin concentration (fig. 4) to approximately 14 ng AI ml⁻¹ min⁻¹. Isoproterenol stimulation of glomeruli superfused with the high potassium Krebs solution increased renin concentration significantly from 14.6 ± 4.4 to 23.4 ± 6.7 (p < 0.01), and further, to 29.8 ± 10.1 before returning toward basal levels by the eighth period.

Discussion

We have used a superfusion system with isolated rat renal glomeruli to study the mechanisms of both basal renin release and release in response to β-adrenergic stimulation using isoproterenol. The current work further supports the theory that the β-adrenergic antagonist, propranolol, can reduce or eliminate isoproterenol-stimulated renin release in a concentration-dependent manner. However, the β-blocker had no effect upon basal renin release.

It is thought that β-adrenergic stimulation of renin release is by means of adenylate cyclase activation which increases cAMP. 13 Churchill and Churchill and Fray have suggested that renin stimulation is a result of a reduction in the cytoplasmic calcium concentration. This may be a function of membrane hyperpolarization, or a stimulation of net calcium efflux linked to a sodium-calcium exchange mechanism.
If cAMP, stimulated by β-adrenergic activation, enhances the sodium-potassium pump, increasing the sodium gradient and thereby enhancing calcium efflux via a favorable gradient for sodium-calcium exchange, the intracellular calcium should decline. Ouabain and low extracellular potassium concentrations inhibit renin, presumably by affecting sodium-potassium ATPase activity. Likewise, low extracellular sodium retards renin release.

By eliminating superfusate calcium to make a gradient favorable to cellular calcium efflux, the magnitude of renin release, either basally or in response to isoproterenol, was not affected, although it did appear prolonged. We did not employ a calcium chelating agent, and consequently, the actual extracellular calcium concentration, though low, is probably greater than that of the superfusate. Reducing extracellular calcium has been reported to inhibit, to have no effect, or to increase renin release from kidney slices and from isolated perfused kidneys.

If calcium efflux, calcium mobilization, or reduced intracellular calcium is associated with renin release, increasing intracellular calcium or upsetting the normal calcium efflux from the cells should inhibit renin release. Increasing the extracellular calcium concentration, and therefore the transmembrane calcium gradient, effectively eliminated isoproterenol-stimulated renin release. Interestingly, basal levels appear unaffected. In vivo intrarenal calcium infusion also inhibits renin release.

Removal of sodium from the incubation medium of kidney slices depresses renin release. The loss of a sodium gradient across the plasma membrane may eliminate the substrate for the sodium-calcium exchange regulating renin release. Removal of calcium from the medium in the absence of sodium does not recover renin, suggesting that simple changes in calcium permeability or cellular concentrations are not the sole mediator of renin release. Reducing extracellular calcium has been shown to inhibit renin release, suggesting that its absence reduces the transmembrane sodium gradient, increasing intracellular calcium via sodium-calcium exchange. However, with elevated superfusate sodium concentration, the basal as well as the isoproterenol-stimulated release of renin remained unchanged. Lyons and Churchill have found that increased extracellular sodium stimulated renin from kidney slices but also depressed renin from cortical cell suspensions. The presence of additional sodium may not be as important as its absence in determining sodium-calcium exchange, and therefore renin release.

Pretreatment of rats with high sodium and DOCA prior to the isolation of glomeruli significantly depressed basal renin release compared to glomeruli from rats fed a normal diet, and abolished the renin response to isoproterenol. This suggests that chronic suppression of renin is carried into the in vitro environment, affecting both basal and stimulated renin release.

When membrane potential and presumably permeability were altered using increased extracellular potassium in the superfusate, basal renin was increased fourfold, but the absolute renin response to isoproterenol was not statistically different from that seen under normal perfusion conditions. Park et al. have suggested basal and stimulated renin release may
originate from different renin pools, which could explain these results. Conversely, the permeability changes may be only affecting basal release while not modifying the response mechanism to \( \beta \)-adrenergic stimulation. Churchill and Churchill\textsuperscript{11} found that both increasing and decreasing extracellular potassium in kidney slices inhibited basal and isoproterenol-stimulated renin release. They suggest that potassium depolarization opens voltage sensitive calcium channels, allowing the entry of calcium and thereby inhibiting renin. The difference between our results and those of the Churchills may be due to the magnitude of depolarization and increased permeability posed in very different in vitro systems. They suggest that intracellular calcium accumulation is required to produce the inhibitory effect of altered potassium concentration,\textsuperscript{24} a condition possibly not altered in our superfusion system over the period studied. Park and Malvin\textsuperscript{14} found that incubation of kidney slices with increased potassium concentration stimulated renin at low calcium concentrations while suppressing it at higher calcium concentrations. The use of a calcium ionophore may stimulate\textsuperscript{17} or inhibit renin,\textsuperscript{17, 26} but in the absence of extracellular calcium, ionophores stimulate renin.\textsuperscript{17, 26}

The specific mechanisms involved in controlling basal and \( \beta \)-adrenergic-mediated renin release remain speculative. In our experiments, low calcium medium, high sodium, and pretreatment with DOCA and salt did not dissociate the two pathways of renin release. However, our results show a disparity of basal and isoproterenol-stimulated renin release with \( \beta \)-blockade, high extracellular calcium, and the alteration of membrane permeability using high potassium. This suggests that renin may be released, at least partially, via different mechanisms under basal conditions and in response to \( \beta \)-adrenergic stimulation.

References

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