Adrenergic Stimulation of Renin Secretion in the Isolated Perfused Rat Kidney

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ABSTRACT

To avoid complicating systemic effects, the influence of adrenergic hormones on renin secretion was examined in the isolated rat kidney perfused with Krebs-dextran solution at a constant mean pressure and flow rate. Isoproterenol and glucagon produced a consistent increase in renin secretion without a change in perfusion pressure and flow. Similarly the increase in renin secretion with norepinephrine, which was associated with intense vasoconstriction and raised perfusion pressure, was still observed after the vasoconstrictive effect was abolished with phenoxybenzamine. Isoproterenol-stimulated renin secretion, unaffected by phenoxybenzamine, was markedly suppressed by dl-propranolol but not by d-propranolol. The failure of dl-propranolol to suppress the renin response to glucagon confirms observations in other tissues and suggests involvement of other receptors or alternative mechanisms. These findings support a direct intrarenal effect of adrenergic hormones on renin secretion, and this effect may be mediated specifically by beta receptors in the case of catecholamines.

KEY WORDS norepinephrine beta receptors renal perfusion pressure isoproterenol propranolol phenoxybenzamine

Considerable evidence implicates the sympathetic nervous system and the catecholamines in the regulation of renin secretion (1–4). In view of the close relation of adrenergic nerve endings to juxtaglomerular cells (5, 6) and the release of renin from renal cortical cell suspensions (7) and slices (8) after the addition of catecholamines, a direct effect of adrenergic hormones on renin secretion seems likely. The recent suggestion that renin secretion provoked by catecholamine infusion or sympathetic nerve stimulation is mediated by beta receptors receives support from studies in dogs (9–11) and man (12), using adrenergic receptor inhibitors. Other studies (13–15) do not substantiate the exclusive role of the beta receptor and show suppression of renin secretion by both alpha- and beta-receptor inhibitors. Interpretation of these findings is complicated by the systemic effects of the pharmacological agents employed and by the consequent operation of other factors known to influence renin secretion. To resolve these objections, we used the isolated rat kidney perfused at a constant pressure and flow rate to investigate the mechanism of renin secretion following stimulation by isoproterenol and norepinephrine. Since glucagon as well as the catecholamines has well-recognized effects on adenyl cyclase activity in renal and other tissues (16), its effect on renin secretion was also determined.

Methods

KIDNEY PERFUSION

Male Wistar rats (350–450 g) maintained on a regular diet were anesthetized with sodium pentobarbital (0.1 mg/g, im) and heparinized (50–100 units, iv). Through a midline incision the abdominal vessels and the left kidney were exposed and beveled Teflon cannulas were inserted in the aorta and the vena cava to the junction with the left renal artery and vein, respectively. This procedure permitted perfusion of the kidney without interruption of blood flow. With perfusion fluid flowing, the aorta and the vena cava were tied above the left renal vessels, allowing selective perfusion of the left kidney in situ. The perfusion fluid was delivered as pulsatile flow at a constant rate (usually 10 ml/min) by a roller pump from a reservoir containing Krebs-dextran solution oxygenated with 95% O2-5% CO2 and maintained at 37°C. Perfusion pressure was monitored by a transducer and a Devices recorder. All pharmacological agents were constantly infused into the arterial cannula at 0.04 ml/min. Mean perfusion pressure, initially high (150–250 mm Hg), fell rapidly to a steady lower level (50–100 mm Hg). Pump efficiency up to 250 mm Hg maintained constant flow rates during pressure changes and steady-state perfusion.
Experiments were conducted during steady-state perfusion, and control collections were obtained before pharmacological stimulation. The total duration of perfusion did not exceed 25 minutes. The perfused kidney gained slightly in weight compared with the nonperfused kidney (1.87 ± 0.03 compared with 1.62 ± 0.08 g wet weight) and showed normal histology.

**RENIN ASSAY**

Timed collections of perfusate were obtained and dialyzed to pH 4.5 over 24 hours at 4°C against 0.16 M phosphate buffers containing disodium ethylenediaminetetraacetate (EDTA) as is done for determinations of plasma renin activity (17). After further dialysis to pH 7.5, the samples were incubated at 37°C with nephrectomized rat plasma (after treatment by the dialysis steps outlined above) as the renin substrate. Neomycin sulfate (0.02%) was added to all samples. Pressor activity was assayed in the anesthetized pentolinium-treated nephrectomized rat, and the renin concentration was expressed as nanogram equivalents of 5-Ile-angiotensin I (Schwartz Bio-medical) generated per milliliter of perfusate per hour of incubation. All samples from one experiment were processed at the same time and assayed in the same rat.

Identity of the incubation product as predominantly angiotensin I was established by radioimmunoassay (18); generated angiotensin levels measured by bioassay closely agreed with the levels measured by angiotensin I immunoassay. Since flow rates remained nearly constant, renin values are expressed as renin concentration without conversion to secretion rates. No pressor activity was generated when the perfusate was incubated without substrate.

**MATERIALS**

Krebs-Ringer's saline (19) with 3.6% dextran (molecular weight, 70,000) was the perfusion fluid. Isopropylnorepinephrine (isoproterenol), norepinephrine tartrate, glucagon HC1, d-fluopropranolol HCl, d-propranolol HCl, and phenoxybenzamine were also used.

**Results**

Figure 1 illustrates the changes in mean perfusion pressure, flow rate, and renin concentration during perfusion in a typical experiment without pharmacological stimulation. Perfusion pressure, although initially high (150-250 mm Hg), fell rapidly to a steady level (50-100 mm Hg), usually within 5 minutes after the beginning of the perfusion. Flow rate and renin concentration remained nearly constant, although renin levels before stimulation varied considerably between experiments (Figs. 2-4). Renin values referred to below compare control values (at least two collections) with maximum values during or after stimulation. All values are means ± se. P values were determined by Student's paired t-test.

**ADRENERGIC STIMULATION AND RENIN SECRETION**

Infusion of isoproterenol (0.006 µg/min g⁻¹) consistently produced increases in renin concentration from 2.5 ± 0.6 to 12.9 ± 2.1 ng/ml hour⁻¹ (P < 0.005) (Fig. 2a); the highest level occurred after at least 2 minutes of infusion. Discontinuation of isoproterenol administration in two experiments reduced renin to control levels, whereas it remained elevated in three of four cases where the infusion was continued. Glucagon (0.6 µg/min g⁻¹) produced a similar significant increase in renin concentration from 3.6 ± 1.7 to 14.7 ± 6.2 ng/ml hour⁻¹ (P < 0.05) which persisted after discontinuation of infusion in four cases (Fig. 2b). In four of six cases, maximal renin concentration was only
Effects of isoproterenol (a), glucagon (b), and norepinephrine (c) at the doses shown on perfusate renin concentration when flow rate was maintained constant. Control refers to the mean of at least two determinations preceding pharmacological stimulation. At the small arrows, infusions were stopped.

ADRENERGIC INHIBITION AND RENIN SECRETION

To determine the effect of adrenergic blockade on renin secretion the inhibitor was infused for a 5-minute period immediately preceding stimulation with catecholamines or glucagon as shown in Figures 3 and 4. Timed collections of perfusate were obtained during the control period, during the infusion of inhibitor, and during the infusion of inhibitor and agonist. In most instances, more collections were obtained after the inhibitor was stopped. In each experiment, the maximal renin concentration observed during combined inhibitor and agonist infusion was compared with that during the infusion of inhibitor alone. Both dl-propranolol (0.6 μg/min g⁻¹) and d-propranolol (0.6 μg/min g⁻¹) produced a small and inconsistent increase in perfusate renin concentration; however, dl-propranolol abolished or markedly reduced the increase in renin concentration observed with isoproterenol infused at 0.005 μg/min g⁻¹ (2.1 ± 0.4 to 3.0 ± 1.2 ng/ml hour⁻¹, P > 0.2) (Fig. 3a). Continuing the infusion of isoproterenol for 5 minutes after dl-propranolol was stopped did not overcome the inhibition. No inhibition was observed with d-propranolol; isoproterenol (0.006 μg/min g⁻¹) produced a significant increase in renin (8.3 ± 3.5 to 27.1 ± 7.5 ng/ml hour⁻¹, P < 0.01) (Fig. 3b). The higher initial renin concentration (8.3 ng/ml hour⁻¹) is due to the marked stimulating effect of d-propranolol alone in one experiment. In contrast, no inhibitory effect of dl-propranolol (2.0 μg/min g⁻¹) on the response to glucagon (0.8 μg/min g⁻¹) was observed even when the dose of propranolol was increased to achieve the molar ratio at which isoproterenol was
inhibited (8.2 μg/min g⁻¹) (Fig. 3c). This high dose of propranolol did not increase renin concentration when it was infused alone for 5 minutes. Infusion of phenoxybenzamine in sufficient amount (0.7 μg/min g⁻¹) to block alpha receptors and to abolish the vasoconstrictive effect and the rise in perfusion pressure following norepinephrine administration did not prevent the increase in renin produced by norepinephrine (2.0 ± 0.6 to 11.3 ± 4.3 ng/ml hour⁻¹, P < 0.05) and isoproterenol (1.2 ± 0.6 to 8.1 ± 1.9 ng/ml hour⁻¹, P < 0.01) (Fig. 4). No consistent effect on renin concentration was apparent when phenoxybenzamine was infused alone. In two cases, the renin response to norepinephrine was considerably higher with phenoxybenzamine than it was without the drug (Figs. 2 and 4), although the variable response and the small numbers make interpretation uncertain. No change in perfusion pressure occurred with propranolol and phenoxybenzamine.

**Discussion**

Adrenergic hormones have a direct intrarenal effect on renin secretion which may occur independently of changes in perfusion pressure and flow. Infusion of isoproterenol and glucagon produced consistent increases in renin concentration without measurable changes in perfusion pressure or flow rate; similarly the increase in renin observed with norepinephrine was not prevented by abolishing its vasoconstrictive action with phenoxybenzamine. Specific involvement of beta receptors in the mediation of renin secretion is suggested by the marked suppression of the renin response to isoproterenol by dL-propranolol, whereas it was essentially unaltered by phenoxybenzamine. No suppression was apparent with d-propranolol,
Infusion of phenoxybenzamine to block alpha receptors did not alter the renin response to isoproterenol (a) or norepinephrine (b). Phenoxybenzamine was infused alone for at least 5 minutes immediately preceding infusion of isoproterenol and norepinephrine. Control refers to the mean of at least two determinations before infusion of phenoxybenzamine. At the small arrows, infusions of phenoxybenzamine, isoproterenol and norepinephrine were stopped.

which has a local anesthetic effect equal to that of dl-propranolol but no significant beta-receptor blocking activity (20). The failure of dl-propranolol, even at high doses, to suppress the renin response to glucagon may indicate involvement of receptor sites other than those for catecholamines (16). A similar dissociation between the effects of propranolol on glucagon and catecholamines has been observed in particulate adenylyl cyclase preparations (21), isolated renal tubules (22), and intact dog hearts (23). The persistent renin-stimulating activity of norepinephrine after alpha-receptor blockade suggests additional beta-receptor activity, demonstrated in its action on the heart (24). The results presented in this paper, together with previous reports of renin release in kidney slices with norepinephrine (8) and in renal cortical cell suspensions with norepinephrine and epinephrine (7), indicate a direct effect of catecholamines on adrenergic receptors related to the renin-producing cell. This view is also supported by studies in the nonfiltering kidney preparation where the renin-stimulating effect of norepinephrine persisted after papaverine treatment to prevent a fall in renal blood flow (25). Studies in the intact animal employing intra-arterial catecholamine infusion (1, 10, 14, 26), renal nerve stimulation (1, 11), and insulin hypoglycemia (9) generally support the concept of adrenergic mediation of renin secretion but are complicated by associated changes in renal blood flow (1, 26), plasma potassium (9-11), and urinary sodium excretion (1). A recent report (27) claiming the ineffectiveness of intrarenal as opposed to intravenous administration of isoproterenol on renin secretion implied involvement of extrarenal rather than intrarenal receptors. However, a more cautious interpretation of the changes in renin secretion observed is indicated by the considerable differences in control renin secretion between the two kidneys and in repeated estimations from the same kidney. Specific involvement of beta receptors, suggested by some studies (9-11), contrast with
other studies that present evidence for both alpha- and beta-receptor involvement (14, 15). The present study was designed to exclude other factors having a conceivable influence on renin secretion. Although perfusion pressure and flow remained constant during infusion of isoproterenol and glucagon, changes in intrarenal flow distribution cannot be discounted. However, such changes probably are not important since norepinephrine and isoproterenol both stimulate renin secretion yet have markedly different effects on intrarenal flow distribution (28). The reported inhibition of proximal tubular sodium reabsorption by isoproterenol (29) draws attention to the renal tubular sodium load as another possible factor in renin release. However, depending on the completeness of reabsorption at distal exchange sites, such inhibition is more likely to suppress than to enhance renin secretion (30, 31). With these reservations, the results presented in this paper indicate a direct intrarenal effect of adrenergic hormones on renin secretion. In view of the well established relation between beta-adrenergic hormones, glucagon, and membrane adenyl cyclase activity, the intracellular formation of 3', 5-cyclic adenosine monophosphate (AMP) may represent an integral step in renin pro-

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