Suppression of Renin Secretion in the Rat Kidney by a Nonvascular \(\alpha\)-Adrenergic Mechanism

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SUMMARY We studied the effect of \(\alpha\)-adrenergic stimulation, using phenylephrine, on basal and isoproterenol-provoked renin secretion in the isolated perfused rat kidney. Infusion of phenylephrine increased renal perfusion pressure and prevented the response in renin secretion to isoproterenol. No suppression of basal secretion was observed. Renal vasoconstriction was abolished, and the response in renin secretion to isoproterenol was restored by \(\alpha\)-adrenoceptor blockade with phenoxybenzamine. In contrast, when renal vasoconstriction was prevented by dihydralazine, suppression of renin release by phenylephrine still occurred. These observations support an inhibitory effect of a nonvascular \(\alpha\)-adrenergic mechanism on renin release. We suggest that the \(\alpha\) receptor mediating this effect is related directly to the juxtaglomerular cell.

Methods

Male Wistar rats (200–300 g), maintained on a regular diet, were anesthetized with sodium pentobarbital (0.1 mg/g, im) and heparinized (50–100 U, 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 by a roller pump from a reservoir containing Krebs-Ringer saline with dextran (mol. wt. 70,000; Pharmacia) 36 g/liter, oxygenated with 95\% \(\text{O}_2\)-5\% \(\text{CO}_2\) and maintained at 37\°C. Perfusion pressure (mm Hg) was continuously recorded, and flow rates (ml/min) were obtained by direct measurement of the venous effluent perfusate. All infusions were given at 0.04 ml/min. After commencing perfusion, we infused either saline (0.16 mol/liter NaCl) or phenylephrine diluted in saline (10.0 nmol/min). Within 3–5 minutes, when renal perfusion pressure clearly was elevated in the phenylephrine experiments, a timed collection of effluent perfusate was obtained for determination of renin concentration. This was designated 0 minutes. At 1 minute, administration of isoproterenol diluted in
saline (0.03 nmol/min) was started and continued for the remaining duration of both experiments. Further collections of perfusate for renin determination were obtained at 3 and 5 minutes and at 8, 10, and 12 minutes after phenylephrine was discontinued at 6 minutes. Experiments with saline or phenylephrine were performed alternately.

Additional experiments were performed with an identical protocol, except that phenoxybenzamine (8.0 nmol/min) was started at the same time as phenylephrine. The design of these experiments is shown in Figures 1 and 2.

In the following experiments, dihydralazine was introduced to modify the vasoconstrictor response to phenylephrine. The effect of dihydralazine alone on renin secretion was examined first. After obtaining a basal collection at 0 minutes, we infused dihydralazine (0.12 μmol/min) at 1 minute and continued until 14 minutes. Control experiments, infusing saline (0.16 mol/liter NaCl) instead of dihydralazine, were performed alternately. Collections were obtained at 4, 8, 10, 12, and 14 minutes. The dihydralazine studies then were repeated with the addition of isoproterenol (0.03 nmol/min) at 5 minutes (Fig. 3). This protocol was repeated in a further study with the addition of phenylephrine (in two experiments, 10 nmol/min, and in four experiments, 2.5 nmol/min) at 2 minutes (Fig. 3). Under these conditions, the prior infusion of dihydralazine prevented the vasoconstrictor action of phenylephrine.

For the determination of renin concentration, the perfusate collections were dialyzed successively to pH 4.5 and 7.5, according to the method of Skinner (1967) for plasma renin activity. After incubation with nephrectomized rat plasma as the renin substrate source (treated as sheep substrate by the...
method of Skinner), further reaction was terminated by heating the samples at 85°C for 5 minutes. The samples then were assayed for angiotensin I by radioimmunoassay (Boyd et al., 1969). All samples from one experiment were processed and assayed at the same time. Renin concentration is expressed in nmol equivalents of Asp\(^1\)-Ile\(^5\) angiotensin I generated per hour per liter of perfusate and converted into secretion rates by multiplying by the flow rate (ml/min). All values shown are mean value ± SEM. Statistical analysis was performed using Student's nonpaired t-test.

**Results**

Administration of the \(\alpha\) agonist, isoproterenol (0.03 nmol/min), resulted in marked stimulation of renin secretion, maximum levels being achieved within 4 minutes of infusion (Fig. 1). Renal perfusion pressure decreased gradually during the course of each experiment, whereas flow rate remained nearly constant. The fall in pressure is similar to that seen in control experiments, with saline infusion in place of isoproterenol, but with markedly less renin release (Vandongen and Peart, 1974a).

Phenylephrine infusion (10 nmol/min) resulted in a consistent elevation in renal perfusion pressure at 0, 3, and 5 minutes (Fig. 1). Basal renin secretion and flow rate were somewhat lower during phenylephrine administration, although these differences were not significant. However, it readily is apparent that the response in renin secretion to isoproterenol (0.03 nmol/min) was reduced considerably and significantly at 3 and 5 minutes (7 ± 3 and 13 ± 2) compared with the previous experiments without phenylephrine (31 ± 5 and 56 ± 9). When the phenylephrine infusion was stopped at 6 minutes, there was a rapid return of perfusion pressure to the levels seen in the nonvasoconstricted kidney (Fig. 1). Nevertheless, renin secretion remained significantly suppressed over this period and approached the values seen in the nonvasoconstricted kidney only at 12 minutes, 6 minutes after phenylephrine was stopped.

The simultaneous infusion of phenoxybenzamine (8.0 nmol/min) abolished the vasoconstrictor response to phenylephrine (10 nmol/min), as shown in Figure 2. The stimulation of renin release by isoproterenol was restored completely under these conditions.

The effect of dihydralazine on renin secretion, renal perfusion pressure, and flow rate was compared with saline (0.16 mol/liter NaCl) and, as is shown in Table 1, no obvious differences were apparent. The rise in renin secretion in saline controls has been observed previously in this preparation (Vandongen and Peart, 1974a).

Infusion of dihydralazine at 1 minute (Fig. 3) did not interfere with the response in renin secretion to isoproterenol introduced at 5 minutes. In the next experiment, also shown in Figure 3, the same procedure was followed with the addition of phenylephrine at 2 minutes. With this infusion sequence, no renal vasoconstriction was observed, and the perfusion pressures and flow rates in these experiments were nearly identical. Notwithstanding, the increase in renin secretion with isoproterenol was abolished completely, in a manner similar to that seen in Figure 1.
Discussion

Suppression of isoproterenol-provoked renin secretion by phenylephrine is largely abolished when renal vasoconstriction is prevented by $\alpha$ blockade with phenoxybenzamine, but not when it is prevented by the renal vasodilator, dihydralazine. These observations confirm and extend earlier findings that point to an inhibitory role of an intrarenal adrenoceptor on renin release (Vandongen and Peart, 1974a). It is evident that only $\beta$-adrenergic stimulation of renin secretion is suppressed, with basal renin levels relatively unaffected, despite similar increases in renal perfusion pressure. This is not due to the shorter duration of renal vasoconstriction, since more prolonged infusion of phenylephrine in vasoconstrictor doses (Vandongen and Peart, 1974b). It readily is apparent that $\alpha$ blockade with phenoxybenzamine abolishes the renin inhibitory and vasoconstrictor effects of phenylephrine. The possibility remains, therefore, that renin secretion is suppressed by a baroreceptor mechanism activated by renal vasoconstriction. However, the persistence of renin inhibition after phenylephrine infusion was stopped made this less likely. Therefore, it is suggested that the inhibition of renin secretion is independent of renal vasoconstriction. The inhibition of renin secretion during isoproterenol infusion by phenylephrine, after complete neutralization of its vasoconstrictor action by dihydralazine, strongly supports this concept.

Since renal tubular function was not studied specifically, possible changes in sodium transport, due to phenylephrine modulating renin release through the macula densa, cannot be ruled out.

The absence of discernible renin stimulation by dihydralazine was surprising, since hydralazine has been shown to provoke renin release in the intact rat (Pettinger et al., 1973). One explanation could be that dihydralazine is a weak stimulus and that its effect is not clearly distinguishable from the basal secretion rate as seen in the controls. The small but consistent rise in basal renin secretion may be due to the fall in perfusion pressure over the period these studies were done. Alternatively, dihydralazine may induce renin release in vivo only, as a result of reflex sympathetic activity and $\beta$-adrenergic stimulation.

From the evidence presented, it would appear that inhibition of renin secretion and renal vasoconstriction by phenylephrine are mediated by a receptor of the $\alpha$-adrenergic type. Since these two effects can be dissociated clearly, it is tempting to speculate on the existence of functional subclasses of $\alpha$ receptors, separately mediating vasoconstriction and renin suppression.

A similar dissociation between renal vasoconstriction and renin inhibition was reported with angiotensin II during graded reduction in the calcium concentration of the medium perfusing the isolated rat kidney (Vandongen and Peart, 1974b). In these experiments, renin suppression still was observed at calcium levels sufficiently low to abolish renal vasoconstriction.

The observations in this study provide evidence for the existence of a distinct nonvascular $\alpha$ receptor on the renin-producing juxtaglomerular cell. Since this structure is derived morphologically from the smooth muscle cell (Barajas and Latta, 1967), certain intracellular components of the contractile process may be present, including microsomal bound calcium. Release of this bound calcium by $\beta$-adrenergic stimulation (Baudouin-Legros and Meyer, 1972) leads to increased free intracellular calcium, inhibition of adenylate cyclase activity (Voliker and Hynie, 1971), and, therefore, the effects of $\beta$-receptor stimulation. Such a mechanism may apply not only to $\alpha$ agonists but to other renal vasoconstrictors which share in common the ability to inhibit renin secretion.
References

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