

Mechanisms of Renal Release of Renin by Electrical Stimulation of the Brainstem in the Cat

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ABSTRACT

Electrical stimulation of the dorsolateral pons for 5-minute periods in anesthetized cats produced a rise in blood pressure of 40%, a reduction in renal blood flow of 28%, and an increase in the rate of renin release (the product of renal plasma flow and the venoarterial difference of plasma renin activity) of more than 100% in intact kidneys. Comparison of an intact kidney with the contralateral denervated one showed that denervation abolished the decrease in renal blood flow and the increase in renin release induced by pontine stimulation. Infusion of phenoxybenzamine into an otherwise intact kidney prevented the decrease in renal blood flow on that side, but brain-induced renin release was at least equal to that from the contralateral kidney in which the vasomotor response remained unchanged. Treatment of the animal with intravenously administered propranolol (1.3–3.0 mg/kg) abolished any rise in renin release, although propranolol accentuated the reduction in renal blood flow during stimulation of the brainstem. We concluded that the increased renal release of renin induced by electrical stimulation of a pressor area of the brainstem was dependent on intact renal nerves and on mechanisms blocked by propranolol but was not dependent on changes in renal blood flow or on renal alpha receptors.

KEY WORDS
renal blood flow

propranolol
adrenergic receptors

phenoxybenzamine
sympathetic nervous system

renal denervation
sympathetic nervous system

■ Several authors have shown that electrical stimulation of the renal nerves (1–5) or some areas of the brain (6, 7) as well as intravenous infusion of catecholamines (1, 4, 8–13) can increase plasma renin activity. All this evidence points to an important role for the sympathetic nervous system in the control of renin release from the kidney (14). However, the meanings and the mechanisms of the neural effect on renin are still largely unclear. It is hard to deduce from most of the published studies whether all the procedures cause an actual increase in renin release; often arterial plasma renin activity is the only measured variable and, when renal vein renin is also measured, there is sometimes no simultaneous monitoring of renal blood flow. As to the mechanisms of the sympathoadrenergic effect on renin, divergent and often opposite conclusions have been reached probably because of the widely different preparations, doses, procedures, and controls employed.

Any direct intrarenal renin-releasing action of catecholamines has been denied (13); it has been claimed (3) and denied (4, 5) that renin release is primarily mediated by changes in renal blood flow. Renin release by sympathetic nerves and catecholamines has variously been attributed to an action on alpha receptors (3), an action on beta receptors (2, 10, 15), or an action on both (11, 16).

The experiments reported in the present paper were planned to contribute toward a clarification of these controversial issues. The sympathetic outflow to the kidneys was activated from a brainstem area so as to yield equal effects on both kidneys, renin release was measured separately from either kidney, and the response of an intact kidney was compared with that of the contralateral kidney in which all neural or all vasomotor effects had been prevented by pharmacological blockade. This study indicated that electrical stimulation of a pressor, renal vasoconstrictor area in the brainstem could markedly increase renin release from the kidneys, that this action was dependent on intact renal nerves but not on simultaneous changes in renal blood flow or on renal alpha receptors, and that the renin-releasing action of neural stimuli could be blocked by intravenously administered propranolol.

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Methods

Cats of either sex (3–4 kg) were fed raw meat containing about 250 mg of sodium on the days preceding an experiment. They were fasted but given water *ad libitum* overnight prior to anesthesia with sodium pentobarbital (35 mg/kg, ip). Light anesthesia was maintained by intermittent intravenous administration of sodium pentobarbital. After tracheal intubation, ventilation was maintained by a Harvard respirator, and the cats were paralyzed by intravenous administration of gallamine triethiodide to avoid respiration disturbances and contraction of skeletal muscle during brainstem stimulation.

Through a midline abdominal incision, electromagnetic flow probes were placed around each renal artery. Polyethylene catheters (1 mm, o.d.) were placed in the left renal vein via the gonadal vein and in the right renal vein via the femoral vein and the inferior vena cava. For denervation experiments, a similar catheter was affixed to one flow probe so that its tip lay dorsal to the renal artery and just lateral to the probe; through this catheter we later injected a small amount of 1% *p*-butylaminobenzoyl-diethyl-aminoethanol (Farmocaine), a local anesthetic structurally similar to tetracaine, which produces a long-lasting effect. For experiments with phenoxybenzamine, another polyethylene catheter was inserted into one renal artery via a femoral artery. The abdominal incision was then closed, the cat was turned into the prone position, and its head was mounted in a standard stereotaxic apparatus. A concentric bipolar electrode assembly with tips 1 mm apart was inserted into the dorsolateral pons. Final position of the tips was selected by trial and error to produce a prominent, bilaterally equal decrease in renal blood flow during brief stimulation with biphasic pulses (2 msec, 50 Hz, 0.5–1.5 ma) delivered by Tektronix series 160 generators. Intensity was maintained at the selected value by intermittently checking the voltage fall across a known resistor on a cathode-ray oscilloscope screen. The exact location of the stimulating electrodes was subsequently identified histologically on serial sections cut along the stereotaxic frontal plane and alternately stained by the Nissl (cells) and Weil (fibers) methods.

The following cardiovascular variables were recorded throughout the experiment on a Grass P7 polygraph and simultaneously monitored on a Hewlett-Packard series 3960 tape recorder. Heart rate was measured using subcutaneous needle electrodes, a Grass model 7P5A electrocardiogram (ECG) amplifier, and a model 7P4A tachometer. The tachometer was calibrated with an accurate square-wave signal generator (Grass model S4A). The instantaneous velocity of flow in each renal artery was recorded by a Statham model 4001 electromagnetic flowmeter and model MDQ non-cannulating probes (1.5–2.0 mm, i.d.). Integration of the velocity signals with Grass model 7P10A integrating amplifiers reset at 2-second intervals allowed simultaneous recording of renal blood flow. The probes were calibrated by perfusing arteries with blood collected in a graduated cylinder during accurately timed intervals. Zero flow was obtained by tightening a snare around the aorta just above the renal arteries and was recorded 1 hour before and just after each 5-minute pontine stimulation. Arterial blood pressure was recorded from a

cannulated femoral artery, using a Statham P23Db transducer and a Grass model 7P1 amplifier, and mean blood pressure was calculated at 2-second intervals by electronic integration through another Grass 7P10A amplifier. Values for renal blood flow, blood pressure, and heart rate shown in the tables are those present at the time of blood sampling for renin activity.

At the beginning of each experiment, brief pontine stimulation (about 10 seconds in duration) was used to demonstrate intact renal nerves as judged by a marked, equal reduction in blood flow to each kidney. In a group of cats we then blocked the nerves to one kidney by injecting Farmocaine through the catheter whose tip had been previously placed adjacent to the renal artery until test pontine stimulation no longer reduced renal blood flow in that kidney. The total amount of Farmocaine injected ranged from 0.3 to 0.7 ml. In other cats, small amounts of phenoxybenzamine were injected intermittently through a catheter in one renal artery until pontine stimulation no longer reduced blood flow to that kidney. The total dose of phenoxybenzamine ranged from 30 to 175 μ g. An unmodified vasoconstrictor response of the contralateral kidney indicated that there was no spread of local anesthetic or phenoxybenzamine far from the site of injection. Cats were rejected if the procedures caused some decrease in the vasoconstrictor response of the contralateral kidney to pontine stimulation.

Injection or infusion of propranolol into a renal artery caused a reduction in heart rate at doses (100 μ g) which did not completely block the renal vasodilator response to intra-arterially administered isoproterenol. We were thus unable to selectively block beta receptors in one kidney without producing an evident effect on the heart; therefore, an unpredictable effect of propranolol on the other kidney was probably unavoidable. Accordingly, propranolol was infused intravenously at a rate of 30–50 μ g/kg min⁻¹ for 30–60 minutes; the total dose of propranolol administered ranged from 1.3 to 3 mg/kg.

In all experiments a resting period of 1 hour was allowed after the last brief pontine stimuli used to place the electrode and to check the renal innervation. We then collected a first set (control) of blood samples for determination of plasma renin activity from each renal vein and from a peripheral artery; immediately thereafter, we started a 5-minute pontine stimulation, during the last minute of which a second set of samples (stimulus) was obtained. In denervation and phenoxybenzamine experiments, another 1 hour of rest was allowed after the end of the stimulation. Recovery samples were then obtained, and in a few cats a second 5-minute stimulation was carried out followed by final sampling for plasma renin activity. In the propranolol experiments, two stimulation trials were always performed just before and during the last 5 minutes of a 1-hour propranolol infusion as described above. Control and stimulus samples for plasma renin activity were obtained as usual immediately before and at the end of each stimulus.

Plasma renin activity was measured by radioimmunoassay for angiotensin I (AI) and was calculated as the difference between the immunoreactive AI formed during a 3-hour plasma incubation at 37°C and that present in an uncubated plasma sample at 4°C. Blood

samples (2 ml) were drawn in iced tubes containing 20 μ liters of 10% ethylenediaminetetraacetate (EDTA), and the plasma was promptly separated by centrifugation in a refrigerated centrifuge and stocked at -20°C after further addition of EDTA (1 mg/ml). The radioimmunoassay used a Schwartz antibody (075103) and followed the method of Haber et al. (17) with the following modifications. Blocking of converting enzyme was achieved by a mixture of 8-hydroxyquinoline (660 μg), dimercaprol (100 μg), and arginine (10 mg) in 0.5 ml of H_2O , brought to pH 6.0 by the addition of phosphoric acid. By this procedure, the pH of the incubation medium was maintained at optimum values for the renin-renin substrate reaction in spite of the high pH (7.8–8.0) of stocked plasma. Generation of AI increased linearly each hour over a 4-hour incubation period in one cat, demonstrating that availability of renin substrate was not a limiting factor. Sensitivity of the method was 0.1 ng, and after incubation the samples were diluted 1:5 and 1:15 to bring the values within the reading range of the method. Reproducibility was excellent: each sample was regularly analyzed in triplicate with very close results, and the range of repeated measurements of the same plasma stocked for periods of 4–18 days was never above 0.4 ng. Renin activity of arterial and renal venous samples was expressed as nanograms of AI formed per milliliter of plasma per hour. The rate of renin release from each kidney was calculated from the venoarterial difference in plasma renin activity (ng/ml hour^{-1}) multiplied by the renal plasma flow (ml/min) and expressed as nanograms per minute with the understanding that nanograms represent AI formed in a 1-hour incubation.

Results were treated statistically by analysis of variance with double classification (18) with specific contrasts between (1) control, stimulus, and recovery values in intact kidneys, (2) control and stimulus values in contralateral denervated or phenoxybenzamine-treated kidneys, (3) control values in intact and denervated or phenoxybenzamine-treated kidneys, (4) percent changes induced by stimulation in intact and contralateral denervated or phenoxybenzamine-treated kidneys, (5) changes induced by two sequential stimuli in intact kidneys, (6) control and stimulus values before and after administration of propranolol, (7) percent changes induced by stimulation before and after administration of propranolol.

Results

CARDIOVASCULAR EFFECTS OF PONTINE STIMULATION

The stimulus always markedly increased mean arterial blood pressure with an almost equal rise in systolic and diastolic values. Blood pressure rose very quickly at the beginning of stimulation, reached maximum values within 15 seconds, declined very slightly thereafter, and at the end of the 5-minute stimulation still averaged 30–50 mm Hg above the prestimulus values. Heart rate also markedly increased (about 50 beats/min) paralleling the changes in blood pressure. Renal blood flow to intact kidneys rapidly fell at the onset of stimula-

tion reaching a minimum of about 15% of its prestimulus value within 20 seconds. These very low levels, however, were seldom maintained for more than 1 minute; blood flow then gradually came back to a level approximating 70–75% of the control value. After the end of stimulation, blood pressure and heart rate rapidly decreased and were generally back to control levels within 1 or 2 minutes, although lower or higher values were sometimes maintained for longer times. Renal blood flow to intact kidneys rose at cessation of stimulation but often not to control values, which were attained only after half an hour at least. The time course of the cardiovascular changes induced by pontine stimulation is illustrated in Figure 1 in which the right kidney is the intact one.

EFFECTIVENESS OF PONTINE STIMULATION ON RENIN RELEASE

A preliminary analysis of 32 stimulation trials in 24 cats was performed to see whether stimulation

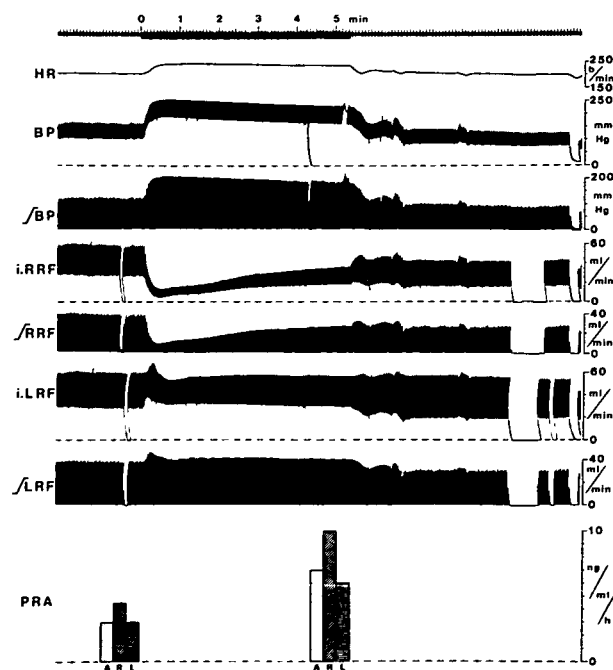


FIGURE 1

Renal denervation. The top tracing records time; pontine stimulation is indicated by the wide portion. From top to bottom are heart rate (HR), phasic arterial blood pressure (BP), mean arterial blood pressure (fBP), right renal blood flow velocity (i.RRF), right renal blood flow (f.RRF), left renal blood flow velocity (i.LRF), and left renal blood flow (f.LRF). The bars indicating plasma renin activity (PRA) also show the time at which blood samples were drawn. A, R, and L indicate arterial, right renal venous, and left renal venous plasma. The left kidney was denervated.

of the pontine area we had selected was capable of inducing an increase in renin release from intact kidneys. With stimulation, the renin activity of renal vein plasma increased from 10.4 ± 1.5 (SE) to 23.9 ± 3.8 ng/ml hour⁻¹ and the venoarterial difference rose from 2.8 ± 0.6 to 11.6 ± 2.8 ng/ml hour⁻¹, so that, in spite of a 28% fall in renal blood flow, renin release markedly and significantly rose from 61 ± 12 to 135 ± 21 ng/min. In 10 trials in which measurements were made 1 hour after pontine stimulation, renal vein renin, venoarterial difference, and renin release had all returned to levels not significantly different from control.

Of the 24 electrode placements, 19 were identified histologically. The stimulating tips were always in the nucleus reticularis parvocellularis, a portion of the lateral pontomedullary reticular formation that belongs to the so-called "pressor" or "vasoconstrictor area" (19).

Pontine stimulation did not always augment the release of renin from intact kidneys. In 3 of the 24 electrode placements, no increase in renin release could be induced. Absence of the renin-releasing effect could not be explained by erroneous positioning of the electrodes; two of the inactive points were in the nucleus reticularis parvocellularis with no apparent segregation from active sites. Nor did the difference in renin release result from different local hemodynamic effects, since we always used electrode placements from which a conspicuous rise in blood pressure and a marked fall in renal blood flow could be induced. Control plasma renin activity and renin release were also within the same range in the 3 cats in which pontine stimulation failed to raise renin release and in the 21 cats in which stimulation was successful. It is possible

that the functional state of the renin-releasing apparatus may be involved in some way in the absent responsiveness of the 3 cats. Among 10 cats rejected because control arterial plasma renin activity was abnormally elevated (above 20 ng/ml hour⁻¹), 6 cats (i.e., almost two-thirds) showed no increase in renin release during pontine stimula-

EFFECT OF DENERVATION ON RENIN RELEASE

Figure 1 illustrates changes in one of a series of cats in which one kidney was intact and the contralateral one was denervated by local anesthesia. Although blood flow to the right innervated kidney fell markedly, blood flow to the left denervated kidney rose at the onset of pontine stimulation and more slightly thereafter, illustrating the autoregulatory response of a denervated bed to a rise in arterial blood pressure (20). As is apparent from the columns at the bottom of Figure 1, pontine stimulation caused a conspicuous increase in arterial plasma renin activity, but the rise in venous plasma renin from the right innervated kidney was renin activity almost doubled. On the other hand, plasma renin activity in the renal vein on the denervated side was even smaller than the arterial value. Thus, pontine stimulation caused an increase in renin release from the innervated kidney but not from the denervated one.

Table 1 summarizes 13 experiments in eight cats with one kidney intact and the contralateral kidney denervated. Immediately before pontine stimulation, renal blood flow, renal venous renin activity, and renin release were equal on the two sides. Just before the end of the 5-minute stimulation, renal blood flow was still significantly and markedly decreased on the intact side, although blood flow to

TABLE 1

Effect of Renal Denervation on Renin Release during Pontine Stimulation

	Intact kidney			Denervated kidney		
	Control	Stimulus	P	Control	Stimulus	P
Renal blood flow (ml/min)	35.5 ± 5.3	26.2 ± 4.2	< 0.001	40.0 ± 4.2	41.9 ± 5.5	NS
Renal venous renin activity (AI ng/ml hour ⁻¹)	18.9 ± 5.1	27.4 ± 7.0	< 0.05	18.6 ± 5.1	18.2 ± 5.0	NS
Venoarterial renin difference (AI ng/ml hour ⁻¹)	4.0 ± 1.6	12.2 ± 4.6	< 0.05	3.7 ± 1.4	3.0 ± 1.3	NS
Renin release (ng/min)	69 ± 28	115 ± 32	< 0.05	66 ± 23	51 ± 16	NS
Mean arterial blood pressure (mm Hg)	125 ± 5	154 ± 10	< 0.01			

Entries are means ± SE for 13 experiments in eight cats. P values refer to differences between means in the preceding two columns: there were no significant differences (NS) between the intact and denervated kidneys without pontine stimulation (control).

the denervated kidney was slightly above prestimulus levels. Renal venous renin activity, venoarterial renin difference, and renin release all markedly increased on the innervated side, but there was no increase in renal venous renin activity or in venoarterial renin difference and an insignificant decrease in renin release from the denervated kidneys.

EFFECT OF PHENOXYBENZAMINE ON RENIN RELEASE

Figure 2 illustrates changes in a cat with one kidney intact and the contralateral one treated with intra-arterially administered phenoxybenzamine (175 μ g). The administered amount of the alpha-receptor blocking drug was large enough to prevent the vasoconstrictive response of the left kidney in which blood flow increased during pontine stimulation. There was no overflow of phenoxybenzamine from the injected kidney, as shown by the marked decrease in blood flow to the contralateral kidney and the conspicuous rise in blood pressure. Although phenoxybenzamine abolished renal vasoconstriction in response to pontine stimulation, it did not interfere with renin release, as is apparent at the bottom of Figure 2.

Table 2 summarizes six experiments in four cats with one kidney intact and the contralateral kidney treated with phenoxybenzamine. Before pontine stimulation, blood flow to the phenoxybenzamine-treated kidney was significantly greater than blood flow to the intact kidney, but renal venous renin activity, venoarterial renin difference, and renin release did not differ significantly on the two sides.

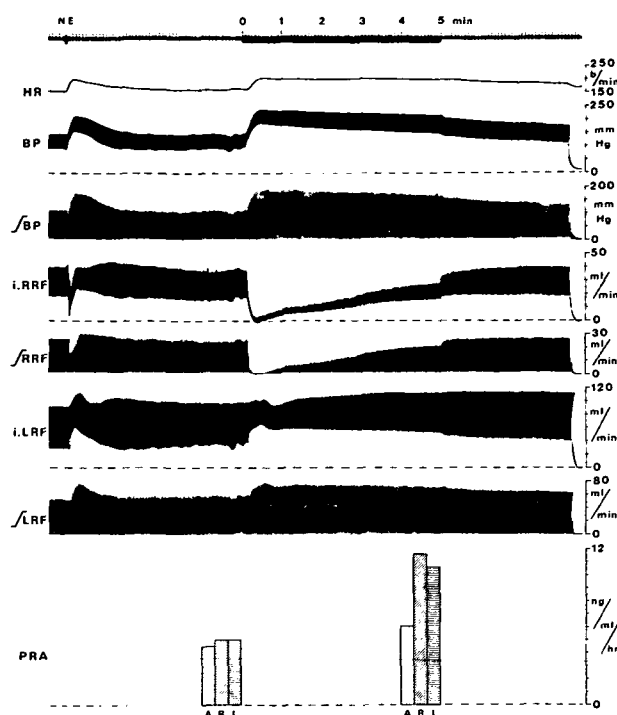


FIGURE 2

Phenoxybenzamine. Tracings and symbols are the same as they are in Figure 1. Phenoxybenzamine (175 μ g) was injected into the left renal artery an hour before the record was made. NE indicates intravenous injection of norepinephrine (1 μ g) to test the efficacy of blockade of the left kidney and to show the absence of blockade of the right kidney.

During pontine stimulation, renal blood flow significantly decreased on the intact side, and blood flow to the phenoxybenzamine-treated kidney rose

TABLE 2

Effect of Phenoxybenzamine on Renin Release during Pontine Stimulation

	Intact kidney			Phenoxybenzamine-treated kidney		
	Control	Stimulus	P	Control	Stimulus	P
Renal blood flow (ml/min)	38.0 \pm 3.7	26.8 \pm 4.7	< 0.001	55.3 \pm 2.5	62.7 \pm 2.3	NS
Renal venous renin activity (AI ng/ml hour ⁻¹)	7.3 \pm 2.4	11.5 \pm 2.8	< 0.01	7.6 \pm 2.5	11.7 \pm 3.2	< 0.05
Venoarterial renin difference (AI ng/ml hour ⁻¹)	1.4 \pm 0.6	4.4 \pm 1	< 0.05	1.8 \pm 0.9	4.4 \pm 1.1	< 0.05
Renin release (ng/min)	33 \pm 19	61 \pm 27	< 0.05	51 \pm 27	139 \pm 39	< 0.05
Mean arterial blood pressure (mm Hg)	122 \pm 9	164 \pm 15	< 0.05			

Entires are means \pm SE of six experiments in four cats. P values refer to differences between means in the preceding two columns. Comparing the intact and phenoxybenzamine-treated kidneys without pontine stimulation (control), renal blood flow was greater (P < 0.05) in the kidneys treated with phenoxybenzamine; the difference in renin release was not significant (NS).

TABLE 3

Changes Induced by Two Sequential Pontine Stimuli in Intact Kidneys

	Stimulus 1	Stimulus 2	P
ΔRenal blood flow (ml/min)	-10 ± 4	-10 ± 2	NS
ΔRenin release (ng/min)	+26 ± 10	+41 ± 18	NS

Entries are means ± SE of six experiments in six cats. *P* values refer to differences between means in the two columns; NS = not significant.

slightly above prestimulus levels. However, renal venous renin activity, venoarterial renin difference, and renin release were markedly and significantly increased on both the intact and the phenoxybenzamine-treated side. Thus, pontine stimulation altered renal blood flow in opposite directions in intact and phenoxybenzamine-treated kidneys, but there was no significant difference in renin release.

Comparison of Tables 1 and 2 shows that pontine stimulation had similar hemodynamic effects in the cats with one kidney denervated or one kidney treated with phenoxybenzamine. However, denervation abolished but phenoxybenzamine did not interfere with renin release during pontine stimulation.

EFFECT OF PROPRANOLOL ON RENIN RELEASE

For the reasons given in Methods, propranolol could not be given intra-arterially in such a way as to restrict its action to one kidney only. Therefore, it was administered intravenously, and we compared renal responses to pontine stimulation before and after propranolol administration.

Preliminary experiments in six cats with one kidney not subjected to any intervention were planned to test whether two sequential pontine

stimuli separated by an hour caused comparable changes in renal blood flow or renin release. Table 3 shows that this condition was fulfilled.

Table 4 summarizes the results of pontine stimulation before and after propranolol administration in eight experiments in eight cats. The marked increase in heart rate caused by pontine stimulation was abolished by propranolol; arterial blood pressure still rose, although from a lower prestimulus level; renal blood flow decreased even more after propranolol administration. Pontine stimulation caused no significant change in renal venous renin activity, venoarterial renin difference, or renin release after propranolol administration.

In the experiments of Table 4, propranolol was infused in doses ranging from 1.3 to 3.0 mg/kg. A preliminary attempt to use smaller doses (1.0 mg/kg) in one cat (not included in Table 4) resulted in a reduced but persistent tachycardia in response to pontine stimulation with an increase in heart rate of 15 beats/min; additional infusion of propranolol (1.6 mg/kg) 1 hour later completely abolished the tachycardia. After the first dose of the drug pontine stimulation conspicuously increased renin release (by about 400 ng/min), but the second dose reduced the increase to only 42 ng/min. Therefore,

TABLE 4

Effect of Propranolol on Renin Release during Pontine Stimulation

	Before propranolol			After propranolol		
	Control	Stimulus	P	Control	Stimulus	P
Renal blood flow (ml/min)	30.8 ± 3.5	23.7 ± 2.6	< 0.01	20.5 ± 1.5	11.4 ± 1.7	< 0.001
Renal venous renin activity (AI ng/ml hour ⁻¹)	10 ± 1.9	39.2 ± 8.6	< 0.01	16.3 ± 3.8	23.5 ± 6.4	NS
Venoarterial renin difference (AI ng/ml hour ⁻¹)	2.8 ± 0.7	18.8 ± 5.6	< 0.05	5.1 ± 1.6	8.7 ± 3.6	NS
Renin release (ng/min)	46 ± 11	182 ± 30	< 0.01	52 ± 16	43 ± 16	NS
Mean arterial blood pressure (mm Hg)	140 ± 4	196 ± 6	< 0.01	111 ± 10	164 ± 13	< 0.01
Heart rate (beats/min)	174 ± 10	226 ± 11	< 0.01	138 ± 8	144 ± 8	NS

Entries are means ± SE of eight experiments in eight cats. *P* values refer to differences between means of the two preceding columns. There were significant differences between the means in the two control columns for renal blood flow (*P* < 0.01), mean arterial blood pressure (*P* < 0.01), and heart rate (*P* < 0.01). NS = not significant.

all subsequent experiments were performed with the larger doses, which always blocked the neural effects on heart rate. No attempt was made to discover the threshold dose for blocking the heart rate response in each experiment to test its action on renin release.

Discussion

Our data showing that brainstem stimulation influences renin release confirm and extend previous reports by Ueda et al. (6) and Passo et al. (7). Although the evidence provided by these authors was limited to an increase in plasma renin activity in a peripheral artery (7) or in a renal vein (6), in our experiments simultaneous measurement of both arterial and renal vein renin and monitoring of renal blood flow allowed the calculation of net renin release and the demonstration that release actually rises in spite of the fall in renal blood flow. There are a few other differences between previous experiments and the present ones. The mesencephalic area stimulated by Ueda et al. (6) apparently gave a combination of sympathetic discharge and secretion of catecholamines, as judged by the persistence of renal vasoconstriction after denervation. A rise in blood epinephrine also occurred in the experiments by Passo et al. (7). Therefore, denervation largely but incompletely abolished the effect on plasma renin activity in one work (6), and occasional small rises in plasma renin activity were observed in denervated kidneys in the other work (7). On the other hand, the pontine area we stimulated appeared to affect the renal circulation through a sympathetic discharge only, since denervation not only reversed the fall in renal blood flow but caused a small increase and not only suppressed the increase in renin release entirely but changed it into a slight decrease. Because this decrease was somewhat variable from cat to cat and statistically insignificant, it is difficult to identify the mechanism whereby it was produced. However, it does not seem unreasonable that it might be due to increased stretch of the afferent arteriole by the raised blood pressure.

Our data also help to clarify the mechanisms by which sympathetic stimuli release renin. Current hypotheses (21, 22) are: (1) activation of renal nerves decreases renal blood flow and glomerular filtration rate in some, e.g., cortical, or in all nephrons with decreased delivery of sodium to the macula densa, (2) decreased stretch of a baroreceptor in the afferent arteriole at the juxtaglomerular apparatus during increased neural ac-

tivity, because of either constriction of smooth muscle cells of the juxtaglomerular arteriole itself or decrease in radius of upstream segments of the afferent arterial system with decreased intraluminal pressure in the more distal juxtaglomerular portion, and (3) direct release of renin from the granules in the juxtaglomerular arteriolar cells where sympathetic endings have been identified (23, 24). Our results clearly favor the third hypothesis. Indeed, renin release increased during brainstem activation of renal nerves both in the intact kidney in which blood flow was decreased and in the phenoxybenzamine-treated kidney in which blood flow was augmented. Although we measured total renal blood flow rather than intrarenal flow distribution, it is known from the work of Carrière (25) that phenoxybenzamine blocks adrenergic effects on both outer cortex and medulla. Therefore, the directionally opposite changes in renal blood flow that we observed in intact and phenoxybenzamine-treated kidneys should have produced directionally opposite changes in both delivery of sodium to the macula densa and stretch of a baroreceptor in the juxtaglomerular afferent arteriole (22). Furthermore, the occurrence of comparable systemic and local hemodynamic changes both in experiments with a denervated kidney and in those with a phenoxybenzamine-treated kidney suggests that the increase in renin release was not related to systemic or local hemodynamics, since renin release was influenced in opposite directions in the two types of experiments.

Our conclusion that renal nerves can release renin by a direct action on the juxtaglomerular cells supports previous observations of Johnson et al. (4) using the nonfiltering kidney. These authors showed that electrical stimulation of the renal nerves in the dog still increased renin release after tubular destruction had been produced and papaverine had been injected with the intention of preventing changes in sodium delivery to the macula densa and stretch of the afferent arteriole. Compared with the results of Johnson and his colleagues (4), our experiments permitted a more complete abolition of renal vasomotor effects through a procedure more selectively limited to one kidney, leaving the contralateral kidney as a useful simultaneous comparison. Moreover, use of the nonfiltering kidney, although a very ingenious preparation, could not indicate how much of the renin release caused by the renal nerves was due to direct rather than indirect mechanisms. Our com-

parison of an intact with a phenoxybenzamine-treated kidney showed that the entire renin release caused by sympathetic stimulation was independent of vasomotor phenomena and directly induced. If there was any change in the phenoxybenzamine-treated kidney, it was toward a greater release than that on the intact side, indicating that the fall in renal blood flow normally occurring during activation of the sympathetic outflow to the kidney would limit rather than stimulate renin release. This conclusion is opposite to that recently reached by Coote et al. (3) in experiments to be discussed below.

Our experiments also provided some clarification of the confused problem of the intrarenal receptors underlying the response of the juxtaglomerular apparatus to sympathoadrenal stimuli. Our observations on phenoxybenzamine-treated kidneys indicated that alpha receptors were not involved, at least if all alpha receptors are defined like the vascular ones as those sites which are blocked by phenoxybenzamine. Our conclusions are supported by previous reports by Passo et al. (16) and by Loeffler et al. (2), although systemic injection of large doses of the drug and the consequent fall in blood pressure and rise in prestimulus renin levels limit the significance of some of the previous experiments. Support also comes from the observation that dibenamine or phenoxybenzamine do not block the renin-releasing action of catecholamines (9, 10, 15). Two divergent reports have to be discussed, however. Coote et al. (3) and Winer et al. (11) found that intravenously administered phentolamine could block renin release induced by renal nerves and by isoproterenol. These experiments are the only ones in which phentolamine has been used as the alpha-receptor blocking agent. It is known, however, that phentolamine has several other properties besides blockade of alpha receptors (26), and recently a beta-receptor stimulating action has also been suggested (27). Furthermore, the very large doses of phentolamine used, at least in the experiments of Coote et al. (3), often caused a very marked increase in prestimulus renin activity; this increase might have been sufficient to prevent a further rise during stimulation. Of course, it cannot be excluded that the hypothetical alpha receptors involved in renin release are more resistant to blockade and require a larger amount of blocking drugs than do vascular alpha receptors. In any case, our observations point out unequivocally that the renin-releasing and the vasomotor effects of neural stimulation

can be completely dissociated by blockade of the vascular alpha receptors.

Our experiments with propranolol provided less satisfactory evidence about the role of intrarenal beta receptors, because we were unable to restrict the action of propranolol to one kidney only, to avoid the extrarenal actions of the drug, and to use the contralateral kidney as a simultaneous comparison. This limitation is common to other works which have employed propranolol. Our finding that this drug can completely block renin release caused by pontine stimulation is consistent with results of all previous experiments which have tested propranolol against stimulation of the brainstem (16) or renal nerves (2, 3) and infusion of catecholamines (9-12, 15): the incomplete abolition observed by Passo et al. (16) clearly resulted from an insufficient dose of propranolol (0.75 mg/kg given over 30 minutes). Propranolol (1 mg/kg) does not completely block the tachycardia produced by electrical stimulation of the mesencephalon in the dog (28), and also in our hands this dose was too low to block the heart rate and renin responses to pontine stimulation. We have no evidence, however, whether threshold doses for blocking the two types of response are equal or whether suppression of renin release requires larger doses than does blockade of tachycardia.

The local anesthetic effect of propranolol (29) seems an unlikely explanation for its abolition of renin release during pontine stimulation. Application of a local anesthetic to the renal nerves abolished renal vasoconstriction, but after propranolol administration the renal vasoconstrictor response to pontine stimulation was accentuated.

Ganong (14) has recently summarized the evidence suggesting that the sympathoadrenal control of renin release is mediated through intrarenal beta receptors. However, a few doubtful aspects need to be clarified before this attractive hypothesis can be considered as an established fact. First of all, it would be necessary to restrict propranolol to one kidney only, otherwise suppression of renin release due to some extrarenal action of propranolol, perhaps alterations in cardiac output affecting humoral mechanisms such as vasopressin (13, 22) which stimulates renin release (22), cannot be ruled out. Reid et al. (13) have stated that the renin-releasing action of small doses of isoproterenol is due to extrarenal actions of this catecholamine, although there is evidence that at least larger doses of isoproterenol can exert a

direct intrarenal influence (15, 30, 31). Finally, the observation by Winer et al. (11, 32) that the renin-releasing action of cyclic adenosine monophosphate (AMP) and of theophylline (known to increase intracellular cyclic AMP) is blocked by propranolol suggests that this drug may act at some intracellular site distal to adrenergic receptors. Quite recently, Reid et al. (33) were unable to confirm the data of Winer et al. on theophylline and the entire question requires further clarification. Until then, it seems unwarranted to define any renin release as neurally mediated simply because it is sensitive to propranolol.

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References

- VANDER, A.J.: Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs. *Am J Physiol* **209**:659-662, 1965.
- LOEFFLER, J.R., STOCKIGT, J.R., AND GANONG, W.F.: Effect of alpha- and beta-adrenergic blocking agents on the increase in renin secretion produced by stimulation of the renal nerves. *Neuroendocrinology* **10**:129-138, 1972.
- COOTE, J.H., JOHNS, E.J., MACLEOD, V.H., AND SINGER, B.: Effect of renal nerve stimulation, renal blood flow and adrenergic blockade on plasma renin activity in the cat. *J Physiol (Lond)* **226**:15-36, 1972.
- JOHNSON, J.A., DAVIS, J.O., AND WITTY, R.T.: Effects of catecholamines and renal nerve stimulation on renin release in the nonfiltering kidney. *Circ Res* **29**:646-653, 1971.
- MARTIN, D.M., AND WHITE, F.N.: Evidence for direct neural release of renin (abstr.). *Fed Proc* **30**:431, 1971.
- UEDA, H., YASUDA, H., TAKABATAKE, Y., IIZUKA, M., IIZUKA, T., IHORI, M., YAMAMOTO, M., AND SAKAMOTO, Y.: Increased renin release evoked by mesencephalic stimulation in the dog. *Jap Heart J* **8**:498-506, 1967.
- PASSO, S.S., ASSAYKEEN, T.A., OTSUKA, K., WISE, B.L., GOLDFIEN, A., AND GANONG, W.F.: Effect of stimulation of medulla oblongata on renin secretion in dogs. *Neuroendocrinology* **7**:1-10, 1971.
- WATHEN, R.L., KINGSBURY, W.S., STROUDER, D.A., SCHNEIDER, E.G., AND ROSTORFER, H.H.: Effects of infusion of catecholamines and angiotensin II on renin release in anesthetized dogs. *Am J Physiol* **209**:1012-1024, 1965.
- UEDA, H., YASUDA, H., TAKABATAKE, Y., IIZUKA, M., IIZUKA, T., IHORI, M., AND SAKAMOTO, Y.: Observations on the mechanisms of renin release by catecholamines. *Circ Res* **27**(suppl. II):II-195-200, 1970.
- TANIGAWA, H., ALLISON, D.J., AND ASSAYKEEN, T.A.: Comparison of the effects of various catecholamines on plasma renin activity alone and in the presence of adrenergic blocking agents. In *Hypertension '72*, edited by J. Genest and E. Koiv. New York, Springer-Verlag, 1972, pp 37-44.
- WINER, N., CHOKSHI, D.S., AND WALKENHORST, W.G.: Effects of cyclic AMP, sympathomimetic amines, and adrenergic receptor antagonists on renin secretion. *Circ Res* **29**:239-248, 1971.
- CHOKSHI, D.S., YEH, B.K., AND SAMET, P.: Effects of dopamine and isoproterenol on renin secretion in the dog. *Proc Soc Exp Biol Med* **140**:54-57, 1972.
- REID, I.A., SCHRIER, R.W., AND EARLEY, L.E.: Effect of extrarenal beta adrenergic stimulation on the release of renin. *J Clin Invest* **51**:1861-1869, 1972.
- GANONG, W.F.: Biogenic amines, sympathetic nerves, and renin secretion. *Fed Proc* **32**:1782-1784, 1973.
- VANDONGEN, R., PEART, W.S., AND BOYD, G.W.: Adrenergic stimulation of renin secretion in the isolated perfused rat kidney. *Circ Res* **32**:290-296, 1973.
- PASSO, S.S., ASSAYKEEN, T.A., GOLDFIEN, A., AND GANONG, W.F.: Effect of alpha and beta adrenergic blocking agents on the increase in renin secretion produced by stimulation of the medulla oblongata in dogs. *Neuroendocrinology* **7**:97-104, 1971.
- HABER, E., KOERNER, T., PAGE, L.B., KLIMAN, B., AND PURNODE, A.: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocrinol* **29**:1349-1355, 1969.
- SNEDECOR, G.W., AND COCHRAN, W.G.: *Statistical Methods*, 6th ed. Ames, Iowa, Iowa State University Press, 1967.
- ROSSI, G.F., AND ZANCHETTI, A.: Brainstem reticular formation: Anatomy and physiology. *Arch Ital Biol* **95**:199-435, 1957.
- IRIUCHIJIMA, J., AND WILSON, M.F.: Sympathetic vasoconstrictor activity to the kidney in carotid occlusion pressor reflex. *Proc Soc Exp Biol Med* **131**:189-192, 1969.
- VANDER, A.J.: Control of renin release. *Physiol Rev* **47**:359-382, 1967.
- DAVIS, J.O.: Control of renin release. *Am J Med* **55**:333-350, 1973.
- BARAJAS, L.: Innervation of the juxtaglomerular apparatus: Electron microscopic study of the innervation of the glomerular arterioles. *Lab Invest* **13**:916-930, 1964.
- WAGERMARK, J., UNGERSTEDT, U., AND LJUNGQVIST, A.: Sympathetic innervation of the juxtaglomerular cells of the kidney. *Circ Res* **22**:149-153, 1968.
- CARRIÈRE, S.: Effect of norepinephrine, isoproterenol and adrenergic blockers upon the intrarenal distribution of blood flow. *Can J Physiol Pharmacol* **47**:199-208, 1969.
- GOODMAN, L.S., AND GILMAN, A.: *Pharmacological Basis of Therapeutics*, 4th ed. New York, The Macmillan Company, 1970, pp 559-560.
- DAS, P.K., AND PARRATT, J.R.: Myocardial and hemodynamic effects of phentolamine. *Br J Pharmacol* **41**:437-444, 1971.
- KONTOS, H.A., AND LOWER, R.R.: Role of beta-adrenergic receptors in the circulatory response to hypoxia. *Am J Physiol* **217**:756-763, 1969.
- BARRETT, A.M., AND CULLUM, V.A.: Biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br J Pharmacol* **34**:43-55, 1968.
- MICHELAKIS, A.M., CAUDLE, J., AND LIDDLE, C.W.: In vitro stimulation of renin production by epinephrine, norepinephrine, and cyclic AMP. *Proc Soc Exp Biol Med* **130**:748-753, 1969.
- VEYRAT, R., AND ROSSET, E.: In vitro renin release by human

- kidney slices: Effect of norepinephrine, angiotensin II and I, and aldosterone. *In* Hypertension '72, edited by J. Genest, and E. Koiv. New York, Springer-Verlag, 1972, pp 37-44.
32. WINER, N., CHOKSHI, D.S., MYUNG, S.Y., AND FREEDMAN, A.D.: Adrenergic receptor mediation of renin secretion. *J Clin Endocrinol* 29:1168-1175, 1969.
33. REID, I.A., STOCKIGT, J.R., GOLDFIEN, A., AND GANONG, W.F.: Stimulation of renin secretion in dogs by theophylline. *Eur J Pharmacol* 17:325-332, 1972.