The Renin Response to Aortic Occlusion Is Enhanced by Stimulation of the Hypothalamus

JAMES P. PORTER

SUMMARY The sympathetic nervous system is an important factor that can induce increased renin secretion by the kidney. In recent years, the notion has arisen that the sympathetic nervous system may also function to set the level of responsiveness of the kidney to nonneural stimuli for renin secretion. However, evidence in favor of this possibility has come primarily from studies employing direct electrical stimulation of renal nerves, and no attempt has been made to determine if central neural sites can also influence the responsiveness of the kidney. In the present study, the ability of hypothalamic activation to enhance the renin response to suprarenal aortic occlusion was investigated. Conscious, freely moving rats with an inflatable cuff placed around the aorta were used to determine the relationship between renal perfusion pressure and plasma renin activity in the control state and during continuous low-level stimulation of the paraventricular nucleus. The stimulation resulted in a rightward shift in the curve that related renal perfusion pressure to plasma renin activity; that is, for any given decrease in renal perfusion pressure, the plasma renin activity was greater during the ongoing stimulation. This rightward shift appeared to be mediated by increased renal nerve activity, since renal denervation prevented the shift. These data indicate that the hypothalamus, which plays an important role in regulating sympathetic activity, is capable of increasing the sensitivity of the kidney to a nonneural stimulus for renin secretion. This effect may become important in certain hypertensive and prehypertensive states where central neural activity is thought to be enhanced. (Hypertension 12: 52-58, 1988)

KEY WORDS • renin • paraventricular nucleus • hypothalamus • hypertension • conscious rat

CONSIDERABLE evidence has accumulated over the past 30 years that suggests that the sympathetic nervous system exerts an important regulatory influence on renin secretion. Activation of the renal sympathetic innervation, whether by electrical or reflex stimulation or by stimulation of sites within the brain, has been associated with increased renin secretion.1-4 Within the past 15 years the notion has arisen that, in addition to directly inducing renin release, the sympathetic nervous system also acts to modulate the responsiveness of the renin-secreting juxtaglomerular cells to nonneural stimuli.5-11 These observations have led to the hypothesis that, in hypertensive or prehypertensive states, where an overactive central nervous system may be responsible for increased renal nerve activity, kidneys release increased amounts of renin in response to normal daily stimuli such as postural changes or changes in dietary sodium.10 This intermittent elevation in circulating renin (and thus angiotensin II) could then contribute to arterial pressure lability. However, there is no direct evidence that nervous activity originating from the brain can alter the responsiveness of the kidney to nonneural stimuli for renin secretion. In the present investigation, this possibility was tested by determining whether electrical stimulation of a central nervous site could enhance the release of renin produced by lowering renal perfusion pressure. Specifically, the effect of low-level electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN) on the renin response to graded suprarenal aortic occlusion was investigated using conscious rats. The PVN was chosen because it is a site that can influence renin release and because it has been shown to contribute to certain forms of hypertension.12-14

The data in the present investigation suggest that low-level stimulation of the PVN can lead to an increased responsiveness of the kidneys to lowered renal perfusion pressure. This effect is mediated by β-adrenergic receptors and is abolished by renal denervation. A portion of this work has been previously reported in abstract form.15

From the Department of Physiology and Biophysics, University of Louisville, Louisville, Kentucky.
Supported by a grant-in-aid from the American Heart Association Kentucky Affiliate.
Address for reprints: James P. Porter, Ph.D., Department of Physiology, Health Sciences Center A-1115, University of Louisville, Louisville, KY 40292.
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Materials and Methods

All experiments were performed using conscious male Sprague-Dawley rats (190–300 g; Harlan, Indianapolis, IN, USA) fed standard rat chow (Purina, St. Louis, MO, USA) and previously prepared in the following manner. After induction of anesthesia with ketamine (140 mg/kg) mixed with acepromazine (1.4 mg/kg i.p.), a catheter was inserted into the lower abdominal aorta through a femoral artery. In the initial experiments, a catheter was also inserted into the left common carotid artery through a midline incision over the trachea. Some rats received an additional catheter in a femoral vein for subsequent infusion of propranolol. All catheters were obturated and tunneled subcutaneously to exit at the back of the neck. Through a midline abdominal incision, an occluding cuff (Bioserv, Frenchtown, NJ, USA), filled with water, was placed around the abdominal aorta proximal to the branch point of either renal artery. In some rats, a miniaturized pulsed Doppler flow probe was placed around a renal artery and sutured in place using 6-0 silk. The tubing from the occluding cuff and the probe-head wires were exteriorized through an incision in the side and then run subcutaneously to exit at the back of the neck. The ends of the wires were soldered to a small two-pronged plug that was anchored to the skull using small screws and cyanoacrylic plastic cement. The animals were then placed in a Kopf stereotaxic apparatus (Tujunga, CA, USA), and a bipolar stimulating electrode (outside diameter 0.25 mm/pole; Plastic Products, Roanoke, VA, USA) was placed into the right PVN using the following coordinates with respect to the bregma: posterior, 1.8 mm; lateral, 0.5 mm; and ventral to the dura, 7.2 mm. The electrode was cemented in place, and the incision was closed around the electrode and probe head-plug. Supplemental doses of anesthetic were given as needed. Postoperatively, each rat was given an intramuscular injection of penicillin G (80,000 units) and a subcutaneous injection of 0.9% saline (3 ml).

Renal Denervation

Some rats underwent bilateral renal denervation at the time of the initial operation. The renal arteries and veins on each side were isolated, and connective tissue was stripped from the vessels. All branches of the renal plexus that were observable using a dissecting microscope were also cut. Finally, the vessels were painted with 10% phenol in absolute ethanol, with care being taken to prevent spread of the solution to the kidney or other vital organs.

Plasma Renin Activity Assay

Blood samples were collected in chilled microcentrifuge tubes containing EDTA (final concentration, 1 mg/ml). The samples were immediately centrifuged at 4 °C, and the plasma was withdrawn and frozen for subsequent assay. Plasma renin activity (PRA) was measured using a modification of a commercially available (New England Nuclear, Boston, MA, USA) radioimmunoassay kit. Plasma (100 μl) was incubated for 1 hour at 37 °C in the presence of dimercaprol and 8-hydroxyquinoline (2 μl each) and a maleate buffer (200 μl; pH 6.0). The angiotensin I generated during this incubation was then determined by radioimmunoassay. The intrassay variability determined in this laboratory is 8.5% (n = 17), and the interassay variability is 11% (n = 9). The assay sensitivity is reported to be 40 pg/ml of angiotensin I per tube.

Histology

At the end of each experiment, animals were anesthetized with urethane (1.25 g/kg i.p.) and perfused through the left cardiac ventricle with 0.9% saline and then 10% formalin. The brains were removed and stored in formalin until later histological processing. In a cryostat, 30-μm sections were obtained through the area of the electrode placement. These sections were mounted on glass slides and stained with cresyl violet. The tissue was examined using a light microscope, and the location of each electrode was determined. Data from animals in which the electrode was determined histologically to end in any part of the PVN were compared with data from animals in which the electrode was placed outside the nucleus.

Data Analysis

Group data were compared using analysis of variance for repeated measures. Post hoc comparisons among means were made using Fisher’s new multiple range test. The slopes of the lines that correlated distal arterial pressure with PRA were determined using least-squares regression analysis. The slopes from two groups of rats were compared using analysis of covariance. In all cases a probability value less than 0.05 was considered significant.

Experimental Protocols

Two to 3 days after operation, the rats were transported in their home cages from the animal care facility to the laboratory. They were placed in a quiet room and connected to the pressure transducer(s) (Century Technology, Inglewood, CA, USA), Doppler flowmeter (University of Iowa Bioengineering, Iowa City, IA, USA), and stimulator (Model S44, Grass, Quincy, MA, USA), and the tubing from the occluding cuff was connected to a water-filled 1-ml syringe operated by a manual micromove (Stoelting, Chicago, IL, USA). All connecting tubes and wires exited the cage in a single bundle, and the rats could move about freely. Blood pressure, heart rate, and renal blood flow were monitored throughout a 45- to 60-minute equilibration period using a Grass Model 7 polygraph. At the end of the stabilization period, the rats underwent
one of the following four experimental protocols. Any one rat was used for only one experiment. All experiments conformed to the guidelines of the institutional animal care and use committee.

Low-Level Stimulation of the Paraventricular Nucleus in 10 Rats

In initial experiments, arterial pressure proximal to the aortic occluding cuff was measured using a carotid catheter and arterial pressure distal to the cuff was measured using the catheter in the lower abdominal aorta. Once the nature of the blood pressure response proximal to the cuff had been characterized, subsequent experiments routinely measured only the distal pressure. Following the equilibration period, a control blood sample (0.25 ml) was withdrawn from the distal arterial catheter. After this and all subsequent blood collections, the removed blood was replaced with an equal volume of 0.9% saline. The occluding cuff was then inflated so as to reduce distal pressure to 110 mm Hg. This pressure was maintained for 5 minutes, and a second blood sample was obtained as before. Distal pressure was then lowered three more times to 95, 80, and 50 mm Hg for 5-minute periods, and blood was withdrawn at the end of each period. After the last blood sample was collected, the occlusion was released and 1 hour was allowed to transpire before continuing the experiment. At the end of the 1-hour period, all variables had returned to baseline values. The stimulator was then turned on, and 150 μA of constant current was delivered to the PVN using a frequency of 5 Hz (0.5 msec duration). In a previous experiment these parameters were shown to be subthreshold for changes in PRA, mean arterial pressure, or renal blood flow. Ten minutes after the beginning of the stimulation period, a second occlusion protocol was performed.

Propranolol Pretreatment in Five Rats

In initial experiments, the effect of D,L-propranolol (Sigma, St. Louis, MO, USA) alone on the relationship between renal perfusion pressure and PRA was investigated. These animals did not have stimulating electrodes or Doppler flow probes. Following the stabilization period, the occlusion protocol already outlined was performed with one modification. In these and all subsequent experiments, three instead of four 5-minute occlusion periods were used. After a 1-hour recovery period propranolol treatment was begun. The treatment consisted of a bolus intravenous injection (1 mg/kg in 0.3 ml) followed by a slow constant infusion (0.3 mg/kg/hr) at a rate of 0.3 ml/hr that lasted throughout the remainder of the experiment. Ten minutes after the start of the propranolol treatment, a second occlusion protocol was performed.

Propranolol plus Stimulation of the Paraventricular Nucleus in Eight Rats

The experiments in these animals were the same as those outlined in the previous section, except that when the propranolol treatment was begun the stimulator was also turned on, so that propranolol and stimulation were present during the second occlusion period.

Renal Denervation in Seven Rats

The experiments performed in these animals were the same as those outlined in the first section, except that the animals had previously undergone bilateral renal denervation. Since this procedure totally prevented the effects of stimulation of the PVN, the denervation was assumed to be adequate and no attempt was made to determine its completeness.

Results

A typical example of four occlusion periods is shown in Figure 1. As the cuff was inflated, pressure decreased distally (as measured by the catheter}

![Figure 1. Representative example of the hemodynamic effects produced by graded suprarenal aortic occlusion during ongoing stimulation of the paraventricular nucleus in a conscious rat. Mean arterial pressure (MAP) proximal to the occluding cuff was measured through a catheter in the carotid artery; distal pressure was measured through a catheter in the femoral artery. At the end of each 5-minute occlusion period, blood samples were collected for measurement of PRA.](http://hyper.ahajournals.org/doi/fig/10.1161/01.HYP.12.1.54)
in the femoral artery). Renal blood flow did not change during the first occlusion, but thereafter it decreased with each subsequent lowering of distal pressure. The pressure proximal to the occluding cuff gradually increased throughout the protocol, while heart rate gradually decreased. Blood samples were collected at the end of each of the 5-minute occlusion periods, and Figure 2 shows the relationship between the distal arterial pressure and PRA in the control state. There was some variation in the level of distal arterial pressure actually attained during each occlusion period, as evidenced by the horizontal standard error bars. Figure 2 also shows that, with ongoing low-level stimulation of the PVN, there was a shift to the right in the relationship between distal arterial pressure and PRA; that is, for pressures of 90 mm Hg or lower, PRA increased more during the stimulation. Regression analysis of each data point revealed that the slope of the line generated during the stimulation period was significantly greater than the slope generated during the control period (Figure 3). The hemodynamic data from these five rats are depicted in Table 1. Renal blood flow remained unchanged during the first reduction in distal pressure but thereafter decreased with each additional lowering. Heart rate decreased significantly as well. The stimulation of the PVN had no effect on any of these responses.

The rightward shift in the pressure-PRA curve was limited to experiments where the stimulating electrode was verified to be in the PVN (Figure 4). In animals where the electrode was outside the nucleus, there was no shift during the stimulation (Figure 5).

Before determining if β-adrenergic blockade with propranolol could prevent the rightward shift in the distal arterial pressure–PRA curve caused by stim-

**Figure 2.** The effect of stimulation of the paraventricular nucleus (150 μA and 5 Hz) on the relationship between distal arterial pressure and PRA. Data were obtained from the first protocol described in Materials and Methods, and each point represents a mean ± SE. Asterisk indicates significant difference between values (p < 0.05).

**Figure 3.** Linear regression analysis of all the data points summarized in Figure 2. Asterisk indicates significant difference between values (p < 0.05).

**Figure 4.** Summary of all electrode placements identified in the present investigation. Filled circles depict placements that ended in the paraventricular nucleus (PVN), and open circles depict placements that were outside the nucleus. Each circle represents the midpoint of the electrode tip, which typically extended 0.15 mm in the rostral and caudal directions. A. Area of the third ventricle 200 to 300 μm rostral to the PVN. B–D. Rostral-caudal extent of the PVN. f = fornix; 3v = third cerebroventricle; AH = anterior hypothalamic area.
FIGURE 5. In animals where the electrode was found to be outside the paraventricular nucleus, no rightward shift in the distal arterial pressure–PRA curve occurred during the stimulation. Data were obtained from animals that underwent the first protocol described in Materials and Methods.

ulation of the PVN, it was necessary to determine the effects of propranolol alone. Figure 6 shows that propranolol treatment resulted in a significant shift to the left of the curve; that is, for any level of renal perfusion pressure attained, PRA increased less with propranolol present.

Figure 7 shows that β-adrenergic blockade prevented the rightward shift of the distal arterial pressure–PRA curve during stimulation of the PVN. The curve was actually shifted to the left, as in Figure 6, but in this case the difference was not statistically significant. Data are only reported from experiments where the electrode was verified to be in the PVN (n = 5).

Bilateral renal denervation also prevented the rightward shift of the distal arterial pressure–PRA curve, as depicted in Figure 8. Again, only data from rats with electrodes in the PVN are presented (n = 4).

FIGURE 6. The effect of propranolol (1 mg/kg bolus followed by 0.3 mg/kg/hr) on the relationship between distal arterial pressure and PRA. Asterisk indicates significant difference between values (p < 0.05).

FIGURE 7. Effect of combined propranolol treatment and stimulation of the paraventricular nucleus (PVN). Control values were obtained after propranolol treatment but before stimulation of the PVN.

Discussion

The sympathetic nervous system is thought to play an important role in the physiological control of renin secretion. It is well known that increases in renal sympathetic activity can lead to a frank increase in renin secretion and subsequent elevation of plasma angiotensin II.1 Evidence presented within the last 15 years has expanded our thinking about how the sympathetic nervous system regulates renin release. As early as 1973, LaGrange et al.3 showed that electrical stimulation of the renal nerves caused a rightward shift in the curve that correlated renal perfusion pressure with renin secretion; that is, for any level of renal perfusion pressure, the renin secretion rate was greater in the presence of ongoing nerve stimulation.3 Stella and Zanchetti7 subsequently showed that, in response to decreased renal perfusion pressure or furosemide administration (two nonneural stimuli), innervated kidneys released more renin than did denervated kidneys. Thames and DiBona8 carried this observa-
tion one step further and showed that direct electrical stimulation of the renal nerves with a very low frequency (which was subthreshold for effects on baseline renin release or other renal functions) could augment the increase in renin produced by lowering renal perfusion pressure to 50 mm Hg. Other investigators have shown that activation of renal \( \beta \)-adrenergic receptors, whether by reflex activation of renal nerves or by epinephrine infusions, can also produce this enhanced responsiveness to nonneural stimuli. Recent evidence suggests that this enhancement by epinephrine requires an intact renal innervation, and the possibility of a presynaptic action by the catecholamine on neurons innervating the kidney has been raised.\(^6\)

Taken together, these data have identified a potential new role for the sympathetic nervous system in controlling renin secretion. Not only can increased sympathetic activity lead to a frank increase in renin secretion; it now seems likely that the sympathetic system also has the potential to regulate the responsiveness of the kidney to other stimuli for renin secretion. This possibility led Johns\(^10\) to hypothesize that, in hypertensive states where sympathetic nervous activity may be increased, plasma levels of renin (and thus angiotensin II) may show an increased fluctuation with normal day-to-day activity. Increased periods of elevated renin could then contribute to the hypertensive disease.

However, in hypertensive states, the increased sympathetic activity may often originate within the central nervous system. To my knowledge, no evidence has been reported to date that directly shows that the central nervous system is organized in such a way that it can influence the responsiveness of the kidney to stimuli for renin secretion. Thus, the present investigation provides the first data that support this possibility. Low-level stimulation of the PVN increased the responsiveness of the kidney to lowered renal perfusion pressure. This effect reached statistical significance only when renal perfusion pressure decreased below the autoregulatory range (see Figure 2 and Table 1). However, the increased slope in the relationship between renal perfusion pressure and PRA suggests that the gain or sensitivity of the system was increased. This finding raises the possibility that, even though statistically undetectable in this study, PRA may be increased more for a given level of perfusion pressure during hypotalamic activation, even within the autoregulatory range.

The mechanism responsible for the increased responsiveness in the kidney is not known. However, in animals without a functioning macula densa, low-level stimulation of the renal nerves did not produce the expected enhancement of responsiveness when renal perfusion pressure was lowered to 50 mm Hg. This finding suggests that alterations in the NaCl load to the macula densa may be involved. However, other mechanisms, including direct effects on the juxtaglomerular cells, may be involved in situations where the reduction in renal perfusion pressure is less drastic.

As blood pressure distal to the occluding cuff decreased, the pressure proximal to the cuff increased. This response means that the baroreceptors in the aortic arch and carotid sinus were exposed to the rising pressure and activation of the baroreceptor reflex likely occurred. This suggestion is supported by the gradual decrease in heart rate that accompanied the increase in proximal pressure. It is also likely that the sympathetic activity in the kidney was also being withdrawn during the occlusion. Thames and DiBona\(^8\) reported that aortic occlusion was accompanied by a small decrease in directly recorded renal nerve activity in anesthetized dogs. Therefore, the enhanced responsiveness observed in the present study during stimulation of the PVN was likely being opposed by the baroreceptor reflex. Apparently the level of prevailing activity to the kidney during the stimulation was still sufficient to augment the responsiveness of the system.

The finding that surgical denervation prevented the augmented increase in PRA in this study suggests that changes in renal nerve activity were responsible for the observed effect. This notion is supported by the observation that propranolol also prevented the enhanced responsiveness. However, these data are also consistent with an alternative hypothesis. It is possible that increased circulating epinephrine accompanied the hypothalamic stimulation. Epinephrine could have then acted presynaptically on the neurons innervating the juxtaglomerular cells to cause release of norepinephrine. In this case, renal denervation and propranolol would both be expected to prevent the increased responsiveness. However, this possibility does not seem likely; the levels of hypothalamic stimulation employed in the present study were low and did not affect cardiovascular or behavioral parameters, and this argues against any notable release of circulating catecholamines. In addition, in a previous experiment, the frank increase in PRA observed after stimulating the PVN with even higher frequencies was completely prevented by renal denervation, suggesting that a residual increase in circulating epinephrine was not present.

The leftward shift in the relationship between renal perfusion pressure and PRA after propranolol is consistent with reports in other animals and supports the notion that the sympathetic nervous system imparts a certain amount of basal sensitivity to the kidney. This would allow the responsiveness of the kidney to be adjusted in either direction depending on central neural outflow.

The PVN is probably not the only site in the central nervous system with the potential to influence the kidney in the manner described in this study. The PVN was chosen because of its anatomical connections with afferent baroreceptor inputs and efferent projections to the sympathetic areas of
the spinal cord.20, 21 In addition, the PVN can induce the kidney to selectively release renin without accompanying changes in renal blood flow or sodium excretion.12 The PVN has been shown to contribute to neurogenic and genetic models of hypertension.13, 14 Certainly other hypothalamic areas, especially the lateral hypothalamus, which has been shown to send extensive neural projections to the medulla and spinal cord, have the potential to exert a similar effect in modulating renin release.22

In summary, electrical stimulation of the PVN in conscious rats enhanced the responsiveness of the kidney to suprarenal aortic occlusion. For a given decrease in pressure to the kidney, greater circulating levels of PRA were attained during the stimulation. This increased responsiveness was prevented by propranolol treatment or by renal denervation. It is suggested that, in certain hypertensive or prehypertensive states where sympathetic activity may be slightly elevated, PRA fluctuates more than normal with daily changes in posture, sodium intake, or state of arousal. These periods of increased PRA could contribute to arterial pressure lability.

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