Renal Nerves Modulate the Secretion of Renin Mediated by Nonneural Mechanisms

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SUMMARY We investigated the role of efferent renal nerve activity in modulating the responses of renin secretion rate to suprarenal aortic constriction and to furosemide administration in female dogs. After the renal nerves had been sectioned, constriction of the suprarenal aorta decreased renal perfusion pressure (from 132 ± 5 to 51 ± 2 mm Hg) and renal blood flow (from 303 ± 21 to 149 ± 18 ml/min) and increased renin secretion rate (from 184 ± 49 to 2012 ± 499 ng/min). Stimulation of the renal nerves at very low frequency (0.25 Hz) had no significant effect on basal renin secretion rate, arterial pressure, renal blood flow, or urinary sodium excretion. Aortic constriction during this low level renal nerve stimulation resulted in a significant augmentation in the renin secretion rate response (from 358 ± 107 to 6988 ± 1600 ng/min), whereas the changes in renal blood flow and renal perfusion pressure were similar to those observed without nerve stimulation. Similarly, low level renal nerve stimulation (0.25 Hz) was found to augment the renin secretion rate response to the intravenous administration of furosemide (1.0 mg/kg bolus followed by 0.017 mg/kg per min). These data show that a very low level of renal nerve activity which by itself does not change arterial pressure, renal blood flow, or urinary sodium excretion, or renin secretion rate, augments the release of renin in response to suprarenal aortic constriction or furosemide. Furthermore, these data provide direct evidence that the renal nerves modulate renin release mediated through nonneural mechanisms. Additional data are presented which show how these observations account for previously reported differences in renin secretion rate responses of innervated and denervated kidneys during aortic constriction and furosemide administration.

THE RELEASE of renin from the kidney has been shown to be largely mediated by three mechanisms (Davis and Freeman, 1976; Reid et al., 1978; Vander, 1967; Zanchetti and Stella, 1975). The first of these is the intrarenal vascular receptor (baroreceptor), which senses changes in renal perfusion pressure and induces an increase in renin secretion when the renal perfusion pressure is decreased (Davis and Freeman, 1976; Reid et al., 1978; Vander, 1967; Zanchetti and Stella, 1975). The second is the macula densa mechanism, mediated by specialized cells in the distal tubule that are sensitive to changes in sodium chloride transport. Factors which tend to reduce distal tubular sodium

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chloride transport increase renin secretion via this mechanism (Davis and Freeman, 1976; Reid et al., 1978). The third mechanism for renin release involves the renal nerves (Davis and Freeman, 1976; Reid et al., 1978; Vander, 1967; Zanchetti and Stella, 1975). Increases in renal sympathetic nerve activity have been shown to evoke increases in renin secretion (Bunag et al., 1966), even in the absence of changes in renal blood flow or glomerular filtration rate (LaGrange et al., 1973). To evaluate the role of each of these mechanisms in the control of renin secretion, each has been studied in a fashion which allowed it to be evaluated independently of the influence of the other two mechanisms. Recent evidence indicates that these vascular, tubular, and sympathetic neural mechanisms controlling renin secretion can, under certain circumstances, function independently of each other (Osborne et al., 1977). Since most investigations have examined one of these three specific mechanisms, there has been limited investigation of possible interactions among them.

Moreover, in spite of all the evidence for neurally
mediated secretion of renin (Davis and Freeman, 1976; Reid et al., 1978; Vander, 1967; Zanchetti and Stella, 1975; Bunag et al., 1986), the relative importance of the renal nerves in the overall control of renin secretion remains incompletely understood. There is little doubt that the renal nerves are important in the control of renin release, especially in views of recent studies in which the release of renin from innervated and contralateral denervated kidneys was determined in anesthetized cats during suprarenal aortic constriction and furosemide administration (Stella et al., 1976; Stella and Zanchetti, 1977) and in conscious dogs during furosemide administration (Grandjean et al., 1978). These studies clearly showed that the innervated kidneys secreted more renin than did the denervated kidneys in response to these interventions (Stella et al., 1976; Stella and Zanchetti, 1977; Grandjean et al., 1978). They further demonstrated that the renal nerves in some way played an important role in determining the amount of renin released in response to interventions that generally have been thought to mediate changes in renin secretion via intrarenal baroreceptor and macula densa mechanisms. There are two possible explanations for the differing renin responses of innervated and denervated kidneys. First, suprarenal aortic constriction and furosemide administration could have caused an increase in renal sympathetic nerve activity and thus an increase in neurally mediated renin secretion. This seems unlikely since aortic constriction usually results in an elevation of arterial pressure above the constriction. This rise in pressure above the constriction would augment the discharge of sinoaortic baroreceptors, resulting in a reflex suppression of renal sympathetic nerve activity (Ninomiya et al., 1971; Kendrick et al., 1972). The second possible explanation is that the prevailing renal nerve activity, even though not augmented by aortic constriction or furosemide administration, may be sufficient to modulate and therefore augment the release of renin from the innervated kidneys. However, there is little direct evidence of an interaction between the renal nerves and the vascular or macula densa mechanisms in the control of renin secretion. In a more general sense, there is little direct evidence that the renal nerves can modulate the release of renin mediated by nonneural mechanisms.

It was thus the major goal of this study to determine whether renin release mediated by nonneural mechanisms could be modulated by the renal nerves. The results show that very low levels of renal nerve activity, which do not change renal blood flow, urinary sodium excretion, or the basal renin secretion rate, augment the release of renin in response to either suprarenal aortic constriction or furosemide administration. Additional data are presented which show how these observations account for differences in renin responses of innervated and denervated kidneys in response to these interventions.

Methods

Female mongrel dogs weighing 15–23 kg were anesthetized with sodium thiopental (30 mg/kg, iv) followed by a-chloralose (80 mg/kg, iv). Supplemental doses of chloralose were given hourly (5–10 mg/kg). Auffed endotracheal tube was inserted, and the dogs were artificially ventilated with air at a frequency of 12 cycles/min and a tidal volume of 20 ml/kg. Arterial Po2, Pco2, and pH were measured at intervals, and pH and Pco2 were maintained between 7.35 and 7.45 and 30 and 40 mm Hg, respectively, by adjustment of the respiratory frequency. Body (rectal) temperature was kept at 37°C by external warming. All dogs received a standard kennel diet for at least 5 days prior to study. Food was withheld for 24 hours prior to study, although free access to water was allowed up to the time of the experiment.

Preparation of the Dogs

The left renal artery, vein, and renal nerves were exposed retroperitoneally via a lumbar incision. Special care was taken to avoid damaging the renal nerves. A small catheter was inserted into the left renal vein via the left gonadal vein to sample renal venous blood for the determination of plasma renin activity. In some experiments (see Protocols) a catheter was inserted in the renal pelvis via the left ureter to collect urine to determine urinary sodium excretion. A mechanical occluder was positioned on the abdominal aorta above the renal arteries and subsequently was used for suprarenal aortic constriction.

Hemodynamic Measurements

Arterial pressure was recorded in all experiments with a fluid-filled catheter advanced to the distal aorta from the left femoral artery. In some experiments arterial pressure was also recorded simultaneously from the brachial artery. Mean arterial pressures were obtained by electronic damping of the pulsatile signal. Measurement of renal arterial blood flow was made with noncannulating electromagnetic flow transducers (Carolina Medical Electronics, Inc.). In each experiment the zero flow signal was obtained by occluding the renal artery downstream from the flow transducer. At the conclusion of each experiment the flow transducer was calibrated in situ by timed volume collection of the dog’s own blood.

Recording of Renal Nerve Activity

A small branch of the left renal nerve was cut as it coursed along the renal artery, and was dissected back toward the main renal nerve bundle (near the left adrenal gland). The nerve was immersed in a pool of mineral oil at approximately 36°C, and effenter nerve traffic was recorded from this small multifiber preparation by platinum electrodes and was amplified with a band pass amplifier (Ortec, model no. 4660). The output of the amplifier was
RENAL NERVES MODULATE NONNEURAL RENIN SECRETION/Thames and DiBona

fed into a frequency/time meter (Ortec, model no. 4672), which generated a standardized voltage pulse for each recorded spike potential whose voltage exceeded a preset voltage level (just above the noise level). The standardized spikes were integrated with a Beckmann resetting voltage integrator, which was calibrated with a square wave generator. The integrated nerve activity, along with arterial pressures and renal blood flows, was displayed on a Beckmann dynograph (model R411).

Renal Denervation and Nerve Stimulation

The renal sympathetic nerves were interrupted in some experiments by sectioning them near the left adrenal gland as they began to project onto the left renal artery. Absence of a change in renal blood flow during electrical stimulation of the left splanchnic nerve or proximal renal nerve bundle (10 V, 1 msec, 10 Hz) was accepted as proof of denervation.

In some experiments the distal cut ends of the renal nerves were placed on platinum electrodes for low level renal nerve stimulation (0.25 Hz, 10 V, 1 msec). All nerve stimulation was carried out with a Grass S9 (Grass Instrument Co.) square wave generator. The stimulator output was calibrated prior to the start of each experiment.

Renin Measurements

Blood samples (4 ml) were withdrawn simultaneously from the distal aorta and renal vein. The samples were collected in commercially available chilled tubes (5 ml, Vacutainer) containing K₂EDTA, and the tubes were centrifuged at 4°C. Sample blood loss was replaced with an equal volume of normal saline (140 mEq NaCl/liter). Renin activity of the separated plasma was measured by radioimmunoassay according to the method of Haber (Haber et al., 1969). The hematocrit was measured from at least two arterial samples, and the renin secretion rate was taken as the product of the venous-arterial renin difference and the renal plasma flow.

Protocols

Low Level Renal Nerve Stimulation

After the left renal nerves had been sectioned (10 dogs), thus rendering the left kidneys denervated, control values for distal aortic and brachial artery mean pressures, renal blood flow, urinary sodium excretion, and renin secretion rate were obtained. Subsequently, the suprarenal aorta was constricted to decrease distal aortic and thus renal arterial pressure to 50 mm Hg. Values for all measured variables were obtained at the end of 5 minutes of aortic constriction and 15 minutes after release of the constriction. The distal cut ends of the renal nerves then were placed on platinum electrodes and stimulated at 0.25 Hz, 1 msec, 10 V, and values were obtained for all measured variables at the end of 10 minutes of nerve stimulation. These parameters were chosen because preliminary experiments in our laboratory and the results of others (LaGrange et al., 1973) have shown that renal nerve stimulation causes renin secretion only at frequencies greater than 0.3 Hz. While low level renal nerve stimulation was maintained, the aorta was again constricted, and values for all measured variables were obtained after 5 minutes of aortic constriction and 15 minutes after release of the constriction and cessation of stimulation. The dogs were randomized so that in half the experiments the aorta was first constricted during low level renal nerve stimulation.

In 12 dogs with renal nerves sectioned, the same parameters were measured before and after the intravenous administration of furosemide, 1 mg/kg bolus followed by a continuous infusion of 0.017 mg/kg per min. Urinary losses were continuously replaced with normal saline (150 mEq NaCl/liter). Values for all measured variables were determined 10 minutes after administration of the bolus of furosemide. Recovery values were obtained 60–90 minutes after discontinuation of the furosemide infusion. The responses to furosemide were then determined during low level renal nerve stimulation (0.25 Hz, 1 msec, 10 V). In four dogs furosemide was administered twice according to the same protocol without low level renal nerve stimulation. It was thus the purpose of these experiments to determine whether very low levels of renal nerve activity could augment the responses in renin secretion rate of acutely denervated kidneys to aortic constriction or furosemide administration.

Responses of Innervated and Denervated Kidneys

In four dogs the nerves to the left kidney were sectioned and a catheter was passed from the right femoral vein to the right renal vein to sample right renal venous blood. The position of the right renal venous catheter was verified at the conclusion of each experiment. The left renal vein was catheterized via the left ovarian vein, and flow probes were positioned on both right and left renal arteries. The nerves to the right kidney were not disturbed. Changes in distal aortic and brachial artery pressure, in left and right renal blood flow, and in renin secretion rate from both the innervated (right) and denervated (left) kidneys were determined during suprarenal aortic constriction and furosemide administration carried out in the manner described above.

Renal Nerve Activity during Aortic Constriction and Furosemide Administration

In 11 dogs a small branch of the left renal nerve was prepared for the recording of effenter renal nerve activity. Changes in mean arterial pressure, renal blood flow, renin secretion rate, and renal nerve activity were determined during aortic constriction and during furosemide administration according to the protocol outlined above. To determine whether the technique of renal nerve dissec-
tion and recording had interrupted most of the innervation of the left kidney, we determined the changes in mean arterial pressure, renal blood flow, and renin secretion rate resulting from high intensity stimulation (10 Hz, 1 msec, 10 V) of the left splanchnic nerve in these 11 dogs.

Data Analysis

Data are presented in the figures and the text as means ± standard error (SE). The statistical significance of the difference in means was evaluated by Student’s t-test for paired observations (Steel and Torrie, 1960). The difference in means was considered significant for \( P < 0.05 \).

Results

Effects of Low Level Renal Nerve Stimulation on Responses to Suprarenal Aortic Constriction

Figure 1 summarizes the responses of the left kidney to suprarenal aortic constriction \((n = 10)\) with and without low level renal nerve stimulation (0.25 Hz). The renal nerves were sectioned in these experiments, so that in the absence of renal nerve stimulation no nerve activity influenced these kidneys. For ease of presentation the data for aortic constriction without renal nerve stimulation are presented first. In these experiments, suprarenal aortic constriction resulted in a large decrease in arterial pressure below the constriction and thus a decrease in renal perfusion pressure (from 132 ± 5 to 51 ± 2 mm Hg), a significant increase in pressure above the constriction (from 131 ± 6 to 150 ± 6 mm Hg), significant decreases in renal blood flow (from 303 ± 21 to 149 ± 18 ml/min) and sodium excretion (from 55.8 ± 13.0 to 0 μEq/min), and a significant increase in renin secretion rate (from 184 ± 49 to 2012 ± 499 ng/min). After release of the constriction all measured variables returned to values that were not different from control. Low level renal nerve stimulation (0.25 Hz) alone did not significantly alter any measured variable. With low level renal nerve stimulation maintained, the aorta was again constricted, and the arterial pressure, renal blood flow, and sodium excretion responses were similar to those observed when the nerves were not stimulated. However, the renin secretion rate increased from 358 ± 107 ng/min during low level renal nerve stimulation alone to 6388 ± 1600 ng/min with aortic constriction and low level renal nerve stimulation. The increase in renin secretion rate was significantly greater (6630 ± 1532 ng/min) when the aorta was constricted during low level nerve stimulation than was observed when the renal nerves were not so stimulated (1828 ± 485 ng/min).

Effects of Low Level Renal Nerve Stimulation on Responses to Furosemide Administration

Figure 2 summarizes the responses of the left kidney \((n = 12)\) for the same measured variables to the intravenous administration of furosemide. With the renal nerves sectioned, furosemide administration resulted in significant increases in urinary sodium excretion (from 80 ± 23 to 757 ± 87 μEq/min) and renin secretion (from 242 ± 59 to 781 ± 185 ng/min). There were no significant changes in arterial pressure or renal blood flow. After 60–90 minutes, mean arterial pressure, urinary sodium excretion, and renin secretion rate returned to near control values. Renal blood flow decreased to 157 ± 13 ml/
RENAI NERVES MODULATE NONNEURAL RENIN SECRETION/Thames and DiBona

Changes in mean arterial pressure (MAP), renal blood flow (RBF), urinary sodium excretion (UNV), and renin secretion rate (RSR) in response to intravenous furosemide (1 mg/kg bolus followed by 0.017 mg/kg per min) administered with and without low level renal nerve stimulation (RNS; 0.25 Hz, 1 msec, 10 V). The renal nerves were sectioned prior to starting the experiment. Renal nerve stimulation alone had no direct effect on baseline values for any measured variable. The renin secretion responses to furosemide administration and concomitant nerve stimulation were significantly greater than were observed with furosemide alone. Data are for left kidney and are shown as mean ± SE. C = control, R = recovery, n = 12.

Responses of Innervated and Denervated Kidneys

Figure 3 summarizes the changes in mean arterial pressure, renal blood flow, and renin secretion rate observed in this study during aortic constriction and during furosemide administration for the innervated right kidney and the denervated left kidney of four dogs. The arterial pressure responses were similar to those previously described. Control renal blood flow tended to be higher in the denervated kidney. After aortic constriction, the renin secretion rate was significantly greater in the innervated than in the denervated kidneys. The increase in renin secretion rate to furosemide was significantly less in the denervated kidneys than in the innervated kidneys. Data are shown as mean ± SE. C = control, R = recovery, n = 4.
vated kidneys, but the difference was not significant. The changes in renal blood flow in the denervated kidneys were similar to those observed in the innervated kidneys. The renin secretion rate of the innervated kidneys increased significantly more during aortic constriction and furosemide administration than did the secretion rate of the denervated kidneys. The difference was most striking for aortic constriction. These findings are in agreement with those previously described for furosemide in cats and dogs (Stella and Zanchetti, 1977; Grandjean et al., 1978) and for aortic constriction in the cat (Stella et al., 1976).

Renal Nerve Activity during Aortic Constriction and Furosemide Administration

It is possible that the increase in renin secretion rate observed in innervated kidneys during aortic constriction or furosemide administration is dependent on an increase in renal sympathetic nerve activity. We examined this possibility by recording efferent renal sympathetic nerve activity during aortic constriction (Fig. 4) and furosemide administration (Fig. 5) in 11 dogs. Changes in arterial pressure, renal blood flow, and renin secretion rate also were determined. Suprarenal aortic constriction (Fig. 4) resulted in changes in mean arterial pressure (above and below the constriction), renal blood flow, and renin secretion rate similar to those previously described. However, efferent renal sympathetic nerve activity decreased from 8.9 ± 0.5 Hz during the control period to 4.4 ± 0.5 Hz during aortic constriction. All parameters promptly returned to control values on release of the occlusion. During furosemide administration (Fig. 5), renal blood flow and renin secretion rate increased significantly, but neither arterial pressure nor renal nerve activity changed.

At the end of the protocol for recording renal nerve activity, the left splanchnic nerve or proximal renal nerve bundle was stimulated at high intensity (10 Hz). This caused significant decreases in renal blood flow (from 296 ± 30 to 200 ± 25 ml/min) and increases in renin secretion rate (from 843 ± 158.6 to 1780.4 ± 604.2 ng/min), indicating that most of the renal nerves were not disturbed by the technique used to record renal nerve activity, and thus that the kidney was functionally innervated.

Discussion

These data show that low level renal nerve stimulation, which by itself had no direct effect on renin secretion rate, renal blood flow, or urinary sodium excretion, greatly augments the renin secretion response of denervated kidneys to aortic constriction and to furosemide administration. If one accepts the widely held view that these interventions (aortic constriction and furosemide) exert their influence during

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\begin{align*}
\text{MAP (mmHg)} & \\
\text{RBF (ml/min)} & \\
\text{RNA (Hz)} & \\
\text{RSR (ng/min)} & 
\end{align*}
\]

\[
\begin{align*}
\text{N = 11} & \\
\text{P < .001} & \\
\text{P < .025} & \\
\text{P < .005} & 
\end{align*}
\]
RENAL NERVES MODULATE NONNEURAL RENIN SECRETION/Thames and DiBona

on renin secretion principally through the intra-renal baroreceptor and macula densa mechanisms (Davis and Freeman, 1976; Corsini et al., 1975), then the present data are consistent with the view that the renin responses mediated by these mechanisms are modulated by renal sympathetic nerve activity. The data in this study thus provide strong support for the concept of an important interaction between the renal nerves and nonneural mechanisms in the overall control of renin secretion.

These data provide direct evidence that the renal nerves modulate renin release mediated through nonneural mechanisms. Other investigators have suggested that the renal nerves could modulate the renin response to aortic constriction and to furosemide (LaGrange et al., 1973; Osborn et al., 1977; Stella et al., 1976; Grandjean et al., 1978; Eide et al., 1974; Slotkoff et al., 1971). However, this suggestion has been based largely on indirect evidence. LaGrange et al. (1973) suggested such a modulatory role for the renal nerves on the basis of augmented renin secretion responses to aortic constriction during renal nerve stimulation. Their study differed importantly from ours in that they stimulated the renal nerves in most of their experiments at frequencies above the threshold for renin secretion. In addition, it is not clear that they decentralized the renal nerves in their study, so that the actual renal nerve activity may have been higher than the apparent evoked activity. Eide et al. showed that β-adrenergic receptor activation with isoproterenol potentiated the renin response to renal arterial constriction. Their study differed from ours in that they stimulated the renal nerves during the course of those experiments.

The protocol for the aortic constriction experiments was of shorter duration, and a decrease in renal blood flow was not observed during the course of those experiments. At times, increases in renal nerve activity could not have accounted for the differential responses of innervated and denervated kidneys. Even though renal nerve activity decreased during aortic constriction, the remaining activity appears to have been sufficient to augment the renin secretion response of the innervated kidney. Even though furosemide administration did not change renin nerve activity, the prevailing nerve activity was sufficient to augment the renin response to furosemide of the innervated kidney. It is likely that the renin response of the innervated kidney would have been further augmented following furosemide had urine volume not been replaced, thus resulting in intravascular volume depletion and increases in renal nerve activity.

Although the renal blood flow tended to decrease during the course of the furosemide experiments, this finding should not alter the interpretation of our results. In several of these experiments, the postfurosemide control flow was close to the control flow observed before furosemide, and the renin secretion response to furosemide in these dogs was augmented by low level renal nerve stimulation. The time-dependent decrease in renal blood flow is commonly observed in experiments of extended duration. In these experiments there was a period of approximately 2 hours between the initial control and the control value obtained following recovery from the initial administration of furosemide. This period was necessary for the diuretic and natriuretic effect of furosemide to abate and for basal conditions to be re-established. More importantly, when furosemide was administered twice without nerve stimulation, the increases in renin secretion rate were significantly greater for the first than for the second furosemide administration, thus indicating that the augmented renin secretion rate during low level renal nerve stimulation was indeed the result of the nerve stimulation. The protocol for the aortic constriction experiments was of shorter duration, and a decrease in renal blood flow was not observed during the course of those experiments.

Renal nerve activity probably varies from minute to minute throughout the day. At times, increases
in renal nerve activity sufficient to cause a direct neural release of renin may occur. Under other circumstances, changes in renal nerve activity may be more modest, but still sufficient to modulate renin responses mediated by other mechanisms. During sodium deprivation there is a contraction of extracellular fluid volume and thus of intravascular volume. Even though arterial pressure may not change, there are alterations in distal tubular sodium handling that are accompanied by an increase in renin secretion, probably mediated in part through the macula densa mechanism (Davis and Freeman, 1976). In addition, the decreased blood volume can reduce the inhibitory influence on the vasomotor center of sensory endings in the cardiopulmonary area (Thames et al., 1978). This would tend to augment renal nerve activity (Clement et al., 1972) and thus the renin response to sodium restriction. Changes in posture and therefore in cardiopulmonary blood volume may play a particularly important role in altering the inhibitory influence of cardiopulmonary receptors (Zoller et al., 1972) and thus of renal nerve activity and renin release (Gordon et al., 1967). It would appear that the renal nerves play an important role in the overall control of renin secretion, either by causing direct neural release of renin, or, possibly more importantly, by modulating the release of renin mediated by non-neural mechanisms. This interaction between the renal nerves and intrinsic renal mechanisms may be important in the minute-to-minute regulation of renin secretion.

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M D Thames and G F DiBona

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