Acute and Chronic Intrarenal Alpha- and Beta-Adrenergic Receptor Stimulation of Renin Release in the Conscious Dog

CARLOS R. AYERS, M.D., RICHARD E. KATHOLI, M.D., ROBERT M. CAREY, M.D., MARTHA R. YANCEY, M.S., AND CURTIS L. MORTON

SUMMARY The effect of continuous intrarenal infusion of norepinephrine, isoproterenol, and methoxamine on renin release was studied in the uninephrectomized conscious dog. Chronic intrarenal infusion of norepinephrine produced a biphasic curve of plasma renin activity (PRA) and a sustained 25 mm Hg increase in mean arterial pressure (MAP). The initial increase in PRA peaked at 3 hours, after which PRA returned to control levels. Alpha- or beta-adrenergic antagonists did not attenuate the initial rise in PRA. The PRA increased again after 48 hours of chronic intrarenal norepinephrine infusion and remained elevated thereafter. The second rise in PRA was increased by 30% with alpha-adrenergic blockade. Chronic intrarenal isoproterenol administration produced a similar increase in PRA, which peaked at 3-5 hours and then returned to control levels. In contrast to norepinephrine, chronic isoproterenol administration did not result in a second increase in PRA. At the end of the chronic isoproterenol infusion period, beta-adrenergic receptor refractoriness was demonstrated, as PRA did not increase significantly in response to a fourfold increase in the dose of isoproterenol. An increase in PRA was produced by acute intrarenal infusion of methoxamine. This increase in PRA was blocked by phentolamine, suggesting a vascular alpha-adrenergic receptor-mediated release of renin. (Hypertension 3: 615-621, 1981)

KEY WORDS • alpha and beta adrenergic receptors • norepinephrine • isoproterenol • methoxamine • renin • prostaglandins

THE mechanism of renin release from the juxtaglomerular cell in response to acute increases in sympathetic tone has been studied extensively. Increases in renal sympathetic efferent nerve activity due to electrical stimulation of the brain,1,2 electrical stimulation with the electrode around the artery or to the distal cut end of the renal nerve,3 tilting,4 cold pressor tests,5 and insulin administration6 result in renin release. Norepinephrine administration into a peripheral vein7 into the renal artery of both intact8 and isolated9 kidney preparations, and to renal cortical slices10 results in renin release by stimulating the β-adrenergic receptors on the cell membrane of the juxtaglomerular cell. The intracellular mediator of this response is probably cyclic AMP.18 Unilateral renal denervation decreases renin concentration, demonstrating the importance of the renal nerves in control of renin production.16-18

Studying mechanisms by which chronically increased sympathetic tone could produce sustained hypertension, we found that chronic intrarenal norepinephrine infusion for 10 days in the uninephrectomized conscious dog resulted in a biphasic plasma renin activity (PRA) curve. There was an initial rise in PRA peaking at 3-5 hours and returning to the control level by 24-48 hours. Thereafter, a second rise in PRA was observed, which persisted as long as the infusion was continued.

The present study was undertaken to define the mechanisms causing this biphasic PRA curve during chronic intrarenal norepinephrine infusion. Since norepinephrine stimulates both α- and β-adrenergic receptors, the role of each receptor was evaluated by infusion of selective agonists and antagonists.
Methods

Animal Preparation

All animals used in this study were female foxhounds selected for their calm, gentle nature; body weight averaged 20.5 ± 2 (SEM) kg. The dogs were prepared for surgery with pentobarbital anesthesia, and the trachea was intubated. A midline laparotomy was performed. By means of a steel guidewire, a Teflon catheter (0.025 cm outer diameter) was inserted through the aortic wall into the left renal artery. The guidewire was removed and the catheter sutured to the aortic wall for hemostasis and stability. A right nephrectomy was performed. Teflon catheters (0.074 cm outer diameter) were inserted into the aorta and inferior vena cava through the right renal artery and vein. The three catheters were exteriorized through a stab wound near the right costovertebral angle, and the laparotomy wound was closed. A canvas jacket was placed around the dog to protect the catheters. The dogs were allowed to recover at least 7 days before the studies began.

Throughout the experiments the dogs were maintained on approximately 80 mEq of sodium per day. This was accomplished by giving food containing less than 5 mEq of sodium per day supplemented by a 2-hour intravenous infusion of 75 mEq of sodium in the form of 0.9% sodium chloride. All animals were allowed to come into sodium balance for 5 days prior to beginning the experiments.

During the experiments the animals were placed in a canvas sling with their feet touching the floor, so that they could stand or rest. Other times, they were kept in individual dog runs in accordance with federal guidelines. Renal artery infusions were given with a Harvard infusion pump during the short experiments (< 5 hours) or for longer experiments with a battery-operated portable Sigmamotor pump weighing 0.47 kg that was secured to the dog's back. The rechargeable batteries were changed every 48 hours, and the pump speed was recalibrated. Guidelines of the American Physiological Society for the humane treatment of laboratory animals were observed.

The PRA was assayed by measuring angiotensin I generated at 37°C and pH 5.7 according to the method of Sealey et al.17

Experimental Protocol

Alpha- and Beta-Adrenergic Receptor Blockade During Intrarenal Norepinephrine Infusion

The dose of norepinephrine used was selected for each dog by means of a dose-response curve. Blood for control peripheral PRA was drawn and sequential 20-minute intrarenal norepinephrine infusions of 0.15, 0.20, 0.25, 0.30, and 0.40 µg/kg/min were given using a Harvard pump; PRA samples were obtained at the end of each infusion period. The dose of norepinephrine that resulted in a four-fold rise in peripheral PRA and a rise in mean arterial blood pressure (MAP) of 20–30 mm Hg was selected as the acute (5-hour) and chronic infusion dose. The mean dose of norepinephrine was 0.24 µg/kg/min.

A loading dose of 0.4 mg/kg of propranolol was given intravenously 15 minutes prior to norepinephrine infusion, and an additional 0.6 mg/kg was given as a constant infusion over a 5-hour period during which time intrarenal norepinephrine was administered. The PRA was monitored during this 5-hour period. This dose of propranolol was twofold greater than the dose sufficient to prevent a significant rise in PRA when 0.12 µg/kg/min of isoproterenol was given directly into the renal artery. This dose of isoproterenol increased PRA to a level equal to that produced by 0.24 µg/kg/min of norepinephrine administered into the renal artery. Phentolamine (1 mg/kg bolus followed by 0.02 mg/kg/min sustained infusion) was given in an attempt to block the acute rise in PRA. This dose of phentolamine was selected because it is in excess of the amount required to block the MAP and PRA response to twice the dose of methoxamine, 4.6 µg/kg/min, required to increase the arterial blood pressure equal to 0.24 µg/kg/min of norepinephrine. This phentolamine dose is also twice the dose required to decrease the blood pressure to normal during intrarenal infusion of 0.24 µg/kg/min of norepinephrine.

The dose of methoxamine was three times that which resulted in an equivalent rise in MAP as with 0.24 µg/kg/min of intrarenal norepinephrine.

Alpha- and Beta-Adrenergic Receptor Blockade During Chronic (4–10 Days) Intrarenal Norepinephrine Infusion

After 4–10 days of continuous norepinephrine infusion into the renal artery, a-adrenergic receptor blockade with phentolamine was carried out using 1 mg/kg as a loading dose and 0.02 mg/kg/min as a continuous infusion during a 2-hour period. Beta-adrenergic blockade with propranolol was produced with a loading dose of 0.4 mg/kg, and an additional total of 0.6 mg/kg was given as a continuous infusion during a 2-hour period. This was twice the dose required for maximum reduction of PRA with propranolol after 10 days. The response of the MAP, heart rate, and PRA were observed.

Acute and Chronic Beta-Adrenergic Receptor Stimulation with Intrarenal Isoproterenol

The renin response to intrarenal infusion of isoproterenol was determined, and the dose that increased PRA approximately fourfold after 30 minutes of infusion (the approximate level achieved with 0.24 µg/kg/min of norepinephrine) was selected as the chronic infusion dose. A mean dose of isoproterenol, 0.12 µg/kg/min, was given continuously into the renal artery of seven uninephrectomized dogs. The PRA, MAP, and heart rate were monitored during this period. At the end of 10 days, the dose of isoproterenol was increased twofold and then fourfold for 1 hour each, and the above observations were repeated.
Intermittent Intrarenal Infusion of Isoproterenol

The dose of isoproterenol was selected as above. The average dose of 0.12 \( \mu g/kg/min \) was infused into the renal artery of four uninephrectomized dogs for 7 hours each day on 4 consecutive days. The PRA and heart rate were measured before and at the end of the infusion period.

Chronic Alpha-Adrenergic Receptor Stimulation with Methoxamine

The dose of methoxamine that raised MAP by 25 mm Hg (simulating the pressure rise obtained with 0.24 \( \mu g/kg/mg \) of norepinephrine) was selected as the infusion dose. A continuous intrarenal infusion of methoxamine at a mean dose of 2.3 \( \mu g/kg/min \) was given to eight dogs for 48 hours. The PRA and MAP were monitored. Renal plasma flow and glomerular filtration rate were measured before and during methoxamine infusion by measuring the clearances of \( H^+ \)-PAH and \( C^{14} \) inulin.

In a separate series of five dogs, methoxamine 4.6 \( \mu g/kg/min \) was infused into the renal artery for 30 minutes. A blood sample for PRA was obtained, and MAP was recorded. An intravenous bolus of phentolamine 1 mg/kg was given, and an additional 1.876 mg/kg of phentolamine was infused over a 90-minute period. The PRA and MAP were measured every 30 minutes.

Statistical Analysis

Student’s \( t \) test for paired observations was used for statistical analysis of the data. Group means are presented with the standard error of the mean (SEM).

Results

Alpha- and Beta-Adrenergic Receptor Blockade During Acute (5-Hour) Intrarenal Norepinephrine Infusion

The same biphasic PRA curve in response to intrarenal norepinephrine infusion as previously described was observed. A similar blood pressure curve was sustained during this 10-day period. The PRA subsequently declined to control levels between 24 and 48 hours. As shown in figure 1, propranolol, 0.6 mg/kg intravenously, did not block the rise in PRA during the initial 5 hours of intrarenal infusion of 0.24 \( \mu g/kg/min \) of norepinephrine. Phentolamine, administered as an intravenous bolus of 1 mg/kg followed by 1.875 mg/kg as a continuous infusion over a 90-minute period, also failed to prevent the rise in PRA with acute intrarenal norepinephrine infusion. These two adrenergic receptor blocking agents were not given concurrently.

Alpha- and Beta-Adrenergic Receptor Blockade During Chronic (4–10 Day) Intrarenal Norepinephrine Infusion

The \( \alpha \)-adrenergic antagonist, phentolamine, was given intravenously over a two hour period during chronic intrarenal norepinephrine infusion. MAP, heart rate and PRA were measured. The MAP decreased immediately from 145 ± 9 to 97 ± 15 mm Hg \( (p < 0.01) \), and the heart rate increased from 106 ± 7 to 172 ± 11 bpm \( (p < 0.01) \). As shown in figure 2, PRA increased from 19 ± 5 to a peak of 27 ± 7 ng/ml/hr \( (p < 0.05) \) at 120 minutes. Propranolol, 1 mg/kg, was given intravenously over a 2-hour period. This was associated with a decrease in PRA from 28 ± 8 to 19 ± 6 ng/ml/hr. The MAP did not change and heart rate decreased from 106 ± 9 to 91 ± 11 beats/min \( (p < 0.01) \).

Acute and Chronic Beta-Adrenergic Receptor Stimulation with Intrarenal Isoproterenol

Isoproterenol was administered as a continuous intrarenal infusion for 10 days, as shown in figure 3. The PRA increased from 3.58 ± 0.59 to a peak of 8.60 ± 0.90 ng/ml/hr \( (p < 0.01) \) at 5 hours. The MAP remained unchanged. The heart rate increased from 112 ± 10 to 154 ± 6 beats/min \( (p < 0.01) \) at 5 hours. At 24 hours, the PRA and heart rate had returned to control levels and remained there for 10 days. On the 11th day the dose of isoproterenol was increased by 100%, then 200%. This increase in \( \beta \)-adrenergic agonist did not increase PRA or heart rate.

![Graph showing PRA response to norepinephrine and isoproterenol.](image-url)
FIGURE 2. Chronic phase of norepinephrine stimulation of renin release. Left: Alpha-adrenergic blockade with phentolamine increased plasma renin activity. Right: Beta-adrenergic blockade decreased plasma renin activity. * p < 0.05. ** p < 0.01.

The control plasma potassium concentration was 4.36 ± 0.65 mEq/liter. With isoproterenol infusion the potassium decreased to 3.61 ± 0.61 mEq/liter (p < 0.01) at 5 hours and 3.73 ± 0.73 mEq/liter (p < 0.05) at 6 days. The plasma sodium was not significantly changed.

Isoproterenol was given intrarenally for 7 hours on 4 successive days in four dogs, as shown in figure 4. There was a significant increase in PRA on each of the 4 days. The heart rate was significantly increased on the fourth day.

Chronic Alpha-Adrenergic Receptor Stimulation with Methoxamine

Figure 5 demonstrates the PRA and MAP responses to continuous intrarenal infusion of methoxamine 2.3 μg/kg/min. The PRA increased from 2.28 ± 0.51 to a peak of 5.35 ± 1.06 ng/ml/hr (p < 0.05) at 3 hours; it then decreased to control levels. The MAP increased from 106 ± 4.5 to a peak of 133 ± 8 mm Hg (p < 0.01) at 3 hours. At the end of 48 hours of infusion, MAP returned to control levels. During
chronic methoxamine administration, glomerular filtration rate decreased from 38 ± 5 to 18 ± 6 ml/min ($p < 0.05$), accompanied by a decrease in renal plasma flow from 172 ± 25 to 97 ± 28 ml/min ($p < 0.05$).

In a separate group of five dogs, shown in figure 6, a larger dose of methoxamine (4.6 μg/kg/min) was infused intrarenally for 30 minutes. There was a marked increase in PRA from 4.9 ± 6 to 33 ± 6 ng/ml/hr ($p < 0.01$). The MAP increased from 120 ± 5 to 164 ± 6 mm Hg ($p < 0.01$). Large doses of phentolamine decreased MAP and PRA to control levels by 120 minutes.

**Discussion**

Chronic intrarenal norepinephrine infusion in the uninephrectomized conscious dog results in a biphasic PRA curve. The purpose of this study was to elucidate the mechanisms of this renin response to chronic sympathetic stimulation. We found that the initial rise in PRA in response to intrarenal norepinephrine was not blocked by propranolol or phentolamine. Our failure to prevent this increase in PRA with these blocking agents suggests activation of other renin-stimulating mechanisms, such as decreased sodium load to the macula densa or alteration in the afferent arteriolar stretch receptor. We have shown previously that there is a 20% to 25% reduction in renal plasma flow associated with a decrease in urinary sodium excretion during the first 24 to 28 hours of intrarenal infusion of norepinephrine. This period of sodium retention appears concurrently with the first peak of the PRA curve. Our observed failure to block renin release with α- and β-adrenergic blocking agents further demonstrates that stimulation of renin release at this concentration of norepinephrine in the intact conscious dog is multifactorial.

Two well-known clinical entities, pheochromocytoma and congestive heart failure, may be associated with normal PRA in the presence of increased circulating norepinephrine levels. These findings, together with our observation that PRA returns to baseline within 24 to 48 hours during continuous intrarenal norepinephrine infusion, suggested to us that juxtaglomerular cell β-adrenergic receptor refractoriness might occur. A relatively pure β-receptor agonist, isoproterenol, was given to verify β-receptor refractoriness. With continued isoproterenol administration, PRA returned to baseline values within 24 to 48 hours and did not change during 8 subsequent days. At this point, a fourfold increase in the dose of isoproterenol failed to increase PRA, thus demonstrating refractoriness of the juxtaglomerular β-adrenergic receptor. With intermittent infusion of isoproterenol, juxtaglomerular β-adrenergic receptor refractoriness was not observed.

With the recent development of radioactive ligands of high specific activity, responsiveness of cell membrane β-adrenergic receptors from a variety of tissues has been studied in several species. With continuous stimulation of the β-receptor, refractoriness occurs
within hours due to a decrease in the number of receptors. A decrease in the number of β-receptors could account for the decrease of PRA to baseline values after 24 to 48 hours of continuous isoproterenol infusion in our experiments. Since the initial rise in PRA during intrarenal norepinephrine infusion does not appear to be solely due to β-adrenergic receptor stimulation, the return to baseline levels of PRA by 24 to 48 hours suggests refractoriness of other non-β-adrenergic receptors, as well. The return of PRA to control levels after 24 to 48 hours of infusing the α-adrenergic agent methoxamine suggests that down regulation of α-adrenergic stimulation of renin release may also occur.

When studying the mechanism of the second rise (4–10 days) in PRA during chronic intrarenal infusion of norepinephrine, we found that large doses of propranolol only decreased PRA by 35%. Together with the demonstrated development of β-adrenergic receptor refractoriness with isoproterenol infusion, this evidence suggests that β-receptor stimulation does not constitute the main mechanism for renin release in the chronic phase. This partial reduction in PRA with α-adrenergic receptor blockade is in keeping with a modulating role for renin release by the sympathetic nervous system. The observation that alpha-adrenergic receptor blockade with phentolamine resulted in an increase in PRA during chronic norepinephrine infusion is consistent with the work of others showing that α2-adrenergic receptor stimulation suppresses renin release. The rise in PRA during phentolamine administration is associated with a decrease in MAP to control levels. It is unknown whether this decrease in MAP to the control level could contribute to the rise in PRA through stimulation of the baroreceptor in the afferent arteriole. It is difficult to incriminate the macula densa as the stimulatory mechanism since our previous studies, performed during intrarenal infusion of norepinephrine, showed initial sodium retention followed by a return to control sodium excretion within 48 hours. In all likelihood, then, the animals were in sodium balance during the 4- to 10-day period when the second increase in PRA occurred. Perhaps an uneven distribution of renal blood flow to the renal cortex, resulting in reduced sodium delivery to the macula densa, could have stimulated renin release. Changes in plasma electrolyte concentration were apparently not important, as the serum sodium concentration did not change and the small change in potassium concentration is unlikely to produce the large increment in PRA observed in these experiments. Thus, the exact mechanism for the increase in PRA between 4 and 10 days of continuous intrarenal infusion of norepinephrine remains unknown.

The role of the α-adrenergic receptors in the control of renin release has been controversial. Early studies indicated that α-adrenergic receptor agonists stimulate renin release by an intracellular mechanism distal to cyclic AMP production. However, this work was not confirmed. Experiments employing α-adrenergic receptor blockade in renal cortical slices, administration of α-adrenergic agonists and antagonists in vivo in rats, and in the isolated perfused kidney indicate that α2-adrenergic receptor stimulation suppresses rather than stimulates renin release. In fact, studies of renal cortical slices indicate that α-adrenergic receptor stimulation of the juxtaglomerular cell with methoxamine does not increase renin release.

The present investigation suggests that the marked increase in renin release with methoxamine is mediated via stimulation of the vascular α1-adrenergic receptor. The present results further suggest that this vascular α1-receptor mechanism is stimulated only with marked reduction in renal blood flow. In summary, intrarenal α-receptor stimulation at low doses of norepinephrine inhibits renin release, possibly by an α2 presynaptic adrenergic receptor. At high doses of norepinephrine, marked renal vasconstriction occurs, and renin release is stimulated secondarily.

References
Acute and chronic intrarenal alpha- and beta- adrenergic receptor stimulation of renin release in the conscious dog.
C R Ayers, R E Katholi, R M Carey, M R Yancey and C L Morton

Hypertension. 1981;3:615-621
doi: 10.1161/01.HYP.3.5.615

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/3/5/615

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/