Effects of Catecholamines and Renal Nerve Stimulation on Renin Release in the Nonfiltering Kidney

By J. Alan Johnson, James O. Davis, and Robert T. Witty

ABSTRACT

The mechanisms whereby catecholamines and renal nerve stimulation increase renin secretion were studied in dogs with nonfiltering kidneys. In six dogs, epinephrine was infused into the renal artery at a rate that decreased renal blood flow to half of the control value. Papaverine was then infused into the renal artery to block the decrease in renal blood flow produced by the catecholamine, and the epinephrine infusion was resumed while the papaverine infusion was continued. In this experiment, renin release increased during infusion of epinephrine alone, but no change occurred with epinephrine during papaverine infusion. The protocol for the experiment on six other dogs was similar except that the infused catecholamine was norepinephrine. In this experiment, norepinephrine increased renin release both prior to and during papaverine infusion. In seven dogs, the effect of electrical stimulation of the renal nerves on renin secretion was studied both before and during the infusion of papaverine into the renal artery; renin release increased strikingly both before and during papaverine infusion. It is suggested that epinephrine increased renin secretion in the nonfiltering kidney by an action on the renal arterioles. In contrast, norepinephrine and renal nerve stimulation apparently increased renin secretion in the nonfiltering kidney by a direct effect on the juxtaglomerular cells. These data provide evidence for specific mechanisms of action of epinephrine, norepinephrine, and the renal nerves in renin release.

KEY WORDS epinephrine norepinephrine papaverine renal baroreceptor macula densa renal blood flow juxtaglomerular cells renin secretion anesthetized dogs

Intravenous and intrarenal arterial infusions of epinephrine or norepinephrine have been reported to increase renin secretion (1, 2). Studies have also suggested that increased sympathetic nerve activity to the kidney plays a role in the control of renin release (1, 3-5). These factors decrease renal blood flow (RBF) and glomerular filtration rate (GFR) (1, 6-11), and decreases in these functions are frequently associated with an increase in renin secretion; therefore, the effects of catecholamines or of renal nerve activity might be mediated through changes in renal hemodynamic function. Also, it is possible that catecholamines and the renal nerves have direct actions on the juxtaglomerular (JG) cells and renal baroreceptors which cause renin release.

In the denervated nonfiltering kidney (12), an increase in renin release occurred in response to hemorrhage or suprarenal aortic constriction in the absence of a functional macula densa and intact renal nerves in adrenalectomized dogs. These studies pointed to the presence of a vascular receptor in the renal arterioles, possibly in the region of the JG cells. Witty et al. (13) provided evidence for a renal baroreceptor mechanism in the denervated nonfiltering kidney by intrarenal arterial infusion of papaverine, a drug which relaxes smooth muscle: infusion of papaverine.
RENIN RELEASE

blocked renin release in response to hemorrhage. To determine the mechanisms whereby epinephrine, norepinephrine, and the renal nerves influence renin secretion, the role of these factors was studied in the nonfiltering kidney before and during papaverine administration. Dogs with a nonfiltering kidney were used to exclude any participation of the macula densa in the renin response to catecholamines or renal nerve stimulation.

Materials and Methods

Nineteen female mongrel dogs weighing 14.2–23.9 kg were used in this study. In each animal, a nonfiltering kidney was produced on the left side under sterile conditions by the technique described by Blaine et al. (12). The left ureter was tied, and a serrefine clamp was placed on the renal artery for 2 hours to produce renal ischemia. Two or three days later, the right kidney was removed through a flank incision, and the intake of food was stopped. The experiment was performed the following day.

On the day of the experiment, the dog was anesthetized with sodium pentobarbital, and the left kidney was exposed by a flank incision. A polyvinyl catheter was placed in the left renal vein by way of the left ovarian vein to collect renal venous blood. A catheter was also placed in the suprarenal aorta via the femoral artery; this catheter was used to obtain arterial blood samples and to measure mean arterial blood pressure. An electromagnetic flow probe was placed around the left renal artery, and RBF was measured with a Carolina square-wave electromagnetic flowmeter. Arterial blood pressure was determined with a Sanborn model 1280 pressure transducer. Both RBF and arterial blood pressure were recorded on a Sanborn model 7700 recorder.

To infuse solutions into the kidney, a 25-gauge needle was attached to a piece of flexible tubing and inserted into the renal artery. The needle was left in place throughout the experiment, and isotonic saline was infused during the control periods. All infusions into the renal artery were at a constant rate of 0.59 ml/min, which was adjusted to achieve a dose that decreased RBF to approximately half of the control level; the average amount infused was 0.4 μg/min. To study the effect of epinephrine on renin release, blood samples were collected at 3 minutes and 6 minutes after the reduced rate of RBF was achieved. The epinephrine infusion was changed to an infusion of isotonic saline which was given for 30 minutes before the blood samples for the recovery period were obtained. After the recovery period, a solution of papaverine in isotonic saline was infused into the renal artery at a rate of 5 mg/min. After 15 minutes of papaverine infusion, blood samples were obtained. Epinephrine was then added to the papaverine solution so that the same amount was delivered to the kidney per minute as in the first part of the experiment, and this mixture was infused into the renal artery for 10 minutes; blood samples were again obtained at 3 minutes and 6 minutes after beginning the catecholamine infusion. Recovery observations were made with papaverine alone which was infused for 30 minutes before the blood samples were collected.

Norepinephrine Infusion.—Six additional animals were used in this experiment. The protocol was the same as that used for the preceding study, with the exception that norepinephrine (Levophed, Winthrop Laboratories) was the catecholamine infused. As before, the concentration of catecholamine in the infused was regulated to obtain a dose that decreased renal blood flow to about 50% of the initial control value, and this same dose of norepinephrine was used in the papaverine solution in the second phase of the experiment. The average amount of norepinephrine infused was 0.06 μg/min.

Renal Nerve Stimulation.—A group of nerves in close proximity to the renal artery and vein were carefully dissected away from the renal vessels at a point about 1.5 inches away from the kidney, and stimulating electrodes were placed on them. Two control samples of arterial and renal venous blood were collected for measurement of renin release in seven dogs. The renal nerves were stimulated electrically with a square-wave stimulator (Grass, model SD-5). Stimulation duration was 10 msec and the frequency was 10 pulses/sec. In each experiment, the stimulus voltage was adjusted to a level that would produce a decided initial fall in RBF; this voltage ranged from 10 to 30 v. Renal nerve stimulation was continued for 25 minutes, and blood samples used to test for renin release were obtained at 5, 15, and 22 minutes during nerve stimulation. For recovery observations, blood samples were obtained 1 hour after cessation of nerve stimulation. Following these observations, a solution of papaverine in isotonic saline was infused into the
FIGURE 1
Effects of intrarenal arterial infusion of epinephrine in dogs with a nonfiltering kidney on renal blood flow (R.B.F.), arterial blood pressure (A.B.P.), and renin secretion rate prior to and during intrarenal arterial infusion of papaverine. Values for R.B.F. and A.B.P. are means. Renin secretion rates are means ± SE. C indicates control periods. R indicates recovery periods.

renin artery at a rate of 3–5 mg/min. After 15 minutes of papaverine infusion, blood samples were again obtained, and the renal nerve stimulation was again initiated at the previous voltage. Blood samples for measurement of renin were obtained at 5 and 22 minutes during the nerve stimulation and papaverine infusion.

At the termination of each experiment, the kidney was examined for the absence of glomerular filtration. The renal capsule was removed, and a solution of lissamine green dye was injected into the suprarenal aorta. Observations with a dissecting microscope showed that the dye failed to appear in the superficial renal tubules in any of the dogs studied, which demonstrates the absence of glomerular filtration in superficial nephrons (12). Several studies performed in our laboratory with this nonfiltering kidney model have shown that nonfiltration is achieved in approximately 95% of the animals.

In all three experiments, 8-ml samples of arterial and renal venous blood were obtained periodically for the determination of plasma renin activity. The samples were cooled to about 4°C, the plasma was separated by centrifugation, and the plasma samples were then frozen. Plasma was prepared for assay for renin by the method of Schneider et al. (14). This consisted of dialyzing 2-ml samples in 0.01M EDTA and then in a phosphate buffer (pH 5.3). After adding NaCl and diisopropylfluorophosphate, the samples were incubated for 3 hours at 37°C. Following enzyme inactivation in a boiling water bath, each sample was diluted to 4 ml with a phosphate buffer solution. The samples were stirred and centrifuged, and the supernatant solution was frozen until assayed. Samples were assayed using the pressor response in the pentobarbital-anesthetized pentolinium-blocked rat, with synthetic angiotensin II (Hypertensin, Ciba) as the standard. The results were expressed as ng angiotensin formed/ml plasma. The rate of renin secretion was calculated by subtracting the arterial plasma concentration of renin activity from the renal venous renin activity, and multiplying this difference by the renal plasma flow. Renin secretion rates are expressed as ng angiotensin/min. Plasma concentrations of sodium and potassium were determined by flame photometry. Hematocrit values were obtained by a microhematocrit method.

Results

Epinephrine Infusion.—Infusion of epinephrine into the renal artery resulted in an immediate decrease in RBF and more than a 100% increase in renin release (Fig. 1). When the catecholamine infusion was stopped, both RBF and renin release returned to the preinfusion level. Only minor changes in mean arterial blood pressure were observed.

The intrarenal arterial infusion of papaverine produced a rapid increase in RBF (Fig. 1). Infusion of epinephrine into the papaverine-dilated kidney produced only a slight decrease in the RBF. During the second part of the experiment when papaverine was administered, arterial blood pressure decreased progressively. The intrarenal arterial infusion of epinephrine and papaverine failed to produce an increase in the rate of renin release, although epinephrine was given at the same rate as in the first part of the experiment. Renin release during the recovery period also remained unaltered. One dog showed the same pattern of response as the other dogs of this series but was unusual in that the control rate of renin release (465 ng/min) was considerably higher than in the other animals, and, during epinephrine infusion, the renin release increased to the very high level of 784 ng/min. These data were excluded from the values used for statistical analysis on the basis that they were an "outlying observation" (15).
Effects of intrarenal arterial infusion of norepinephrine in dogs with a nonfiltering kidney on renal blood flow (R.B.F.), arterial blood pressure (A.B.P.), and renin secretion rate prior to and during intrarenal arterial infusion of papaverine. Values for R.B.F. and A.B.P. are means. Renin secretion rates are means ± SE. C indicates control periods. R indicates recovery periods.

Therefore, all data from this dog were excluded from statistical calculations, and renin secretion, RBF, and arterial blood pressure data from this animal are not included in Figure 1. Student's t-test for paired observations (15), when applied to the value for renin release during epinephrine infusion compared to the average value for the control and recovery periods, revealed that prior to papaverine administration the infusion of epinephrine produced an increase in renin release that was statistically significant (P<0.01); similar statistical testing for the values obtained during papaverine infusion failed to reveal a significant change in renin release during epinephrine administration.

Norepinephrine Infusion.—Infusion of norepinephrine into the renal artery produced a decrease in RBF similar to the effect observed in the previous experiments with epinephrine (Fig. 2). This was accompanied by an increase in renin release (P<0.05). When the norepinephrine infusion was terminated, RBF and renin release returned to near the control level. Mean arterial blood pressure remained unchanged during these observations. Infusion of papaverine during the second half of the experiment increased RBF, and the superimposed infusion of norepinephrine at the same rate as used in the first part of the experiment was accompanied by a slight decrease in the RBF. The infusion of norepinephrine together with papaverine resulted in an increase in renin release (P<0.01) of a magnitude similar to that seen during the first part of the experiment. Cessation of the norepinephrine infusion was followed by a slow decline in RBF and arterial blood pressure and a return of renin release to the control level. One dog showed the same response pattern as the other five dogs in this series; however, the infusion of norepinephrine during papaverine administration produced an abnormally large increase in renin secretion (1352 ng/min). This value was excluded during the tests for statistical significance on the basis that it represents an outlying observation (15). The renin release, RBF, and the mean arterial blood pressure data from this dog are not included in Figure 2.

Effects of renal nerve stimulation in dogs with a nonfiltering kidney on renal blood flow (R.B.F.), arterial blood pressure (A.B.P.), and renin secretion rate prior to and during intrarenal arterial infusion of papaverine. Values for R.B.F. and A.B.P. are means. Renin secretion rates are means ± SE. C indicates control periods. R indicates recovery periods.
Renal Nerve Stimulation.—Stimulation of the renal nerves prior to papaverine administration resulted in an immediate decrease in RBF and an increase in renin release for each period (Fig. 3). On cessation of nerve stimulation, RBF increased to near the control level, and the rate of renin release fell to prestimulation levels. When each period was tested against the average of the initial control and recovery values by the nonparametric signed rank test of Wilcoxon (16), this increase in renin release was statistically significant for all three observations (5 minutes, \( P < 0.01 \); 15 minutes, \( P < 0.05 \); 22 minutes, \( P < 0.01 \)). Arterial blood pressure was not affected by renal nerve stimulation. Following the infusion of papaverine, RBF increased in some dogs, but the change was not statistically significant for the group. Superimposed renal nerve stimulation decreased RBF in some animals but, again, the decline was not significant. Renal nerve stimulation during papaverine infusion also produced a significant (\( P < 0.05 \)) increase in renin release at 5 minutes. Arterial pressure decreased progressively after initiation of the papaverine infusion.

Discussion

A variety of stimuli are known to increase the secretion of renin. These include hemorrhage (17–20), suprarenal aortic constriction (21, 22), constriction of the renal artery (23), and sodium depletion (24–27). It has been suggested (28, 29) that the increased renin secretion in these conditions is secondary to a decrease in stretch of the renal afferent arterioles and JG cells (baroreceptor hypothesis) or results from a change in the sodium load or concentration reaching the macula densa (macula densa hypothesis). Perhaps both mechanisms are involved. Recent reviews by Vander (28) and by Davis (29) presented the details of these hypotheses.

Several investigators have shown that the infusion of epinephrine or norepinephrine, either intravenously (1, 8–11) or into the renal artery (2, 10), resulted in a decrease in RBF and GFR. It has also been demonstrated that catecholamine infusion increased renin release (1, 2). This response might reflect, at least in part, renal afferent arteriolar constriction and stimulation of the baroreceptor mechanism, or the effect could be secondary to the decrease in GFR which alters the sodium load or concentration reaching the macula densa. It is also possible that catecholamines act through some additional mechanism to increase renin release. It is conceivable that epinephrine and norepinephrine have a direct action on some site in the kidney, such as the JG cells, which effects renin release independent of the baroreceptor or macula densa mechanisms. Indeed, the observations of Otsuka et al (30), Assaykeen et al (31) and Winer et al (32) are consistent with a direct action on adrenergic receptors in the JG cells. Previous studies have, however, failed to provide definitive information about a specific locus of action of these hormones.

The conclusions from the present experiments are contingent on the effectiveness with which the nonfiltering kidney eliminates the macula densa mechanism from participating in the control of renin secretion and on the ability of papaverine to block the baroreceptor mechanism of renin release. As previously mentioned, the lack of appearance of lissamine green dye in the surface tubules following its injection into the suprarenal aorta is evidence of nonfiltration. This provided a convenient method for checking each kidney preparation, and provided convincing evidence that filtration was not occurring in the superficial tubules. Recent studies from this laboratory (33) have shown that the extraction ratios for creatinine were indistinguishable from zero for nonfiltering kidneys after reopening the ureter and that the clearance of creatinine was very low (average of 2.2 ml/min in eight dogs). These findings show that this type of kidney preparation is, indeed, nonfiltering.

Experiments from our laboratory by Blaine et al (34) have demonstrated that in adrenalectomized dogs with denervated nonfiltering kidneys a hemorrhage of 20 ml/kg body weight resulted in increased renin release.
These findings indicate that some mechanism other than the macula densa, the renal nerves, or changes in the circulating catecholamines is capable of altering renin release; this mechanism presumably is the baroreceptor mechanism in the renal afferent arterioles. It was postulated that inhibition of the contractility of the renal vasculature might block this baroreceptor mechanism. Because papaverine is known to relax vascular smooth muscle (35), this compound was used in an attempt to examine this hypothesis. Recent studies by Witty et al (13) have revealed that, in dogs with denervated nonfiltering kidneys in which papaverine was infused into the renal artery, a hemorrhage of 20 ml/kg body weight failed to alter the rate of renin release. These results strongly suggest that papaverine inhibited the baroreceptor mechanism for the control of renin secretion.

In the present study, use of the nonfiltering kidney excluded the possible effect of catecholamines on the macula densa mechanism. Prior to the administration of papaverine, epinephrine and norepinephrine each produced an increase in the release of renin. These results show that each of these substances is capable of stimulating renin release by mechanisms other than those involving the macula densa. In the second phase of the experiments, the administration of papaverine was used to block the baroreceptor mechanism for the control of renin release. It appears, therefore, that the increase in renin release which occurred during norepinephrine and papaverine infusion in the nonfiltering kidney was mediated by a nonbaroreceptor and a non-macula-densa mechanism. A likely possible mechanism is a direct action of norepinephrine on the JG cells. In contrast, the intrarenal infusion of epinephrine failed to increase renin release in the nonfiltering kidney during papaverine infusion, which suggests that epinephrine increases renin secretion in the normal kidney by acting through the baroreceptor or the macula densa mechanism or both and that a direct effect on the JG cells does not occur.

The rate of catecholamine infusion used in the present study ranged from 0.3 to 0.6 µg/min for epinephrine and from 0.3 to 1.5 µg/min for norepinephrine. From the control renal plasma flow rates, it was calculated that the concentration of the catecholamines in the renal arterial plasma at the beginning of the initial experimental period averaged 11.1 µg/liter (range 5.5 to 16.1 µg/liter) for epinephrine and 14.1 µg/liter (range 6.2 to 20.8 µg/liter) for norepinephrine. As the normal arterial plasma concentrations of these catecholamines in the dog are approximately 1.0 and 1.6 µg/liter for epinephrine and norepinephrine, respectively, when measured fluorometrically by the trihydroxyindole method (36), the concentrations achieved in the renal arterial plasma in the present experiments are considerably above the normal levels. However, Manger et al (36) found arterial plasma concentrations of epinephrine of 18–47 µg/liter and of norepinephrine of 11–19 µg/liter at 60 minutes following severe hemorrhage in dogs. Therefore, the amounts of catecholamines used in the present experiments produced calculated plasma levels which are achieved under certain in vivo conditions.

A variety of experimental approaches have suggested that the renal nerves play a role in renin release (1, 3-5, 37-39). In one of the first reports, Vander (1) observed an increase in renal venous renin activity accompanying a decrease in renal plasma flow and urinary sodium excretion during apparent renal nerve stimulation in anesthetized dogs. However, nerve stimulation was achieved by means of loop electrodes placed around the renal artery, and thus it is questionable whether the responses obtained were due solely to renal nerve stimulation or whether a direct electrical stimulation of the renal artery produced the effect.

In contrast, it has been well established that renal nerve stimulation produces a decrease in RBF (7, 16, 40, 41). Thus, it is reasonable to suggest that the increase in renin secretion observed during renal nerve stimulation in the normal dog is mediated, at least in part, by
renal baroreceptor or macula densa mechanisms or both. However, in the present study, renal nerve stimulation during papaverine administration in the nonfiltering kidney still produced an increase in renin release and, as pointed out above, papaverine inhibits vasoconstriction of the renal arterioles. These studies suggest, therefore, that renal nerve stimulation, like norepinephrine infusion, increased renin secretion by acting through mechanisms other than the renal baroreceptor or macula densa mechanisms. A likely possible explanation for the present data is that a direct influence in the renal nerves ending in the JG cells causes a renin release. Recent work by Witty et al (13) has led to similar conclusions about the action of the renal nerves. It was observed that a hemorrhage of 20 ml/kg body weight produced a marked increase in renin release in dogs with nonfiltering papaverine-treated kidneys when the renal nerve supply was intact, but this forcing in similar preparations with denervated kidneys failed to show a change in the rate of release of renin.

The present studies demonstrate, therefore, that renal nerve stimulation, as well as the infusion of norepinephrine into the renal artery, stimulated renin secretion by acting through mechanisms other than the baroreceptor or macula densa and possibly by a direct action on the JG cells. On the other hand, the data show that epinephrine increased renin secretion solely by an action on the renal arterioles and, presumably, on the baroreceptor mechanism in the nonfiltering kidney. The present observations provide further evidence that the secretion of renin is governed by a very complex control system in which the renal nerves and circulating catecholamines play a role.

Acknowledgment

The technical assistance of Mr. Robert Monroe, Mrs. Ellen Roper, and Mr. John Haas is gratefully acknowledged. The authors thank Dr. Kenneth G. Kohlstaedt of Eli Lilly and Co., Indianapolis, for generously supplying the papaverine used in this study. The authors also appreciate the gift of Levophed bitartrate from Winthrop Laboratories, Division of Sterling Drug Inc., Rensselaer, N. Y.
Effects of Catecholamines and Renal Nerve Stimulation on Renin Release in the Nonfiltering Kidney

J. Alan Johnson, James O. Davis and Robert T. Witty

_Circ Res._ 1971;29:646-653
doi: 10.1161/01.RES.29.6.646

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1971 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/29/6/646