Epinephrine Enhances Neurogenic Vasoconstriction in the Rat Perfused Kidney

PAUL QUINN, KAZIMIERZ R. BORKOWSKI, AND MICHAEL G. COLLIS

SUMMARY Epinephrine has been implicated in the genesis of some forms of hypertension. We have investigated the effects of epinephrine on vasoconstrictor responses evoked by adrenergic stimuli in the isolated perfused rat kidney. Low concentrations of epinephrine (2.5 - 5 x 10^{-7} M) increased the amplitude of vasoconstrictor responses evoked by electrical stimulation of the renal adrenergic nerves. These concentrations of epinephrine had no effect on the basal perfusion pressure of the kidney or on the amplitude of vasoconstrictor responses evoked by exogenous norepinephrine. The potentiating effect of epinephrine persisted after infusion of the amine had ceased. Kidneys that had been perfused with 3H-epinephrine accumulated radioactivity, which could then be released by renal nerve stimulation. Cocaine (3 x 10^{-3} M) reduced the renal accumulation of 3H-epinephrine and abolished both the persistent potentiating effect of the amine and the release of radioactivity evoked by subsequent nerve stimulation. The potentiating effect of epinephrine infusion was abolished by the beta selective adrenergic receptor antagonist ICI 118,551 (3 x 10^{-8} M), but not by the beta selective adrenergic receptor antagonist atenolol (10^{-6} M). These results indicate that concentrations of epinephrine that can be achieved during acute stress can enhance the amplitude of neurogenic vasoconstrictor responses. This effect appears to be mediated via a prejunctional beta-adrenergic receptor. The persistent nature of this effect may be due to the neuronal accumulation and subsequent release of epinephrine. (Hypertension 7: 47-52, 1984)

KEY WORDS • epinephrine • vasoconstriction • rat perfused kidney • sympathetic neurotransmission

E PINEPHRINE has been shown to enhance the exocytotic release of 3H-norepinephrine from adrenergic nerves in some isolated tissues after pretreatment with inhibitors of neuronal uptake.1 Majewski and Rand2 have proposed that this effect could amplify vasoconstrictor responses evoked by adrenergic nerve activity. There have been few studies, however, in which this hypothesis has been tested directly by examination of the effects of epinephrine on adrenergic vasoconstrictor responses.

In a previous study,3 we showed that epinephrine could selectively enhance the pressor responses evoked by stimulation of the sympathetic nerves in the pithed rat. In the present study, we have examined the effects of epinephrine on adrenergic vasoconstrictor responses in vitro. We performed these studies using the isolated perfused kidney of the rat, since alterations in the renal vascular resistance may play an important role in the long-term control of blood pressure and in the pathogenesis of hypertension.4,5

Methods

We used 4- to 6-month-old female normotensive Wistar-Kyoto (WKY) rats that weighed 225 to 275 g.

Isolated Perfused Kidney Preparation

Kidneys were isolated from rats as described by Collis and Vanhoutte6 and perfused at a constant flow (6 ml/min) with Krebs-Ringer solution of the following composition (mmol): NaCl, 118.2; K Cl, 4.7; Mg SO4, 1.2; KH2PO4, 1.2; Ca Cl2, 2.5; Na HCO3, 25; glucose, 5.0; which contained ethylenediaminetetra-acetic acid (EDTA) (1 mg/liter), ascorbic acid (10 mg/liter), and dextran (Sigma Chemical Company, St. Louis, MO) (36 g/liter; average molecular weight, 81,600). The solution was maintained at 37 °C and bubbled with 95% O2 + 5% CO2. Perfusion pressure was measured via a pressure transducer (Bell and Howell, C.E.C. Instrumentation Ltd., Basingstoke, England) and displayed on a chart recorder (Lectromed MX2, St. Oven, Jersey Channel Islands). Vasocon-
stricter responses were evoked by electrical stimulation of the renal nerves via platinum electrodes with the use of parameters that activate adrenergic nerves in this preparation (0.5 to 5 Hz, 12 V, 1 msec, 10-second trains). Responses were also evoked by close intraarterial injection of norepinephrine (Sigma Chemical Company) (6.25 to 200 ng in 20 μl of saline). Responses were measured as the increase in perfusion pressure from the basal perfusion pressure immediately preceding the response. Dose and frequency response curves were repeated in the presence of epinephrine (Sigma Chemical) (10^{-9} M to 10^{-8} M), and these results were expressed as a percentage of the control vasoconstrictor response. The effect of epinephrine on vasoconstrictor responses was further examined after pretreatment with atenolol (Tenormin, ICI Pharmaceuticals, Macclesfield, England) (10^{-6} M), ICI 118551 (3 × 10^{-8} M), and cocaine (3 × 10^{-5} M).

3H-Epinephrine Labeling of Kidneys

After a 45-minute equilibration period, the kidneys were perfused with 3H-epinephrine (5 × 10^{-9} M, 72.9 Ci/mmol) (New England Nuclear, Boston, MA) for 25 minutes, and a 10-minute washout period was allowed before stimulation of the renal nerves.

Measurement of 3H Efflux

During a period of 45 minutes, the kidney was stimulated at 5-minute intervals (3 Hz, 12 V, 1 msec, 10-second trains). Prior to stimulation, two consecutive 12-second collections of perfusate were taken to determine basal tritium overflow. During and after stimulation, three consecutive 12-second collections were made to determine the efflux of 3H evoked by electrical stimulation, as an indicator of the efflux of epinephrine.

The experiment was repeated in kidneys perfused with Krebs solution containing cocaine (3 × 10^{-5} M) or ICI 118551 (3 × 10^{-8} M). These drugs were added 30 minutes before 3H-epinephrine perfusion and were present for the duration of the experiment.

AquaSol (New England Nuclear), a universal liquid scintillation cocktail, was added to aliquots (1.2 ml) of the perfusate, and a stiff gel formed after shaking. Radioactivity (counts/min/aliquot) was determined by liquid scintillation counting on a Philips Liquid Scintillation Counter (Eindhoven, The Netherlands). The results were expressed as the total stimulus-evoked increase in the efflux of radioactivity.

Tissue Radioactivity

Total tissue radioactivity was measured in the kidneys at the end of the experiment. The tissues were chopped, and 9 ml of xylene-based scintillation medium (P.C.S., Amersham International, Amersham, England) tissue solubilizer was added and allowed to stand at 37 °C for 48 hours. Then 250 μl of the resulting solute was transferred to a counting vial, and 40 μl of a 1:3 dilution of concentrated HCl plus 4 ml of P.C.S. (Amersham) liquid scintillant were added. To-
hanced by the highest concentration of epinephrine (10^{-8} M) used (Figure 2).

The epinephrine-induced (5 \times 10^{-9} M) enhancement of vasoconstrictor responses evoked by renal nerve stimulation was significantly attenuated 10 minutes after cessation of the epinephrine infusion. However, the amplitude of the responses remained significantly higher than those obtained prior to epinephrine infusion (Figure 3).

Kidneys that had been perfused with \textsuperscript{3}H-epinephrine (5 \times 10^{-9} M) and then perfused for 60 minutes with normal Krebs solution accumulated significant amounts of radioactivity (Table 1). Stimulation of the renal nerves to these kidneys (3 Hz, 10-second train every 5 minutes) produced a consistent and significant increase in the efflux of radioactivity (Figure 4).

Effect of Atenolol and ICI 118551

The presence of atenolol, a selective \beta_{1}-adrenergic receptor antagonist (10^{-6} M), or ICI 118551 (3 \times 10^{-8} M), a selective \beta_{2}-adrenergic receptor antagonist, caused a slight enhancement of the vasoconstrictor responses to renal nerve stimulation and injected norepinephrine (Figures 5 and 6). The subsequent epinephrine-induced (5 \times 10^{-9} M) enhancement of vasoconstrictor responses evoked by renal nerve stimulation was unaffected in the presence of atenolol (Figure 5). The epinephrine-induced enhancement was abolished, however, in the presence of ICI 118551 (Figure 5). Epinephrine (5 \times 10^{-9} M) had no effect on the amplitude of vasoconstrictor responses evoked by exogenous norepinephrine in the absence or presence of the \beta_{2}-adrenergic-receptor-blocking drugs (Figure 6).

The ICI 118551 (3 \times 10^{-8} M) had no significant effect on the accumulation of radioactivity in the kidneys that had been perfused with \textsuperscript{3}H-epinephrine (Table 1). The efflux of radioactivity from these kidneys evoked by renal nerve stimulation was significantly reduced (p < 0.05) but not abolished by ICI 118551 (Figure 4).
### Table 1. Total Tissue Radioactivity of Kidneys after Perfusion with $^{3}H$-Epinephrine ($5 \times 10^{-9}$ M) in the Absence or Presence of Cocaine or of ICI 118551

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total tissue radioactivity (counts $\times 10^5$/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.51 $\pm$ 0.4</td>
</tr>
<tr>
<td>ICI 118551 ($3 \times 10^{-8}$ M)</td>
<td>5.90 $\pm$ 0.17</td>
</tr>
<tr>
<td>Cocaine ($3 \times 10^{-5}$ M)</td>
<td>2.07 $\pm$ 0.38*</td>
</tr>
</tbody>
</table>

*Significant difference ($p < 0.001$, analysis of variance) between the treated group ($n = 6$) and the control group ($n = 6$).

### Effect of Cocaine

In the presence of cocaine ($3 \times 10^{-5}$ M), epinephrine ($5 \times 10^{-9}$ M) produced a significant enhancement of the amplitude of responses evoked by renal nerve stimulation (Figure 3). This potentiating effect of epinephrine was significantly less than that which occurred in the absence of cocaine (Figure 3). In the presence of cocaine, the potentiating effect of epinephrine was abolished 10 minutes after cessation of the perfusion with the catecholamine (Figure 3).

Cocaine ($3 \times 10^{-5}$ M) significantly reduced the accumulation of radioactivity and kidneys that had been perfused with $^{3}H$-epinephrine (Table 1). Stimulation of the renal nerves (3 Hz) in these kidneys did not evoke a significant increase in the efflux of radioactivity (Figure 4).

### Discussion

The experiments described in this paper demonstrate that low concentrations of epinephrine increase the amplitude of vasoconstrictor responses evoked by adrenergic nerve stimulation. The degree of facilitation of neurogenic vasoconstrictor responses was related to the concentration of the amine used. The concentration required to cause a statistically significant enhancement was slightly above the normal plasma level in this strain of rat, but within the range of concentrations that occur during acute stress. Thus, it is likely that neurogenic vasoconstriction in the intact animal will be enhanced by circulating epinephrine during stress.

The persistent nature of the facilitary effect of epinephrine in the kidney indicates that neurogenic vasoconstriction in vivo may remain enhanced after a stress-induced elevation of plasma epinephrine levels has subsided. This persistent effect appears to be due to the accumulation of epinephrine at some site in the kidney and its subsequent release during nerve stimulation. Since cocaine caused a marked reduction in the epinephrine content of the kidney, it is likely that most of the accumulation of the amine occurs via the neuronal uptake system. Both the release of tritium on nerve stimulation and the persistent enhancement of vasoconstrictor responses were abolished by cocaine. Thus, it appears that the persistent effect of epinephrine is due to the release of the catecholamine that has

![Figure 4](http://hyper.ahajournals.org/)

**Figure 4.** Efflux of radioactivity induced by renal nerve stimulation (3Hz, 10-second train) from isolated perfused rat kidneys ($n = 6$) after infusion of $^{3}H$-epinephrine. Effect of pretreatment with cocaine ($3 \times 10^{-5}$ M) or with ICI 118551 ($3 \times 10^{-4}$ M). Control efflux = stippled bars at the left; efflux from kidneys pretreated with cocaine ($3 \times 10^{-5}$ M) = cross-hatched bars in the center; efflux from kidneys pretreated with ICI 118551 ($3 \times 10^{-4}$ M) = diagonally ruled bars at the right. In the presence of cocaine (for the duration of the experiment), renal nerve stimulation did not evoke a significant increase in the efflux of radioactivity. Significant difference from the efflux of radioactivity evoked by renal nerve stimulation in control conditions is indicated by * $p < 0.05$; ** $p < 0.01$. Vertical bars indicate SEM.
been stored in the adrenergic nerve terminals of the kidney. The facilitory effect of epinephrine in the perfused rat kidney may be mediated by a prejunctional β-adrenergic receptor. A prejunctional site of action is suggested by the observation that low concentrations (2.5 — 5 × 10⁻⁹ M) of epinephrine enhanced the amplitude of neurogenic vasoconstrictor responses without affecting the basal perfusion pressure of the kidney or its responses to exogenous norepinephrine. The highest concentration of epinephrine (10⁻⁵ M) not only enhanced responses to nerve stimulation but also enhanced those evoked by low doses of norepinephrine. This postjunctional effect may have accounted for the exaggerated enhancing effect of this concentration of epinephrine on the responses evoked by the lowest frequency of nerve stimulation used (0.5 Hz). The existence of a prejunctional facilitatory β-adrenergic receptor in the rat kidney has also been demonstrated by a study of the effects of salbutamol on the stimulus-evoked overflow of ³H-norepinephrine.⁹

The β₂-adrenergic-receptor-selective antagonist ICI 118551¹⁰ abolished the facilitative effect of epinephrine on the amplitude of vasoconstrictor responses evoked by intra-arterial norepinephrine in the isolated perfused rat kidney (n = 6) in the absence and presence of epinephrine (5 × 10⁻⁹ M). Control dose-response curve (O), dose-response curve in the presence of epinephrine (5 × 10⁻⁹ M) (●). Data are expressed as means ± SEM.
evoked by renal nerve stimulation. By contrast, the \( \beta_1 \)-selective antagonist atenolol\(^\text{11}\) did not. ICI 118551 also reduced the stimulus-evoked efflux of radioactivity from kidneys that had been perfused with tritiated epi-
ephine. This latter effect cannot have been due to inhibition of the neuronal uptake of epinephine by the drug, since the renal accumulation of tritium was not altered. Consequently, this effect of ICI 118551 must be due to blockade of a prejunctional \( \beta \)-adrenergic receptor that facilitates the release of adrenergic neurotransmitter.

The pharmacological classification of the prejunctional \( \beta \)-adrenergic receptor on the adrenergic nerves has been controversial. The receptor is apparently blocked by the nonselective antagonist propranolol,\(^\text{12}\) the \( \beta_1 \)-selective antagonist metobrolol,\(^\text{13}\) and the \( \beta_2 \)-selective antagonist butoxamine.\(^\text{14}\) In the present study, the most selective \( \beta_2 \)-adrenergic receptor an-
tagonist available, ICI 118551,\(^\text{10}\) reduced the stimulus-
evoked efflux of tritium from the kidney and inhibited the epinephrine-induced selective enhancement of vasoconstrictor responses evoked by renal nerve stimulation. Consequently, the prejunctional \( \beta_2 \)-adrenergic re-
ceptor present in the rat kidney appears to be of the \( \beta_2 \) subtype.

Cocaine reduced, but did not abolish, the facility effect of the epinephrine infusion. Since this effect is qualitatively similar to that of ICI 118551, it could be argued that cocaine blocks \( \beta_2 \)-adrenergic receptors. There is no evidence to support this proposition. It is therefore likely that this effect of cocaine is due to an inhibition of neuronal uptake. Thus, it appears that epinephrine derived from two sources can stimulate the prejunctional \( \beta_2 \)-adrenergic receptor. First, circulating epinephrine can directly stimulate the receptor. Second, it can be stimulated by epinephrine that has been taken up by the adrenergic nerve ending and subsequently released during nerve stimulation. The facilitatory effect of epinephrine that persists after the catecholamine infusion has ceased must be due solely to the latter (neuronal) source of epinephrine. This suggestion is supported by the observation that cocaine not only abolishes the persistent facility effect of epinephrine but also abolishes the stimulus-evoked efflux of the labeled catecholamine.

The present paper supports evidence that suggests that epinephrine may be important in the genesis of some forms of hypertension. Majewski et al.\(^\text{15}\) have shown that rats implanted with a slow-release depot preparation containing epinephrine develop a sus-
tained elevation in blood pressure. It has also been reported\(^\text{9}\) that bilateral adrenal demedullation of 4-
week-old spontaneously hypertensive rats significantly attenuates the development of hypertension. The

results of the present study demonstrate that an acute release of epinephrine can cause a persistent facilita-
tion of neurogenic vasoconstrictor responses. This ef-
fect may help to explain the prohypertensive action of this catecholamine.

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