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^-5 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 β1-selective adrenergic receptor antagonist ICI 118,551 (3 x 10^-8 M), but not by the β2-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 β2-adrenergic receptor. The persistent nature of this effect may be due to the neuronal accumulation and subsequent release of epinephrine.

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Key Words: epinephrine • vasoconstriction • rat perfused kidney • sympathetic neurotransmission

Epinephrine 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. Majewski and Rand 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, 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.

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 Vanhoutte and perfused at a constant flow (6 ml/min) with Krebs-Ringer solution of the following composition (mmol): NaCl, 118.2; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 25; glucose, 5.0; which contained ethylenediaminetetraacetic 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-
striectior 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 x 10⁻⁸ M), and cocaine (3 x 10⁻⁵ M).

³H-Epinephrine Labeling of Kidneys

After a 45-minute equilibration period, the kidneys were perfused with ³H-epinephrine (5 x 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 x 10⁻⁵ M) or ICI 118551 (3 x 10⁻⁸ M). These drugs were added 30 minutes before ³H-epinephrine perfusion and were present for the duration of the experiment.

Aquadol (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.

Statistical Methods

Statistical analysis of data was by Student’s paired and unpaired t tests, and by analysis of variance (ANOVA), as appropriate. Significance was accepted when p < 0.05.

Results

Effect of Epinephrine

The amplitude of vasoconstrictor responses evoked by renal nerve stimulation and by exogenous norepinephrine was reproducible for the duration of the experiment. Continuous infusion of epinephrine (10⁻⁹—5 x 10⁻⁹ M) had no significant effect on basal perfusion pressure of the kidneys (72.6 ± 1.3 mm Hg, n = 24). However, a higher concentration of epinephrine (10⁻⁴ M) caused a significant (p < 0.05) increase in basal perfusion pressure to 80.6 ± 1.3 mm Hg (n = 6). Epinephrine infusion (2.5 x 10⁻⁴ to 10⁻⁸ M) caused a concentration-related enhancement of the amplitude of vasoconstrictor responses induced by renal nerve stimulation (Figure 1), while responses induced by exogenous norepinephrine were only slightly en-
Enhanced 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 \(\beta\)-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).

**Figure 2.** Effect of epinephrine on the amplitude of vasoconstrictor responses evoked by intra-arterial injection of norepinephrine in the isolated perfused kidney of the rat (n = 6). Epinephrine (10^{-9} M) = stippled bars at left; epinephrine (2.5 \times 10^{-9} M) = cross-hatched bars second from left, epinephrine (5 \times 10^{-9} M) = diagonally ruled bars third from left; epinephrine (10^{-8} M) = open bars at the right. Vasoconstrictor responses were measured as the increase in perfusion pressure from the basal pressure immediately preceding the response. 100% represents the amplitude of the vasoconstrictor response evoked in control solution (in the absence of epinephrine). \(* = p < 0.05; \** = p < 0.01.

Vertical bars indicate SEM.

**Figure 3.** Effect of epinephrine (5 \times 10^{-9} M) on the amplitude of vasoconstrictor responses evoked by renal nerve stimulation in the isolated perfused rat kidney (n = 6) in the absence (Group 1) and presence (Group 2) of cocaine (3 \times 10^{-5} M). Epinephrine (5 \times 10^{-9} M) = stippled bars at the left; 10 minutes after cessation of epinephrine infusion = cross-hatched bars second from left, epinephrine (5 \times 10^{-9} M) infusion in the presence of cocaine (3 \times 10^{-5} M) = diagonally ruled bars third from left; 10 minutes after cessation of epinephrine infusion (cocaine still present at 3 \times 10^{-5} M) = open bars at the right. Vasoconstrictor responses were measured as the increase in perfusion pressure from the basal pressure immediately preceding the response. Group 1: 100% represents the amplitude of the vasoconstrictor response evoked in control solution. Group 2. 100% represents the control response evoked in the presence of cocaine. \(* = p < 0.05; \** = p < 0.01; \*** = p < 0.001. Vertical bars indicate SEM.
Table 1. Total Tissue Radioactivity of Kidneys after Perfusion with $^3$H-Epinephrine (5 x 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 x 10^5/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.51 ± 0.4</td>
</tr>
<tr>
<td>ICI 118551 (3 x 10^{-4} M)</td>
<td>5.90 ± 0.17</td>
</tr>
<tr>
<td>Cocaine (3 x 10^{-5} M)</td>
<td>2.07 ± 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 x 10^{-5} M), epinephrine (5 x 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 x 10^{-5} M) significantly reduced the accumulation of radioactivity in 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 facilitory 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...
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 \( \beta \)-adrenergic receptor. A prejunctional site of action is suggested by the observation that low concentrations (2.5 — 5 \( \times \) 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\(^{-8} \) 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 facilitory \( \beta \)-adrenergic receptor in the rat kidney has also been demonstrated by a study of the effects of salbutamol on the stimulus-evoked overflow of \(^3\)H-norepinephrine.\(^9\)

The \( \beta_2 \)-adrenergic-receptor-selective antagonist ICI 118551\(^{10}\) abolished the facilitative effect of epinephrine on the amplitude of vasoconstrictor responses.
evoked by renal nerve stimulation. By contrast, the β1-selective antagonist atenolol did not. ICI 118551 also reduced the stimulus-evoked efflux of radioactivity from kidneys that had been perfused with tritiated epinephrine. This latter effect cannot have been due to inhibition of the neuronal uptake of epinephrine by the drug, since the renal accumulation of tritium was not altered. Consequently, the effect of ICI 118551 must be due to blockade of a prejunctional β-adrenergic receptor that facilitates the release of adrenergic neurotransmitter.

The pharmacological classification of the prejunctional β-adrenergic receptor on the adrenergic nerves has been controversial. The receptor is apparently blocked by the nonselective antagonist propranolol,12 the β1-selective antagonist metoprolol,13 and the β1-selective antagonist butoxamine.14 In the present study, the most selective β1-adrenergic receptor antagonist available, ICI 118551,19 reduced the stimulus-evoked efflux of tritium from the kidney and inhibited the epinephrine-induced selective enhancement of vasconstrictor responses evoked by renal nerve stimulation. Consequently, the prejunctional β1-adrenergic receptor present in the rat kidney appears to be of the β1 subtype.

Cocaine reduced, but did not abolish, the facilitory effect of the epinephrine infusion. Since this effect is qualitatively similar to that of ICI 118551, it could be argued that cocaine blocks β1-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 β1-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 facilitatory 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.15 have shown that rats implanted with a slow-release depot preparation containing epinephrine develop a sustained elevation in blood pressure. It has also been reported9 that bilateral adrenalectomy 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 facilitation of neurogenic vasoconstrictor responses. This effect may help to explain the prohypertensive action of this catecholamine.

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