Effects of Endothelin on Neuroeffector Junction in Mesenteric Arteries of Hypertensive Rats

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The effect of endothelin, a novel vasoconstrictor peptide, on the adrenergic neuroeffector junction was investigated in isolated perfused mesenteric arteries of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. The vasoconstrictor responses to periarterial sympathetic nerve stimulation and exogenous norepinephrine were determined. Infusion of endothelin-1 increased the baseline perfusion pressure dose dependently to similar extents in the two strains. A subpressor dose of endothelin-1 (10^{-10} M) enhanced the pressor response to norepinephrine; its effect was greater in WKY rats than in SHR. Endothelin-1 (10^{-12} to 10^{-8} M) attenuated the pressor response to sympathetic nerve stimulation, and the degree of inhibition tended to be less in SHR than in WKY rats. Higher doses (3\times10^{-9} and 10^{-8} M) of endothelin-1 enhanced the pressor response to nerve stimulation in both WKY rats and SHR. Endothelin-1 inhibited norepinephrine release from rat mesenteric arteries; the inhibition was significantly less in SHR than in WKY rats. These results suggest that endothelin enhances the responsiveness of \(\alpha\)-adrenergic receptors to catecholamines, whereas it inhibits presynaptic adrenergic neurotransmission. Thus, endothelin can interact with the neuroeffector junction in addition to having a vasoconstricting effect in peripheral vessels. The difference in the mode of modulation by endothelin at the vascular neuroeffector junction in SHR from that in WKY rats might explain the maintenance of hypertension. (Hypertension 1990;15:739–743)

Endothelin was isolated from conditioned medium of cultured porcine aortic endothelial cells.\(^1\) Recently, three forms of endothelin, named ET-1, ET-2, and ET-3, were identified by screening a human genomic library.\(^2\) The extremely potent vasoconstrictor action of endothelin and the wide distribution of its binding sites,\(^3\) including arteries, brain, and kidneys, suggest that it might be important in control of blood pressure as well as in the pathogenesis of hypertension. Recently, we found that ET-1 also has a neuromodulatory action in normotensive rats.\(^4,5\)

To evaluate the contribution of endothelin to the maintenance of hypertension in the present study, we investigated its direct pressor action, its effect on the pressor responses evoked by exogenous norepinephrine and by periarterial nerve stimulation, and its effects on the release of norepinephrine from sympathetic nerve endings in perfused mesenteric arteries of spontaneously hypertensive rats (SHR).

Methods

Animals

SHR (12 weeks old) and age-matched Wistar-Kyoto (WKY) rats (Charles River Japan, Atsugi, Japan) weighing 289±3 and 300±5 g, respectively, and maintained on standard rat chow were used in the study. The systolic blood pressure and pulse rate of the rats were measured by the tail-cuff method with a programmable sphygmomanometer (TS-100, Riken Kaihatsu, Tokyo, Japan) 1 day before the operation.

Mesenteric Artery Preparation

Mesenteric arteries were prepared for perfusion as described previously.\(^6\) Briefly, the rats were anesthetized with pentobarbital sodium (30 mg/kg i.p.) and treated with heparin (1,300 units/kg i.v.). The superior mesenteric artery was cannulated with PE-90 tubing, flushed with 15 ml Krebs-Ringer solution, and isolated by cutting around the intestinal border of the mesentery. The whole preparation was placed in a container with a water jacket maintained at 37°C and perfused with Krebs-Ringer solution of the following composition (mM): NaCl 118.4, KCl 4.7,
CaCl₂·2H₂O 2.5, MgSO₄·7H₂O 1.2, NaH₂PO₄·2H₂O 1.2, NaHCO₃ 25, and glucose 11.1. This solution was maintained at 37° C and aerated with a mixture of 5% CO₂ and 95% O₂ to obtain a pH of 7.4. The tissues of two rats, one SHR and one WKY, were perfused simultaneously at a constant flow of 4.5 ml/min with a peristaltic pump (MP-6001, Tokyo Rika, Tokyo, Japan). Test drugs were infused with a microinfusion pump (501B, ATOM, Tokyo, Japan). The perfusion pressure was recorded with a pressure transducer (TP-300T, Nihon Kohden Electronic Co., Tokyo, Japan) connected to a polygraph (MM-6000, Nihon Kohden Electronic Co.). All experiments were done after an equilibration period of 30 minutes.

Direct Pressor Action of Endothelin-1

Changes in baseline perfusion pressure were monitored before and after infusion of cumulatively applied ET-1 (10^{-13} to 10^{-9} M) in the mesenteric arteries of both SHR and WKY rats. We defined increase in perfusion pressure as the difference from the baseline.

Exogenous L-Norepinephrine Response

L-Norepinephrine (Sigma Chemical Co., St. Louis, Missouri) as a single dose of 50, 100, or 200 ng in 10 μl Krebs-Ringer solution was injected into the arterial cannula with a microsyringe, and the pressor response was then determined as the increase in perfusion pressure.

Periarterial Nerve Stimulation

Postganglionic sympathetic nerve fibers were stimulated by bipolar platinum electrodes placed around the proximal end of the superior mesenteric artery. Biphasic square-wave pulses of 2-msec duration and supramaximal voltage (30 V) were delivered by an electric stimulator (SEN-3201, Nihon Kohden Electronic Co.) for 30 seconds at frequencies of 4, 8, or 16 Hz at 4-minute intervals. We confirmed the neural basis of the pressor response mediated by stimulation of arterial adrenergic nerve by abolition of the response after perfusion of guanethidine (10^{-4} M) and tetrodotoxin (10^{-7} M) and by suppression of the response after perfusion of bunazosin (10^{-9} M), an α₁-blocker.

Measurement of [³H]Norepinephrine Efflux

Mesenteric tissue isolated from rats was placed in 5 ml Krebs-Ringer solution aerated with 5% CO₂ and 95% O₂ and containing 5.7×10^{-4} M ascorbic acid. The tissue was incubated with 20 μCi [³H]norepinephrine (specific activity 31 Ci/mmol, final concentration 0.1 μM) (Amersham, Buckinghamshire, UK) for 90 minutes at 37° C to label the neuronal norepinephrine stores. After washout with [³H]norepinephrine-free Krebs-Ringer solution for 30 minutes, the perfusate was collected every 1 minute. Spontaneous and stimulation-evoked release of radioactivity was monitored in a liquid scintillation counter after addition of 10 ml of a scintillation mixture (ACS II, Amersham, Buckinghamshire, UK) to each sample. Periarterial nerve stimulation (PNS) (8 Hz) was applied for 30 seconds at 16-minute intervals. The infusion of endothelin was started 14 minutes before PNS and continued throughout application of PNS. The [³H]norepinephrine efflux was expressed as a percentage of the amount of [³H]norepinephrine in the tissue. The net [³H]norepinephrine efflux during each stimulation was calculated by subtracting the spontaneous efflux just before application of PNS from the average [³H]norepinephrine efflux in two 1-minute samples after application of PNS. The net [³H]norepinephrine efflux in the presence of ET-1 was calculated as a percentage of the control value.

Drugs

Synthetic ET-1 (Peptide Institute, Osaka, Japan) was made up as stock solution (10^{-4} M) in distilled water and stored in aliquots at −20° C. Just before use for infusion, the stock solution was diluted with Krebs-Ringer solution containing 0.05% bovine serum albumin.

Statistical Analysis

Values are shown as mean±SEM. Statistical significance was assessed by analysis of variance followed by Dunnett’s multiple range test for individual comparisons of means, and p values of less than 0.05 were regarded as significant. Comparisons between groups were analyzed by Student’s t test after adjustment of p values using Bonferroni’s method.

Results

The systolic blood pressure, pulse rate, and pressor responses to PNS and norepinephrine are summarized in Table 1. The systolic blood pressure of SHR was significantly higher than that of WKY rats. There was no significant difference between the baseline perfusion pressures in SHR and WKY rats. The vasoconstrictor responses of the mesenteric arteries evoked by both PNS (8 Hz) and norepinephrine (100 ng) injection were greater in SHR than in WKY rats.

Infusion of ET-1 at concentrations of more than 3×10^{-10} M caused pronounced increase in the perfusion pressure; however, there was no significant difference between the pressor responses of the arteries of SHR and WKY rats (Figure 1). Figure 2 shows the endothelin-induced potentiation of the pressor responses to norepinephrine (100 ng) in SHR and WKY rats. The percentage of changes in each rat was calculated by dividing the magnitude of the pressor response in the presence of ET-1 at different concentrations by the magnitude of the baseline pressor response. A subpressor dose of 10^{-10} M ET-1 enhanced the pressor responses in both strains. The degree of potentiation was greater in WKY rats than in SHR at all doses. The potentiation of the pressor response to exogenous norepinephrine observed with two different doses of endothelin (10^{-10} and 3×10^{-10} M) was also observed at lower (50 ng) and higher (200 ng) doses and was dose dependent in both strains. Endothelin, however, attenuated the pressor response to PNS only at subpressor doses,
whereas at higher doses, it caused pronounced dose-dependent enhancement of the pressor response to PNS (Figure 3). Its attenuating effect tended to be less in SHR than in WKY rats. ET-1 at $10^{-11}$ M suppressed the pressor response to each frequency of PNS (4, 8, and 16 Hz) in both strains. Figure 4 shows the effect of ET-1 on $[^{3}H]$norepinephrine efflux evoked by PNS (8 Hz). Mesenteric preparation of the two strains took up the same amount of tritiated norepinephrine (mean total tissue counts were approximately $2.6 \times 10^6$ cpm). ET-1 inhibited norepinephrine release from sympathetic nerve endings of rat mesenteric arteries in both strains; however, its inhibitory effect was significantly less in SHR than in WKY rats ($p<0.05$).

**Discussion**

We showed in the present study that endothelin, when given alone, caused a pronounced increase in the perfusion pressure of mesenteric arteries in SHR and WKY rats and that the extents of increase were similar in the two strains. ET-1 also potentiated the norepinephrine-induced increase in the pressure in a dose-dependent manner in both strains. At lower concentrations, it suppressed the PNS-induced increase in pressure, whereas at higher concentrations, it enhanced the increase in pressure. The degrees of potentiation and suppression were different in the two strains, suggesting that caution is required when interpreting the results.

We observed that infusion of ET-1 into isolated perfused rat mesenteric arteries caused a pronounced increase in the perfusion pressure. The minimum pressor dose and the magnitude of the pressor effect on resistant vessels of SHR were almost the same as the dose and pressor effect on vessels of WKY rats. There are recent reports that the pressor response to ET-1 is more prominent in WKY rats than in SHR in the conscious state, whereas the sensitivity of the renal artery to ET-1 is greater in SHR than in WKY rats. No consistent results on the direct pressor action of ET-1 in SHR and WKY rats, however, have been reported.

We confirmed that, even at subpressor doses, ET-1 enhanced the pressor responses evoked by exogenous norepinephrine administration in SHR and WKY rats. Norepinephrine and endothelin appear to share common mechanisms of action in vascular smooth

### TABLE 1. Systolic Blood Pressure and Baseline Perfusion Pressure and Pressor Responses to Periarterial Nerve Stimulation and Exogenous Norepinephrine Injection in Isolated Perfused Mesenteric Arteries of Rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>147±4</td>
<td>197±58*</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>409±24</td>
<td>468±14</td>
</tr>
<tr>
<td>Baseline perfusion pressure (mm Hg)</td>
<td>21.6±1.3</td>
<td>22.7±1.1</td>
</tr>
<tr>
<td>Pressor response to PNS (8 Hz) (mm Hg)</td>
<td>8.6±1.4</td>
<td>18.0±3.0*</td>
</tr>
<tr>
<td>NE (100 ng) (mm Hg)</td>
<td>14.0±1.7</td>
<td>21.2±1.6*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; PNS, periarterial nerve stimulation; NE, norepinephrine.

$^{*}p<0.05$ vs. WKY.

Figure 1. Plotting of dose-response curves for effect of endothelin-1 on perfusion pressure in mesenteric arteries of spontaneously hypertensive rats (n=6) (●●) and Wistar-Kyoto rats (n=6) (○○). Points represent mean±SEM.

Figure 2. Plotting showing comparison of dose dependences of the effect of endothelin-1 on pressor response of mesenteric arteries to exogenous norepinephrine (100 ng) in spontaneously hypertensive rats (n=6) (●●) and Wistar-Kyoto rats (WKY) (n=6) (○○). Points represent mean±SEM. $^{*}p<0.05$, $^{* *}p<0.01$ vs. each vehicle control.

$^\dagger p<0.05$, $^\dagger ^\dagger p<0.01$ vs. WKY.
The present study also showed that at subpressor doses, ET-1 inhibited the pressor response of both SHR and WKY rat mesenteric arteries to electrical sympathetic nerve stimulation, whereas at pressor doses, it enhanced the pressor response. The enhancement of the pressor response to PNS by the higher doses of ET-1 might be due to postjunctional potentiation, as seen in the endothelin-induced enhancement of the pressor response to exogenous norepinephrine. The inhibitory effect of the lower doses of ET-1 on pressor response to PNS was probably mediated through inhibition of norepinephrine release from nerve endings because ET-1 (10^-10 M) decreased [H]norepinephrine efflux in both strains. This observation is consistent with recent reports that ET-1 inhibits the nerve stimulation-induced release of [H]norepinephrine in isolated perfused Sprague-Dawley rat mesenteric artery and guinea pig femoral artery and pulmonary artery. Endothelin also reportedly stimulates the release of prostaglandins from guinea pig and rat lung, and Sprague-Dawley rat mesenteric arteries. Prostaglandin E2 (PGE2) is known to inhibit the release of norepinephrine by a prejunctional mechanism. The released prostaglandin, however, does not contribute to inhibition of the pressor response to PNS.

Neuropeptide Y has some similarities to endothelin and might provide a clue to the mode of action of endothelin. Neuropeptide Y probably coexists and cooperates with norepinephrine in perivascular nerve fibers in causing sympathetic activation. This peptide also causes prejunctional inhibition of norepinephrine release, which results in conservation of norepinephrine for release, determined as [H]norepinephrine from nerve endings and postsynaptic enhancement of the pressor response to exogenous norepinephrine. In the present study, the inhibition of norepinephrine release, determined as [H]norepinephrine from nerve endings by endothelin, was weaker in SHR than in WKY rats, was weaker in SHR than in WKY rats. This might have been because norepinephrine release in the mesenteric vasculature is enhanced in adult SHR. The endothelin-induced potentiation of the postsynaptic α-adrenergic receptor was greater in WKY rats than in SHR, and as a result at higher doses ET-1-induced enhancement of the pressor response to PNS to similar extents in the two strains. Interestingly, the inhibition of norepinephrine release in the mesenteric artery by neuropeptide Y was also less in SHR than in WKY rats.

Muscle cells; that is, endothelin reportedly activates phosphatidylinositol turnover and protein kinase C, resulting in vasoconstriction through increase of intracellular calcium. α-Adrenergic agonists also activate phospholipase C, resulting in an elevation of intracellular calcium. Endothelin might act synergistically on the postsynaptic α-adrenergic receptor. This enhancing effect was smaller in SHR than in WKY rats. The reason for this difference in the magnitude of enhancement of the norepinephrine-induced pressor response could be explained by supposing that the plasma concentrations of endothelin in SHR and WKY rats can be different, and consequently, the number or affinity of endothelin receptors can be less in SHR than in WKY rats. Resolution to this problem requires further experimentation.
Although it appears unlikely that a direct vasoconstrictor effect of endothelin is involved in maintenance of hypertension in SHR, endothelin enhances α-adrenergic receptor responsiveness postjunctionally and inhibits norepinephrine release prejunctionally in isolated perfused mesenteric arteries of SHR and WKY rats, suggesting that it acts on the adrenergic neuroeffector junction as well as on vascular smooth muscle cells. The difference in its mode of modulation of the vascular neuroeffector junction in SHR from that in WKY rats might explain sustained hypertension in this strain.

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