Interleukin-2 Causes Endothelium-Dependent Contractions to Arachidonic Acid

Chantal M. Boulanger and Paul M. Vanhoutte

The present experiments were designed to investigate the effect of interleukin-2 on the response to arachidonic acid in rings with and without endothelium from Wistar-Kyoto (WKY) and spontaneously hypertensive rat (SHR) aortas. In control rings, arachidonic acid induced contractions of WKY aorta that were not different between preparations with and without endothelium. Incubation with interleukin-2 (10 units/mL) for 6 or 18 hours augmented the response to arachidonic acid in rings with, but not in those without, endothelium from WKY rat aortas. In the WKY aorta, both the endothelium-dependent and endothelium-independent contractions to arachidonic acid observed after incubation with interleukin-2 were abolished by indomethacin and ridogrel (a thromboxane-endoperoxide receptor antagonist and a thromboxane synthase inhibitor) but were not affected by dazoxiben (a thromboxane synthase inhibitor). Interleukin-2 did not augment the vascular reactivity of WKY aortic smooth muscle to activation of the thromboxane-endoperoxide receptor with U46619. In aortas from SHRs, arachidonic acid evoked endothelium-dependent contraction; interleukin-2 did not modify the response to arachidonic acid in preparations with and without endothelium. These data demonstrate that 1) endothelium-dependent contractions to arachidonic acid are observed in SHR but not in WKY rat aortas; 2) interleukin-2 induces endothelium-dependent contractions to arachidonic acid in the WKY aorta that are mediated by an augmented release of a metabolite of cyclooxygenase, different from thromboxane A₂ but activating thromboxane-endoperoxide receptors; and 3) interleukin-2 does not affect the endothelium-dependent and endothelium-independent response to arachidonic acid in the SHR aorta. (Hypertension 1993;21:289–293)

KEY WORDS • prostaglandin synthase • prostaglandin endoperoxides • endothelium • aorta • rats, inbred WKY • rats, inbred SHR • arachidonic acids • interleukin-2

Vascular endothelial cells participate in the regulation of vascular smooth muscle tone by releasing relaxing or contracting factors or both. In spontaneously hypertensive rats (SHRs), the impairment of endothelium-dependent relaxations to acetylcholine is due to the release of an endothelial contracting factor concomitant with that of endothelium-derived relaxing factor. The contracting factor released by endothelial cells from the SHR is a product of the metabolism of arachidonic acid by cyclooxygenase. It is different from prostacyclin, thromboxane A₂, or superoxide anions. This contracting factor is likely to be prostaglandin H₂, an endoperoxide precursor of other prostaglandins formed during activation of prostaglandin H synthase cyclooxygenase. The expression and activity of prostaglandin H synthase are augmented in cultured endothelial cells after stimulation with interleukin-2, a cytokine released during activation of T lymphocytes. The purpose of the present study was to examine the effect of interleukin-2 on endothelium-dependent and endothelium-independent responses to arachidonic acid in aortas from normotensive rats and SHRs.

Methods

Blood Vessels

Experiments were performed on thoracic aortas obtained from age-matched (19–24 weeks) and weight-matched (290–365 g) male SHRs and normotensive Wistar-Kyoto (WKY) rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.). The mean systolic blood pressure, measured by the tail-cuff method, was 211.6±5.5 (n=10) and 147.5±2.2 (n=15) mm Hg for SHRs and WKY rats, respectively. The animals were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg), and the aortas were removed and placed into cold modified Krebs-Ringer solution of the following composition (mM): NaCl 118.3, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, CaEDTA 0.026, and glucose 11.1 (control solution, pH 7.4). The blood vessels were cleaned of fat and connective tissue and cut into rings (4–5 mm long). In some preparations, the endothelium was removed mechanically by inserting the tip of a forceps into the lumen and gently rolling the preparation back and forth on a paper towel wetted with control solution.

The rings were then placed in 12-well multiwell plates (one ring per well) with minimal essential medium (1...
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Figure 1. Line graphs show contractions to arachidonic acid in control preparations with and without endothelium from Wistar-Kyoto (WKY) rat (left panel) and spontaneously hypertensive rat (SHR) (right panel) aortas. Experiments were performed in the presence of nitro-L-arginine (10⁻⁴ M). Responses are shown as mean±SEM and expressed as percent of maximal contraction to phenylephrine (3x10⁻⁵ M). In WKY aortas, contractions to phenylephrine in rings with and without endothelium were 2.3±0.4 and 1.7±0.3 g, respectively (n=6). In SHR aortas, maximal contractions to phenylephrine in rings with and without endothelium were 2.2±0.1 and 1.9±0.2 g, respectively (n=7; NS). *Statistically significant (p<0.05) difference between rings with and without endothelium.

Control Response
Arachidonic acid (10⁻⁶ to 10⁻⁴ M) induced contractions of rings without endothelium from SHR than WKY aorta (Figure 1). The presence of the endothelium did not affect the response to arachidonic acid in the WKY aorta but increased that observed for a high concentration of arachidonic acid (10⁻⁴ M) in the SHR aorta (Figure 1).

Materials
Arachidonic acid (sodium salt), bovine serum albumin fraction V, indomethacin, phenylephrine, and thrombin were purchased from Sigma Chemical Co., St. Louis, Mo., and nitro-L-arginine from Aldrich Chemical Co. Inc., Milwaukee, Wis. Human recombinant interleukin-2 (10 units/mL) was purchased from Boehringer, Mannheim, FRG, and U46619 from Cayman Chemical Co. Inc., Ann Arbor, Mich. R 68070 (ridogrel) was obtained from Janssen Pharmaceuticals, Beerse, Belgium, and dazocibene (UK 37248) from Pfizer, Groton, Conn. Minimum essential medium, penicillin, and streptomycin were obtained from Whittaker Bioproducts, Walkersville, Md. Plasteware was purchased from Costar Corp., Pleasanton, Calif.

Concentrations are expressed in molar concentrations. All drugs were prepared daily in distilled water and stored at +4°C until use. Indomethacin was dissolved in the presence of Na₂CO₃ and sonicated before use. The final concentration of Na₂CO₃ was 2x10⁻⁴ M. A stock solution of ridogrel (5x10⁻³ M; 1 mL) was prepared in distilled water and alkalinized with one drop of 1N NaOH. Interleukin-2 aliquots (10,000 units/mL) were kept frozen at -70°C in phosphate-buffered saline solution supplemented with bovine serum albumin (0.1%).

Results

Statistical Analysis
Data are expressed as percent of a maximal contraction evoked by 3x10⁻⁵ M phenylephrine. Results are given as mean±SEM, and n represents the number of experiments on rings from different rats. The ED₅₀ represents the negative logarithm of the molar concentration of agonist causing 50% of the maximal contraction. Statistical analysis was performed by using Student's paired t test and analysis of variance. A value of p<0.05 was considered statistically significant.
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Figure 2. Line graphs show effect of interleukin-2 (10 units/mL; 2–18 hours incubation) on response to arachidonic acid in rings with endothelium from Wistar-Kyoto (WKY) rat (left panel) and spontaneously hypertensive rat (SHR) (right panel) aortas. Experiments were performed in the presence of nitro-L-arginine (10^-4 M). Responses are shown as mean±SEM and expressed as percent of maximal contraction to phenylephrine (3×10^-5 M). In WKY aortas, contractions to phenylephrine were 2.3±0.4 g (control; n=14). In WKY preparations incubated with cytokine, maximal contractions to phenylephrine were 2.1±0.2 g (2 hours incubation; n=4), 2.1±0.2 g (6 hours incubation; n=6), and 3.0±0.4 g (18 hours incubation; n=4). In SHR aortas, contractions to phenylephrine were 3.1±0.4 g (control; n=10), 2.4±0.3 g (interleukin-2, 6 hours; n=6), and 3.1±0.3 g (interleukin-2, 18 hours; n=4). *Statistically significant (p<0.05) difference between rings with and without endothelium.

Effect of Interleukin-2

Incubation of WKY aortas with interleukin-2 (10 units/mL, 6–18 hours) augmented the response to arachidonic acid in preparations with (Figure 2) but not in those without endothelium (Table 1). No effect of interleukin-2 (10 units/mL) was observed after 2 hours of incubation (Figure 2).

Incubation with interleukin-2 (10 units/mL, up to 18 hours) did not significantly affect the response to arachidonic acid of rings both with and without endothelium from SHR aortas (Figure 2 and Table 1). Incubation with higher concentrations of interleukin-2 (30 units/mL) did not augment the response to arachidonic acid in rings with endothelium compared with that of rings without endothelium (n=3; data not shown).

In rings with and without endothelium from WKY aorta, interleukin-2 did not significantly affect the ED_50 and maximal response to activation of the thromboxane-endoperoxide receptor with U46619 (Table 2).

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Maximum*</th>
<th>Area under the curve†</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY control</td>
<td>29.7±13.1</td>
<td>58.2±38.4</td>
</tr>
<tr>
<td>WKY+IL-2</td>
<td>34.0±16.7</td>
<td>67.6±50.6</td>
</tr>
<tr>
<td>SHR control</td>
<td>85.6±12.0</td>
<td>194.9±26.3</td>
</tr>
<tr>
<td>SHR+IL-2</td>
<td>89.3±8.1</td>
<td>221.0±20.6</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rat; SHR, spontaneously hypertensive rat; IL-2, interleukin-2 (10 units/mL and 6 hours of incubation); n=6 aortas. Values are mean±SEM.

*Response to arachidonic acid (10^-4 M) expressed as percent of maximal contraction to phenylephrine (3×10^-5 M). In WKY aortas, maximal contraction to phenylephrine was 2.0±0.2 g (control) and 2.3±0.5 g (cytokine-treated). In SHR aortas, maximal contractions to phenylephrine were 2.0±0.2 and 2.3±0.2 g for control and interleukin-2-treated preparations, respectively.

†Expressed in arbitrary units.

Inhibitors

Indomethacin (10^-5 M) and ridogrel (5×10^-5 M, an antagonist of thromboxane-endoperoxide receptors and an inhibitor of thromboxane synthase) abolished the response to arachidonic acid in rings with and without endothelium from WKY aortas after incubation with interleukin-2 (Figure 3). The response to arachidonic acid in rings with and without endothelium was not decreased significantly after incubation with dazoxiben (10^-4 M, an inhibitor of thromboxane synthase) (Figure 3).

Discussion

The present experiments demonstrate that incubation with interleukin-2 induces endothelium-dependent...
contractions to arachidonic acid in the aorta from normotensive rats but not from SHRs. This effect is likely to be mediated by a product of cyclooxygenase different from thromboxane A2 but activating thromboxane-endoperoxide receptors of vascular smooth muscle.

Arachidonic acid is the substance with which endothelium-dependent contractions were first described in canine veins, a response that is mediated by products of cyclooxygenase. In untreated rings from WKY rats, the presence of the endothelium did not affect the response to arachidonic acid. This suggests either that little endothelial arachidonic acid metabolites are formed in the WKY aorta or that these products do not exhibit potent vasoactive properties in this strain. By contrast, the presence of the endothelium augmented the contractions to arachidonic acid of rings in the SHR aorta. The augmentation of the contraction to arachidonic acid by the endothelium in SHR but not in WKY aorta is consistent with the release of a contracting factor produced during activation of cyclooxygenase and resembles similar findings obtained with acetylcholine and serotonin.

The response to arachidonic acid was more pronounced in rings without endothelium from SHR aorta than that from WKY aorta. This observation is consistent with earlier findings that smooth muscle cells of the SHR produce more, or are more sensitive to, products of cyclooxygenase than those of the WKY rat.

Incubation of WKY aorta with interleukin-2 increased the endothelium-dependent response to arachidonic acid but did not affect its direct contractile effect on smooth muscle. It is likely that this effect is mediated by an augmented production of a cyclooxygenase metabolite and not by an increased sensitivity of the vascular smooth muscle to activation of the thromboxane-endoperoxide receptors. This conclusion is prompted by the facts that the endothelium-dependent contractions to arachidonic acid are abolished by an inhibitor of cyclooxygenase and by an antagonist of thromboxane-endoperoxide receptors, whereas incubation with interleukin-2 does not affect the response of the smooth muscle to the thromboxane analogue U46619.

It is unlikely that the endothelium-dependent contractions to arachidonic acid are mediated by thromboxane, because the thromboxane synthase inhibitor dazoxiben did not affect the response to the fatty acid in WKY aortas with endothelium incubated with interleukin-2. A similar conclusion has been reached for the endothelium-dependent contractions evoked by acetylcholine in SHR aortas. These endothelium-dependent contractions may be due to the release of superoxide anions produced during activation of cyclooxygenase, although endothelium-dependent contractions in SHR aorta are insensitive to scavengers of superoxide anions. Another possible interpretation could be that endoperoxides mediate the endothelium-dependent contractions observed in WKY aorta after incubation with the cytokine, because they have been implicated in endothelium-dependent contraction observed in blood vessels from hypertensive rats. Finally, lipoygenase metabolites (such as 15-HETE) can be ruled out as ultimate mediators of the response observed in the present study, because the endothelium-dependent contractions to arachidonic acid are prevented by an inhibitor of cyclooxygenase.

The present findings suggest that interleukin-2 induces the production of a contracting factor in preparations with endothelium from the WKY aorta. The fact that, in the present study, the potentiation by interleukin-2 of the response to arachidonic acid was not observed immediately but required several hours of incubation is in line with this interpretation. A likely explanation is that interleukin-2 augments the cyclooxygenase activity in endothelial cells of the WKY rat. This interpretation is supported by the induction of both prostaglandin H synthase messenger RNA expression and activity in cultured human and bovine endothelial cells during stimulation with the cytokine.
hypothesis were true, the present results would suggest that the induction of cyclooxygenase activity by interleukin-2 occurs only in the endothelial cells but not in the smooth muscle of the WKY rat aorta, because the cytokine did not affect the contractions to arachidonic acid in rings without endothelium. The present experiments do not rule out the possibility that the cytokine augments the activity of lipoxygenases, monoxygenases, or both, as this was observed in other cell systems.  

These metabolites of arachidonic acid could be metabolized further into endoperoxides by the cyclooxygenase activity and thus could induce contraction of the vascular smooth muscle.  

Interleukin-2 did not affect the endothelium-dependent as well as the direct contractile effect of arachidonic acid in the SHR aorta. The absence of induction of the arachidonic acid cascade in the SHR by interleukin-2 could be explained either by a different regulation of this pathway in WKY rat and SHR or by the presence of already fully induced enzymes in the SHR. The different regulation of arachidonic acid metabolism in WKY and SHR aorta may help to explain why endothelium-dependent contractions are more prominent in arteries of the SHR than in those of the WKY rat.  

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References  

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