Endothelium-Derived Contracting Factors in Resistance Arteries of Young Spontaneously Hypertensive Rats Before Development of Overt Hypertension

Michael Jameson, Fu-Xiang Dai, Thomas Lüscher, Joseph Skopec, Alice Diederich, and Dennis Diederich

Vascular relaxations are impaired in adult spontaneously hypertensive rats (SHRs) because of increased production of an endothelium-derived, cyclooxygenase-dependent contractile factor or factors. To test the hypothesis that alterations in endothelial function precede and contribute to the development of overt hypertension in SHRs, we compared in myographs endothelium-mediated relaxations of mesenteric resistance arteries from 4-week-old SHRs and Wistar-Kyoto (WKY) rats. Acetylcholine (10⁻⁸ to 10⁻⁴ M) induced comparable relaxations in SHR and WKY arteries precontracted (ED₅₀) with norepinephrine. In arteries obtained from SHRs but not from WKY rats, relaxations were replaced by contractile responses with higher concentrations of acetylcholine (10⁻⁹ to 10⁻⁷ M). The contractile responses were endothelium dependent, were augmented by nitro L-arginine (10⁻⁴ M), and were prevented by pretreatment with indomethacin (10⁻⁵ M) or 3-amino-1,2,4-triazole (10⁻³ M), an inhibitor of superoxide anion production via the cyclooxygenase pathway. Inhibition of thromboxane synthetase (CGS-13080, 5x10⁻⁵ M) and antagonism of prostaglandin H₂/thromboxane A₂ receptors (SQ-29,548, 5x10⁻⁵ M) failed to block the contractile response to acetylcholine in SHR arteries. Acetylcholine-mediated relaxations were significantly impaired in mesenteric arteries from 16-week-old SHRs but not from WKY rats. Endothelium-independent relaxations produced by sodium nitroprusside and contractile responses to norepinephrine and endothelin were comparable in arteries from SHRs and WKY rats of all ages. In summary, endothelium-dependent relaxations of mesenteric arteries from "prehypertensive" SHR rats were impaired by the production of a contractile factor (or factors) that appears to be superoxide anions.

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KEY WORDS • acetylcholine • endothelium-derived relaxing factor • endothelins • free radicals • indomethacin • vascular resistance

An increase in peripheral resistance is a characteristic finding in established essential hypertension in humans and in genetic strains of spontaneously hypertensive rats (SHRs). Peripheral resistance may increase as a result of either enhanced contractility or impaired relaxation of vascular smooth muscle of resistance arteries. Endothelial cells modulate underlying vascular smooth muscle tone by releasing en-
Methods

Experimental Animals

Male SHRs and WKY rats of 4, 16, and 28 weeks of age were obtained from Harlan Laboratories, Boston. All rats were maintained four per cage at a constant temperature (24±1°C), with a 12-hour dark/light cycle and on standard rat chow. All procedures followed were in accordance with institutional guidelines. Systolic blood pressures were measured in conscious, prewarmed, restrained rats by the tail-cuff method using plethysmography and a physiograph recorder (model 11 TC, Innovators in Instrumentation, Woodland Hills, Calif.). The median of four to five successive measurements was used as the estimate of blood pressure. Blood pressures and acetylcholine responses reported for 4–10-week-old SHRs were derived from a single group of SHRs obtained at age 4 weeks; blood pressures for the actual rats used in the vascular studies are presented. Rats were anesthetized with ether. The mesentery was removed en bloc through an abdominal incision and placed in cold Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 22, edetate calcium disodium 0.026, and glucose 11. Small mesenteric arteries (first, second, and third branches as a unit) were dissected free under a dissecting microscope with care taken to avoid stretching and trauma to the vessel. A 20-μm-diameter wire was inserted into the lumen of the vessel, and a 2-mm distal segment (second branch in 4-week-old and third branch in older rats) of the vessel was transected with the wire in place. For removal of endothelium, the remaining vessel was cannulated and perfused with 0.5% 3-(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) for 45 seconds followed by Krebs bicarbonate solution; a 2-mm-long segment of the distal end was prepared as above.

Experimental Setup

The vessel segments were positioned in myographs (Living Systems, Burlington, Vt.) filled with Krebs-Ringer solution and were maintained at 37°C and pH 7.40–7.45 by aeration with 5% CO2-95% O2. The vessel was perfused with 0.5% CHAPS, 3,1,2,4-triazole (AT), N0-nitro-L-arginine (L-NA), and L-arginine HCl were obtained from Sigma Chemical Co., St. Louis, Mo. 1,3-Dimethyl-2-thiourea was purchased from Aldrich Chemical Co., Milwaukee, Wis. CGS-13080 (thromboxane synthetase inhibitor) was a gift from CIBA-GEIGY, Summit, N.J. SQ-29,548, a prostaglandin H2/thromboxane A2 receptor antagonist,16 was a gift from Squibb, Princeton, N.J. Endothelin-1, obtained from Peptides International, Inc., Lexington, Ky., was dissolved in H2O and diluted for use in 0.1% Triton X-305 containing 0.2 mg/ml bovine serum albumin. Indomethacin was dissolved in equimolar NaHCO3. All other drugs were dissolved in H2O. Drug concentrations are expressed as final molar (M) bath concentrations.

Calculations and Statistics

Concentration–response curves for acetylcholine and sodium nitroprusside were obtained in a cumulative fashion (10-9 to 10-4 M) after stabilization of a preconstriction of the vessel with norepinephrine (50% of maximal contraction, ED50 dose). Individual data points, ED50 (expressed as negative logarithm of the concentration of agonist required to produce 50% relaxation, pD2), the area under the concentration–response curve, and the maximal relaxation were used for comparative analyses. In rings in which ED50 could not be calculated, a value of >4 was used. Results are presented as mean±SEM; n refers to the number of rats from which vessels were studied. Statistical evaluation was performed using Student’s t test for paired or unpaired observations or one-way analysis of variance. Means were considered significantly different when the two-tailed probability was <0.05.

Results

Blood Pressure, Weights, and Vessel Dimensions

Table 1 summarizes mean±SEM values for blood pressures and body weights. Blood pressures were measured at 4, 6, and 10 weeks of age in a single group of SHRs obtained at 4 weeks of age to more clearly define the increase of blood pressure as a function of age. Mean values for blood pressures were higher in SHRs than in WKY rats at all ages. However, the small increases (6 mm Hg differences) in mean blood pressures in SHRs noted at 4 and 6 weeks of age were not significantly different from those of WKY rats. Blood pressures of 10-week-old and older SHRs were significantly higher than those of WKY rats (p<0.05). The increase in blood pressures from age 6 to 10 weeks averaged 11.5 and 2 mm Hg per week for SHRs and WKY rats, respectively (p<0.05). Mean±SEM values for lumen diameters of the vessels from SHRs and WKY rats, respectively (p<0.05). Mean±SEM values for lumen diameters of the vessels from SHRs and WKY rats, respectively (p<0.05).
TABLE 1. Blood Pressure as a Function of Age in Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (weeks)</th>
<th>n</th>
<th>Weight (g)</th>
<th>Systolic BP (mm Hg)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>4</td>
<td>10</td>
<td>52±6</td>
<td>114±3</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>4</td>
<td>16</td>
<td>48±5</td>
<td>120±3</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>WKY</td>
<td>6</td>
<td>6</td>
<td>120±4</td>
<td>120±6</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>6</td>
<td>16</td>
<td>108±5</td>
<td>126±5</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>WKY</td>
<td>10</td>
<td>8</td>
<td>205±8</td>
<td>128±7</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>10</td>
<td>8</td>
<td>185±7</td>
<td>174±7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>WKY</td>
<td>16</td>
<td>8</td>
<td>360±16</td>
<td>142±5</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>16</td>
<td>8</td>
<td>340±20</td>
<td>195±7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WKY</td>
<td>28</td>
<td>8</td>
<td>392±8</td>
<td>148±7</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>28</td>
<td>8</td>
<td>383±4</td>
<td>214±9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BP, blood pressure; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Values are mean±SEM; p value compares systolic BP of SHRs with WKY rats at each age group using Student's t test.

WKY rats were 128±4 versus 133±10 µm and 152±5 versus 148±6 µm at 4 and 16 weeks of age, respectively.

Endothelium-Dependent Responses

Acetylcholine induced prompt relaxations of mesenteric arteries obtained from 4-week-old WKY rats and SHRs (maximum relaxation, 85±3% and 78±3%; pD2, 7.5±0.1 and 6.7±0.4; n=14 and 10, respectively) (Figure 1). However, as the acetylcholine concentration was increased above 10⁻⁷ M, the relaxations were replaced by powerful contractions in SHR arteries. Contractions in response to acetylcholine were not observed in vessels obtained from WKY rats of any age. In WKY control arteries, arachidonic acid (10⁻⁹, 10⁻⁷, and 10⁻⁵ M) produced small contractions in norepinephrine-activated arteries with endothelium (10±6%, 11±6%, and 28±11%, respectively; n=3) as well as without endothelium (6±2%, 13±10%, and 34±12%, respectively; n=5). The magnitude of the contractile response to acetylcholine in SHR arteries was not affected by the addition of arachidonic acid (10⁻⁷ M) to the bathing medium (n=3, data not shown). Prior removal of the endothelium abolished acetylcholine-induced relaxations of mesenteric resistance arteries from both WKY rats and SHRs as well as the contractile response noted in SHR vessels (n=5, data not shown).

Histamine (10⁻⁵ to 10⁻⁴ M) also produced a contractile response in SHR arteries but not in WKY arteries (Figure 2). As with acetylcholine, the responses to histamine were endothelium dependent; prior removal of the endothelium blocked histamine-induced relaxations in both SHR and WKY arteries and the contractile response in SHR arteries.

Mediators of Endothelium-Dependent Relaxations

Relaxations induced by acetylcholine were not dependent on the production of prostaglandins. Preincubation of SHR vessels with indomethacin (10⁻⁵ M) increased relaxations by blocking the contractile response induced by acetylcholine (Figures 1 and 3). After inhibition of cyclooxygenase, acetylcholine-induced relaxations were essentially identical in SHR and WKY mesenteric resistance arteries (pD2 values, 7.6±0.2 versus 7.5±0.1, respectively; p>0.1). Addition of Histamine [- log M]
of hemoglobin (10⁻³ M) to the bathing solution reversed relaxations induced by acetylcholine (10⁻⁷ M) in both WKY and SHR vessels; maximum relaxations decreased from 88% and 80% to 10% and 12% for WKY and SHR vessels, respectively (n=5). Precontraction of the SHR arteries with norepinephrine was required for the development of endothelium-dependent contractions to acetylcholine. In quiescent SHR vessels, the maximum contractions induced by acetylcholine (10⁻⁹ to 10⁻⁴ M) averaged <5% of the maximum response to norepinephrine (n=5).

Pretreatment of SHR mesenteric arteries with L-NA (10⁻⁴ M) for 30 minutes before addition of acetylcholine abolished relaxations and markedly potentiated the contractile response to the muscarinic agonist (Figure 3). Indomethacin added along with L-NA abolished the contractile response to acetylcholine (Figure 3). In additional experiments, deferoxamine (10⁻³ M) or sodium salicylate (10⁻⁴ M), scavengers of hydroxyl radicals, also failed to block the contractile response to acetylcholine (Figure 3). Similarly, pretreatment of SHR arteries with superoxide dismutase (100 units/ml), a cell-impermeable scavenger of superoxide anions, also failed to block the relaxations induced by acetylcholine (10⁻⁷ M) in both WKY and SHR arteries (Figure 3). Differences noted were not significant.

Mediators of Endothelium-Dependent Contractions

Additional experiments were performed to determine the mediator or mediators of the cyclooxygenase and endothelium-dependent contractions in SHR vessels. Preincubation of SHR resistance arteries with the thromboxane synthetase inhibitor (CGS-13080, 5×10⁻⁵ M) or with the prostaglandin H₂/thromboxane A₂ receptor antagonist (SQ-29,548, 5×10⁻³ M) failed to block the contractile response induced by acetylcholine. Experiments with SQ-29,548, presented in Figure 4, demonstrate small but insignificant increases in relaxations and pD₂ values (7.0±0.4 versus 6.0±0.4, p<0.213) in vessels preincubated with the receptor inhibitor. The potential role of superoxide or hydroxyl radicals or both as mediators of the contractile response was tested using AT (10⁻³ M), the inhibitor of superoxide production via cyclooxygenase,¹⁸-²⁰ and the cell-permeable hydroxyl radical scavenger diethylthiourea (10⁻⁴ M). Preincubation of arteries from 4-week-old SHR with AT abolished the contractile response to acetylcholine (Figure 5), whereas diethylthiourea pretreatment had essentially no effect on the contractile response to the muscarinic agonist. The pD₂ values for control, AT-, and dimethylthiourea-treated SHR vessels (6.7±0.4, 6.1±0.4, and 7.0±0.6, respectively) were not significantly different (p>0.2 for both AT and dimethylthiourea versus control). In additional experiments, deferoxamine (10⁻³ M) or sodium salicylate (10⁻⁴ M), scavengers of hydroxyl radicals, also failed to block the EDCF released by acetylcholine in SHR arteries (n=3, data not shown). Similarly, pretreatment of SHR arteries with superoxide dismutase (100 units/ml), a cell-impermeable scavenger of superoxide anions, also failed to block the relaxations induced by acetylcholine (10⁻⁷ M) in both WKY and SHR arteries (Figure 3). Differences noted were not significant.
to block the contractile response to acetylcholine (n=4, data not shown).

**Effect of Age**

Acetylcholine-mediated responses were essentially identical in arteries from 4- and 8-week-old SHRs (Figure 6). In vessels from 16-week-old SHRs, acetylcholine-induced relaxations were impaired because of an increase in the contractile response to higher concentrations of the agonist. Preincubation of arteries from 16-week-old SHRs with indomethacin (Indo, 10⁻⁵ M) improved relaxations and blocked the contractile response to acetylcholine. *Significantly different from 4-week-old SHR arteries.

**Vascular Smooth Muscle Contractility**

Contractions induced by norepinephrine (10⁻⁷ to 10⁻⁴ M) and by endothelin (10⁻¹⁰ to 10⁻⁷ M) were not significantly different between arteries from 4-week-old SHRs and WKY rats. The pD₂ values for norepinephrine and endothelin were 5.4±0.1 and 7.7±0.2 for arteries from SHRs (n=10) and 5.3±0.1 and 7.7±0.1 for WKY arteries (n=10), respectively.

**Discussion**

The present study demonstrates that acetylcholine induces endothelium-dependent contractions in isolated mesenteric resistance arteries of 4-week-old SHRs, whereas no such response was observed in WKY rats. Endothelium-dependent contractions have previously been demonstrated in mesenteric resistance arteries of adult, hypertensive SHRs.8,11-14 Contractions to acetylcholine were not observed in perfused (arteriograph system) SHR mesenteric arteries,6,10 suggesting that flow may downregulate the cyclooxygenase pathway. Endothelial dysfunction, characterized by production of one or more EDCFs in response to both acetylcholine and histamine, preceded an accelerated phase of blood pressure increase between weeks 6 and 10 in the SHR. Other investigators have described a “developmental phase” of hypertension noted between weeks 5 and 13 in SHRs.22-24 In the present study, mean systolic pressures were 6 mm Hg higher in SHRs than in WKY rats at 4 weeks of age (120±3 versus 114±3 mm Hg, respectively; p<0.06); at 10 weeks of age, systolic pressures were 46 mm Hg higher in SHRs (p<0.005). Are 4-week-old SHRs in fact “prehypertensive”? There are conflicting reports in published literature (for review, see Reference 25). In one of the most definitive studies addressing this issue, Lai et al.
leagues compared direct intra-arterial measurements of systolic and diastolic pressures in SHRs and WKY rats at 2–4 weeks of age. Indirect measurements of systolic pressure (tail-cuff method) were also compared with direct measurements in 4-week-old rats. By direct measurements, systolic and diastolic pressures did not differ in SHRs and WKY rats at 2 or 3 weeks of age; both values were significantly elevated in SHRs at 4 weeks of age. Values obtained from indirect measurements for systolic pressure of 4-week-old rats corresponded closely with those obtained from direct measurements (123±12 versus 131±3 mm Hg for SHRs; 114±6 versus 119±3 for WKY rats, respectively). Of interest for the present study, systolic pressures by the indirect method were not significantly different; in addition, the actual values were quite similar to those noted in the present study. Because significant increases in systolic pressures were noted only after 6 weeks of age in SHRs in the present study, we suggest that at 4 weeks of age, the SHRs were in fact “prehypertensive.”

Production of the EDCF before the development of hypertension suggests that the response is not a result of endothelial injury produced by hypertension but rather is related to a preexistent alteration or alterations of the cyclooxygenase pathway of SHR endothelial cells. Indeed, inhibition of cyclooxygenase or removal of the endothelium blocked the EDCF response to acetylcholine. The nature of the EDCF released by histamine awaits further studies.

A putative EDCF released by acetylcholine from SHR mesenteric resistance arteries reversed the endothelium-dependent relaxation induced by the muscarinic agonist. The latter response must be mediated by endothelium-derived nitric oxide, as it was prevented by L-NA and hemoglobin. After inhibition of cyclooxygenase, acetylcholine-mediated relaxations of SHR and WKY arteries were essentially identical. Thus, the impaired relaxations in SHR arteries were not due to a decreased production of nitric oxide but rather were due to the concomitantly released factor reversing its effects.

Endothelium-dependent contractions produced by acetylcholine have been demonstrated in canine basilar arteries, in the pulmonary artery of rabbits, in canine and human veins, in the aorta and renal artery of SHRs, and more recently in the aorta of very old WKY rats. Under all conditions, the contractile responses induced by acetylcholine were blocked by endothelium-dependent relaxations. We found no support for enhanced nitric oxide production by either cyclooxygenase inhibition or by AT enhanced nitric oxide-mediated relaxations. The inability of superoxide anions to penetrate the endothelial cells producing the superoxide radicals per se serve this function. Failure of superoxide dismutase to block the contractile response in SHR arteries may be due to the inability of this large molecule to penetrate the endothelial cells producing the superoxide radicals. Superoxide anions may alter endothelial function by several mechanisms. Superoxide accelerates the inactivation of nitric oxide, thereby opposing nitric oxide-mediated modulation of basal as well as activated vascular tone. In some vessels, superoxide serves as a vasoconstricting agent, seemingly independent of its effect on nitric oxide. Additionally, superoxide anions can react with nitric oxide radicals to form peroxynitrite. Peroxynitrite itself may serve as a damaging radical and also may be further metabolized to the highly reactive hydroxyl radicals. Endothelial-dependent responses attributed to hydroxyl radicals have been described in cerebral arteries of cats and in the aorta of rats. Thus, enhanced endothelial production of superoxide anions may impair endothelial and vascular smooth muscle function via multiple mechanisms. Based on results of studies in this report, the major effect of superoxide (EDCF) appears to be “inactivation” of nitric oxide: inhibition of superoxide production by either cyclooxygenase inhibition (indomethacin) or by AT enhanced nitric oxide-mediated relaxations.

The nature of the putative cyclooxygenase-dependent constricting substance released by acetylcholine from the endothelium of SHR mesenteric resistance arteries has been partially defined using specific inhibitors of cyclooxygenase products. Products of the cyclooxygenase pathway known to produce vascular contractions include prostaglandin H2, thromboxane A2, prostaglandin F2α, and in the rat, prostaglandin E2. Inhibition of thromboxane synthetase (CGS-13080) and blockade of prostaglandin H2/thromboxane A2 receptors (SQ-29,548) had no effect on the contractile response; thus, it is unlikely that either thromboxane A2 or prostaglandin H2 is involved. The rapidity with which the contractile responses to acetylcholine developed or disappeared tends to exclude prostaglandin F2α as the responsive factor. The inability of ketoconazole to block the contractile response suggests that products of the P-450 epoxygenase pathway, such as 5,6-epoxyeicosatrienoic acid or 20-hydroxyecosatetraenoic acid, which may be further metabolized to endoperoxide derivates via the cyclooxygenase pathway, are not responsible for the response. Inhibition of the contractions to acetylcholine in SHR arteries by AT, an agent that inhibits superoxide production by the cyclooxygenase pathway, suggests that superoxide anions may mediate the response. The present observations do not exclude the possibility that AT may function indirectly as a cyclooxygenase inhibitor. As pointed out below, the cyclooxygenase activity of prostaglandin endoperoxide synthase is activated by free radicals. AT may inhibit activation of cyclooxygenase indirectly by altering the production of free radicals required to activate cyclooxygenase. We have no direct evidence that superoxide radicals per se serve this function. Failure of superoxide dismutase to block the contractile response in SHR arteries may be due to the inability of this large molecule to penetrate the endothelial cells producing the superoxide radicals. Superoxide anions may alter endothelial function by several mechanisms. Superoxide accelerates the inactivation of nitric oxide, thereby opposing nitric oxide-mediated modulation of basal as well as activated vascular tone. In some vessels, superoxide serves as a vasoconstricting agent, seemingly independent of its effect on nitric oxide. Additionally, superoxide anions can react with nitric oxide radicals to form peroxynitrite. Peroxynitrite itself may serve as a damaging radical and also may be further metabolized to the highly reactive hydroxyl radicals. Endothelial-dependent responses attributed to hydroxyl radicals have been described in cerebral arteries of cats and in the aorta of rats. Thus, enhanced endothelial production of superoxide anions may impair endothelial and vascular smooth muscle function via multiple mechanisms. Based on results of studies in this report, the major effect of superoxide (EDCF) appears to be “inactivation” of nitric oxide: inhibition of superoxide production by either cyclooxygenase inhibition (indomethacin) or by AT enhanced nitric oxide-mediated relaxations. We found no support for enhanced production of hydroxyl radicals serving as the EDCF. Dimethylthiourea, an effective scavenger of hydroxyl radicals that readily enters the cellular milieu where reactive radicals are generated, as well as sodium salicylate and deferoxamine, all failed to block the contractile response or to significantly improve endothelium-de-
dependent relaxation. Similarly, the potentiation of the contractile response by L-NA, an inhibitor of nitric oxide production, suggests that peroxynitrite formation is not involved in the contractile response. The fact that acetylcholine did not evoke endothelium-dependent contractions in quiescent preparations, but reversed the relaxations to acetylcholine in precontracted vessels, would suggest that the radicals formed after activation of cyclooxygenase function by inactivation of nitric oxide rather than by production of direct contractions. Contrasting findings were noted in the aorta of the SHR, where endothelium-dependent contractions have been ascribed to the formation of prostaglandin H2, \(^{36,54}\) and where acetylcholine causes pronounced endothelium-dependent contractions in quiescent preparations. \(^{11,14,55}\) Contrasting findings are also noted in renal resistance arteries of the SHR, where acetylcholine induces cyclooxygenase- and endothelium-dependent contractions in precontracted arteries that are prevented by blockade of prostaglandin H2/thromboxane A2 receptors with SQ-29,548. \(^{55}\) A characteristic shared by the different contractile factors noted in SHR arteries is their cyclooxygenase dependence. It is conceivable that the explanation for the different mediators of the endothelium-mediated contraction in SHR arteries lies in differences in substrates for the prostaglandin H synthase reaction or in the capacities of the arteries to form nitric oxide.

Prostaglandin H synthase (prostaglandin endoperoxide synthase) is a hemoprotein that has two inseparable activities, namely, cyclooxygenase responsible for the oxidation of arachidonic acid to the hydroperoxide prostaglandin G2, and hydroperoxidase responsible for the peroxidation of prostaglandin G2 to prostaglandin H2. \(^{56-58}\) Free radical species formed by the peroxidase reaction (synthase tyrosyl radical), by abstracting an allylic hydrogen from the substrate polyunsaturated fatty acid, may initiate the cyclooxygenase reaction by producing a fatty acyl radical capable of reacting with molecular oxygen. \(^{59,59}\) Indomethacin inhibits cyclooxygenase by producing one or more perturbations of the hydroperoxide-induced radical species required to activate cyclooxygenase. \(^{59}\) Recent studies suggest that nitric oxide (or the derivative nitroxy anion) may function as an antioxidant that prevents activation of cyclooxygenase. \(^{60,61}\) Nitric oxide can form a nitric oxide–heme adduct with the iron atom of cyclooxygenase, thereby converting the ferric-enzyme to the ferrous-inactive form of the enzyme. The ferrous forms of cyclooxygenase are inactive and need to be activated to the ferric form by traces of hydroperoxides or hydrogen peroxide \(^{62}\) to function. Endothelial cells with limited production of nitric oxide may be less capable of regulating cyclooxygenase activity and indirectly the resulting endoperoxide (prostaglandin H2) and superoxide (or other radical) production. Prostaglandin H synthase can also generate superoxide radicals when acting on arachidonic acid in the presence of NADH or NADPH. \(^{56}\) Superoxide anions accelerate the breakdown of nitric oxide \(^{44,45}\) but may also combine with nitric oxide to produce the peroxynitrite anion (ONOO\(^{-}\)) \(^{47-50}\) \(^{52-53}\) and serve as precursors for hydroxyl radicals. \(^{48,49,51}\)

Membrane fatty acid metabolism is accelerated in SHR arteries \(^{52,62}\) point mutations in the phospholipase-\(\delta\) gene in SHRs may be causally related to the augmented phospholipase-\(\delta\) activity noted in SHRs. \(^{64}\) Augmentation of phospholipase-\(\delta\) activity, by increasing intracellular calcium, could favor vasoconstriction. Similarly, acetylcholine, by increasing cellular calcium, \(^{44}\) may further increase arachidonic and phospholipid metabolism in SHR endothelial cells. Products of the activated prostaglandin H synthase pathway may function directly as contractile factors (prostaglandin H2, thromboxane A2, superoxide radicals) or indirectly as contractile factors by inactivation of nitric oxide (superoxide anions). Acetylcholine in all likelihood does not serve as a physiological regulator of in vivo endothelial cell vasoactive metabolism. The relevance of the present in vitro studies arises from the fact that this muscarinic receptor agonist may serve as a useful probe for the examination of functional alterations in membrane receptors in SHR endothelial cells that may at least contribute to the development of hypertension in this genetic model.

In summary, endothelium-dependent relaxations induced by acetylcholine are impaired in mesenteric resistance arteries of prehypertensive as well as hypertensive SHRs. Acetylcholine releases an endothelium-derived factor that reverses the effects of nitric oxide in the SHR, whereas no such substance is produced in resistance arteries obtained from normotensive WKY rats. The production of this factor requires the activity of cyclooxygenase, as indomethacin normalizes endothelium-dependent relaxations in resistance arteries of the SHR. In contrast to the aorta or renal resistance arteries of SHRs, the cyclooxygenase product or products interfering with the effects of nitric oxide in SHR mesenteric arteries is not prostaglandin H2 or thromboxane A2 but rather are oxygen-derived free radicals that are most likely superoxide anions. Enhanced production of endothelium-derived free radicals in the SHR may contribute to the development of hypertension, especially because this defect precedes the accelerated increase in blood pressure in this genetic model of hypertension.

References

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