Decreased Endothelium-Dependent Hyperpolarization to Acetylcholine in Smooth Muscle of the Mesenteric Artery of Spontaneously Hypertensive Rats

Koji Fujii, Mitsuhiro Tominaga, Susumu Ohmori, Kazuo Kobayashi, Tokushi Koga, Yutaka Takata, and Masatoshi Fujishima

The endothelium-dependent vascular relaxation to acetylcholine (ACh) in spontaneously hypertensive rats (SHR) may be impaired because of an imbalance of endothelium-derived relaxing factor and contracting factor. However, the role of the endothelium-dependent hyperpolarization remains undetermined. We examined the ACh-induced hyperpolarization and its contribution to relaxation in arteries of SHR. Membrane potentials were recorded from the mesenteric artery trunk of 6–8-month-old male SHR and also Wistar-Kyoto (WKY) rats. Endothelium-dependent hyperpolarization to ACh was unaffected by Nω-nitro-L-arginine, indomethacin, or glibenclamide; was reduced by tetraethylammonium or high K+ solution; and was enhanced by low K+ solution or methylene blue, thereby indicating that hyperpolarization is not mediated by nitric oxide (endothelium-derived relaxing factor) but is presumably mediated by a hyperpolarizing factor and is due to an opening of K+ channels that probably differ from the ATP-sensitive ones. Hyperpolarizations to ACh were markedly reduced in SHR compared with findings in WKY rats (maximum, 8±1 versus 17±1 mV). In addition, under conditions of depolarization with norepinephrine (10−5 M), the ACh-induced hyperpolarization was even less and transient in SHR, while it was large and sustained in WKY rats (6±1 versus 29±2 mV). Endothelium-dependent relaxations to ACh in arterial rings precontracted with 10−5 M norepinephrine were far less in SHR than in WKY rats, even in the presence of indomethacin. Furthermore, high K+ solution showed smaller inhibitory effects on the relaxations in SHR than in WKY rats. Endothelium-independent hyperpolarizations and relaxations to cromakalim, a K+ channel opener, were similar between SHR and WKY rats. It would thus appear that the endothelium-dependent hyperpolarization to ACh is reduced in SHR and this would, in part, account for the impaired relaxation to ACh in SHR mesenteric arteries. (Circulation Research 1992;70:660–669)

KEY WORDS • membrane potential • endothelium-derived hyperpolarizing factor • acetylcholine • vascular smooth muscle • spontaneously hypertensive rats

Stimulation of arterial tissues with acetylcholine (ACh) results in membrane hyperpolarization of vascular smooth muscle cells, a response that requires the presence of intact endothelial cells.1–5 In a bioassay system, the perfusate or superfusate of the endothelium-intact vessel stimulated with ACh produced hyperpolarization in the denuded arterialized vessel,6,17 thereby suggesting that hyperpolarization is mediated by a humoral factor(s). Endothelium-dependent relaxation, first reported by Furchgott and Zawadzki,7 appears to be mainly mediated by endothelium-derived relaxing factor (EDRF),8 a compound perhaps identical to nitric oxide (NO) or a nitroso compound related to NO.9,10 However, endothelium-dependent vasorelaxants, including NO, do not necessarily elicit hyperpolarization.11–13 In addition, hyperpolarizations and accompanying increases in the rubidium-86 efflux rate produced by ACh were unaffected either by methylene blue or by hemoglobin,5,14 both inhibitors of the action of EDRF.15 These observations led to the suggestion that hyperpolarization is generated by an endothelium-derived substance that is distinct from NO/EDRF.4,14,16,17 Endothelium-dependent hyperpolarization may also contribute to relaxation by blunting voltage-dependent mechanisms.3,5 Endothelium-dependent vascular relaxation to ACh is impaired in patients with hypertension18 and in various models of experimental hypertension.19–22 However, in spontaneously hypertensive rats (SHR), Löscher et al.19,22 and our group23 have shown that, in certain arteries, relaxations induced by ACh normalized after exposure to indomethacin. It was therefore suggested that impaired endothelium-dependent relaxations to ACh in the SHR may be due to a simultaneous release of cyclooxygenase-dependent contracting factor rather than to a reduced release of EDRF. However, little is known concerning the possible role of endothelium-dependent hyperpolarization...
ization in hypertension. The present study was designed to evaluate endothelium-dependent hyperpolarization to ACh and its contribution to relaxation in arteries of SHR and Wistar-Kyoto (WKY) rats. Characteristics of endothelium-dependent hyperpolarization were also studied.

Materials and Methods
Preparation of Arteries
Six- to 8-month-old male SHR and WKY rats, which had been maintained in the Institute of Experimental Animals in Kyushu University, were used. Rats were fed standard rat chow and had free access to tap water. Systolic blood pressure was measured by the tail-cuff method. The rats were stunned by a blow to the head and then decapitated. The main branches of the superior mesenteric arteries were excised and placed on a plate containing cold Krebs' solution. The arteries were then cleaned of adherent connective tissues and cut into 3- and 1-mm rings for electrophysiological and tension experiments, respectively. In some rings, the endothelium was removed by gentle rubbing of the intimal surface with polyethylene tubing. The presence or absence of the endothelium was confirmed by scanning electron microscopic examinations.23

Membrane Potential Recording
Transverse strips prepared by cutting along the longitudinal axis of the rings were placed in an experimental chamber (capacity, 2 ml) with the endothelial layer up. Tissues were carefully pinned to the rubber bed fixed at the bottom of the chamber and were then superfused with 36°C Krebs' solution bubbled with 95% O2-5% CO2 (pH 7.3–7.4) at a rate of 3 ml/min and were allowed to equilibrate for at least 60 minutes before the start of the recordings. Membrane potential was recorded as described elsewhere.24 Briefly, glass capillary microelectrodes filled with 3 M KCl and with tip resistances of 50–80 MΩ and tip potentials of less than 4 mV were impaled into the smooth muscle cell from the endothelial side. Criteria for a successful impalement were an abrupt drop in voltage on entry of the microelectrode into the cell, stable membrane potential for at least 2 minutes, and a sharp return to zero on withdrawal of the electrode. Changes in membrane potentials produced by ACh, norepinephrine, and cromakalim were all measured from the continuous recordings and not by reimpalement of microelectrodes after addition of these agents. No attempt was made to simultaneously record mechanical responses. Electrical responses were monitored on an oscilloscope (VC-11, Nihon Kohden Co. Ltd., Tokyo) and recorded (RJG-4002, Nihon Kohden).

Force Measurements
Rings were placed in an experimental chamber (capacity, 2 ml). Two fine stainless-steel wires were placed through the lumen of the ring; one was anchored and the other was connected to the mechanotransducer (UL-10GR, Shinkoh Co. Ltd., Nagano, Japan). An optimal resting tension of 1.0 g was applied to the rings. The rings were superfused with Krebs' solution (36°C) and allowed to equilibrate for at least 60 minutes before the start of the recordings. The rings were repeatedly challenged with 40 mM KCl until the challenge gave no further increase in response; then the rings were exposed to 10-3 M norepinephrine, which produced a near maximum response in arteries of both strains of rats. After the contraction had reached a steady level, increasing doses of ACh or cromakalim were added to the bath to induce relaxation. Norepinephrine was applied, in most cases, for a total of three times with or without pretreatments with other agents, each application being separated by at least a 60-minute washout period. In such cases, similar magnitudes of contraction and relaxation were elicited by norepinephrine and ACh, respectively, in the time control study. Responses were displayed on a pen writing recorder (model 3056, Yokogawa Hokushin Electric, Japan).

Solutions and Drugs
The ionic millimolar composition of Krebs' solution was as follows: Na+ 137.4, K+ 5.9, Mg2+ 1.2, Ca2+ 2.5, HCO3- 15.5, H2PO4- 1.2, Cl- 134, and glucose 11.5. Concentrations of K+ were modified by replacing NaCl with KCl. Drugs used were ACh chloride, norepinephrine hydrochloride, indomethacin, N(G)-nitro-L-arginine (L-NNA), and glibenclamide (Sigma Chemical Co., St. Louis, Mo.); methylene blue and tetraethyiammonium chloride (Wako Pure Chemical, Osaka, Japan); N(G)-monomethyl-L-arginine (L-NMMA) (Calbiochem Corp., La Jolla, Calif.); and cromakalim (Beechem Pharmaceuticals, Harlow, UK). Indomethacin was dissolved in 10-2 M Na2CO3, cromakalim in 90% ethanol, L-NNA in 0.2N HCl, and glibenclamide in dimethyl sulfoxide. Other drugs were dissolved in distilled water. All drugs were further diluted by 1,000 times or more in normal or modified Krebs' solution to give final bath concentrations. Solvents used to dissolve drugs did not, by themselves, affect electrical and mechanical responses at their final bath concentrations.

Statistics
Results are given as mean±SEM. Dose–response curves of hyperpolarizations and relaxations were analyzed by two-way analysis of variance followed by unpaired Student’s t test. Other variables were compared using unpaired or paired Student’s t test. Values of p<0.05 were considered significant.

Results
Blood pressure, body weight, and resting membrane potential of the mesenteric artery trunks of 6–8-month-old SHR and WKY rats are summarized in Table 1. Resting membrane potentials of the superior mesenteric artery with an intact endothelium were slightly depolarized in SHR compared with those in age-matched WKY rats. Removal of the endothelium did not significantly affect the membrane potential; thus, the difference in the membrane potential between SHR and WKY rats was still evident. This would mean that the difference did not arise from any alteration in endothelial function. The reason for the difference is unclear.

ACh produced sustained hyperpolarizations of the smooth muscle membrane (Figures 1–3). Hyperpolarizations were abolished by the removal of the endothelium but were not affected by L-NNA (3×10-5 M) or L-NMMA (10-4 M) (data not shown), compounds that
inhibit the formation of NO from L-arginine25,26 (Figure 1). Methylene blue (3x10^-5 M), a soluble guanylate cyclase inhibitor, slightly augmented the hyperpolarizations, probably because of its membrane-depolarizing effect (Figure 1). Hyperpolarizations were reduced by 10 mM tetraethylammonium (a K+ channel blocker) or by high extracellular K+ (20 mM) solution in which the K+ equilibrium potential may be less negative than in normal K+ solution27 but were enhanced by low K+ solution (1 mM) in which the K+ equilibrium potential may be more negative than in normal K+ solution27 (Figure 2). Glibenclamide (10^-5 M), a specific blocker of ATP-sensitive K+ channels,28,29 had no effect on hyperpolarizations to ACh (Figure 2). These results may suggest that hyperpolarization is not mediated by NO/EDRF but presumably by another endothelium-derived substance and is caused by an opening of K+ channels that probably differ from the ATP-sensitive K+ channels.

Endothelium-dependent hyperpolarizations to ACh, applied at the resting state of the membrane, were significantly decreased in amplitude in the SHR compared with those in the WKY rats (Figures 3 and 4). Indomethacin (10^-5 M) did not affect hyperpolarizations to ACh. Thus, the difference between SHR and

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**TABLE 1. Body Weight, Blood Pressure, and Resting Membrane Potential in Spontaneously Hypertensive and Wistar-Kyoto Rats**

<table>
<thead>
<tr>
<th></th>
<th>No. of animals</th>
<th>Body wt (g)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Resting membrane potential of superior mesenteric artery (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td>With endothelium</td>
</tr>
<tr>
<td>SHR</td>
<td>24</td>
<td>316±6</td>
<td>239±4*</td>
<td>-47.2±0.7 (24)</td>
</tr>
<tr>
<td>WKY</td>
<td>25</td>
<td>339±7</td>
<td>146±2</td>
<td>-49.7±0.5 (28)</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. Rats were 6–8 months old. Numbers in parentheses indicate the number of cells from four to 10 animals. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

*p<0.001; **p<0.05 compared with values of age-matched WKY.
WKY rats was unaltered after treatment with indomethacin (Figure 4). In arteries without an endothelium, ACh produced virtually no change in the membrane potential regardless of the rat strain (Figure 4).

Norepinephrine (10^{-5} M) depolarized the membrane to a similar extent in the SHR and WKY rats (Figures 5A and 5B). However, in the presence of norepinephrine, ACh (10^{-3} M) elicited only a small and transient hyperpolarization in the SHR. Consequently, the norepinephrine-induced depolarization was, in large part, maintained (Figure 5A). On the other hand, in the WKY rats, ACh produced a large and sustained hyperpolarization and repolarized the membrane to a more negative level than the resting membrane potential (Figure 5A). Results from six animals in each strain are summarized in Figure 5B.

Arterial rings with an intact endothelium precontracted with 10^{-5} M norepinephrine relaxed dose dependently by increasing doses of ACh in arteries from WKY rats (Figure 6). On the other hand, only minimal relaxations were elicited in SHR tissues. Pretreatment with indomethacin markedly enhanced the relaxations to ACh in the SHR, indicating a simultaneous release of vasoconstrictor substances through cyclooxygenase pathway in the SHR (Figure 6). Relaxations to ACh in WKY tissues were also significantly, but to a much lesser extent, enhanced by indomethacin.30 The intriguing finding was that even in the presence of indomethacin, the magnitudes of relaxations to ACh were far less in the SHR than in the WKY rats (Figures 6 and 7). Presumably, another mechanism is linked to the impaired relaxation in the SHR in addition to the release of constrictor substances.

L-NNA or methylene blue applied in the presence of indomethacin enhanced the response to norepinephrine by about 20–50%, suggesting a basal release of EDRF
by norepinephrine. This enhancement tended to be larger in the WKY rats than in the SHR, but the difference did not reach statistical significance. Methyl-

![Graph showing hyperpolarizations to acetylcholine and effects of indomethacin (10^-5 M) and endothelium removal on acetylcholine-induced hyperpolarizations in the mesenteric artery of 6-8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Indomethacin was applied to the bath at least 30 minutes before the exposure to acetylcholine. Indomethacin (10^-5 M) had no effect on the membrane potential. *p<0.001 compared with WKY control; †p<0.05, ††p<0.001 compared with WKY with indomethacin.

![Figure 3. Hyperpolarizations produced by acetylcholine (ACh, 3×10^-4 M to 1×10^-3 M), applied at the resting state of the membrane, in the mesenteric artery with intact endothelium of 6-8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). All recordings for each strain were from the same preparation.](image-url)

eine blue, which inhibits the formation of cGMP, a mediator of EDRF-induced relaxation, lowered relaxations to lower doses of ACh (Figure 6) but had little effect on relaxations to higher doses of ACh (≥10^-6 M) (Figure 6). This effect of methylene blue might possibly be determined by the balance of the enhanced hyperpolarizations (Figure 2) and the inhibited production of cGMP by this agent shown in other studies. Higher doses (≥10^-5 M) of methylene blue were not used because they evoked large depolarizations and contractions. Because we did not measure vascular cGMP contents, incomplete inhibition of guanylate cyclase cannot be totally ruled out.

High K+ solution (20 mM) generally reduced relaxations to ACh in WKY rats but had little inhibitory effect on the relaxations in SHR (Figure 7). On the other hand, L-NNA markedly inhibited relaxations to ACh in SHR, in most cases leaving only small and transient relaxations, while in WKY rats, relaxations to higher doses of ACh were only partially inhibited by L-NNA. Consequently, in the presence of L-NNA together with indomethacin, relaxations to ACh were markedly smaller in SHR than in WKY rats (Figure 7). The combined application of 20 mM K+ solution and L-NNA virtually abolished relaxations to ACh in both strains (Figure 7).

Cromakalim, a K+ channel opener, produced endothelium-independent hyperpolarization of the smooth muscle membrane, which was virtually abolished by 10^-3 M glibenclamide (data not shown). Hyperpolarizations to cromakalim (10^-5 M) were almost identical between SHR and WKY rats (Figure 8) (30.0±2.0 mV [n=4] for SHR versus 29.0±1.9 mV [n=4] for WKY rats). Cromakalim (10^-2 M) also induced similar relaxations in SHR and WKY vessels precontracted with 10^-5 M norepinephrine (89.6±2.5% of preexisting tension [n=6] for SHR, 89.9±1.7% [n=6] for WKY rats).

**Discussion**

We report here the first evidence that endothelium-dependent hyperpolarizations to ACh were decreased...
in the mesenteric artery of adult SHR compared with findings in age-matched WKY rats. Endothelium-dependent relaxations to ACh were also reduced in the SHR even in the presence of indomethacin, which blocks the formation of cyclooxygenase-dependent substances. High K+ solution (20 mM), which reduces hyperpolarizations to ACh, showed greater inhibitory effects on relaxations to ACh in WKY rats than in SHR. On the other hand, L-NNA markedly inhibited relaxations to ACh in SHR and to a lesser extent in WKY rats.

**Figure 5.** Panel A: Hyperpolarization to acetylcholine (ACh, 10^{-5} M) applied in the presence of norepinephrine (10^{-5} M) in the mesenteric artery of 6–8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Note a small and transient hyperpolarization in SHR and a large and sustained one in WKY. Panel B: Bar graph summarizing the degree of depolarizations to norepinephrine (10^{-5} M) and hyperpolarizations to ACh (10^{-5} M) in the presence of norepinephrine. *p<0.001 compared with WKY.

**Figure 6.** Graphs showing relaxations induced by increasing doses of acetylcholine and effects of indomethacin (10^{-5} M) and methylene blue (3x10^{-6} M) on acetylcholine-induced relaxations in the mesenteric artery of 6–8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Relaxations were expressed as percentages of the 10^{-5} M norepinephrine-induced contraction. Before the second challenge of norepinephrine, indomethacin was present in the bath for at least 30 minutes. In addition, methylene blue was added to the bath 15 minutes before the third challenge of norepinephrine. *p<0.005, **p<0.001 compared with WKY with indomethacin; †p<0.01, ††p<0.001 compared with WKY with indomethacin+methylene blue; ‡p<0.05 compared with values with indomethacin of each strain.
periods indicated was M) L-NNA (3×10⁻⁵ M), but with control in 6–8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Relaxations were expressed as percentages of the 10⁻² M norepinephrine–induced contraction. Indomethacin (10⁻⁵ M) was present throughout the experiment. Before the second challenge of norepinephrine, either high K⁺ solution (20 mM) or L-NNA (3×10⁻⁵ M) was applied to the bath for 15 minutes. Before the third challenge of norepinephrine, both high K⁺ solution (20 mM) and L-NNA (3×10⁻³ M) were applied to the bath for 15 minutes. High K⁺ (20 mM) solution did not, by itself, produce any contractions but enhanced the norepinephrine-induced contractions by about 10% in both SHR and WKY. *p<0.005, **p<0.001 compared with control (+indomethacin) of each strain; †p<0.05, ††p<0.001 compared with control (+indomethacin) of each strain.

Figure 7. Graphs showing relaxations induced by increasing doses of acetylcholine and effects of high K⁺ solution (20 mM), N⁰-nitro-L-arginine (L-NNA, 3×10⁻⁵ M), and combined application of both the solution and agent on acetylcholine-induced relaxations in the mesenteric artery of 6–8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Relaxations were expressed as percentages of the 10⁻² M norepinephrine–induced contraction. Indomethacin (10⁻⁵ M) was present throughout the experiment. Before the second challenge of norepinephrine, either high K⁺ solution (20 mM) or L-NNA (3×10⁻⁵ M) was applied to the bath for 15 minutes. Before the third challenge of norepinephrine, both high K⁺ solution (20 mM) and L-NNA (3×10⁻³ M) were applied to the bath for 15 minutes. High K⁺ (20 mM) solution did not, by itself, produce any contractions but enhanced the norepinephrine-induced contractions by about 10% in both SHR and WKY. *p<0.005, **p<0.001 compared with control (+indomethacin) of each strain; †p<0.05, ††p<0.001 compared with control (+indomethacin) of each strain.

Figure 8. Hyperpolarizations produced by cromakalim (10⁻³ M) in the mesenteric artery with intact endothelium of 6–8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Cromakalim was applied for the periods indicated by horizontal bars.

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**Figure 8.** Hyperpolarizations produced by cromakalim (10⁻³ M) in the mesenteric artery with intact endothelium of 6–8-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Cromakalim was applied for the periods indicated by horizontal bars.
depolarizing substance(s) by ACh; 2) a direct depolarizing effect of ACh on the smooth muscle; 3) reduced responsiveness of the smooth muscle to hyperpolarizing agents; 4) impaired synthesis, release, or diffusion of a putative endothelium-derived hyperpolarizing factor; and finally 5) hyperpolarization is mediated by a mechanism other than a humoral factor(s) and this process is impaired. In the SHR, there appeared to be, indeed, an enhanced release of cyclooxygenase-dependent products by ACh. However, hyperpolarizations to ACh were not affected by indomethacin, thereby excluding the possibility of a release of cyclooxygenase products being capable of producing depolarization. Involvement of other endothelium-derived substances, such as endothelin, cannot be excluded, however unlikely from the time course of the response.

In vascular tissues without an endothelium, ACh produced virtually no change in the membrane potential of either rat strain used. This means that the direct depolarizing effect of ACh on the smooth muscle is unlikely to explain the reduced hyperpolarization in the SHR. Endothelium-independent hyperpolarizations to cromakalim were identical between SHR and WKY rats. Thus, the responsiveness of smooth muscle cells to hyperpolarizing agents did not seem to be decreased in the SHR. This possibility, however, cannot be totally dismissed because K+ channels responsible for hyperpolarization to ACh appeared to be distinct from those to cromakalim, as judged by the sensitivity to glibenclamide. Endothelial cells themselves could also hyperpolarize in response to ACh. This raised the possibility of electrotonic transmission of hyperpolarization from endothelial cells to the underlying smooth muscle cells via gap junctions and impairment of this process in hypertension. However, clear evidence that hyperpolarization to ACh was transferable by a humoral factor(s) and the recent report indicating poor electrical and dye couplings between endothelial and smooth muscle cells make this possibility unlikely. Taken together, we prefer to attribute decreased hyperpolarization in the SHR to impaired synthesis, release, or diffusion of a hyperpolarizing factor.

Endothelium-dependent relaxations to ACh were also markedly impaired in the SHR, even in the presence of the cyclooxygenase inhibitor indomethacin. Hyperpolarization per se has been shown to be an important mediator of smooth muscle relaxation of the monkey coronary artery. We propose that the impaired relaxation to ACh in the SHR is, at least in part, due to the decreased endothelium-dependent hyperpolarization. First, the maintained phase of norepinephrine-induced contractions has been shown to be mainly dependent on Ca2+ entry through voltage-dependent Ca2+ channels. In the mesenteric artery of both SHR and WKY rats, 10–5 M norepinephrine produced a potent depolarization, presumably activating voltage-dependent Ca2+ channels. In the WKY rats, ACh hyperpolarized the membrane to a level more negative than the resting membrane potential, most likely closing voltage-dependent Ca2+ channels. On the other hand, in the SHR, because hyperpolarization to ACh was small and transient, the greater part of norepinephrine-induced depolarization persisted. Assuming that a similar change in membrane potential is occurring under conditions used in the tension experiment, this difference in membrane potential between SHR and WKY rats should result in a substantial difference in the level of voltage-dependent Ca2+ influx, thereby in tension.

Second, in the mesenteric artery of normotensive WKY rats, endothelium-dependent relaxations to higher doses of ACh were only partially inhibited by L-NNA, a potent inhibitor of NO formation from l-arginine, while relaxations to ACh were generally reduced by high K+ solution, which reduced hyperpolarizations to ACh. A similar resistance of ACh-induced vasodilatation to L-NMMA or L-NNA was also noted by other investigators. The combined application of high K+ solution and L-NNA virtually abolished relaxations. L-NNA was without effect on ACh-induced hyperpolarizations in this artery. It would thus appear that the relaxation to ACh in the rat mesenteric artery is mediated through both the l-arginine pathway and hyperpolarization. In SHR arteries, the effect of high K+ solution on relaxations was of little significance, while that of blocking the l-arginine pathway by L-NNA appeared to be more pronounced. Furthermore, the differences in relaxation between SHR and WKY rats were still evident in the presence of L-NNA. These results suggest that it is the hyperpolarization-induced component of relaxation that is mainly attenuated in SHR.

Third, the smooth muscle cells of SHR mesenteric arteries may be able to relax to a greater extent than that achieved with ACh in response to hyperpolarization, because the near maximal relaxations to 10–5 M cromakalim, which mostly depended on hyperpolarization, were the same between SHR and WKY rats. These observations, overall, led to the notion that the impaired endothelium-dependent relaxation to ACh in the SHR is, at least in part, caused by the decreased hyperpolarization.

Previous studies on the SHR mostly indicated that after the cyclooxygenase inhibition, endothelium-dependent relaxations to ACh are not impaired. The discrepancy in the results may arise from several factors. First, dependence of the contraction on the voltage-dependent mechanism might be greater in our experiments because we used a dose of norepinephrine that produced a potent depolarization. The contribution of hyperpolarization to relaxation may increase when voltage-dependent Ca2+ influx is high. Second, the degree and time course of hyperpolarization to ACh itself may well differ from tissue to tissue. Thus, the overall contribution of hyperpolarization to relaxation might vary depending on the experimental condition and could be surprisingly large as noted in the present study.

It remains to be clarified whether the impaired hyperpolarization to ACh in the adult SHR arteries might be a cause or a result of hypertension. Age-related changes in hyperpolarization to ACh are currently under investigation. Nevertheless, if endothelium-dependent hyperpolarization does play a physiological role, the diminished protective effect of this mechanism against depolarization could have a crucial influence on the course of hypertension, particularly in view of the experimental evidence of pressure-dependent depolarization of the vascular smooth muscle membrane. Responses to platelet-derived substances as well as responses in resistance vessels are also under investigation.
In conclusion, endothelium-dependent hyperpolarization to ACh, presumably mediated by an endothelium-derived hyperpolarizing factor, is reduced in the mesenteric artery of adult SHR. Endothelium-dependent relaxation to ACh is also impaired. We suggest that a decreased hyperpolarization may account, at least in part, for the impaired relaxation to ACh in SHR mesenteric arteries.

Acknowledgments

We thank Beecham Pharmaceuticals, Harlow, UK, for the gift of cromakalim and Miss M. Ohara for helpful comments.

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_Circ Res._ 1992;70:660-669
doi: 10.1161/01.RES.70.4.660

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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