Activation of Endothelial L-Arginine Pathway in Resistance Arteries
Effect of Age and Hypertension

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In conduit arteries, nitric oxide is formed from L-arginine in the endothelium and released after stimulation with acetylcholine. The contribution of the L-arginine pathway and the effects of age and hypertension on endothelium-dependent vascular regulation were studied, using a video dimension analyzer, in pressurized and perfused mesenteric resistance arteries of 8- and 16–20-week-old Wistar-Kyoto and spontaneously hypertensive rats. Norepinephrine and phenylephrine caused contractions, which were similarly augmented after removal of the endothelium. N°-Monomethyl-L-arginine, an inhibitor of nitric oxide formation, augmented the contraction, but less than endothelial removal. Acetylcholine caused endothelium-dependent relaxations that were much more pronounced with intraluminal than with extraluminal application. N°-Monomethyl-L-arginine, methylene blue, and hemoglobin only partially inhibited the response. With aging, the endothelium-dependent inhibition of the response to norepinephrine decreased in Wistar-Kyoto rats; in spontaneously hypertensive rats this inhibition was smaller as compared with age-matched Wistar-Kyoto rats. In Wistar-Kyoto rats, the difference between intraluminal and extraluminal activation became more pronounced in adult rats. In the adult but not the young spontaneously hypertensive rats, the response to intraluminal but not extraluminal acetylcholine was reduced as compared with Wistar-Kyoto rats. Thus, in mesenteric resistance arteries of the rat, nitric oxide is released from L-arginine under basal conditions and after stimulation with acetylcholine but only in part accounts for endothelium-dependent responses. With aging and hypertension, the inhibitory effects of the endothelium against norepinephrine-induced contractions decrease. In hypertension, the intraluminal but not extraluminal activation of the release of endothelium-derived relaxing factors is impaired. (Hypertension 1990;15:170–179)

The endothelium covers the inner surface of all blood vessels and has been recognized to play an important role in the local regulation of vascular smooth muscle tone. In 1980, Furchgott and Zawadzki reported that the relaxations evoked by acetylcholine in the aorta of the rabbit depend on the presence of endothelial cells. Besides acetylcholine many other vasodilators or physical stimuli trigger endothelium-dependent relaxations of different species including humans. The endothelium also can reduce the effects of vasoconstrictor substances either through the basal or (as in the case of norepinephrine and serotonin in certain blood vessels) stimulated release of endothelium-derived relaxing factor. Recently, evidence has been provided that nitric oxide may account for the biological activity of endothelium-derived relaxing factor; indeed, in the intact rabbit aorta nitric oxide is released after stimulation with acetylcholine. The most likely precursor from which nitric oxide is formed in endothelial cells is L-arginine. In the rabbit and rat aorta and in certain large human arteries, endothelium-dependent relaxations to acetylcholine can be inhibited by N°-monomethyl-L-arginine (L-NMMA) and restored by the addition of L-arginine. These observations imply that the formation of nitric oxide in endothelial cells may play a role in vascular regulation. However, little is known about the endothelial regulation of small resistance arteries and whether differences exist in the intraluminal and extraluminal activation of the endothelial cells.
In the thoracic aorta of genetically hypertensive rats, endothelium-dependent relaxations to acetylcholine are depressed. An altered endothelial regulation of hypertensive blood vessels would be of primary pathophysiological importance if it would occur in that part of the circulation where peripheral vascular resistance is regulated. The present study in pressurized and perfused mesenteric resistance arteries of the rat was designed to evaluate: 1) the importance of the L-arginine pathway as a mediator of endothelium-dependent relaxations, 2) differences between intraluminal and extraluminal activation of the endothelium, and 3) the effects of age and hypertension on these responses.

Methods

Experimental Animals

Male Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), 8 (young) or 16–20 weeks (adult) of age, were obtained from Charles River Wiga GmbH, Sulzfeld, FRG. Systolic blood pressure was measured in conscious rats by the tail-cuff method. An average of three readings was assessed; values above 200 mm Hg were read as 200 mm Hg. Blood pressure was 121±3 mm Hg and 134±3 mm Hg in 8- and 16–20-week-old WKY rats and 147±4 mm Hg and 187±3 mm Hg in 8- and 16–20-week-old SHR, respectively (p<0.005 versus age-matched WKY rats).

Experimental Setup

In rats anesthetized with pentobarbital (50 mg/kg i.p.), the entire mesenterium was removed and placed into cold modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118.0, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, edetate calcium disodium 0.026, glucose 11.1 (Krebs solution). A 3–4 mm long segment of the third branch of the mesenteric artery was carefully dissected and cleaned of adhering adipose tissue under a dissection microscope. The artery was transferred to an arteriograph chamber filled with oxygenated (95% O2 and 5% CO2) Krebs solution. Krebs solution circulated from a 300 ml oxygenated reservoir through the arteriograph chamber at a flow rate of 50 ml/min. Temperature was continuously monitored to maintain the vessel environment at 37±0.5°C.

The chamber contained two glass microcannulas, of which one was fixed (afferent cannula) and the other was mounted on a manipulator to facilitate positioning (afferent cannula) (Figure 1). The proximal end of the artery was cannulated with the afferent cannula and secured with a surgical nylon suture (diameter 25 μm). The distal end was attached to the inside of the efferent cannula. The artery was perfused with Krebs solution containing 1.0% albumin from bovine serum. A small catheter was located in the afferent cannula and was connected to a pressure transducer for measurement of transmural pressure. In some experiments, drugs were administered intraluminally through this catheter (Figure 1). Flow through the vascular segment was measured repeatedly at the efferent cannula and the concentrations of the drugs required were calculated and adapted accordingly under each experimental condition.

In some experiments, the endothelium was removed by intraluminal perfusion with 0.5% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) for 30 seconds. The presence or absence of the endothelium was confirmed by the presence or absence of a relaxation to acetylcholine (10-6 to 10-5 M). The contractions evoked by 100 mM KCl before and after removal of the endothelium did not differ either in WKY rats (71±2% and 73±1% decrease in intraluminal diameter, respectively; n=6) or in SHR (71±2% and 71±3%, respectively; n=6; NS).

The arteriograph was placed on a stage of a microscope that had a video camera attached to the viewing tube. The signal derived from the video image of the vessel was processed by an electronic system (Living Systems Instrumentation, Burlington, VT) for continuous measurement and recording of intraluminal diameter and wall thickness.

Arterial responses were measured at a transmural pressure of 30 mm Hg. This pressure was found to be optimal for contraction of the mesenteric resistance arteries in all groups of rats as assessed by repeated exposures to 100 mM KCl at various transmural pressures (15–120 mm Hg). The intraluminal flow rate was 0.2–0.9 ml/min.

After 1 hour of equilibration, the arteries were contracted with 100 mM KCl several times at 30-minute intervals until successive contractions remained constant. Some arteries were perfused intraluminally with 1) indomethacin (10-3 M, 30 minutes) to block the vascular production of prostaglandins, 2) L-NMMA
(10^{-4} \text{ M} \text{ or} \ 10^{-3} \text{ M}, 30 \text{ minutes}) \text{ to inhibit the endoge-}

nous production of nitric oxide from \text{l}-arginine,^{15-19} 3) \text{ hemoglobin (10^{-3} \text{ M}, 10 \text{ minutes}) \text{ to inactivate nitric}

oxide,^{5,20,21}\text{ or} \ 4) \text{ methylene blue (3\times10^{-7} \text{ M}, 30 \text{ minutes})}

to inhibit soluble guanylate cyclase.^{22,23} \text{ Higher concentrations (}>3\times10^{-7} \text{ M}) \text{ of methylene blue}

resulted in a complete loss of vascular reactivity of the blood vessels (data not shown). \text{ All inhibitors used were present in}

the perfusion solution throughout the experiments.

**Protocols**

Concentration–response curves to norepinephrine were obtained in each artery by extraluminal cumulative application of the drug. The contraction was expressed as percent decrease in intraluminal vascular diameter. The diameter at the resting level was taken as 100%. To study relaxations, arteries were precontracted by a half-maximal concentration of norepinephrine (ED_{50} value). When a steady contraction was established, acetylcholine was added intraluminally or extraluminally in a cumulative manner and changes in intraluminal diameter were measured. The relaxation was expressed as percent increase in the intraluminal diameter obtained during the contraction to norepinephrine.

**Drugs**

The following drugs were used: acetylcholine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (Sigma), indomethacin (Sigma), methylene blue (Auer Bittman Soulié AG, Basel, Switzerland), \text{N}^0\text{-monomethyl-L-arginine acetate (Calbiochem Cor-}

poration, Lucerne, Switzerland), and \text{l}-norepinephrine and phenylephrine (Sigma).

**Calculations and Statistics**

Data are given as mean±SEM. The concentration of an agonist causing half maximal contraction or relaxation was calculated for each experiment separately and expressed as negative log molar (pD\text{2} value). In each set of experiments, \( n \) equals the number of animals studied. Statistical evaluation was done by paired and unpaired Student’s \( t \) test and by analysis of variance followed by Scheffe’s \( F \) test. Means were considered significantly different when the \( p \) value was less than 0.05.

**Results**

**Vascular Dimensions in Normotensive and Hypertensive Rats**

The intraluminal diameter and wall thickness of the studied vessels were 207±6 \text{ \mu m} \text{ and} \ 33±1 \text{ \mu m} \text{ in} 16-20\text{-week-old WKY rats and 198±6 \mu m} \text{ and} \ 27±1 \text{ \mu m} \text{ in} 8\text{-week-old WKY rats, respectively. In both age groups, the wall thickness was greater in SHR (16-20-week-old, 39±1 \mu m; 8-week-old, 31±1 \mu m; \( p<0.005 \text{--}0.05 \) than in that of age-matched WKY rats, whereas the intraluminal diameter of SHR (16-20-week-old, 195±5 \mu m; 8-week-old, 185±7 \mu m) was not significantly different from that of age-matched WKY rats.

**Endothelium-Dependent Responses in Normotensive Rats**

In quiescent mesenteric resistance arteries with endothelium, \text{l}-NMMA (10^{-4} \text{ to} \ 10^{-3} \text{ M}) \text{ did not cause con-}

tractions (\( n=8 \), data not shown).

**Norepinephrine**

In mesenteric resistance arteries with endothelium of 16–20-week-old WKY rats, intraluminal application of norepinephrine (3\times10^{-7} \text{ and} \ 10^{-6} \text{ M}) \text{ evoked significantly greater con-}

tractions than extraluminal application of the catecholamine (Figure 2, left panel; \( n=6; \ p<0.01 \)). Removal of the endothelium significantly augmented the contractions to norepinephrine both with intraluminal and extraluminal application (Figure 2, right panel and Figure 3, left panel). The pD\text{2} value of extraluminal norepinephrine (3\times10^{-7} \text{ to} \ 10^{-4} \text{ M}) \text{ was 5.7±0.1}

before and 6.1±0.1 after removal of the endothelium (concentration shift, 2.9-fold; \( p<0.005 \)). The maximal response was smaller in the presence (74±3%) than in the absence of the endothelium (Figure 3, left panel; 87±1%; \( p<0.01 \)).

Increasing concentration of \text{l}-NMMA enhanced the maximal response to extraluminal norepineph-}

rine in arteries with endothelium from 59±3% to 78±5% (10^{-4} \text{ M}; \ p<0.01) and 74±1% (10^{-3} \text{ M}; \text{p}<0.05 versus control), respectively (Figure 4, left panel). Although the pD\text{2} value of norepinephrine was not changed by the inhibitor, the area under the concentration–response curve was increased by \text{l}-NMMA 10^{-4} \text{ M} (\( p<0.05 \)) and 10^{-3} \text{ M} (\( p<0.005 \)). In contrast, indomethacin did not affect the contrac-}

tions (\( n=4 \), data not shown).

**FIGURE 2.** Bar graphs showing effects of intraluminal and extraluminal application of norepinephrine in rat mesenteric resistance arteries with endothelium (left panel) or without endothelium (right panel). Contractions were significantly more pronounced in preparations without as compared with those with endothelium (\( p<0.005 \)). Intraluminal application of the catecholamine evoked significantly greater contractions than extraluminal application of norepinephrine in preparations both with and without endothelium. (**p<0.05; ***p<0.005)
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Figure 3. Line graphs showing contractions induced by extraluminal norepinephrine in pressurized mesenteric resistance arteries with and without endothelium obtained from 16-20-week-old Wistar-Kyoto (WKY) rats (left panel) and spontaneously hypertensive rats (SHR) (right panel). In both animals, removal of endothelium significantly augmented contractions induced by norepinephrine (p<0.05 to 0.005). Shift caused by removal of endothelium was smaller in SHR (1.4-fold) than in WKY rats (2.9-fold).

Figure 4. Line graphs showing effects of N\(^{\text{3}}\)-monomethyl-L-arginine (L-NMMA) on the contractions induced by extraluminal norepinephrine in pressurized mesenteric resistance arteries from 16-20-week-old Wistar-Kyoto (WKY) rats (left panel) and spontaneously hypertensive rats (SHR) (right panel). In both animals, L-NMMA augmented the response to norepinephrine (p<0.05 to 0.003). Enhancement of maximal response to norepinephrine was smaller in SHR than in WKY rats (p<0.05).

Figure 5. Bar graphs showing inhibitory effects of the endothelium against contractions induced by intraluminally applied norepinephrine (left panel) or phenylephrine (right panel). Contractions to both α-adrenergic agonists were significantly augmented in absence as compared with presence of endothelium. Augmentation of contractions induced by endothelium removal was comparable under both conditions. WKY, Wistar-Kyoto rats. *p<0.05; **p<0.005.

Figure 6. Tracings of endothelium-dependent relaxations to extraluminal acetylcholine in pressurized mesenteric resistance artery obtained from 16-20-week-old Wistar-Kyoto rats. In preparations contracted with norepinephrine (NE), acetylcholine evoked potent relaxations in the presence of the endothelium (top panel; vascular diameter, 225 μm) but not in the absence of the endothelium (lower panel; vascular diameter, 201 μm). The inhibitor of nitric oxide formation N\(^{\text{3}}\)-monomethyl-L-arginine (L-NMMA) inhibited but did not prevent relaxations induced by muscarinic agonist (middle panel).

Phenylephrine. Intraluminally applied phenylephrine (10\(^{-6}\), 3x10\(^{-7}\), and 10\(^{-6}\) M) evoked concentration-dependent contractions that were also significantly augmented after removal of the endothelium (Figure 5, right panel; p<0.05-0.005; n=5). The effect of endothelium removal on the contractions induced by phenylephrine was comparable to that obtained when norepinephrine (10\(^{-7}\), 3x10\(^{-7}\), and 10\(^{-6}\) M) was used to contract the blood vessels (Figure 5, left panel; p<0.005; n=5-6).

Endothelium-dependent relaxation

Extraluminal activation. In 16-20-week-old WKY rats, extraluminal acetylcholine (10\(^{-9}\) to 10\(^{-6}\) M) caused concentration-dependent relaxation in resis-
FIGURE 7. Line graphs showing effects of intraluminally applied \textit{N}^6\textit{monomethyl-L-arginine (L-NMMA) on endothelial-dependent relaxations to extraluminal acetylcholine in pressurized mesenteric resistance arteries obtained from 16-20-week-old Wistar-Kyoto (WKY) rats (left panel) and spontaneously hypertensive rats (SHR) (right panel). Increasing concentrations of L-NMMA inhibited but did not fully prevent response to acetylcholine in both animals (p<0.05-0.005).

FIGURE 8. Line graphs showing effects of intraluminally applied hemoglobin on endothelial-dependent relaxations induced by extraluminal acetylcholine in pressurized mesenteric resistance arteries of Wistar-Kyoto (WKY) rats (left panel) and age-matched spontaneously hypertensive rats (SHR) (right panel). Hemoglobin reduced relaxations to acetylcholine in both animals (concentration shift 2.5-fold; p<0.05) but did not prevent the response.

FIGURE 9. Line graphs showing effects of intraluminally applied methylene blue (3×10^{-7} M) on endothelial-dependent relaxations to extraluminal acetylcholine in pressurized mesenteric resistance arteries of 16-20-week-old Wistar-Kyoto (WKY) rats (left panel) and age-matched spontaneously hypertensive rats (SHR) (right panel). Methylene blue significantly reduced sensitivity and maximal response to muscarinic agonist (p<0.05-0.01) but did not prevent relaxation in both rat strains.

Effect of Age in Normotensive Rats

Contraction to norepinephrine. In arteries without endothelium, the contractions to norepinephrine did not differ in 8-week-old and 16-20-week-old WKY rats. The presence of the endothelium inhibited the contraction to norepinephrine in young WKY rats (pD_{2} value, 6.7±0.2; concentration shift, 7.1-fold; p<0.05 versus control) as 10^{-3} M L-NMMA and reduced the relaxation at the highest concentration of the muscarinic agonist (57±11%; p<0.005 versus control; p<0.01 versus 10^{-4} M L-NMMA). The sensitivity to acetylcholine was reduced by hemoglobin (10^{-5} M; pD_{2} value, 7.2±0.1 and 6.8±0.1; concentration shift, 2.5-fold; p<0.05; Figure 8, left panel). Methylene blue (3×10^{-7} M) caused a similar change in the sensitivity (pD_{2} value, 7.6±0.2 and 6.8±0.2; concentration shift, 6.3-fold; p<0.05) and reduction in the relaxation at 10^{-4} M acetylcholine (97±2% and 62±10%; p<0.05) as L-NMMA at 10^{-3} M (Figure 9, left panel). The concentration shift at pD_{2} value caused by either L-NMMA, hemoglobin, or methylene blue was not statistically different.

Intraluminal activation. Intraluminal acetylcholine caused more pronounced relaxations than did extraluminal acetylcholine (Figure 10, left panel). The pD_{2} value was 8.6±0.1 with intraluminal and 7.4±0.2 with extraluminal application of the muscarinic agonist (concentration shift, 13.5-fold; p<0.005) and the maximal response averaged 98±1% and 88±3%, respectively (p<0.05).
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FIGURE 10. Line graphs showing effects of extraluminal and intraluminal acetylcholine in pressurized mesenteric resistance arteries contracted with norepinephrine. In both Wistar-Kyoto (WKY) rats (left panel) and spontaneously hypertensive rats (SHR) (right panel), intraluminal activation of the endothelium caused a strikingly more powerful relaxation (concentration shift, 13.5-fold and 5.4-fold). Relaxations obtained with extraluminal activation of the endothelium was comparable in both animals, whereas the endothelium-dependent relaxations obtained with intraluminal activation were reduced in SHR as compared with WKY rats (p<0.05).

inhibition of the maximal response, however, was more pronounced in young (Figure 11, left panel; 83±1% and 63±2%; p<0.005) than in adult WKY rats (Figure 3, left panel; 87±1% and 74±3%).

Endothelium-dependent relaxation. As in adult rats, in young WKY rats the relaxation induced by intraluminal acetylcholine occurred at a lower concentration and was more pronounced than the relaxation induced by extraluminal acetylcholine (pD2 value, 8.2±0.1 and 7.4±0.2; p<0.005), whereas the maximal response did not differ (98±1% and 99±1%; NS) under both conditions (Figure 12, left panel). However, the concentration shift at pD2 value between the relaxations induced by intraluminal and extraluminal acetylcholine was greater in adult WKY rats (Figure 10, left panel; concentration shift, 13.5-fold) than in young WKY rats (Figure 12, left panel; 6.6-fold; p<0.05). This difference was due to a small shift to the left of the concentration–response curve with intraluminal acetylcholine, whereas the response to extraluminal activation did not change. The maximal response to extraluminal acetylcholine was greater in young rats (99±1%) as compared with adult WKY rats (88±3%; p<0.01), whereas the maximal response to intraluminal acetylcholine did not differ.

Endothelium-Dependent Responses in Hypertensive Rats

Contraction to norepinephrine

Endothelium removal. In adult SHR, removal of endothelium only minimally augmented the contractions to norepinephrine (Figure 3, right panel). The pD2 value was 5.7±0.1 and 5.8±0.1 in the presence and absence of the endothelium, and the maximal response averaged 76±3% and 83±2%, respectively (NS). The area under the concentration–response curve, however, was increased slightly after removal of the endothelium (p<0.05). The shift caused by removal of endothelium was smaller in SHR (1.4-fold) than in WKY rats (2.9-fold).

L-NMMA. The maximal responses to norepinephrine of the arteries with endothelium in adult SHR was also enhanced by treatment with L-NMMA 10-4 M (74±1% and 78±1%; p<0.05) and 10-3 M (80±2%; p<0.05) (Figure 4, right panel). The
enhancement of the maximal responses was smaller in adult SHR than in age-matched WKY rats (p<0.05). Indomethacin did not affect the contraction (n=4, data not shown).

Endothelium-dependent relaxation

Extraluminal activation. Extraluminally applied acetylcholine induced concentration-dependent relaxation of arteries of adult SHR, and the relaxation was partially inhibited by L-NMMA (Figure 7, right panel). The area under the curve was significantly smaller in the presence of 10^{-4} (p<0.05 and 10^{-3} M L-NMMA (p<0.01) than under control conditions. The maximal response to acetylcholine was also reduced by 10^{-7} M L-NMMA from 96±2% to 82±6% (p<0.05). The inhibitory effect of L-NMMA tended to be smaller in adult SHR (concentration shift at pD_2 value by 10^{-3} M L-NMMA, 2.3-fold) than in age-matched WKY rats (13.5-fold; p<0.05). This was due to reach statistical significance. Hemoglobin (10^{-5} M) reduced the sensitivity to acetylcholine in adult SHR (pD_2 value, 7.4±0.1 and 7.0±0.1; concentration shift, 2.5-fold; p<0.05; Figure 8, right panel) but not the maximal response. Methylene blue (3x10^{-7} M) reduced the sensitivity to acetylcholine (pD_2 value, 7.5±0.2 and 7.1±0.2, respectively; NS; area under the curve, p<0.05) as well as the maximal relaxation evoked by the muscarinic agonist (95±1% and 73±6%, respectively; p<0.01) to a comparable degree as in WKY rats (Figure 9; n=4). The concentration shift at pD_2 value by L-NMMA, hemoglobin, or methylene blue was not different. Indomethacin (10^{-5} M) did not affect the relaxation in SHR (n=4, data not shown).

Intraluminal activation. The relaxation induced by intraluminal acetylcholine was greater than that with extraluminal application (pD_2 value, 8.1±0.2 and 7.3±0.1; p<0.005; Figure 10, right panel). The shift between the concentration-response curves to intraluminal and extraluminal acetylcholine was smaller in adult SHR (5.4-fold) than in age-matched WKY rats (13.5-fold; p<0.05). This was due to reduced relaxations to intraluminal acetylcholine in the SHR (p<0.05 versus WKY rats), whereas the relaxation induced by extraluminal acetylcholine did not differ between the two groups.

Effect of age in hypertensive rats

Norepinephrine. Removal of the endothelium augmented the response to norepinephrine in young SHR (Figure 11). The pD_2 value was 5.8±0.1 and 6.4±0.3 in arteries with and without endothelium (p<0.05). The contractions of the arteries with endothelium did not differ in young and adult SHR, but contractions of arteries without endothelium were greater in young SHR than in adult SHR (Figure 3, right panel and Figure 11, right panel). Accordingly, the concentration shift at pD_2 value by removal of the endothelium was 4.5-fold in young SHR but only 1.4-fold in adult SHR.

The contractions of the arteries without endothelium were not different between young WKY rats and SHR, and the concentration shift at pD_2 value obtained by removal of the endothelium was similar in WKY rats (3.0-fold) and SHR (4.5-fold; Figure 11). In contrast, in preparations with endothelium the maximal contractions were greater in young SHR than in young WKY rats (Figure 11; p<0.005).

Acetylcholine. The relaxations induced by both intraluminal and extraluminal acetylcholine and the concentration shift at pD_2 value did not differ in young and adult SHR (Figure 10, right panel and Figure 12, right panel). In young SHR, the relaxations induced by both intraluminal and extraluminal acetylcholine (pD_2 value, 8.3±0.2 and 7.5±0.1; concentration shift, 7.2-fold; p<0.005; maximal response, 99±1% and 99±1%; NS) were not different from those of young WKY rats (Figure 12), whereas the response to intraluminally applied acetylcholine was reduced in adult SHR as compared with adult WKY rats (Figure 10; see Endothelium-dependent relaxation section).

Discussion

The present study demonstrates that endothelium-derived nitric oxide is released under basal conditions and after stimulation with acetylcholine in pressurized mesenteric resistance arteries of the rat; however, the L-arginine pathway accounts only in part for endothelium-dependent responses in resistance arteries. The relaxation induced by acetylcholine was much more pronounced with intraluminal than with extraluminal activation of the endothelial cells. The inhibitory effects of the endothelium against contractions to norepinephrine were reduced in hypertension and decreased with aging. In adult SHR, intraluminal but not extraluminal acetylcholine caused smaller relaxations than in age-matched WKY rats.

Extraluminal acetylcholine induced concentration-dependent relaxation in mesenteric resistance arteries over the same concentration range as in large arteries, indicating that the endothelium may play a key role in the regulation of vascular tone in resistance arteries. This observation confirms previous reports from other investigators and our own group. Originally, it was thought that acetylcholine released from parasympathetic nerve endings could not reach the endothelium to cause the release of endothelium-derived relaxing factor. However, the present observation, as those of others, demonstrates that the muscarinic agonist can activate endothelial cells from the adventitial side. Thus, acetylcholine released from cholinergic nerve endings may contribute to the regulation of vascular tone via an endothelium-dependent mechanism. Indeed, in the gastric mucosa of the rat, both vagal stimulation and intraluminally infused acetylcholine increase blood flow.
dependent relaxation than extraluminal application. It is likely that a lesser amount of acetylcholine reaches the endothelium with extraluminal than with intraluminal application, as it has to diffuse through the adventitia, the media, and the subendothelial layer including the basal membrane to reach the endothelium. Breakdown of acetylcholine by acetylcholinesterase may occur during this process33-36 or the basal membrane may act as a physical barrier preventing acetylcholine in part from reaching the endothelium. Alternatively, the abluminal membrane of endothelial cells may contain less muscarinic receptors than the luminal surface. Whether acetylcholine can reach the endothelium from the luminal side is unknown. However, certain endothelial cells possess the enzyme choline acetyltransferase and are thus capable of synthesizing the muscarinic agonist.37-39 It has been proposed that endothelial cells may release acetylcholine under certain conditions (i.e., hypoxia and cell injury) and thereby evoke the release of endothelium-derived relaxing factors via a paracrine mechanism.37

L-NMMA, a specific inhibitor of nitric oxide formation from L-arginine, inhibited the relaxation induced by acetylcholine demonstrating that nitric oxide is synthesized from L-arginine in the endothelium of rat mesenteric resistance arteries and released after stimulation with acetylcholine. This observation confirms previous reports demonstrating that endothelium-dependent relaxations induced by acetylcholine can be inhibited by L-NMMA in large conduit arteries of experimental animals15,17 and humans.19 However, in rat mesenteric resistance arteries, the inhibition of the relaxation by L-NMMA was smaller than that obtained in most large arteries. This may be related to: 1) large stores of L-arginine in the endothelium of resistance arteries making it difficult to completely inhibit the L-arginine pathway with L-NMMA, 2) nitric oxide formation from a source distinct from L-arginine, or 3) the concomitant release of endothelium-derived relaxing factors other than nitric oxide. The fact that even high concentrations of L-NMMA did not prevent the relaxations induced by acetylcholine strongly argues against an incomplete inhibition of the endothelial L-arginine pathway. Indeed, particularly in the SHR, the maximal inhibition was achieved with 10^-4 M of L-NMMA with no further effect at higher concentrations of the false substrate. Furthermore, the scavenger of nitric oxide hemoglobin40 and the inhibitor of soluble guanylate cyclase methylene blue inhibited the relaxation to a comparable extent as did L-NMMA. Thus, the endothelium of rat mesenteric resistance arteries most likely releases other endothelium-derived relaxing factors distinct from nitric oxide.41,42 Prostacyclin can be excluded as a contributing factor, as indomethacin did not affect the relaxations induced by acetylcholine. The presence of the endothelium markedly inhibited the contractile effects of both extraluminal and intraluminal norepinephrine in mesenteric resistance arteries of the rat. Because this endothelium-dependent inhibition of norepinephrine-induced contractions also occurred with extraluminal application of the catecholamine, this cannot be related to physical properties of the endothelium or breakdown of norepinephrine by monoxydase present within the cells. A contribution of endothelium-derived prostacyclin can also be excluded, as indomethacin did not augment the contractions induced by norepinephrine. Although L-NMMA did augment the contractions induced by norepinephrine, removal of the endothelium caused a slightly more pronounced shift to the left of the concentration-response curve to the catecholamine. Thus, the endothelium must continuously release nitric oxide and possibly also small amounts of a second endothelium-derived relaxing factor to inhibit the contractile effects of norepinephrine. The release of endothelium-derived nitric oxide during contractions induced by norepinephrine does not involve activation of a1-adrenergic receptors on the endothelium,7-9 as removal of the endothelium augmented the contractions induced by the a1-adrenergic agonist phenylephrine to a similar degree as those to norepinephrine. It remains to be clarified, however, whether endothelium-derived relaxing factors are spontaneously released or to what extent intraluminal shear stress exerted by the perfusate or the contractile process itself stimulate their release. The more pronounced contractions to intraluminally applied norepinephrine as compared with extraluminal application of the agonist both in preparations with and without endothelium confirms the results of others43 and most likely is related to a greater neuronal uptake of the catecholamine on the adventitial surface of the blood vessel wall.

In normotensive rats, aging augmented the maximal contractions induced by norepinephrine in preparations with endothelium only, suggesting that the inhibitory effects of the endothelium against contractions induced by the catecholamine decrease with age. In contrast, the difference between intraluminal and extraluminal activation of the endothelial cells with acetylcholine was more pronounced in adult as compared with young WKY rats, and this was in large part due to the more powerful effects of intraluminal acetylcholine. This indicates that, in normotensive rats, maturation is associated with a growing importance of the intraluminal activation of the endothelial cell.

The relaxation induced by acetylcholine (both intraluminal and extraluminal) did not differ in young WKY rats and SHR. In contrast, the relaxation induced by intraluminal, but not extraluminal, acetylcholine was impaired in adult SHR as compared with age-matched WKY rats. Thus, while in the WKY rats intraluminal activation of the endothelial cells becomes more prominent with maturation and aging, this process does not occur in hypertensive animals. In fact, in the SHR the relaxations to both intraluminal and extraluminal acetylcholine tended to decrease with age. Most likely, the increase in arterial pressure occurring during maturation and

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aging in the SHR specifically leads to functional alterations of the luminal surface of the endothelium, which is most exposed to mechanical forces, and in turn leads to reduced endothelium-dependent relaxations in response to intraluminal acetylcholine.

In the aorta and in mesenteric resistance arteries of the SHR studied in organ chambers (with recording of isometric tension), the impaired endothelium-dependent relaxations to acetylcholine can be normalized by inhibitors of cyclooxygenase.14,18,21,31,55 In the aorta of the SHR, this is related to the release of a cyclooxygenase-dependent, endothelium-derived contracting factor at higher concentrations of the muscarinic agonist (i.e., $10^{-6}$ to $10^{-5}$ M).6,22,24 However, in the present experiment and previous studies in which pressurized mesenteric resistance arteries were used,24 acetylcholine up to $10^{-6}$ M did not induce endothelium-dependent contractions. The possibility that cyclooxygenase products inhibit the relaxation induced by acetylcholine rather than causing frank contractions can be excluded, as indomethacin did not affect the response to the muscarinic agonist in WKY rats and in SHR. Thus, in the SHR the response to acetylcholine differs in pressurized arteries exposed to flow and in ring preparations. Possibly, flow or intraluminal pressure inhibit the production of the contracting factor, at least with acetylcholine as an agonist. Alternatively, the release of the endothelium-derived contracting factor may come into play in older animals. Indeed, in mesenteric rings of SHR, endothelium-dependent contractions increase with age.45

In the SHR, the inhibitory effects of the endothelium at higher concentrations of norepinephrine was already reduced in young rats as compared with age-matched WKY rats. In the adult SHR, the enhancement of the response to norepinephrine by l-NMMA was smaller than in age-matched WKY rats. In the adult SHR, the release of endothelium-derived nitric oxide must be reduced in hypertension. In addition, the basal release of the endothelium-derived relaxing factor distinct from nitric oxide must also be impaired in the SHR.

In conclusion, the endothelium of mesenteric resistance arteries of the rat is capable of forming nitric oxide from L-arginine, but the pathway only accounts in part for the inhibitory role of the endothelium. Intraluminal activation of the endothelium causes much more striking responses than extraluminal activation. With aging, the inhibitory effects of the endothelium against contractions induced by norepinephrine decrease, whereas the endothelium-dependent relaxations induced by acetylcholine become more pronounced, particularly during intraluminal stimulation. In contrast, in hypertension the inhibitory effects of the endothelium against contractions induced by norepinephrine are reduced as compared with age-matched controls, and the intraluminal activation of the endothelial cells with acetylcholine becomes impaired. These functional changes of the endothelium in hypertensive resistance arteries may play a role in the maintenance of increased peripheral vascular resistance that occurs in established hypertension.

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