Effects of Aging and Hypertension on Endothelium-Dependent Vascular Relaxation in Rat Carotid Artery

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We evaluated the effects of aging and hypertension on endothelium-dependent relaxation of rat common carotid arteries using 14-week-old (young) and 11-month-old (old) Wistar-Kyoto rats (WKY) and age-matched spontaneously hypertensive rats (SHR). Isometric tension of common carotid artery ring segments was measured. With a resting tension of 2.0 g determined from the baseline tension-contraction curves, precontraction was induced by 10^-7 M 5-hydroxytryptamine and endothelium-dependent relaxation was measured by application of either acetylcholine or adenosine 5'-triphosphate (ATP). Mean arterial blood pressure was 73.1 ± 3.0 mm Hg in WKY and 110.0 ± 3.1 mm Hg in SHR. These baseline values were significantly different. Acetylcholine-induced maximal relaxations were 70.1 ± 2.6% of the 5-hydroxytryptamine-induced contraction in young WKY, 45.6 ± 2.1% in old WKY, 35.1 ± 1.8% in young SHR, and 21.4 ± 2.5% in old SHR. On the other hand, ATP-induced relaxations were 52.0 ± 3.2%, 35.7 ± 3.8%, 21.7 ± 3.5%, and 17.0 ± 1.8% in the groups, respectively. Acetylcholine-induced relaxations were significantly different between WKY and SHR, young and old, independently. On the other hand, ATP-induced relaxations were also significantly different between young and old WKY, although no significant difference was observed between young and old SHR. The fact that endothelium-dependent relaxation of a cephalic artery is impaired in old rats and in hypertensive rats suggests that aging and hypertension are risk factors that may augment the disturbance of the cerebral circulation in pathologic conditions. (Stroke 1988;19:892–897)

Since Furchgott and Zawadski first reported the obligatory role of endothelial cells in the relaxation of arterial smooth muscle caused by acetylcholine (ACh), the role of the endothelium in vasodilator responses to several vasoactive agents has been demonstrated. The effects of aging and hypertension on vascular reactivity in general have been extensively studied. In addition, there are several reports examining the influence of aging or hypertension on endothelium-dependent vasodilatations. In these studies, ACh-induced relaxation of rat mesenteric artery was unaffected by age, whereas hypertension impaired endothelium-dependent relaxation in experimental animals. There are at present, however, no reports of the effects of aging or hypertension on the endothelium-dependent relaxations of cephalic arteries. This is surprising considering the major effects that aging and hypertension are thought to exert on the cerebral circulation.

Recently Freeiman et al demonstrated that atherosclerosis impairs endothelium-dependent vascular relaxation to ACh and thrombin in monkeys. The common carotid artery, which is often affected by disorders such as atherosclerosis, has a great influence on cerebral circulation. Therefore, it seems pertinent to investigate the effects of aging and hypertension on the endothelium-dependent vascular relaxation in a cephalic artery such as the common carotid artery. Our experiments were designed to demonstrate these effects using young and old Wistar-Kyoto rats (WKY) and young and old spontaneously hypertensive rats (SHR).

**Materials and Methods**

**Animal and arterial preparations.** Male WKY weighing 210–420 g and male SHR (Charles River...
Labs, Boston, Massachusetts) weighing 280–420 g were used. Young rats were 14 weeks old and old rats were 11 months old in each strain. Rats were anesthetized with 36 mg/100 g i.p. chloral hydrate. The right femoral artery was exposed and cannulated with a PE-50 polyethylene tube (Becton Dickinson, Towson, Maryland) that was connected to a Hewlett-Packard 1290A pressure transducer (Palo Alto, California) and a Hewlett-Packard 78353B amplifier to monitor blood pressure. After the blood pressure became stable, the cannula was removed and the rat was killed by exsanguination. All handling of the rats conformed to the University of Virginia Animal Research Committee’s regulations. The cervical common carotid artery was removed and placed in a dissecting chamber filled with a modified Krebs solution of the following millimolar composition: NaCl 120, KCl 4.5, MgSO$_4$ 1.0, NaHCO$_3$ 27.0, KH$_2$PO$_4$ 1.0, CaCl$_2$ 2.5, and dextrose 10.0. The common carotid artery was dissected free from surrounding tissues and cut into 3-mm-long rings. In certain rings, the endothelium was removed by the following procedure. The arterial ring was placed in a chamber filled with Krebs solution. A 30-gauge needle connected to the gas supply (95% O$_2$-5% CO$_2$) was introduced into the lumen of the artery. A gentle stream of gas was passed through the vessel lumen for 10 minutes to dry the endothelium.$^{23}$ The vascular lumen was then filled with Krebs solution, and the endothelial cells were removed by gentle rubbing with a PE-20 polyethylene tube. The lumen was then irrigated several times with Krebs solution. Specimens with or without endothelium were suspended between L-shaped stainless steel rods in an organ bath with a 10 ml working volume and bubbled with the 95% O$_2$-5% CO$_2$ gas mixture. The pH of the solution ranged from 7.40 to 7.50. The preparations were allowed to equilibrate at 37°C for 90 minutes before use. Resting tension was adjusted to 2.0 g for each group of arterial rings, which was determined from the tension-development curves to be optimal (Figure 1). Isometric tension was recorded using a Grass FT.03 force-displacement transducer (Quincy, Massachusetts) and displayed on a Soltec 3418 chart recorder (San Fernando, California). The contractile response to 40 mM KC$_1$ was first obtained on each arterial ring by repeated washings. An equilibration period of 30 minutes was allowed between each series of experiments. After confirming a consistent contractile response to KC$_1$, experiments with 5-hydroxytryptamine (5-HT) were begun. Because the vessel must be optimally precontracted for relaxation studies,$^{23}$ submaximal tone was induced with $10^{-5}$ M 5-HT under the resting tension of 2.0 g as mentioned above. This condition was then used for all subsequent relaxation studies. ACh or adenosine 5'-triphosphate (ATP) was then added in a cumulative fashion. The relaxation induced by ACh or ATP was expressed as a percentage of the tonic phase of the contraction induced by $10^{-5}$ M 5-HT. In the relaxation studies with ATP, the arterial rings were pretreated with $10^{-6}$ M 8-phenyltheophylline (8-PT), an adenosine antagonist, 5 minutes before application of $10^{-5}$ M 5-HT to exclude the effect of endothelium-independent relaxation by adenosine.$^{23}$ Papaverine ($10^{-6}$ M to $10^{-4}$ M) was added in a cumulative fashion to observe an endothelium-independent vascular relaxation.

**Drugs.** KCl, 5-HT, ACh, papaverine, ATP, and 8-PT were obtained from Sigma Chemical Co., St. Louis, Missouri. To make stock solutions, all drugs were dissolved in distilled water except 5-HT, which was dissolved in 0.1N HCl with 0.1% ascorbic acid. The drugs were diluted in Krebs solution before use such that volumes of <0.1 ml were added to the organ bath.

**Statistical analysis.** The data were expressed as mean ± standard error of the mean (SEM). The dose–response curves were analyzed using the general linear models procedure for analysis of variance (ANOVA) of the SAS computer program, followed by Scheffé’s test. The values were considered to be significantly different if $p$ was <0.05.

**Results**

**Blood pressure.** Mean arterial blood pressure of SHR was significantly higher than that of WKY in both young and old rats (Table 1).

**TABLE 1. Characteristics of Rats Used to Study Endothelium-Dependent Relaxation**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Age</th>
<th>Body weight (g)</th>
<th>Mean arterial blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar-Kyoto</td>
<td>14 weeks</td>
<td>247±9</td>
<td>73.4±2.9</td>
</tr>
<tr>
<td></td>
<td>11 months</td>
<td>374±9</td>
<td>77.4±2.6</td>
</tr>
<tr>
<td>Spontaneously hypertensive</td>
<td>14 weeks</td>
<td>310±10</td>
<td>108.8±4.2</td>
</tr>
<tr>
<td></td>
<td>11 months</td>
<td>399±7</td>
<td>111.2±5.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM ($n=12$).
Contraction induced by 5-HT. 5-HT caused potent vasoconstriction of arterial rings in all groups of rats. There was no significant difference between young and old rats of either strain, but there was a significant difference between strains (Figure 2, left). When contraction was expressed as percent of that induced by 40 mM KCl, however, the maximal contraction induced by 5-HT was not significantly different between strains (Figure 2, right).

Endothelium-dependent relaxation. ACh (10⁻⁸ M to 10⁻³ M) and ATP (10⁻⁷ M to 10⁻⁴ M) evoked dose-dependent relaxation in rat common carotid artery rings precontracted by 10⁻⁵ M 5-HT. There was no vasodilation caused by ACh or ATP observed in the arterial rings without endothelium. Figure 3 shows the endothelium-dependent relaxation caused by ACh. Furthermore, no vasoconstriction occurred in the endothelium-denuded vessels treated with 10⁻⁸ M to 10⁻⁵ M ACh or with 10⁻³ M to 10⁻⁴ M ATP.

Effects of aging and hypertension on vasodilation induced by ACh. Maximal vasodilation was induced by 10⁻⁸ M ACh in arterial rings of all groups of rats (Figure 4). Maximal relaxations expressed as percent of contraction induced by 10⁻⁵ M 5-HT were 70.1 ± 2.6% in young WKY, 45.6 ± 2.1% in old WKY, 35.1 ± 1.8% in young SHR, and 21.4 ± 2.5% in old SHR; these values are significantly different. The dose-response curves to ACh-induced vasodilation were also significantly different between young and old rats in either strain as well as between strains. Our findings demonstrate that both aging and hypertension impair the vasodilation induced by ACh; furthermore, these effects are independent.

Effects of aging and hypertension on vasodilation induced by ATP. ATP induced maximal vasodilation at 10⁻³ M (Figure 5) except in old SHR, which showed maximal relaxation at 10⁻⁴ M (application of 10⁻³ M ATP elicited vasoconstriction, data not shown). Maximal relaxations expressed as percent of contraction induced by 10⁻³ M 5-HT were 52.0 ± 3.2% in young WKY, 35.7 ± 3.8% in old WKY, 21.7 ± 3.5% in young SHR, and 17.0 ± 1.8% in old SHR. There was a significant difference in the degree of vasodilation between young and old WKY; however, no significant difference was observed between young and old SHR.
between young and old SHR. On the other hand, there was a significant difference between age-matched WKY and SHR.

**Vasodilation induced by papaverine.** Papaverine (10^-6 M to 10^-4 M) evoked dose-dependent relaxation in rat common carotid arterial rings precontracted by 10^-5 M 5-HT (Figure 6). Arterial rings in all groups of rats were maximally relaxed by 10^-4 M papaverine: 92.7 ± 1.2% in young WKY, 95.1 ± 1.0% in old WKY, 97.0 ± 0.7% in young SHR, and 94.8 ± 1.1% in old SHR. No significant differences were observed among these groups. Furthermore, no significant difference between the dose–response curves for each group was observed.

**Discussion**

Our study demonstrates that 1) ACh-induced relaxation is significantly greater in young than old rats and in normotensive than hypertensive rats, 2) ATP-induced relaxation is significantly greater in young than old normotensive rats, 3) vasodilation induced by papaverine shows no significant difference among all groups of rats, and 4) 5-HT–induced vasoconstriction is greater in hypertensive than normotensive rats but among normotensive rats there is no significant difference due to aging. In our experiments, however, the maximum contraction induced by 5-HT was not significantly different among the groups when contraction was expressed as percent of that induced by 40 mM KCl.

Although it is widely accepted that aging and hypertension affect vascular relaxation to \( \beta \)-adrenergic stimulants,5,7,8,12 relaxation induced by nonspecific vascular smooth muscle relaxants (such as nitroprusside, sodium nitrite, or papaverine) is thought to be unrelated to age8,11,17 or hypertension.19–21 There are, however, a few conflicting reports stating that hypertension can affect vascular relaxation induced by nitroglycerin14 and papaverine.24

In our experiments, 10^-4 M papaverine elicited maximal relaxation of common carotid arterial rings without significant differences among the groups tested and without significant differences in the dose–response curves. Therefore, it is likely that a decrease in the relaxations induced by ACh and ATP, which are known to be endothelium-dependent, is due to specific impairment of the responsiveness of endothelium-dependent relaxation mechanisms rather than to other nonspecific changes in vascular reactivity. In our experiments with ATP, we pretreated the arteries with 8-PT, an adenosine antagonist, to avoid endothelium-independent relaxations23 produced by the ATP degradation products adenosine monophosphate and adenosine. Thus, it is likely that decreases in the relaxation of rat common carotid arterial rings induced by ACh or ATP associated with aging or hypertension are related to changes in the endothelium itself. Several investigators have demonstrated that endothelium-dependent relaxation is impaired with hypertension in the thoracic aorta18–20,25 and femoral artery18 of rats. No impairment in this response with age was noted, however, when relaxation induced by ACh was studied in the rat mesenteric artery.17 The disparity with our results may be related to the different vascular sites studied.

Six major mechanisms for the impairment of endothelium-dependent vasodilation by aging and hypertension can be postulated: 1) a decrease in the number of receptors to vasoactive agents on the endothelial cell membrane, 2) a decrease in the affinity of receptors on the endothelial membrane to vasoactive agents, 3) a decrease in the production or release of endothelium-derived releasing factor(s) (EDRF), 4) a decrease in the responsiveness of the smooth muscle to EDRF, 5) an exaggeration of endothelium-dependent contraction in old and/or hypertensive rats, or 6) a decrease in the production of an endothelium-dependent component of prostaglandin(s) or a decreased sensitivity of those prosta-
taglandin(s) to the smooth muscle. It has been shown that there is a remodeling of the rat aortic endothelial layer and changes in cell density and surface morphology in rats with aortic coarctation or experimental deoxycorticosterone acetate hypertension. Therefore, these morphologic changes may be related to impairment of the endothelium-dependent relaxation. Bossaller et al demonstrated, by examining atherosclerotic rats, that the relaxation induced by ACh was suppressed, whereas no changes in relaxation were observed in reaction to the calcium ionophore A23187. Bossaller et al speculated that the impairment of endothelium-dependent relaxation was due to a decrease in the sensitivity of the endothelium to ACh instead of to a decrease in EDRF. Our findings also suggest that a decrease in the endothelial receptors' affinity for ACh and ATP is also a possible cause of decreased endothelium-dependent relaxation. Furthermore, Van de Voorde and Leusen demonstrated, with the aid of a bioassay technique, that decreased ACh-induced relaxation in hypertensive rats was not due to a decrease in the release of EDRF but rather to an impaired coupling between the endothelium and the smooth muscle. On the other hand, there are reports demonstrating that endothelium produces not only a relaxing factor but also a constricting factor in hypertensive rats and furthermore, that ACh and ATP elicited a much greater contraction of endothelium-denuded artery in hypertensive than in normotensive rats. We did not observe contraction of endothelium-denuded vessels in either hypertensive or normotensive rats in our experimental model in response to ACh or ATP (data not shown). Therefore, we do not consider augmentation of contraction by ACh and ATP in hypertensive rats to be a major cause of the decrease in endothelium-dependent relaxation produced by hypertension. Several investigators have described that ACh or ATP release prostaglandin(s) from the endothelium. To release prostaglandin(s) from the endothelium, however, a higher concentration of ACh and a longer period of incubation than those used in our study are needed. Therefore, this possibility may be minimal.

We did not identify the main cause of the impairment of endothelium-dependent vasodilation by aging and hypertension. However, our experiments do confirm that endothelium-dependent vascular relaxation is suppressed by either aging or hypertension in the cervical carotid of rats. However, ACh and ATP showed slightly different effects on vascular dilation; ATP-induced relaxation was not affected by aging in hypertensive rats. Although the reason these two drugs act differently is unclear at present, there is a possibility that the EDRF released by ATP is different from that released by ACh. This has been previously postulated by De Mey and Vanhoucke in the canine femoral artery.

Although the physiologic role of endothelium-dependent relaxation still remains unclear, our results suggest that both aging and hypertension may affect the cerebral circulation. These are considered to be two of the causes for the decreasing cerebral circulation in older people. The relative loss of endothelium-dependent vasodilatation may, at least in part, explain these relations.

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