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

Kazuhiro Hongo, MD, Tadayoshi Nakagomi, MD, Neal F. Kassell, MD, Tomio Sasaki, MD, Michael Lehman, BA, Dennis G. Vollmer, MD, Tetsuya Tsukahara, MD, Hisayuki Ogawa, MD, and James Torner, PhD

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^{-5} 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 Freiman 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

From the Department of Neurosurgery, University of Virginia School of Medicine, Charlottesville, Virginia.

Address for correspondence: Neal F. Kassell, MD, Department of Neurosurgery, Box 212, Medical Center, University of Virginia, Charlottesville, VA 22908.

Received June 30, 1987; accepted February 3, 1988.
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 Krebs solution. A 30-gauge needle connected to the gas supply (95% O2-5% CO2) 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 chamber with a 10 ml working volume and bubbled with the 95% O2-5% CO2 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 KCl was first obtained on each arterial ring followed by repeated washings. An equilibration period of 30 minutes was allowed between each series of experiments. After confirming a consistent contractile response to KCl, experiments with 5-hydroxytryptamine (5-HT) were begun. Because the vessel must be optimally precontracted for relaxation studies, a 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^{-3} M 5-HT to exclude the effect of endothelium-independent relaxation by adenosine.23 Papaverine (10^{-5} 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 Scheffe'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>108.6 ± 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).
5-Hydroxytryptamine (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^{-7} M to 10^{-4} M ATP.

**Effects of aging and hypertension on vasodilation induced by ACh.** Maximal vasodilation was induced by 10^{-6} 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^{-5} 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 in old SHR.

---

**Figure 2.** Left: Dose-response curves to 5-HT. Contractile force is expressed in grams. Each point represents mean±SEM (n=10). Right: Dose-response curves to 5-HT. Contractile force is expressed as ratio of contraction to 5-HT compared with that induced by 40 mM KCl. Each point is mean±SEM (n=10). ○, young Wistar-Kyoto rats (WKY); ●, old WKY; ○, young spontaneously hypertensive rats (SHR); ■, old SHR.

**Figure 3.** Effect of acetylcholine (ACh) on 10^{-5} M 5-hydroxytryptamine (5-HT)-induced contraction of rat common carotid arterial rings with and without endothelium. Numbers with arrows indicate negative exponents of molar concentrations of ACh. W, washout.

**Figure 4.** Dose-response curves of effect of acetylcholine (ACh) on 10^{-5} M 5-hydroxytryptamine (5-HT)-induced contraction of common carotid arterial rings. Each point represents mean±SEM (n=12). ○, young Wistar-Kyoto rats (WKY); ●, old WKY; ○, young spontaneously hypertensive rats (SHR); ■, 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^{-4} M to 10^{-4} M) evoked dose-dependent relaxation in rat common carotid arterial rings precontracted by 10^{-3} 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 β-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 age 8,11,17 or hypertension. 19–21 There are, however, a few conflicting reports stating that hypertension can affect vascular relaxation induced by nitroglycerin 14 and papaverine. 24
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 artery 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 Vanhoutte 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 vasodilation may, at least in part, explain these relations.

Acknowledgments

The authors thank Dr. Alex Loeb, Department of Pharmacology, University of Virginia, for methodological advice, Sarah Hudson and Grace Asban for technical assistance, and Lucille Staiger for manuscript preparation.

References

3. Cherry PD, Furchgott RF, Zawadski JV, Jothianandan D: Role of endothelial cells in relaxation of isolated arteries by bradykinin. Proc Natl Acad Sci USA 1982;77:2106-2110
18. Konishi M, Su C: Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. Hypertension 1983;5:881-886


**KEY WORDS** • cerebral arteries • platelet aggregation • subarachnoid hemorrhage • cats
Effects of aging and hypertension on endothelium-dependent vascular relaxation in rat carotid artery.

K Hongo, T Nakagomi, N F Kassell, T Sasaki, M Lehman, D G Vollmer, T Tsukahara, H Ogawa and J Torner

doi: 10.1161/01.STR.19.7.892

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/19/7/892

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/