Endothelium-Dependent Responses of Cerebral Blood Vessels During Chronic Hypertension

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Acetylcholine produces less dilatation of pial arterioles in stroke-prone spontaneously hypertensive rats (SHRSP) than in normotensive (WKY) rats. Responses of cerebral vessels to acetylcholine and bradykinin appear to involve different mechanisms. Our first goal was to determine whether responses of pial arterioles to bradykinin are impaired in SHRSP. Diameter of pial arterioles (20–60 μm) was measured using intravital microscopy in WKY rats and SHRSP (9–12 months old). Superfusion of bradykinin (3×10⁻⁷ M) dilated pial arterioles by 35±6% (mean±SEM) in WKY rats, but only 21±3% in SHRSP (p<0.05 versus WKY rats). Both nitric oxide (5×10⁻⁷ M) and nitroglycerin (10⁻⁵ M) produced similar vasodilatation in WKY rats and SHRSP. Our second goal was to determine whether alteration of postreceptor mechanisms contributes to impairment of endothelium-dependent cerebral vasodilatation in SHRSP. Calcium ionophore A23187 (10⁻⁵ M) produced more vasodilatation in WKY rats than in SHRSP (32±8% versus 9±4%, p<0.05). Responses to A23187 (10⁻³ M) were inhibited by indomethacin (46±13% versus 15±5%, p<0.05) in WKY rats, whereas responses to A23187 (10⁻⁶ M) were potentiated modestly by indomethacin (−3±2% versus 4±2%, p<0.05) in SHRSP. Thus, 1) chronic hypertension impairs responses to two distinct endothelium-dependent dilators in pial arterioles, 2) responses of pial arterioles to nitric oxide are not altered during chronic hypertension, 3) impairment of endothelium-dependent responses in chronic hypertension involves an alteration in postreceptor mechanisms, and 4) impaired responses to A23187 in chronic hypertension may be due in part to co-release of a constrictor substance through the cyclooxygenase pathway. (Hypertension 1991;17:612–618)

Dilatation of cerebral arterioles in response to acetylcholine and bradykinin appears to involve different mechanisms. Dilatation of cerebral arterioles in response to bradykinin is inhibited by scavengers of oxygen radicals,¹⁻³ which suggests that the mediator is an oxygen-derived free radical. In contrast, dilatation of cerebral arterioles in response to acetylcholine is not altered by scavengers of oxygen radicals and is impaired by generation of oxygen radicals.³⁻⁵

Dilator responses of pial arterioles to acetylcholine are impaired during chronic hypertension.⁶ The first goal of this study was to determine whether responses of pial arterioles to bradykinin, as well as acetylcholine, are impaired during chronic hypertension.

Several mechanisms may account for impaired endothelium-dependent dilatation of hypertensive vessels.⁷ Production of an endothelium-derived contracting factor (EDCF), which counteracts the dilator effect of endothelium-derived relaxing factor (EDRF), or impaired coupling between endothelium and vascular muscle cells may lead to impaired endothelium-dependent vasodilatation. In addition, impaired endothelium-dependent vasodilatation could be due to reduced synthesis or release of EDRF or reduced responsiveness of vascular muscle to EDRF. Nitric oxide or a related compound appears to be an EDRF that is released in response to many agonists in several vascular beds,⁸⁻¹¹ although nitric oxide may not be an EDRF for acetylcholine in cerebral arterioles.⁵,¹² The second goal of this study was to determine whether responses of cerebral arterioles to nitric oxide are altered during chronic hypertension.

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The synthesis or release of EDRF is calcium-dependent. The calcium ionophore A23187, which elevates intracellular calcium concentration ([Ca\(^{2+}\)], produces endothelium-dependent dilatation in pial arterioles by a receptor-independent mechanism. The third goal of this study was to determine, with calcium ionophore, whether impaired endothelium-dependent vasodilatation during chronic hypertension involves an altered postreceptor mechanism.

In pial arterioles of mice, endothelium-dependent dilatation in response to A23187 is cyclooxygenase dependent. In addition, several agonists that release EDRF can also release an EDCF, especially in spontaneously hypertensive rats (SHR). Synthesis and release of EDCF, which impairs the vasodilator response to EDRF, is inhibited by indomethacin. The fourth goal of this study was to determine whether indomethacin alters responses to A23187 in pial arterioles of Wistar-Kyoto (WKY) rats and stroke-prone SHR (SHRSP).

**Methods**

**Preparation of Animals**

Male WKY rats (n=33) and SHRSP (n=31) (9–12 months old) were anesthetized with pentobarbital sodium (50 mg/kg i.p.). After completion of tracheotomy, animals were ventilated mechanically with room air and supplemental oxygen. Gallamine triethiodide (15–30 mg/kg i.v.) was used for skeletal muscle paralysis. Supplemental anesthetic and skeletal muscle relaxant were administered as needed (pentobarbital sodium, 10–20 mg·kg\(^{-1}\)·hr\(^{-1}\) i.v.; gallamine triethiodide, 5–10 mg·kg\(^{-1}\)·hr\(^{-1}\) i.v.).

A catheter was placed in the left femoral vein for injection of drugs. The femoral arteries were cannulated for measurement of arterial blood pressure and for withdrawal of blood for measurement of arterial blood gases and pH. To visualize the microcirculation of the cerebrum, a cranial window was prepared over the right parietal cortex. A cranial window was filled with this artificial CSF and nitric oxide was examined responses of cerebral arterioles to nitroglycerin (10\(^{-6}\) M and 10\(^{-5}\) M).

**Experimental Protocol**

Cerebral vessels were superfused with artificial CSF for 30 minutes before testing responses of arterioles to the agonists. In each rat, we studied responses of the largest pial arteriole in the cranial window to application of agonists. All drugs were dissolved in artificial CSF and then superfused over the cerebral vessels. Application of vehicle did not affect vessel diameter. With the exception of A23187, the diameter of cerebral arterioles was measured immediately before application of other agonists and every 20–30 seconds for 2–4 minutes during application of agonists. Steady-state responses to agonists were reached within 1–2 minutes after the application. Values obtained at steady state are reported in this study. Because responses of pial arterioles to A23187 are relatively slow, diameter of cerebral arterioles was measured after continuous superfusion with A23187 for 10 minutes.

First, we examined responses of cerebral arterioles to bradykinin (10\(^{-7}\) M and 3×10\(^{-7}\) M) in WKY rats and SHRSP. To determine whether impaired dilatation of cerebral arterioles in SHRSP was related to nonspecific impairment of vasodilatation, we also examined responses of cerebral arterioles to nitroglycerin (10\(^{-6}\) M and 10\(^{-5}\) M).

Second, we examined the effect of nitric oxide (1.5×10\(^{-7}\) M and 5×10\(^{-7}\) M) on diameter of cerebral arterioles. To prepare nitric oxide, artificial CSF was continuously bubbled with 100% nitrogen for at least 20 minutes before application. A gas-tight syringe was filled with this artificial CSF and nitric oxide was then dissolved within this syringe.

Third, we tested responses of cerebral arterioles to the calcium ionophore A23187 (10\(^{-6}\) M and 10\(^{-5}\) M) to determine whether impaired endothelium-dependent responses involve an altered postreceptor mechanism. A stock solution of A23187, prepared in 100% dimethyl sulfoxide, was diluted with artificial CSF before application. Diameter of cerebral arterioles was measured after continuous superfusion with A23187 for 10 minutes. Vehicle (0.1% dimethyl sulfoxide in artificial CSF) did not affect baseline diameter.

Fourth, we also examined effects of indomethacin on responses of cerebral arterioles to A23187. Responses of pial arterioles to A23187 (10\(^{-6}\) M and 10\(^{-5}\) M), nitroglycerin (10\(^{-5}\) M), and arachidonic acid (200 \(\mu\)g/ml) were compared with and without treatment of indomethacin (10 mg/kg).

Bradykinin, indomethacin, A23187, and dimethyl sulfoxide were purchased from Sigma Chemical Co., St. Louis, Mo. Nitroglycerin and nitric oxide were from Du Pont Pharmaceuticals, Wilmington, Del. and Matheson Gas Products, Secaucus, N.J., respectively.

**Statistical Analysis**

An unpaired t test was used to compare values between different groups of animals. Paired t test was used to compare differences in the same animal. A value of \(p<0.05\) was considered to be significant.
Results

Control Conditions

Mean arterial pressure was 97±2 mm Hg (mean±SEM) in WKY rats and 192±3 mm Hg in SHRSP (p<0.05 versus WKY rats). Baseline diameter of pial arterioles was significantly less in SHRSP than in WKY rats (41±1 μm in WKY rats versus 32±1 μm in SHRSP). We have shown previously that the number of arterial branching points from the circle of Willis to the area exposed by the craniotomy is similar in WKY rats and SHRSP. Thus, although the vessel diameter is smaller in SHRSP, we examined responses of cerebral arterioles in WKY rats and SHRSP that are of equivalent hierarchy.

Responses of Cerebral Vessels

Dilatation of cerebral arterioles in response to bradykinin was significantly less in SHRSP than in WKY rats (Figure 1). Vasodilator responses to nitroglycerin were similar in WKY rats and SHRSP (Figure 2). Because responses to nitroglycerin were preserved in SHRSP, impaired responses to bradykinin are not related to nonspecific impairment of vasodilatation in SHRSP.

Nitric oxide produced dose-related dilatation of cerebral arterioles that was similar in WKY rats and SHRSP (Figure 3). Thus, responses to this potential EDRF are not altered in cerebral arterioles during chronic hypertension.

Dilatation of cerebral arterioles in response to A23187 was significantly less in SHRSP than in WKY rats (Figure 4). These data suggest that impairment of endothelium-dependent responses during chronic hypertension may involve an altered postreceptor mechanism.

Dilatation of cerebral arterioles in response to arachidonic acid was inhibited by indomethacin in both WKY rats (16±5% versus 1±1%, p<0.05, n=8) and SHRSP (21±10% versus 2±3%, p<0.05, n=4). Cerebral vasodilatation in response to A23187 (10⁻⁵ M) was inhibited by indomethacin in WKY rats, whereas vasodilatation in response to A23187 (10⁻⁶ M) was slightly potentiated by indomethacin in SHRSP (Figure 5). Indomethacin did not affect vasodilatation produced by nitroglycerin in WKY rats or SHRSP (Figure 5). These data suggest that, in pial arterioles of WKY rats, dilatation in response to A23187 is partially cyclooxygenase dependent. These data also suggest that...
impaired vasodilatation in response to A23187 in SHRSP may be partly due to co-release of an EDCF through the cyclooxygenase pathway.

**Discussion**

There are four new findings in this study. First, dilatation of cerebral arterioles in response to bradykinin is impaired in SHRSP. Impaired responses to bradykinin are not related to nonspecific alteration in dilator mechanisms in vascular muscle in SHRSP because dilatation in response to nitroglycerin was similar in WKY rats and SHRSP. Second, responses of cerebral arterioles to a potential EDRF, nitric oxide, are not impaired in SHRSP. Third, dilatation of cerebral arterioles in response to the calcium ionophore A23187 is impaired in SHRSP, which suggests that altered postreceptor mechanisms contribute to impairment of endothelium-dependent vasodilatation during chronic hypertension. Fourth, in pial arterioles of normotensive rats, vasodilatation in response to A23187 is partially cyclooxygenase dependent. Also, impaired cerebral vasodilatation produced by A23187 in SHRSP may be partly due to co-release of an EDCF through the cyclooxygenase pathway.

**Responses to Vasodilators**

We examined reactivity of cerebral arterioles in WKY rats and SHRSP to bradykinin and A23187. In mice, cerebral vasodilator responses to bradykinin and A23187 in vivo are inhibited after injury to endothelial cells with the light-dye or laser-dye techniques, which suggests that responses to bradykinin and A23187 are dependent on intact endothelium in cerebral arterioles. Our preliminary data also suggest that responses of cerebral arterioles to bradykinin are endothelium-dependent in rats.

In pial arterioles of several species, topical application of bradykinin produces dilatation. In cats and mice, bradykinin-induced cerebral vasodilatation appears to be mediated by generation of oxygen radicals. Our preliminary study showed that bradykinin-induced cerebral vasodilatation in rats was almost completely inhibited by catalase.

Cerebral vasodilatation produced by acetylcholine, however, is not mediated by an oxygen radical. Dilatation of pial arterioles in response to acetylcholine in rats is inhibited by N'-monomethyl L-arginine which suggests that nitric oxide may play an important role in cerebral vasodilatation produced by A23187.
acetylcholine. Thus, our findings suggest that chronic hypertension impairs at least two different endothelium-dependent mechanisms since responses to both acetylcholine and bradykinin are impaired.

In a previous study, we found that responses of cerebral arterioles to nitroglycerin are normal in SHRSP.6 Because the animals used in the present study (9–12 months old) were somewhat older than those used previously (6–8 months old), we again examined responses to nitroglycerin. Responses of cerebral arterioles to nitroglycerin were preserved in SHRSP, which suggests that endothelium-independent responses of cerebral arterioles are not impaired in SHRSP.

Nitric oxide has been proposed to be the EDRF released by many agonists from endothelial cells of several vascular beds, including the basilar artery, but not from pial arterioles of cats. In this study, nitric oxide produced similar responses in WKY rats and SHRSP. Thus, the ability of cerebral arterioles to respond to a potential EDRF, as well as to nitroglycerin, is not impaired in SHRSP. In addition, in cerebral arterioles of rats, our preliminary data showed that bradykinin-induced dilatation is mediated by hydrogen peroxide and that responses to hydrogen peroxide are similar in WKY rats and SHRSP. These findings, taken together, suggest that impairment of endothelium-dependent cerebral vasodilatation in chronic hypertension is not due to decreased responsiveness of smooth muscle to EDRF.

Because nitric oxide is unstable when exposed to oxygen, it is possible that we underestimated effects of nitric oxide in the present study in which an open cranial window was used. Because experimental conditions were identical for WKY rats and SHRSP, however, the influence of oxygen presumably was similar in both groups. Thus, the finding that responses to nitric oxide were similar in WKY rats and SHRSP probably was not affected by exposure to oxygen in the cranial window.

Nitric oxide has been proposed as the terminal activator of guanylate cyclase by nitrosodilators, including nitroglycerin. The generation of nitric oxide from nitroglycerin, however, requires the presence of thiol compounds. The observation that nitric oxide is more potent than nitroglycerin in this study might be related to the concentration of endogenous thiol compounds that is available in cerebral arterioles for the generation of nitric oxide from nitroglycerin.

Mechanisms of Impaired Responses

Several mechanisms could account for impaired endothelium-dependent dilatation of cerebral arterioles in hypertensive rats. Reduced synthesis or release of EDRF or reduced responsiveness of vascular smooth muscle to EDRF could lead to impaired endothelium-dependent vasodilatation. Preserved responses to nitroglycerin and nitric oxide (present study) and hydrogen peroxide in SHRSP suggest that impairment of endothelium-dependent responses in cerebral arterioles is not due to nonspecific impairment of responsiveness of vascular smooth muscle to EDRF but may be due instead to impaired synthesis or release of EDRF.

Several agonists that release EDCF also can release an EDCF, especially in SHR. Synthesis of EDCF, which impairs the dilator effect of EDRF, is inhibited by indomethacin. In rats, cerebral vasodilatation in response to bradykinin is inhibited by indomethacin. Thus, we were unable to test the possibility that impaired vasodilatation induced by bradykinin in SHRSP is due in part to co-release of EDCF through the cyclooxygenase pathway. In our preliminary study, dilator responses of pial arterioles to bradykinin were almost completely abolished by catalase in both WKY rats and SHRSP. If cerebral arterioles in SHRSP produce both EDRF and EDCF in response to bradykinin, we would anticipate that bradykinin would produce cerebral vasoconstriction in SHRSP after the dilator response to EDRF is inhibited with catalase. These findings, taken together, suggest that impaired responses to bradykinin are not due to co-release of a vasoconstrictor prostaglandin. In deoxycorticosterone acetate–salt hypertensive rats, impaired endothelium-dependent vasodilatation appears to be related to impaired coupling between endothelium and vascular muscle and is not related to diminished release of EDRF by vessels. In the present study, we cannot exclude the possibility that decreased endothelium-dependent...
vasodilatation in response to bradykinin in SHRSP is due in part to impaired coupling between endothelium and vascular muscle.

The synthesis or release of EDRF is a Ca\textsuperscript{2+}-dependent process.\textsuperscript{13–15} The calcium ionophore A23187 produces endothelium-dependent relaxation in several vascular beds, including that of the cerebrum.\textsuperscript{16} It is likely that A23187 increases intracellular [Ca\textsuperscript{2+}]\textit{i} of endothelial cells and produces endothelium-dependent relaxation by enhancing the release of EDRF.

In this study, A23187 produced less cerebral vasodilatation in SHRSP than in WKY rats. Because A23187 increases intracellular [Ca\textsuperscript{2+}]\textit{i} by a mechanism that is receptor-independent, the findings suggest that an altered postreceptor mechanism may contribute to impairment of endothelium-dependent cerebral vasodilatation during chronic hypertension.

Indomethacin inhibited cerebral vasodilatation produced by A23187 in WKY rats. This finding suggests that cerebral vasodilatation in response to A23187 is partially cyclooxygenase dependent. In addition, because vasodilatation in response to A23187 is potentiated by indomethacin in SHRSP, these data also suggest that impaired vasodilatation produced by A23187 in SHRSP may be due in part to co-release of a contracting factor through the cyclooxygenase pathway. The exact identity of this contracting factor is not yet known.

In thoracic aorta of SHR and mesenteric arteries of SHRSP, release of the cyclooxygenase-dependent contracting factor in response to acetylcholine is not affected by inhibitors of thromboxane synthetase.\textsuperscript{18,30,33} In addition, endothelium-dependent contraction produced by acetylcholine in SHR may involve stimulation of thromboxane A\textsubscript{2}/prostaglandin H\textsubscript{2} receptors by prostaglandin H\textsubscript{2}.\textsuperscript{32}

**Conclusion and Implications**

First, because acetylcholine and bradykinin produce cerebral vasodilatation through different endothelium-dependent mechanisms, our findings suggest that chronic hypertension affects at least two distinct endothelium-dependent mechanisms. Second, responses of cerebral arterioles to both nitroglycerin and the potential EDRF nitric oxide are preserved in SHRSP, but endothelium-dependent dilatation is impaired. These findings suggest a functional abnormality of endothelium in SHRSP. Third, impaired responses to A23187 in SHRSP suggest that impaired endothelium-dependent cerebral vasodilatation involves an altered postreceptor mechanism. In addition, impaired cerebral vasodilatation in SHRSP in response to A23187 may be due in part to co-release of an EDCF.

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