Mechanisms of Impaired Endothelium-Dependent Cerebral Vasodilatation in Response to Bradykinin in Hypertensive Rats

Shuh-Tsong Yang, MD; William G. Mayhan, PhD; Frank M. Faraci, PhD; and Donald D. Heistad, MD

Bradykinin produces less dilatation of pial arterioles in stroke-prone spontaneously hypertensive rats than in normotensive Wistar-Kyoto rats. The goals of this study were to determine the mediator of bradykinin-induced dilatation in cerebral arterioles of rats and to determine whether responses to this mediator are altered in hypertensive rats. Diameter of pial arterioles (20–65 μm) was measured using intravital microscopy in 18 normotensive and 17 hypertensive rats. Superfusion of 3×10^{-7} M bradykinin dilated pial arterioles by 53±4% (mean±SEM) in normotensive rats but only 33±6% in hypertensive rats (p<0.05 versus normotensive rats). Vasodilatation in response to bradykinin was almost completely inhibited by 280 units/ml catalase in both normotensive and hypertensive rats (n=7 and n=7, respectively) whereas 150 units/ml superoxide dismutase (n=6 and n=5, respectively) and 1 mM deferoxamine (n=5 and n=5, respectively) did not attenuate bradykinin-induced vasodilatation. These findings suggest that hydrogen peroxide is the mediator of bradykinin-induced dilatation in cerebral arterioles of rats. We also examined responses of cerebral arterioles to hydrogen peroxide in five normotensive and six hypertensive rats. Dilator responses of cerebral arterioles to 3.2×10^{-5} M to 1.6×10^{-4} M hydrogen peroxide did not differ in normotensive and hypertensive rats, which suggests that impaired dilatation of cerebral arterioles in response to bradykinin is not related to altered responsiveness of smooth muscle to an endothelium-derived relaxing factor. In summary, our findings suggest that hydrogen peroxide mediates bradykinin-induced cerebral vasodilatation in rats and that impaired cerebral vasodilatation in response to bradykinin during chronic hypertension is not due to altered responsiveness of smooth muscle to hydrogen peroxide. (Stroke 1991;22:1177–1182)

Cerebral vasodilator responses to acetylcholine and bradykinin in vivo are abolished after selective injury to endothelial cells.1,2 Thus, endothelium-dependent mechanisms apparently mediate dilator responses of cerebral arterioles to these agonists.

Dilatation of cerebral arterioles in response to acetylcholine and bradykinin appears to involve two distinct mechanisms. Bradykinin-induced dilatation of cerebral arterioles can be inhibited by scavengers of oxygen radicals,3–5 which suggests that the mediator is an oxygen-derived free radical. Acetylcholine-induced dilatation of cerebral arterioles is not inhibited by scavengers of oxygen radicals but is impaired by generation of oxygen radicals.4,6,7 In pial arterioles of cats and mice, hydroxyl radical appears to mediate dilatation produced by bradykinin.3,5

Chronic hypertension impairs responses of cerebral arterioles to acetylcholine and bradykinin.8,9 Several mechanisms could account for the impaired endothelium-dependent responses of hypertensive cerebral vessels. Impaired endothelium-dependent vasodilatation may be due to reduced synthesis or release of endothelium-derived relaxing factor (EDRF), to reduced responsiveness of vascular
smooth muscle to EDRF, or to release of an endothelium-derived contracting factor.

We first examined the mediator responsible for dilatation produced by bradykinin in cerebral arterioles of rats. We then compared responses of cerebral arterioles to this mediator in normotensive Wistar-Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) to determine whether impaired responses to bradykinin in SHRSP are related to altered responsiveness of smooth muscle to the EDRF for bradykinin.

Materials and Methods

Eighteen male WKY and 17 male SHRSP 8–10 months old were anesthetized with 50 mg/kg i.p. pentobarbital sodium. After completion of a tracheotomy, the rats 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 (10–20 mg/kg/hr i.v. pentobarbital sodium, 5–10 mg/kg/hr i.v. gallamine triethiodide) were administered as needed.

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. The cranial window was suffused with artificial cerebrospinal fluid (CSF) bubbled continuously (pH=7.35±0.01, Pco₂=42±1 mm Hg, and Po₂=79±3 mm Hg in WKY and pH=7.35±0.01, Pco₂=42±1 mm Hg, and Po₂=75±2 mm Hg in SHRSP; mean±SEM). Temperature was maintained at approximately 38°C. Arterial blood gases and pH were monitored periodically and maintained within normal limits (pH=7.46±0.01, Pco₂=35±1 mm Hg, and Po₂=107±4 mm Hg in WKY and pH=7.48±0.01, Pco₂=35±1 mm Hg, and Po₂=112±2 mm Hg in SHRSP).

Pial arteriolar diameter was measured using a video image shearing device (model 907, Instrumentation for Physiology and Medicine, Inc., San Diego, Calif.).

Cerebral vessels were superfused with artificial CSF for 30 minutes before application of agonists. In each rat, we studied responses of the largest pial arteriole present in the cranial window. All drugs were dissolved in artificial CSF and then superfused over the cerebral vessels. Application of vehicle did not affect vessel diameter.

The diameter of cerebral arterioles was measured immediately before application of 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 application. Values obtained at steady state are reported.

The goal of the first part of this study was to determine the mediator that is responsible for bradykinin-induced dilatation in cerebral arterioles of rats. Responses of cerebral arterioles to bradykinin were tested in the presence of one of the following agents administered topically: catalase (280 units/ml), superoxide dismutase (150 units/ml), and deferoxamine (1 mM). The doses of the agents that were used are based on previous studies of cerebral vessels.4,5 The cranial window was pre-treated with either catalase, superoxide dismutase, or deferoxamine for 20 minutes prior to application of bradykinin. Responses of pial arterioles to the endothelium-independent dilator nitroglycerin were also tested in the presence of either catalase, superoxide dismutase, or deferoxamine.

Responses of cerebral arterioles to 3.2×10⁻⁵ M to 1.6×10⁻⁴ M hydrogen peroxide were also determined. Our preliminary studies indicated that responses of cerebral arterioles to hydrogen peroxide were relatively slow and reached a steady state 8–10 minutes after application. Thus, we measured the diameter of cerebral arterioles after continuous superfusion with a given concentration of hydrogen peroxide for 10 minutes.

Effects of catalase, superoxide dismutase, and deferoxamine on bradykinin-induced responses were analyzed with a paired t test. Responses of cerebral arterioles to hydrogen peroxide in WKY and SHRSP were compared with an unpaired t test. A probability value of less than 0.05 was considered to be significant. Results are reported as mean±SEM.

Results

Mean arterial blood pressure was 94±3 mm Hg in WKY and 196±5 mm Hg in SHRSP (p<0.05 versus WKY). Baseline diameter of pial arterioles was significantly less in SHRSP than in WKY (36±3 μm in SHRSP versus 44±4 μm in WKY, p<0.05). We have reported previously that the number of arterial branching points from the circle of Willis to the area exposed by the craniotomy is similar in WKY and SHRSP.10 Thus, in this study we examined

<table>
<thead>
<tr>
<th>Agent</th>
<th>n</th>
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<tr>
<td>Catalase (280 units/ml)</td>
<td>7</td>
<td>46±3</td>
<td>45±4</td>
</tr>
<tr>
<td>WKY</td>
<td>7</td>
<td>36±2</td>
<td>36±2</td>
</tr>
<tr>
<td>SHRSP</td>
<td>6</td>
<td>45±6</td>
<td>42±5</td>
</tr>
<tr>
<td>Superoxide dismutase (150 units/ml)</td>
<td>5</td>
<td>36±3</td>
<td>37±3</td>
</tr>
<tr>
<td>Deferoxamine (1 mM)</td>
<td>5</td>
<td>38±3</td>
<td>38±3</td>
</tr>
<tr>
<td>WKY</td>
<td>5</td>
<td>35±3</td>
<td>34±3</td>
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Values are mean±SEM. WKY, Wistar-Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats.
responses of cerebral arterioles that are of equivalent hierarchy.

Catalase, superoxide dismutase, and deferoxamine did not affect the baseline diameter of pial arterioles (Table 1). Dilator responses to bradykinin in cerebral arterioles were almost completely inhibited by catalase in both WKY and SHRSP (Figure 1). In contrast, catalase had little effect on responses to nitroglycerin (Figure 2). Neither superoxide dismutase nor deferoxamine inhibited cerebral vasodilatation produced by bradykinin (Figures 3 and 4). In fact, both superoxide dismutase and deferoxamine tended to potentiate the effect of bradykinin, especially in WKY. These data suggest that dilator responses to bradykinin in cerebral arterioles of rats are dependent on formation of the oxygen-derived molecule hydrogen peroxide.

Bradykinin at $10^{-7}$ and $3 \times 10^{-7}$ M produced less cerebral vasodilatation in SHRSP (15±3% and 33±6%, respectively) than in WKY (30±4% and 53±4%, respectively; p<0.05 versus SHRSP). Responses to nitroglycerin were not impaired in SHRSP (control conditions, Figure 2). Hydrogen peroxide produced dilatation of cerebral arterioles in both WKY and SHRSP (Figure 5). Responses to hydrogen peroxide were similar in WKY and SHRSP. This finding suggests that responses of cerebral arterioles to the mediator responsible for bradykinin-induced dilatation is not impaired during chronic hypertension.

Discussion

There are two major findings in this study. First, dilatation of cerebral arterioles induced by bradykinin is mediated by hydrogen peroxide in rats. Second, responses of cerebral arterioles to hydrogen peroxide are not impaired in SHRSP. Thus, our findings suggest that impaired cerebral vasodilatation in response to bradykinin during chronic hypertension is...
not due to altered responsiveness of smooth muscle to the factor that mediates cerebral vasodilator responses to bradykinin.

In mice, cerebral vasodilator responses to bradykinin in vivo are abolished after injury to endothelial cells with the light-dye or laser-dye techniques. This finding suggests that responses to bradykinin 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.

Bradykinin-induced cerebral vasodilatation was almost completely inhibited by catalase, which scavenges hydrogen peroxide. Hydroxyl radical, which appears to mediate cerebral vasodilatation in response to bradykinin in cats and mice, can be generated from hydrogen peroxide through either the Fenton reaction or the Haber-Weiss reaction. Our findings with catalase suggest that either hydrogen peroxide or hydroxyl radical mediates bradykinin-induced cerebral vasodilatation in rats.

The presence of superoxide anion, hydrogen peroxide, and iron are required for the Haber-Weiss reaction. Both superoxide dismutase, which scavenges superoxide anion, and deferoxamine, which chelates iron, can inhibit the Haber-Weiss reaction by maintaining low concentrations of superoxide anion and iron, thus inhibiting the generation of hydroxyl radical. High concentrations of deferoxamine may also scavenge hydroxyl radical directly. Because neither superoxide dismutase nor deferoxamine inhibited cerebral vasodilatation induced by bradykinin, our findings suggest that bradykinin-induced cerebral vasodilatation in rats is not mediated by hydroxyl radical and that it may be mediated by hydrogen peroxide. The concentrations of catalase, superoxide dismutase, and deferoxamine that were used in this study are similar to or higher than those that would be expected in vivo.
The mechanism by which both superoxide dismutase and deferoxamine tend to potentiate bradykinin-induced vasodilatation in cats and mice.4,5

The explanation for the finding that cerebral vasodilatation in response to bradykinin is mediated by different oxygen-derived molecules in rats and cats or mice is not clear. Differences between species is one possible explanation. In the cerebral microcirculation of cats, for example, it has been suggested that superoxide anion and hydrogen peroxide also may contribute to vasodilatation induced by bradykinin.15,16 Hydrogen peroxide, but not hydroxyl radical, also appears to mediate dilatation in other vascular beds in rats and dogs in response to generation of oxygen-derived free radicals by the xanthine oxidase reaction.17,18

The mechanism by which both superoxide dismutase and deferoxamine tend to potentiate bradykinin-induced vasodilatation is not clear. Because superoxide dismutase facilitates the generation of hydrogen peroxide and deferoxamine prevents the formation of hydroxyl radical from hydrogen peroxide, it is possible that the presence of superoxide dismutase or deferoxamine results in a higher concentration of hydrogen peroxide and thus potentiated bradykinin-induced vasodilatation.

In the coronary circulation, hydrogen peroxide is able to trigger the release of EDRF.17 In the same vascular bed, superoxide anion inactivates the EDRF released by acetylcholine, and thus superoxide dismutase prolongs the half-life of EDRF.19 If this is also the case in the cerebral circulation, it is possible that hydrogen peroxide triggers the release of EDRF by bradykinin and that superoxide dismutase as well as deferoxamine potentiate responses of cerebral arterioles to bradykinin by inactivating superoxide anion, which destroys EDRF. Deferoxamine has been reported to react with superoxide anion.20 This hypothetical model of the interaction between oxygen radicals and endothelium-dependent relaxation of vascular smooth muscle was discussed in detail by Rubanyi.21

In the present study, hydrogen peroxide produced similar dose-dependent vasodilatation in WKY and SHRSP (Figure 5). Vasodilatation produced by low concentrations of hydrogen peroxide, which did not produce a maximal response, was approximately equal in WKY and SHRSP. These data indicate that similar responses to hydrogen peroxide in normotensive and hypertensive rats were not due to application of maximal concentrations of hydrogen peroxide.

Reduced synthesis or release of EDRF or reduced responsiveness of vascular smooth muscle to EDRF could lead to impaired endothelium-dependent vasodilatation during chronic hypertension. In the present study, we tested responses of cerebral arterioles to hydrogen peroxide, which appears to mediate dilatation in response to bradykinin in rats, in both WKY and SHRSP. Our data indicate that responses of cerebral vessels to this mediator are not impaired in SHRSP. If hydrogen peroxide is the EDRF for bradykinin in cerebral arterioles of rats, preserved responses to hydrogen peroxide in SHRSP suggest that impairment of endothelium-dependent responses is not due to impaired responsiveness of vascular smooth muscle to the EDRF. If hydrogen peroxide is not the EDRF for bradykinin but triggers the release of an EDRF, preserved responses to hydrogen peroxide in SHRSP may suggest that the ability of endothelial cells to release EDRF in response to hydrogen peroxide and the responsiveness of smooth muscle to EDRF are not impaired in SHRSP. In either case, our findings with hydrogen peroxide suggest that impaired endothelium-dependent cerebral vasodilatation in response to bradykinin during chronic hypertension is not related to impaired responsiveness of smooth muscle to the EDRF for bradykinin.

It is possible that impaired diffusion of EDRF from endothelium to vascular smooth muscle, related to subendothelial thickening in chronic hypertension, may impair endothelium-dependent vasodilatation. However, endothelium-dependent relaxation in the aorta of spontaneously hypertensive rats is normal in the presence of indomethacin, which suggests that the morphological change is of minor importance in this model of hypertension.22 Furthermore, the barrier between endothelium and smooth muscle may not be as important in arterioles as in large vessels. Nevertheless, we cannot exclude the possibility that impaired diffusion of EDRF contributes to impaired vasodilatation in SHRSP.

Impaired endothelium-dependent vasodilatation during chronic hypertension may be due in part to release of a contracting factor. Several agonists that release EDRF can also release an endothelium-derived contracting factor, especially in spontaneously hypertensive rats. In the present study, dilator responses of cerebral arterioles to bradykinin were almost completely abolished by catalase in both WKY and SHRSP. If cerebral arterioles in SHRSP produce both EDRF and endothelium-derived contracting factor 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. Thus, our findings suggest that impaired responses to bradykinin in SHRSP are not due to corelease of an endothelium-derived contracting factor.

References

Key Words • bradykinin • free radicals • hydrogen peroxide • rats
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