Responses of Cerebral Arterioles to Adenosine 5'-Diphosphate, Serotonin, and the Thromboxane Analogue U-46619 During Chronic Hypertension

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SUMMARY The goal of this study was to determine whether responses of cerebral arterioles to products released by platelets are impaired in stroke-prone spontaneously hypertensive rats (SHRSP). The diameter of pial arterioles was measured during suffusion with adenosine 5'-diphosphate (ADP), serotonin, and the thromboxane analogue U-46619, using intravital microscopy in normotensive Wistar-Kyoto rats (WKY) and SHRSP (7-10 months old). Responses of cerebral arterioles to ADP and serotonin were profoundly impaired in SHRSP. ADP (10^{-6} M) increased pial arteriolar diameter 17 ± 3% (mean ± SE) in WKY and only 4 ± 1% in SHRSP. Serotonin (10^{-5} M) increased pial arteriolar diameter 15 ± 2% in WKY and, in contrast, reduced the diameter 13 ± 1% in SHRSP. Nitroglycerin produced a similar dilatation of cerebral arterioles in WKY and SHRSP, suggesting that impairment of dilatation in SHRSP in response to ADP and serotonin was not related to nonspecific impairment of vasodilatation in SHRSP. The thromboxane analogue U-46619 produced a similar constriction of arterioles in WKY and SHRSP. We also examined the possibility that impaired dilator responses of cerebral arterioles in SHRSP in response to ADP and serotonin may be related to production of a cyclooxygenase vasoconstrictor substance. Indomethacin (10 mg/kg i.v.) partially restored dilator responses to ADP and serotonin in SHRSP, without altering responses in WKY. Thus, we speculate that vasoactive substances released by platelets may release a prostanoid constrictor substance from cerebral vessels of SHRSP and thereby predispose SHRSP to cerebral ischemia and, perhaps, stroke. (Hypertension 12: 556-561, 1988)

KEY WORDS • endothelium-dependent responses • endothelium-derived relaxing factor • nitroglycerin • platelet products • indomethacin • brain • stroke • rats

PLATELET aggregation may contribute to transient cerebral ischemia and, perhaps, stroke. Platelets contain large amounts of serotonin, adenosine 5'-diphosphate (ADP), and thromboxane that are released during platelet aggregation.1-3 The responses of aortic rings to products released by platelets are altered during chronic hypertension. The relaxation of thoracic aorta in response to ADP is impaired,4 and there is enhanced sensitivity of vascular muscle to serotonin in hypertensive rats.5-8 Experiments in vitro suggest that enhanced contraction of thoracic aorta4 and coronary arteries9 from hypertensive animals in response to serotonin may be related to altered endothelial function. Removal of the endothelium4 or pretreatment with indomethacin9 reduces contraction of vascular muscle in response to serotonin. These findings suggest that a constrictor factor may be released from endothelium by way of the cyclooxygenase pathway in hypertensive animals in response to serotonin.

Recently, we have found that responses of cerebral arterioles in vivo to endothelium-dependent agonists are altered in stroke-prone spontaneously hypertensive rats (SHRSP).10 Acetylcholine produced dilatation of cerebral arterioles in normotensive Wistar-Kyoto rats (WKY) but did not change the diameter of arterioles in SHRSP. Serotonin produced dilatation of cerebral arterioles in WKY and, in contrast, constricted cerebral arterioles in SHRSP. The first goal of this study was to test the hypothesis that dilator responses of cerebral arterioles to...
products that are released by platelets are impaired in SHRSP. We compared responses of cerebral arterioles in WKY and SHRSP to ADP and serotonin. We also tested whether constrictor responses of cerebral arterioles to thromboxane are augmented in SHRSP. The second goal of this study was to test the hypothesis that impaired dilator responses of cerebral arterioles to products released by platelets in SHRSP may be related to production of a cyclooxygenase constrictor substance by cerebral vessels in SHRSP.

Materials and Methods

Preparation of Animals

Male WKY (n = 13) and SHRSP (n = 14), 7 to 10 months old, were anesthetized (pentobarbital sodium, 50 mg/kg body weight i.p.), and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. Skeletal muscle paralysis was induced with gallamine triethiodide (10 mg/kg i.v.). Supplemental anesthesia was administered intravenously at a dose of 10 to 20 mg/kg/hr. A catheter was placed into a femoral vein for injection of drugs, and a femoral artery was cannulated for measurement of arterial blood pressure.

To visualize the microcirculation of the cerebral, a craniotomy was prepared over the right parietal cortex. The cranial window was suffused with artificial cerebrospinal fluid that was bubbled continuously (WKY: pH, 7.43 ± 0.01; partial pressure of CO₂ [Paco₂], 47 ± 1 mm Hg; partial pressure of O₂ [Pao₂], 53 ± 4 mm Hg; SHRSP: pH, 7.32 ± 0.01; Paco₂, 47 ± 1 mm Hg; Pao₂, 59 ± 2 mm Hg; values are means ± SE). The temperature of the suffusate was maintained at 38 °C. Gases and pH of the artificial cerebrospinal fluid were constant throughout the experiment. Arterial blood gases were monitored and maintained within normal limits throughout the experiment (WKY: pH, 7.43 ± 0.01; Paco₂, 36 ± 1 mm Hg; Pao₂, 152 ± 12 mm Hg; SHRSP: pH, 7.41 ± 0.01; Paco₂, 38 ± 1 mm Hg; Pao₂, 148 ± 7 mm Hg).

The pial arteriolar diameter was measured using a video image-shearing device (Model 907, Instrumentation for Physiology and Medicine, San Diego, CA, USA).

Experimental Protocol

Cerebral vessels were superfused with artificial cerebrospinal fluid for 30 minutes before the responses of arterioles to the agonists were tested. We examined responses of cerebral arterioles to ADP (10⁻⁵ M and 10⁻⁴ M), serotonin (10⁻⁶ M and 10⁻⁵ M), and the thromboxane analogue U-46619, 15(S)-hydroxy-11α,9α(epoxy-methano)prosta-5Z, 13E-dienoic acid (10⁻⁴ M and 10⁻⁷ M). To determine whether impaired dilatation of cerebral arterioles in SHRSP was related to nonspecific impairment of vasodilatation in SHRSP, we also examined the responses of cerebral arterioles in WKY and SHRSP to nitroglycerin (10⁻⁶ M and 10⁻⁵ M). Drugs were mixed in artificial cerebrospinal fluid and then superfused over the cerebral microcirculation. The application of vehicle did not affect vessel diameter. The application of agonists was randomized. In each rat, we studied the responses of the largest pial arteriole exposed by the craniotomy to the application of agonists. The diameter of cerebral arterioles was measured immediately before the application of agonists and every 20 to 30 seconds for 3 to 5 minutes during the application of agonists. Steady state responses to agonists were reached within 1 to 2 minutes after application, and the diameter of cerebral arteries returned to control within 1 to 2 minutes after the application of agonists was stopped.

In some rats, we examined the possibility that impaired responses of cerebral arterioles in SHRSP may be related to production of a cyclooxygenase constrictor substance by the endothelium of SHRSP. To test this possibility, we examined the responses of cerebral arterioles in WKY and SHRSP to the agonists before and 20 to 30 minutes after an intravenous infusion of indomethacin (10 mg/kg). The efficacy of the indomethacin was demonstrated by inhibition of the dilatation of cerebral arterioles to topical application of arachidonic acid (100 μg/min), as described previously.

Statistical Analysis

An unpaired t test was used to compare values between different groups of animals. A p value of 0.05 was considered significant.

Results

Control Conditions

Mean arterial pressure was 107 ± 3 mm Hg in WKY and 194 ± 3 mm Hg in SHRSP. The diameter of pial arterioles was 44 ± 3 μm in WKY and 34 ± 2 μm in SHRSP (p < 0.05). 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 and SHRSP. Thus, in this study, we examined responses of cerebral arterioles that are of equivalent hierarchy.

Responses to Agonists

ADP produced dilatation of cerebral arterioles in WKY and SHRSP (Figure 1). Dilatation of cerebral arterioles in response to ADP, however, was significantly less in SHRSP than in WKY (see Figure 1). In terms of relative potency, the response of cerebral arterioles to 10⁻³ M ADP in WKY was greater than the response to 10⁻⁴ M ADP in SHRSP; this suggests that ADP is more than 10 times more potent in WKY than SHRSP. Thus, responses of cerebral arterioles to ADP are profoundly impaired in SHRSP as compared with WKY.

Serotonin produced dilatation of cerebral arterioles in WKY (Figure 2). In contrast, serotonin
produced constriction of arterioles in SHRSP (see Figure 2). Thus, in SHRSP there is a reversal of responses to serotonin from vasodilatation to vasoconstriction.

Nitroglycerin (10^-6 M and 10^-3 M) dilated cerebral arterioles by 15 ± 1 and 27 ± 3% in WKY, and by 17 ± 3 and 31 ± 3% in SHRSP (p > 0.05). Thus, impaired cerebral vasodilatation in SHRSP in response to ADP and serotonin cannot be explained by nonspecific impairment of the ability of cerebral arterioles to dilate in SHRSP.

The thromboxane analogue U-46619 produced constriction of cerebral arterioles in WKY and SHRSP (Figure 3). The responses of cerebral arterioles to U-46619 were not different in WKY and SHRSP.

**Responses After Indomethacin**

Intravenous infusion of indomethacin did not affect the resting diameter of cerebral arterioles. The resting diameter in WKY was 40 ± 3 μm before indomethacin versus 40 ± 2 μm after indomethacin (p > 0.05); the diameter in SHRSP was 37 ± 4 μm before indomethacin versus 37 ± 5 μm after indomethacin (p > 0.05). The efficacy of indomethacin in WKY and SHRSP was determined by examining the responses of cerebral arterioles to topical application of arachidonic acid. Arachidionate (100 μg/min) dilated cerebral arterioles by 16 ± 2% in WKY and 11 ± 1% in SHRSP. Indomethacin (10 mg/kg) completely inhibited the dilatation of cerebral arterioles in response to topical application of arachidonate in WKY and SHRSP (1 ± 1%; p < 0.05 vs response before indomethacin).

In WKY, indomethacin did not alter the responses of cerebral arterioles to ADP (Figure 4) or serotonin (Figure 5). The responses of cerebral arterioles to nitroglycerin (10^-3 M) also were not altered by indomethacin in WKY (21 ± 1% before vs 27 ± 5% after indomethacin; p > 0.05).

In SHRSP dilatation of cerebral arterioles in response to ADP was greater following treatment with indomethacin (see Figure 4). Constriction of cerebral arterioles in SHRSP in response to serotonin before infusion of indomethacin was converted to vasodilatation after infusion of indomethacin (see Figure 5). Dilator responses of cerebral arterioles in SHRSP to nitroglycerin (10^-3 M) were not altered after indomethacin (25 ± 6% before vs 26 ± 3% after indomethacin; p > 0.05).
after indomethacin; \( p > 0.05 \). These findings suggest that impaired dilator responses of cerebral arterioles to ADP and serotonin in SHRSP may be related to the synthesis of a cyclooxygenase constrictor substance by cerebral vessels in SHRSP.

**Discussion**

There are three major findings in this study. First, dilator responses of cerebral arterioles to ADP and serotonin are profoundly impaired in the cerebral microcirculation of SHRSP as compared with WKY. We have previously reported that dilator responses of cerebral arterioles to serotonin are impaired in SHRSP as compared with WKY.\(^{10}\) Second, constrictor responses of cerebral arterioles to the thromboxane analogue U-46619 are similar in WKY and SHRSP. Third, the impaired dilatation of cerebral arterioles in SHRSP in response to ADP and serotonin may be related to the production of a cyclooxygenase constrictor substance in SHRSP, presumably by the endothelium.

**Consideration of Methods**

We examined the reactivity of cerebral arterioles in WKY and SHRSP to ADP and serotonin, which release endothelium-derived relaxing factor (EDRF) in a variety of vascular preparations. We also examined responses of cerebral arterioles to nitroglycerin, which produces vasodilatation independently of the release of EDRF. There are no data, however, to establish that the agonists used in the present study are endothelium-dependent or endothelium-independent in cerebral arterioles in rats. On the basis of responses of cerebral arterioles in other species,\(^{14}\) thoracic aorta in rats,\(^{4}\) and coronary arteries in dogs\(^{15, 16}\) it seems unlikely that there is an unusual role of the endothelium in the modulation of responses of cerebral arterioles in rats to the agonists used in the present study.

**Response to ADP**

ADP and adenosine 5'-triphosphate (ATP) are contained in platelets and are released when platelets aggregate. ADP is a potent endothelium-dependent vasodilator.\(^{14, 17}\) The relaxation of canine coronary arteries in response to platelets is inhibited by apyrase, which hydrolyzes ATP and ADP.\(^{18}\) Thus, aggregation of the platelets appears to produce vascular relaxation primarily by the release of ADP and ATP.

The relaxation of thoracic aorta in response to ADP is impaired in spontaneously hypertensive rats (SHR).\(^{4}\) We have extended the findings of the previous study\(^{4}\) with the observation that dilatation of cerebral arterioles in response to ADP is impaired in SHRSP. Thus, altered vasodilatation in response to ADP during chronic hypertension is not confined to large arteries but also involves arterioles. Our findings suggest that the impairment of responses may be particularly profound in cerebral arterioles.

**Response to Serotonin**

In several vascular beds, serotonin produces constriction of large arteries and dilatation of small arterioles. However, exceptions to this concept have been reported in the cerebral circulation. The application of serotonin to pial vessels in cats has been reported to produce constriction of both arteries and arterioles.\(^{19}\) In other studies in cats, however, application of serotonin produced constriction of cerebral arteries but dilatation of arterioles.\(^{20}\) In rats, serotonin produced minimal constriction of penetrating intracerebral arterioles in vitro but dose-related dilatation of cerebral arterioles in vivo.\(^{10, 22}\) Thus, the responses of cerebral arterioles are complex, and the factors accounting for differences in response to the application of serotonin are not clear.

Platelets contain large amounts of serotonin, which is released during platelet aggregation.\(^{1-3}\) Previous studies have shown increased sensitivity of aorta and cerebral blood vessels to serotonin in SHR and deoxycorticosterone acetate–salt hypertensive rats.\(^{5-8}\) The enhanced sensitivity of vascular muscle to serotonin in hypertensive rats could not be explained by structural alterations in vascular muscle and may be related to changes in the calcium sensitivity of vessels and/or decreases in calcium binding sites that are responsible for membrane stabilization.\(^{5, 8}\)

Recent studies suggest that the enhanced constriction of arteries from hypertensive animals in response to serotonin may not be related to direct effects of serotonin on vascular muscle but may be related to altered endothelial function.\(^{4, 9}\) Serotonin produces more constriction of thoracic aorta\(^{4}\) and coronary blood vessels\(^{9}\) in SHR than in WKY. The contraction of thoracic aorta and coronary blood vessels in SHR in response to serotonin was significantly decreased after removal of the endothelium\(^{4}\) and following infusion of indomethacin\(^{9}\) in SHR. These
findings suggest that the endothelium in SHR may release a constrictor factor in response to serotonin.

In the present study and in a previous study,10 we found that dilator responses of cerebral arterioles to serotonin are abolished in cerebral arterioles of SHRSP and that serotonin produces constriction of cerebral arterioles in SHRSP. Although we cannot exclude the possibility that constriction of cerebral arterioles in SHRSP may be related to an increased sensitivity of vascular muscle to serotonin in SHRSP, we speculate that differences in response to serotonin in WKY and SHRSP are related to altered endothelial function in SHRSP.

Response to Thromboxane

Thromboxane, like ADP and serotonin, is contained in platelets and is released when platelets aggregate. The present study is the first, to our knowledge, to examine whether responses of blood vessels to thromboxane are altered during chronic hypertension.

Evidence concerning the role of endothelium in responses of blood vessels to thromboxane is not consistent. The contraction of canine and porcine coronary arteries in response to U-46619 was not different following removal of the endothelium.23 Recent studies, however, have shown that the contraction of rabbit coronary arteries24 and rat aorta25 in response to thromboxane is modulated by the endothelium. The contraction of coronary arteries24 and aorta25 was greater following removal of the endothelium or application of hemoglobin than with intact endothelium. We are not aware of any studies that have examined the role of endothelium in the modulation of responses of cerebral blood vessels to thromboxane.

In the present study, we predicted that if constrictor responses of cerebral arterioles to thromboxane are modulated by release of an EDRF, constriction of cerebral arterioles would be greater in SHRSP than WKY. However, we observed similar constriction of cerebral arterioles in response to thromboxane in WKY and SHRSP. We cannot determine from our findings whether cerebral arterioles in WKY and SHRSP do not release an EDRF in response to thromboxane or whether the release of an EDRF in response to thromboxane is similar in WKY and SHRSP.

Mechanism of Impaired Responses to ADP and Serotonin

We have considered three mechanisms that may account for the impaired dilatation of cerebral arterioles in response to ADP and serotonin in SHRSP. First, there may be diminished release of EDRF from cerebral arterioles of SHRSP in response to ADP and serotonin. Previous studies have shown that dilatation of blood vessels in response to ADP14, 17 and serotonin15, 16 is dependent on an intact endothelium. Studies by Van de Voorde and Leusen26 using bioassay techniques, however, have shown that impaired dilatation of aorta in response to acetylcholine in SHR is not related to diminished release of EDRF.

Second, the impaired responses of cerebral arterioles to ADP and serotonin in SHRSP may be related to chronic production of oxygen radicals, produced by the cyclooxygenase pathway, to inactivate EDRF. Oxygen radicals that are produced during acute hypertension abolish the dilatation of cerebral arterioles in response to acetylcholine.27 Dilator responses of cerebral arterioles are restored after acute hypertension by topical application of superoxide dismutase and catalase. In the present study, we found improvement of dilatation of cerebral arterioles in response to ADP and reversal of vasoconstriction in response to serotonin in SHRSP following treatment with indomethacin. Thus, it is possible that indomethacin inhibits the production of oxygen radicals, produced by the cyclooxygenase pathway, in SHRSP. We are not aware, however, of evidence that production of oxygen radicals is increased during chronic hypertension.

Third, we considered the possibility that impaired responses of cerebral arterioles to ADP and serotonin in SHRSP may be related to the production of a cyclooxygenase constrictor substance in SHRSP. Previous studies have implicated a role for a cyclooxygenase constrictor substance in the altered responses of thoracic aorta4 and coronary arteries9 to serotonin in SHR. Serotonin produced greater contraction of thoracic aorta in SHR than in WKY.4 Furthermore, serotonin produced less contraction of thoracic aorta in SHR after removal of the endothelium. In another study, serotonin increased coronary blood flow in WKY but decreased coronary blood flow in SHR.9 The altered response of coronary blood vessels in SHR to serotonin could be reversed with indomethacin.

In the present study, we found that impaired dilatation of cerebral arterioles in response to ADP in SHRSP could be restored toward that observed in WKY by indomethacin. In addition, the constriction of cerebral arterioles in SHRSP in response to serotonin could be reversed to vasodilatation by indomethacin. These findings suggest that the endothelium in SHRSP may release a constrictor substance in response to ADP and serotonin.

Implications

Recent evidence suggests that platelets from hypertensive patients and animals are more reactive to stimuli producing platelet aggregation,28 release more serotonin,29, 30 adhere more readily to vascular endothelium,31 have a decreased survival time,32, 33 and have an augmented turnover as compared with platelets from normotensive patients and animals.34, 35 Furthermore, aggregating platelets produce greater contraction of thoracic aortas in hypertensive rats than in normotensive rats.4

We speculate that altered responses of cerebral arterioles to vasoactive substances released by plate-
lets may be of particular importance in the cerebral circulation. When platelets aggregate at plaques in the carotid arteries and release ADP and serotonin, the impairment of vasodilator responses during chronic hypertension may predispose to cerebral ischemia and, perhaps, to stroke. Thus, the findings in this study may have important implications for mechanisms by which chronic hypertension predisposes to stroke.

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