The goal of this study was to determine whether responses of cerebral vessels to intravascular administration of serotonin are altered in stroke-prone spontaneously hypertensive rats. We measured pressure in pial arterioles and cerebral blood flow in normotensive and hypertensive rats during intra-atrial infusion of serotonin. In normotensive rats, pial arteriolar pressure was 48±3 mm Hg (mean±SEM) and cerebral blood flow was 48±5 ml/min/100 g under control conditions. Intr-atrial infusion of serotonin (5 and 50 μg/kg/min for 5 minutes) produced only minimal changes in pial arteriolar pressure (−3±4 and −4±4 mm Hg, respectively) and did not alter cerebral blood flow. In hypertensive rats, pial arteriolar pressure was 95±9 mm Hg and cerebral blood flow was 57±4 ml/min/100 g under control conditions. In contrast to normotensive rats, intra-atrial infusion of serotonin (5 and 50 μg/kg/min for 5 minutes) in hypertensive rats profoundly decreased pial arteriolar pressure (−29±7 and −44±4 mm Hg, respectively) without altering cerebral blood flow. To determine whether altered responses of cerebral arterioles to serotonin in hypertensive rats were related to nonspecific increases in vascular reactivity, we examined the effects of angiotensin in normotensive and hypertensive rats. Responses to angiotensin (1 and 3 μg/kg/min i.v. for 5 minutes) were not potentiated in hypertensive rats. Thus, constrictor responses of cerebral vessels to intravascular serotonin are potentiated in hypertensive rats. We speculate that when serotonin is released by platelets, augmented vasoconstrictor responses to serotonin may have important implications for the pathogenesis of cerebral ischemia, and perhaps stroke, during chronic hypertension. (Hypertension 1990;15:872–876)

Chronic hypertension alters the morphology and function of vascular muscle and endothelium and also appears to affect blood platelets. Platelets from hypertensive patients and animals are more reactive to stimuli that produce aggregation and adhere more readily to endothelium when compared with platelets from normotensive patients and animals. Furthermore, aggregating platelets produce greater contraction of thoracic aorta in hypertensive rats than in normotensive rats. Platelets contain large amounts of serotonin that are released during aggregation. We have recently shown that responses of cerebral arterioles to topical application of serotonin are altered in stroke-prone spontaneously hypertensive rats (SHRSP). In normotensive Wistar-Kyoto (WKY) rats, topical application of serotonin produced dilatation of cerebral arterioles, but in contrast, topical application of serotonin produced constriction of cerebral arterioles in SHRSP.

Because the release of serotonin from platelets during aggregation occurs into the vascular lumen and because vascular responses to abluminal and luminal application of serotonin may be different, the goal of our study was to determine whether responses of the cerebral circulation to intravascular administration of serotonin are altered during chronic hypertension.

Methods

Preparation of Animals

Male SHRSP (n=11) and WKY (n=11) rats (7–10 months old) were anesthetized (50 mg/kg body wt i.p. pentobarbital sodium) and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. Skeletal muscle paralysis was obtained with gallamine triethiodide (5–10 mg/kg i.v.). Supplemental anesthesia was administered at a dose of 10–20 mg/kg/hr i.v.

A catheter was inserted into a femoral vein for injection of drugs, and a femoral artery was cannu-
lated for measurement of arterial blood pressure. Both brachial arteries were cannulated for withdrawal of reference blood samples. The heart was exposed through a thoracotomy, and a double-lumen cannula was placed in the left atrial appendage for injection of microspheres and serotonin.

To visualize the microcirculation of the cerebrum, a craniotomy was prepared over the right parietal cortex.14 The cranial window was suffused with artificial cerebral spinal fluid, which was bubbled continuously to maintain pH and gases within normal limits. Temperature of the suffusate was maintained at 38°C. Gases and pH of the artificial cerebral spinal fluid were constant throughout the experiment. Arterial blood gases were monitored and were maintained within normal limits throughout the experiment (pH 7.40±0.02, Pco2 of 39±1 mm Hg, and Po2 of 111±15 mm Hg for WKY rats; pH 7.39±0.02, Pco2 of 38±2 mm Hg, Po2 of 130±25 mm Hg for SHRSR).

Measurement of Microvascular Pressure and Cerebral Blood Flow

Pressure in pial arterioles (30–50 μm in diameter) was measured with a micropipette connected to a servo-null pressure measuring device (model 5, Instrumentation for Physiology & Medicine, Inc., San Diego, California). Pipettes were sharpened to a beveled tip of 2–4 μm in diameter, filled with 1.5 M sodium chloride, and then inserted into the lumen of arterioles with a micromanipulator (model MM-33, Brinkmann Instruments, Inc., Westbury, New York). Pial arteriolar pressure was measured continuously under control conditions and during intra-atrial infusion of serotonin or intravenous infusion of angiotensin in SHRSR and WKY rats. Insertion of the micropipette did not cause bleeding or produce spasm of the arterioles.

Cerebral blood flow was measured by injection of microspheres (15 μm in diameter) into the left atrium in 10 seconds. The microspheres were labeled with 82Sc, 86Sr, 113Sn, and 153Gd. Each vial of microspheres was shaken vigorously for 3–5 minutes before injection. Starting 10 seconds before injection of microspheres and continuing for 1 minute after injection, reference arterial blood samples were withdrawn at 0.21 ml/min from each brachial artery.

At the end of the study, the rats were killed with an injection of saturated potassium chloride. The brain was removed and placed in buffered formalin for 1–3 days. Tissue samples and reference arterial blood samples were counted using a sodium-iodine well-type gamma counter (model 300, Beckman Instruments, Inc., Fullerton, California). Isotope separation was performed by using standard techniques. Cerebral blood flow (ml/min/100 g) was calculated as

\[
\text{CBF} = \frac{(C_r \times 100 \times Q_r)}{C_c},
\]

where CBF is cerebral blood flow, Q, is the reference sample flow rate, and C and C, are counts in tissue and reference samples, respectively. Large artery resistance was calculated as follows: (aortic pressure – pial arteriolar pressure)/ cerebral blood flow to the cerebral. We have described the methods of measurement of microvascular pressure, cerebral blood flow, and resistance of large cerebral arteries in detail previously.15–18

Experimental Protocol

In WKY rats (n=7), we measured systemic arterial pressure, pial arteriolar pressure, and cerebral blood flow under control conditions and during intra-atrial injection of two doses of serotonin (5 and 50 μg/kg/min for 5 minutes).

In SHRSR (n=7), a similar protocol as described above for WKY rats was followed. Systemic arterial pressure, pial arteriolar pressure, and cerebral blood flow were measured under control conditions and during injection of two doses of serotonin (5 and 50 μg/kg/min for 5 minutes).

We also examined whether altered responses of cerebral vessels to serotonin in SHRSR were related to nonspecific increases in vascular reactivity in SHRSR. Systemic arterial pressure and pial arteriolar pressure were measured under control conditions and during intravenous injection of two doses of angiotensin II (1 and 3 μg/kg/min for 5 minutes) in SHRSR (n=4) and WKY rats (n=4). Increases in systemic arterial pressure in response to angiotensin were prevented by withdrawal of blood from the femoral artery.

Statistics

Statistical analysis was performed by analysis of variance to compare control and intervention values. Analysis of variance was used to compare values between groups of rats (SHRSR vs. WKY rats). A value of p<0.05 was considered to be significant.

Results

Control Conditions

Mean arterial pressure and pial arteriolar pressure were significantly higher in SHRSR than in WKY rats (Table 1) under control conditions. The pressure gradient between aorta and pial arteriole was significantly greater in SHRSR (86±6 mm Hg) compared with WKY rats (44±4 mm Hg). Thus, although mean arterial pressure was about 90 mm Hg higher in SHRSR than in WKY rats, pial arteriolar pressure was only 47 mm Hg higher in SHRSR than in WKY rats. Values for cerebral blood flow were similar in SHRSR and WKY rats, and resistance of large arteries was greater in SHRSR than in WKY rats during control conditions (Table 1). This finding indicates that large arteries attenuate increases in pressure in the cerebral microcirculation under control conditions.

Responses to Serotonin

The difference between aortic and pial arteriolar pressure did not change significantly in WKY rats during infusion of the low dose of serotonin but did increase modestly during infusion of the high dose of serotonin (Table 1, Figure 1). In contrast, the difference between aortic and pial arteriolar pressure...
TABLE 1. Cerebral Hemodynamics in Wistar-Kyoto and Stroke-Prone Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>5 µg/kg/min</th>
<th>50 µg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wistar-Kyoto rats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>93±4</td>
<td>92±6</td>
<td>103±5</td>
</tr>
<tr>
<td>Pial arteriolar pressure (mm Hg)</td>
<td>48±3</td>
<td>45±2</td>
<td>44±2</td>
</tr>
<tr>
<td>Change in pressure (mm Hg)</td>
<td>44±4</td>
<td>47±7</td>
<td>60±7*</td>
</tr>
<tr>
<td>Cerebral blood flow (mL/min/100 g)</td>
<td>48±5</td>
<td>42±4</td>
<td>43±6</td>
</tr>
<tr>
<td>Large artery resistance (mm Hg/mL/min/100 g)</td>
<td>0.9±0.1</td>
<td>1.2±0.1</td>
<td>1.6±0.1*</td>
</tr>
<tr>
<td><strong>Stroke-prone spontaneously hypertensive rats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>181±6†</td>
<td>175±10†</td>
<td>197±16†</td>
</tr>
<tr>
<td>Pial arteriolar pressure (mm Hg)</td>
<td>95±9†</td>
<td>66±8†</td>
<td>52±7†</td>
</tr>
<tr>
<td>Change in pressure (mm Hg)</td>
<td>86±6†</td>
<td>119±3†</td>
<td>145±12†</td>
</tr>
<tr>
<td>Cerebral blood flow (mL/min/100 g)</td>
<td>57±4</td>
<td>60±6</td>
<td>59±5</td>
</tr>
<tr>
<td>Large artery resistance (mm Hg/mL/min/100 g)</td>
<td>1.4±0.1</td>
<td>1.1±0.2†</td>
<td>2.7±0.3†</td>
</tr>
</tbody>
</table>

Values are mean±SEM under control conditions and during infusion of serotonin into the left atrium. Change in pressure refers to pressure gradient between aorta and pial arteriole.

*p<0.05 vs. control conditions.

†p<0.05 vs. Wistar-Kyoto rats.

Serotonin produced only a minimal decrease in pial arteriolar pressure in WKY rats but produced a profound decrease in pial arteriolar pressure in SHRSP (Figure 2). Cerebral blood flow was not altered during infusion of serotonin in SHRSP and WKY rats.

In WKY rats, infusion of the low dose of serotonin did not alter resistance of large arteries, but infusion of the high dose of serotonin produced a moderate increase in large artery resistance (Figure 3). In SHRSP, there was a much greater increase in large artery resistance than was observed in WKY rats during infusion of serotonin (Table 1).

Thus, responses of the cerebral circulation to intravascular infusion of serotonin are potentiated in SHRSP compared with those in WKY rats.

FIGURE 1. Bar graphs showing effect of intra-atrial infusion of serotonin on the pressure gradient from aorta to pial arterioles (30-50 µm diameter) in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). Values are mean±SEM. *p<0.05 vs. WKY rats.

FIGURE 2. Bar graphs showing effect of intra-atrial infusion of serotonin on pial arteriolar pressure in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP). Values are mean±SEM. *p<0.05 vs. WKY rats.

Responses to Angiotensin

To test the specificity of altered responses of the cerebral circulation to serotonin in SHRSP, we examined the effects of angiotensin in SHRSP and WKY rats. Responses of the cerebral circulation to angiotensin were similar in SHRSP and WKY rats. The change in pressure between aorta and pial arteriole was similar in SHRSP and WKY rats during infusion of angiotensin. Furthermore, the change in pial arteriolar pressure in response to angiotensin was similar in SHRSP and WKY rats (Figure 4). Thus, potentiated constrictor responses of the cerebral circulation to serotonin in SHRSP cannot be explained by a nonspecific increase in sensitivity of vascular muscle to vasoconstriction.

Discussion

The present study is the first to examine the effects of intravascular administration of serotonin on the cerebral circulation during chronic hypertension. The major new finding of the present study is that constrictor responses of large cerebral arteries to intravascular administration of serotonin are potentiated during chronic hypertension. This finding may have important implications for the pathogenesis of
Serotonin produced constriction of large cerebral arteries particularly in SHRSP, which reduced microvascular pressure but did not decrease cerebral blood flow. These findings suggest that cerebral blood flow does not change during infusion of serotonin because small vessels downstream from the site of pressure measurement dilate in response to the decrease in pial arteriolar pressure. The advantage of our experimental approach is that it allowed us to detect augmented responses of large cerebral arteries in hypertensive rats in response to serotonin that would not have been detected if only cerebral blood flow, and not cerebral microvascular pressure, had been measured.

The precise site of constriction of large arteries in SHRSP during infusion of serotonin is not known. Serotonin may constrict large extracranial arteries or large intracranial arteries proximal to the pial arterioles in which pressure was measured. Chronic hypertension has been reported to augment responses of the carotid artery and the basilar artery to serotonin in vitro. Although the precise site of constriction has not been defined, our study indicates that chronic hypertension augments constrictor responses of large cerebral arteries to serotonin in vivo.

We considered the possibility that altered responses of the cerebral circulation to serotonin in SHRSP may be related to a non-specific increase in vascular reactivity in SHRSP. To test this possibility, we examined responses in SHRSP and WKY rats to angiotensin. In contrast to those observed during infusion of serotonin, we found that responses of the cerebral circulation to angiotensin were similar in SHRSP and WKY rats. Thus, this finding suggests that altered responses of the cerebral circulation in SHRSP may be somewhat specific for serotonin.

We measured blood flow to the entire cerebral hemisphere and pressure in a single pial arteriole and used these measurements to calculate resistance. This approach could reduce the precision of our calculation of resistance. In a previous study, however, we measured blood flow to the region of the cerebrum that was perfused by the pial artery in which we had measured pressure and in the remainder of the cerebrum. We found that perfusion of the region supplied by the pial artery was similar to that for the entire cerebral hemisphere. Although these previous studies were done using cats, and we cannot exclude the possibility that it may be different for rats, it appears that our calculation of resistance is valid.

Doses of serotonin used in the present study were chosen based on previous studies that examined the sensitivity of the cerebral circulation in normal and atherosclerotic monkeys to infusion of serotonin. These previous studies have shown that injections of doses of serotonin similar to those used in the present study produce levels of serotonin that closely resemble those measured during partial occlusion of a coronary artery with a thrombus present. Thus, blood levels of serotonin that were achieved in the present study probably are similar to those that occur in pathophysiological states.

Consideration of Previous Studies

Previous studies in vitro have shown an increased sensitivity of vascular muscle to serotonin in spontaneously hypertensive rats (SHR) and deoxycorticosterone acetate-salt hypertensive rats. The mechanism of enhanced sensitivity of vascular muscle to serotonin in hypertensive animals is unclear. Evidence suggests that this enhanced sensitivity of vascular muscle to serotonin cannot be explained by structural alterations of vascular muscle but may be related to changes in calcium sensitivity of vessels or decreases in calcium binding sites required for membrane stabilization.

Other studies, however, suggest that enhanced constriction of arteries from hypertensive rats in response to serotonin may not be related to direct effects of serotonin on vascular muscle but may be related to altered endothelial function in hypertensive rats. In one study, serotonin produced more constriction of the thoracic aorta in vitro in SHR than in WKY rats. Contraction of the thoracic aorta in response to serotonin was significantly decreased after removal of the endothelium. This finding suggests that the endothelium in SHR may release a constrictor substance in response to serotonin. In another study, altered responses of the coronary circulation in SHR to serotonin could be reversed with indomethacin. This finding suggests that a cyclooxygenase constrictor substance is released from coronary vessels in SHR in response to serotonin.

Recently, we found that responses of cerebral arterioles to topical application of serotonin are altered in SHRSP compared with those in WKY rats. Topical application of serotonin produced modest dilatation of cerebral arterioles in WKY rats but constriction of cerebral arterioles in SHRSP.
We found that responses of cerebral arterioles in SHRSP to topical application of serotonin could be restored almost to those observed in WKY rats after treatment with indomethacin. Thus, the mechanism of impaired responses of cerebral arterioles in SHRSP in response to topical application of serotonin appears to be related to the production of a cyclooxygenase constrictor substance, presumably from the endothelium. Thus, although the precise mechanism for altered responses of blood vessels to serotonin during chronic hypertension is not entirely clear, we suggest that an alteration in endothelial function may play an important role.

Implications

Platelets contain large amounts of serotonin that are released during aggregation. Evidence suggests that platelets from hypertensive patients and animals are more reactive to stimuli that produce aggregation, release more serotonin, adhere more readily to endothelium, have a decreased survival time, and have an augmented turnover when compared with platelets from normotensive patients and animals. Furthermore, responses of blood vessels to topical application of serotonin are profoundly altered during chronic hypertension.

We suggest that the release of serotonin into the vascular lumen during platelet aggregation on plaques in carotid arteries may be of particular importance in the cerebral circulation. We speculate that, in the presence of an existing stenosis or partial obstruction of a cerebral artery by an embolus, a reduction in microvascular pressure may contribute to the pathogenesis of cerebral ischemia, and perhaps stroke, during chronic hypertension.

References


Key Words: serotonin • blood flow • vasoconstriction • brain • stroke • rat studies
Cerebral vasoconstrictor responses to serotonin during chronic hypertension.
W G Mayhan and F M Faraci

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