Angiotensin Converting Enzyme Inhibition and the Upper Limit of Cerebral Blood Flow Autoregulation: Effect of Sympathetic Stimulation


The effect of stimulation of the cervical sympathetic ganglia on the upper limit of cerebral blood flow (CBF) autoregulation was studied in normotensive Wistar-Kyoto rats (WKY) and in spontaneously hypertensive rats (SHR) following intravenous administration of the angiotensin converting enzyme inhibitor captopril (10 mg/kg). CBF was measured using the intracarotid $^{133}$Xe injection method in halothane/nitrous oxide anaesthetized WKY and SHR. Arterial blood pressure was raised stepwise by the intravenous infusion of noradrenaline. Toward the end of the study, Evans blue was injected and the brains examined for gross blood-brain barrier breakdown. In SHR, sympathetic stimulation reextended the upper limit of CBF autoregulation, which was at a mean arterial blood pressure level of 120-139 mm Hg in the control group of eight SHR and above 170 mm Hg in the stimulated group of nine SHR. In the group of nine WKY subjected to sympathetic stimulation, the upper limit of CBF autoregulation was reached at a mean arterial blood pressure level of 110-129 mm Hg as opposed to 90-109 mm Hg in a previous unstimulated group of WKY. In the two groups subjected to sympathetic stimulation, there was no extravasation of Evans blue in any of the brains. In the control group of SHR, in which there had been marked increases in CBF, three out of eight brains had foci with extravasation of the dye. It is concluded that in normotensive and in hypertensive rats sympathetic stimulation attenuates the downward shift of the upper limit of CBF autoregulation, which is known to accompany intravenous administration of captopril. (Circulation Research 1989;64:1197-1204)

Cerebral blood flow (CBF) autoregulation keeps CBF constant over a wide range of perfusion pressure. This is mediated by caliber changes in the small arteries and arterioles. Decreased systemic arterial pressure leads to dilation of small arteries and arterioles, whereas increased blood pressure leads to a constriction. Below the lower limit of autoregulation, the dilation is insufficient to maintain CBF, which then falls with falling arterial perfusion pressure. Above the upper limit of autoregulation, there is forceful dilation of the resistance vessels, the arterioles, and CBF increases.1-4

A possible role of the renin-angiotensin system in the regulation of cerebral blood flow was recently demonstrated, when it was found that captopril, a potent angiotensin converting enzyme (ACE) inhibitor, shifts the limits of CBF autoregulation to lower blood pressure levels, thus improving tolerance to acute hypotension but impairing tolerance to acute hypertension.5-7 This effect of captopril on the limits of CBF autoregulation was present after acute administration of the drug in normotensive as well as in spontaneously hypertensive rats.5,6 Although it was hypothesized that the effect could be caused by release of the angiotensin II-induced tonus on the larger cerebral arteries, the mechanism by which ACE inhibition affects CBF autoregulation remains unsolved.

The sympathetic nerves that innervate the cerebral vessels arise primarily from the superior cervical ganglia.3 Many studies have shown that they constrict the larger cerebral vessels and exert a protective effect against breakthrough of autoregulation of CBF during acute hypertension3,8,9: the

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limits of CBF autoregulation are shifted to higher blood pressure levels. The effect of sympathetic activation during concomitant ACE inhibition has not previously been examined.

The present study was performed to determine whether the downward shift in the upper limit of CBF autoregulation, which is known to accompany captopril administration, would be affected by concomitant sympathetic stimulation during acute hypertension in normotensive and in spontaneously hypertensive rats (SHR).

Materials and Methods

Experiments were carried out in 34 three-month-old male SHR and Wistar-Kyoto rats (WKY) weighing between 290 and 390 g and assigned to the following groups (the results from group 2A were reported previously): group 1A (SHR, control, sham-operated) n=8; group 1B (SHR, stimulated) n=9; group 2A (WKY, control) n=8; and group 2B (WKY, stimulated) n=9.

Surgery

Anesthesia was induced with halothane 4%, and the rats were tracheotomized. Anesthesia was maintained with halothane 0.8% in 70% N\textsubscript{2}O - 30% O\textsubscript{2} by controlled ventilation at normocapnia. The animals were paralyzed with suxamethonium (~40 mg/kg i.v.). Both femoral veins and arteries were cannulated. Body temperature was kept close to 37° C by a rectal-thermistor heating lamp, and mean arterial pressure (MAP) was continuously monitored. After each CBF measurement the arterial PCO\textsubscript{2}, pH, and P0\textsubscript{2} were measured using conventional microelectrodes (Radiometer, Copenhagen, Denmark). One femoral vein catheter was used for transfusion of blood from a donor rat in order to replace loss of blood from blood sampling, and the other was used for drug infusion. The rats were heparinized (~4,000 IU/kg i.v.) and prepared for the intra-arterial \textsuperscript{133}Xe injection method for CBF measurement\textsuperscript{10,11}. The right common carotid artery was exposed near the carotid bifurcation, and all extracerebral branches were ligated to minimize extracerebral distribution of the \textsuperscript{133}Xe. A catheter was introduced in the external carotid artery with the tip placed at the carotid bifurcation. The scalp and temporal muscle were deflected on the right side. The cervical sympathetic trunks were identified and exposed bilaterally after isolation from vagal tissue. A delicate pair of silver electrodes was attached to the superior sympathetic ganglia on both sides. After the operation, which typically lasted 100 minutes, the animals were allowed to rest for at least 30 minutes.

CBF Measurement

For each CBF measurement a 20-30-μl saline bolus containing \textsuperscript{133}Xe (10 mCi/ml) was injected into the right internal carotid artery. The washout was followed by an external collimated sodium iodide crystal detector placed over the skull ipsilateral to the injection site. CBF was determined from the washout curve by the initial slope method\textsuperscript{12} and calculated relative to the baseline CBF. At each CBF measurement the following parameters were recorded: MAP, CBF (ml/100 g×min and percent baseline), arterial CO\textsubscript{2} tension (PacO\textsubscript{2}), arterial O\textsubscript{2} tension (Pao\textsubscript{2}), arterial pH (pH\textsubscript{a}), and body temperature (TP).

Autoregulation Study

Two to five resting CBF measurements were performed in each rat. The values were averaged for the determination of the baseline CBF level. Then, before the induction of hypertension, captopril (10 mg/kg i.v.) in 200–300 μl saline was infused over 1 minute. Blood pressure was allowed to stabilize for 15 minutes and then was manipulated to demonstrate the autoregulation curve: From the postcaptopril level (approximately 80 mm Hg in SHR and 50 mm Hg in WKY) blood pressure was raised stepwise to the highest obtainable level (approximately 170 mm Hg in SHR and 130 mm Hg in WKY) by the infusion of noradrenaline intravenously. CBF was measured at 10–20 mm Hg intervals.

Sympathetic Stimulation

In groups 1B and 2B, both superior cervical ganglia were stimulated electrically at supramaximal frequency (10 Hz). Stimulation was started when MAP had increased to 120 mm Hg in SHR and to 80 mm Hg in WKY and was continued throughout the rest of the experiment. Stimulation parameters (5–20 V, 0.05–1 msec) were adjusted to produce prompt and maximal dilatation of the pupils. CBF was not measured during the initial 5 minutes after stimulation had begun. The period of stimulation typically lasted 40 minutes. The animals in group 1A were all sham-operated, and electrodes were attached to the ganglia, but no electrical stimulation was performed during the experiment. Since the results from this group did not differ significantly from the previously published group of captopril-treated (but unstimulated) SHR,\textsuperscript{5} it was concluded that the sham operation per se did not influence the results. Therefore, the results from the WKY in group 2B were compared only with a previous captopril-treated (but unstimulated) group of WKY,\textsuperscript{5} group 2A.

Blood-Brain Barrier

At the end of the experiment Evans blue 2.5% (2 ml/kg i.v.) was administered to delineate possible major lesions of the blood-brain barrier (BBB): Six brains from group 1A, 8 brains from group 1B, and 9 brains from group 2B were successfully perfused for later gross neuropathological examination: The chest was opened, and a large cannula was inserted into the ascending aorta via the left ventricle. Saline was then infused after incision of the right atrium and clamping of the descending aorta to wash out the intravascular dye. The rat was decapitated, and the brain was removed, frozen in isopentane over acetone, and...
TABLE 1. Baseline Values

<table>
<thead>
<tr>
<th>n</th>
<th>SHR (control)</th>
<th>SHR (stimulated)</th>
<th>WKY (stimulated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>CBF (ml/100 g x min)</td>
<td>82±12</td>
<td>86±16</td>
<td>80±10</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>123±11</td>
<td>127±14</td>
<td>74±17*</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>38.3±1.2</td>
<td>38.8±1.6</td>
<td>39.8±2.4</td>
</tr>
<tr>
<td>pHa</td>
<td>7.38±0.03</td>
<td>7.40±0.02</td>
<td>7.39±0.02</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>125±14</td>
<td>139±15*</td>
<td>142±24</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>37.2±1.0</td>
<td>37.1±0.6</td>
<td>37.6±0.7*</td>
</tr>
</tbody>
</table>

SHR; spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; CBF, cerebral blood flow; MAP, mean arterial pressure; PaCO₂, arterial CO₂ tension; pHa, arterial pH; PaO₂, arterial O₂ tension. All values are presented as mean±SD.

Group IB vs. 1A and group 2B vs. IB by Student two-sample t test for unpaired data. *p<0.05.

Statistics

The one-way analysis of variance followed by the Dunnett multiple comparison test was used for statistical comparisons within each group of rats. The mean CBF value (indicated as percent baseline) in each MAP range was compared with the mean CBF in the reference MAP range defined as the range of MAP where CBF was closest to the plateau, that is, 80–99 mm Hg in group 1 and 50–69 mm Hg in group 2. For all other parameters, comparisons were made between the values in each MAP range and the baseline value for the same parameter. The Student two-sample t test for unpaired data was used to compare baseline values in group 1A and 1B and in group 1B and 2B (Table 1). Differences were accepted as significant at p<0.05.

The autoregulation curves in Figures 2, 3, and 5 were determined by the repetitive fitting of two regression lines: one horizontal line through the mean CBF value of all measurements corresponding to mean arterial pressures below a given value and one sloped regression line through all data sets with MAP above the given value. For each given MAP value, the combined sum of squares for the two lines was calculated. One set of regression lines was calculated for each 1 mm Hg increment in MAP. The set of two lines yielding the minimum sum of squares was chosen, and the upper limit of CBF autoregulation was defined as the MAP value in the intersection of the two lines.

Results

Baseline

Mean baseline MAP was 74 mm Hg in WKY and 125 mm Hg in SHR, but there was no significant difference in baseline CBF (Table 1). Although there were slight differences in the baseline arterial PO₂, PCO₂, and TP (Table 1), these parameters did not change significantly during the autoregulation study, except at extreme hypertension toward the end of the study (Table 2). These alterations were too small to explain any difference in CBF autoregulation in the groups studied.

Table 2. Data From Autoregulation Study for All Variables Observed in Each 20 mm Hg Range of Mean Arterial Blood Pressure

<table>
<thead>
<tr>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>CBF (% baseline)</th>
<th>PaCO₂ (mm Hg)</th>
<th>pHa</th>
<th>PaO₂ (mm Hg)</th>
<th>TP (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>15</td>
<td>80–99</td>
<td>96±8</td>
<td>39.0±1.8</td>
<td>7.37±0.03</td>
<td>124±9</td>
</tr>
<tr>
<td>control</td>
<td>16</td>
<td>100–119</td>
<td>109±17</td>
<td>39.8±1.8</td>
<td>7.36±0.04</td>
<td>123±8</td>
</tr>
<tr>
<td>n=8</td>
<td>11</td>
<td>120–139</td>
<td>140±61*</td>
<td>39.3±2.3</td>
<td>7.37±0.02</td>
<td>119±11</td>
</tr>
<tr>
<td>13</td>
<td>140–159</td>
<td>207±79†</td>
<td>41.2±2.2†</td>
<td>7.36±0.05</td>
<td>116±10</td>
<td>37.0±0.4</td>
</tr>
<tr>
<td>7</td>
<td>160–179</td>
<td>219±46†</td>
<td>41.6±3.2†</td>
<td>7.31±0.04†</td>
<td>107±16</td>
<td>36.8±0.7</td>
</tr>
<tr>
<td>SHR</td>
<td>16</td>
<td>80–99</td>
<td>101±10</td>
<td>39.6±1.7</td>
<td>7.39±0.03</td>
<td>136±17</td>
</tr>
<tr>
<td>stimulated</td>
<td>20</td>
<td>100–119</td>
<td>103±10</td>
<td>38.5±2.1</td>
<td>7.40±0.04</td>
<td>132±17</td>
</tr>
<tr>
<td>n=9</td>
<td>16</td>
<td>120–139</td>
<td>98±18</td>
<td>38.3±1.6</td>
<td>7.38±0.03</td>
<td>139±20</td>
</tr>
<tr>
<td>16</td>
<td>140–159</td>
<td>105±12</td>
<td>38.8±2.6</td>
<td>7.38±0.04</td>
<td>130±21</td>
<td>37.1±0.6</td>
</tr>
<tr>
<td>16</td>
<td>160–179</td>
<td>109±16</td>
<td>41.0±3.1†</td>
<td>7.36±0.05†</td>
<td>121±18</td>
<td>37.3±0.6</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>50–69</td>
<td>100±21</td>
<td>39.5±1.8</td>
<td>7.39±0.02</td>
<td>138±16</td>
</tr>
<tr>
<td>stimulated</td>
<td>13</td>
<td>70–89</td>
<td>101±12</td>
<td>41.9±2.1</td>
<td>7.37±0.02</td>
<td>141±16</td>
</tr>
<tr>
<td>n=9</td>
<td>10</td>
<td>90–109</td>
<td>108±7</td>
<td>41.4±2.2</td>
<td>7.36±0.02*</td>
<td>139±22</td>
</tr>
<tr>
<td>12</td>
<td>110–129</td>
<td>123±22*</td>
<td>38.3±2.9</td>
<td>7.38±0.03</td>
<td>132±23</td>
<td>38.0±0.4</td>
</tr>
</tbody>
</table>

n, number of observations; MAP, mean arterial pressure; CBF, cerebral blood flow; PaCO₂, arterial CO₂ tension; pHa, arterial pH; PaO₂, arterial O₂ tension; TP, body temperature; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. The analysis of variance and the Dunnett multiple comparison test were used to compare data in each MAP range to baseline values. *p<0.05. †p<0.01.
The present study demonstrates that stimulation of the cervical sympathetic pathways attenuates the effect of captopril on the upper limit of cerebral blood flow autoregulation. Sympathetic activation is known to extend the upper limit of CBF autoregulation, whereas ACE inhibition lowers the upper limit. The new finding of the present study is that even in the presence of the potent ACE inhibitor, captopril, sympathetic activation reextends (at least partially) the upper limit of CBF autoregulation. The effect was most pronounced in SHR compared with WKY, which could be explained by a lower sensitivity of the cerebral arteries to activation of the sympathetic nervous system in WKY.
The intra-arterial $^{133}$Xe injection method is a convenient method for rapid and repetitive CBF studies in rats. Despite the lack of regional and bilateral information, the method is advantageous for studying the autoregulation of global CBF. Examination of the brains for extravasation of Evans blue is a gross and insensitive method for evaluating BBB defects. In this study, it only served as a tool to reveal possible foci with major evident BBB breakdowns, which was only present in the unstimulated group of SHR where CBF had increased to high levels. That there were no blue-stained foci in the stimulated groups of rats does not exclude more delicate BBB lesions.

Sympathetic Nervous System and CBF

Electrical stimulation of the cerebral sympathetic pathways has little or no effect on resting CBF at normotension and normocapnia. Although an initial decrease in CBF has been observed, blood flow escapes toward normal in 3–5 minutes. The large pial arteries remain constricted throughout the stimulation, whereas the intraparenchymal vessels begin to compensatory dilate about 3 minutes after the initial constriction, thus keeping resting CBF constant. In the present study, CBF was not measured during the first 5 minutes after the start of stimulation. The effect of bilateral stimulation is greater than the effect of unilateral stimulation because, although the distribution of the innervation is largely ipsilateral, there is overlap in basal and medial areas of the brain at the circle of Willis. To obtain maximal response to sympathetic stimulation we stimulated bilaterally in the present study.

During drug-induced acute hypertension to above the upper limit of autoregulation, electrical stimulation of the cerebral sympathetic pathways reduces the increase in CBF and the extent of disruption of the BBB, because under these conditions the cerebral vessels do not escape from sympathetic constrictor effects. This has been observed in the rat using the arteriovenous oxygen difference method and later in other species using microspheres or the xenon-injection method for measuring CBF. Similar results have been observed when sympathetic nerves are activated physiologically during severe hypertension. Conversely, after denervation of the sympathetic pathways the upper limit of CBF autoregulation is shifted to lower blood pressure levels. In the present study, no attempt was made to reconfirm the effect of sympathetic stimulation on the upper limit of CBF autoregulation because in untreated rats we are unable to reach the upper limit even without sympathetic stimulation.

ACE Inhibition and CBF

Recently we studied the effect of the ACE inhibitor captopril on the limits of CBF autoregulation in SHR and in WKY: both the upper and the lower limits of CBF autoregulation were shifted to lower blood pressure levels and the autoregulatory plateau shortened. The upper limit was shifted 50–60 mm Hg: from >200 mm Hg to 130–149 mm Hg in SHR and from >150 mm Hg to 90–109 mm Hg in WKY. Although the mechanism remains unsolved, this effect was interpreted to be a consequence of
compensatory autoregulatory constriction of the small resistance vessels in the brain following a captopril-induced dilatation of the large resistance vessels. The small vessels would then be better able to dilate during a blood pressure decrease but less able to further constrict during a blood pressure increase. The captopril-induced dilatation of the large vessels was interpreted to be a result of impairment of the vasoconstrictory tonus induced by angiotensin II.3-6

ACE Inhibition and the Sympathetic Nervous System

ACE inhibition seems to elicit fewer sympathetic counterregulatory responses (such as tachycardia) than other vasodilators, which are being used in the treatment of hypertension. It has therefore been proposed that ACE inhibition interferes with sympathetic activity. Evidence from animal studies suggests that angiotensin II facilitates adrenergic neurotransmission at the vascular neuroeffector junction.31 After captopril, pressor responses to sympathetic stimulation are abolished32,33 and more efficiently in SHR than in normotensive rats.34

Captopril-treated patients have physiological and pharmacological evidence of diminished sympathetic activity.35 The question still remains whether reduced sympathetic activity is involved in the antihypertensive effect of captopril.36 A recent study, however, suggests that the known interaction between the renin-angiotensin and the sympathetic nervous system observed in animals is probably of little significance in humans.37

The present study demonstrated that sympathetic activation attenuates the effect of captopril on the upper limit of CBF autoregulation. This result could be explained by two different mechanisms: 1) The previously shown effect of captopril on the upper limit of CBF autoregulation could be interpreted as a consequence of impaired constriction by angiotensin II, an effect independent of the sympathetic nervous system. In acute hypertension the constrictor effect of sympathetic stimulation on the larger cerebral arteries then counteracts this dilatory effect of captopril. 2) Alternatively, the effect of captopril on the limits of CBF autoregulation could be interpreted simply as a result of the decreased sympathetic activity that is induced by ACE inhibition. Therefore sympathetic reactivation would tend to reextend the upper limit. There are two reasons why the latter cannot be the only mechanism responsible for the effect of captopril on CBF autoregulation. First, in a previous report,38 there was no effect of phenoxybenzamine on CBF autoregulation, indicating that the initial sympathetic activity in the rat preparation is very low. Secondly, even in rats subjected to surgical denervation of the cervical sympathetic nerves, captopril affects CBF autoregulation.39

Clinical Relevance

Although no studies with chronic treatment schedules have been performed, the effect of ACE inhi-
bition on the lower limit of CBF autoregulation might be beneficial in the treatment of hypertension and might be the reason why this drug is not associated with the side effect of orthostatic hypotension. In a study of the effect of captopril on CBF in congestive heart failure, it was concluded that CBF was well preserved despite severe reduction of the blood pressure. This finding was confirmed by similar results in other groups of patients with arterial hypertension and congestive heart failure.4,42 The leftward shift in the upper limit of CBF autoregulation could be expected to lead to a greater risk of cerebral hemorrhage at extreme rises in arterial blood pressure. In the awake human, however, increases in blood pressure are often associated with or caused by increased sympathetic activity, which according to the results of the present study may reextend the upper limit of autoregulation. In the clinical setting, ACE inhibition therefore is unlikely to result in a shift of the upper limit of autoregulation.

In conclusion, the present study demonstrates that in normotensive as well as in hypertensive rats stimulation of the cervical sympathetic pathways during concomitant ACE inhibition counteracts the leftward shift of the upper part of the CBF autoregulation curve, which is known to accompany ACE inhibition.

References


**KEY WORDS** • angiotensin converting enzyme • angiotensin II • cerebral circulation • sympathetic nervous system • captopril
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G Waldemar, O B Paulson, D I Barry and G M Knudsen

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