Influence of Antihypertensive Treatment With Budralazine on Autoregulation of Cerebral Blood Flow in Spontaneously Hypertensive Rats

Satoru Tanaka, Makoto Tanaka, PhD, and Akira Akashi, PhD

We studied the effect of chronic antihypertensive treatment with budralazine on the lower blood pressure limit of cerebral blood flow autoregulation using spontaneously hypertensive rats. Cerebral blood flow in the parietal cortex and caudate nucleus was measured to determine the lower limit using the hydrogen clearance method. The lower limit in both cerebral regions was significantly higher in 10 untreated spontaneously hypertensive rats than in 10 Wistar-Kyoto rats. The upward-shifted lower limit was restored to close to normal in the caudate nucleus and was partially restored in the parietal cortex of nine rats by 9 weeks of treatment with the high dose (50–68 mg/kg/day) of budralazine, which kept blood pressure constant at approximately normotension during the treatment period; the lower limit was slightly restored in both cerebral regions of seven rats by 4 weeks of treatment with the high dose. However, 9 weeks of treatment with the low dose (19–27 mg/kg/day) of budralazine, which produced moderate continuous hypotension in nine rats, did not apparently influence the lower limit. Our results suggest that long-term antihypertensive therapy with budralazine reduces the upward-shifted lower blood pressure limit of cerebral blood flow autoregulation toward normal and that the restoration induced by budralazine depends on the degree of blood pressure reduction as well as on the duration of the therapeutic period. (Stroke 1989;20:1724–1729)

In chronic hypertension, the lower blood pressure limit of cerebral blood flow (CBF) autoregulation is raised. This hypertension-induced increase has been observed in both animals1–3 and humans,4 presumably due to arteriolar wall hypertrophy to protect the vascular tissue from increased perfusion pressure.5–9 Recent studies have shown that long-term antihypertensive treatment restores the lower limit of CBF autoregulation to close to the normal level in hypertensive rats10,11 as well as in patients.12 However, little is known about the possible relations between the degree of restoration and the magnitude of the hypotensive effect induced by antihypertensive drugs and the duration of their therapeutic period.

Budralazine, a phthalazine antihypertensive drug, has been shown to produce a long-lasting antihypertensive effect through direct vasodilation of the peripheral blood vessels in hypertensive animals13–16 and patients.17 The drug is also reported to increase CBF in cats18 and spontaneously hypertensive rats (SHR)18 and to prevent hypertension and reduce the incidence of stroke in salt-loaded SHR.20 Our study was designed to examine the effect of chronic antihypertensive treatment with budralazine on the adapted CBF autoregulation in SHR. The rats were treated with two doses of the drug for 9 weeks to estimate the influence of the magnitude of budralazine’s antihypertensive activity. In addition, to determine the influence of the duration of the therapeutic period, SHR were treated for two periods with the high dose of the drug.

Materials and Methods

We used 49 male SHR 6 months old and 10 age-matched Wistar-Kyoto rats (WKY). The rats were housed in a room with controlled temperature (23° C), humidity (55%), and light/dark cycle (07:00/19:00 hr). The rats were maintained on food (Funabashi Farm Co., Japan) and water available ad libitum.

In Experiment 1, 28 SHR were studied; 18 received chronic antihypertensive treatment with budralazine (Daiichi Pharmaceutical Co., Ltd.,
Tokyo, Japan) for 9 weeks. The drug was added to powdered food at one of two concentrations adjusted to the daily intake of the rats. The absolute doses of budralazine taken by the rats were calculated from the amount of food consumed. The 10 WKY were used as normotensive controls. The 28 SHR were divided randomly into three groups: the high dose (50–68 mg/kg/day, n=9) group in which systolic blood pressure was continuously maintained at normotensive levels, the low dose (19–27 mg/kg/day, n=9) in which systolic blood pressure was maintained at levels intermediate between those of untreated SHR and WKY, and the untreated group (n=10) in which powdered food alone was given. Blood pressure was measured weekly by the tail-cuff method using a W+W blood pressure recorder (Scientific Instruments Inc., Basel, Switzerland), and each rat was weighed when blood pressure was measured. After 9 weeks of treatment, CBF was measured in all four groups.

In Experiment 2, 14 SHR were studied as in Experiment 1, but for 4 weeks. Seven SHR received the high dose of budralazine so that systolic blood pressure was reduced to normotensive levels. The remaining seven SHR received powdered food alone. Blood pressure and body weight were measured at least once a week during the treatment period. After 4 weeks of treatment, CBF was measured in both groups.

In Experiment 3, a single oral dose of budralazine (40 mg/kg) was administered to seven SHR 3 hours before CBF was measured. This dose of budralazine produces approximate normotension from 3 to 8 hours later. Seven control SHR received powdered food alone. Blood pressure and body weight were measured at least once a week during the treatment period. After 4 weeks of treatment, CBF was measured in both groups.

CBF was measured by the hydrogen clearance technique. After anesthesia with 30 mg/kg i.v. sodium pentobarbital and 2% halothane, the rats were tracheotomized, paralyzed with 6 mg/kg i.v. gallamine triethiodide (May & Baker Ltd., Dagerham, England), and artificially ventilated with a gas mixture (30% O2-70% N2O) using a respirator (Model 681, Harvard Apparatus, Millis, Massachusetts). Both femoral arteries were cannulated with PE 50 polyethylene catheters, one for continuous monitoring of mean arterial blood pressure (MAP) and the other for sampling of blood gases and for removal of blood to reduce MAP. A femoral vein was cannulated for the administration of additional gallamine triethiodide (3–6 mg/kg) when necessary. Teflon-coated platinum electrodes were placed in the parietal cortex (1.5 mm below the brain surface) and in the caudate nucleus according to the brain atlas of König and Klippel. The reference Ag-AgCl electrode was inserted under the skin. Hydrogen was added at a concentration of 5–10% to the gas mixture, and the hydrogen clearance curve was recorded on a pen recorder (Model U-228, Unique Medical Co., Ltd., Tokyo, Japan). CBF was calculated from the clearance curve by the initial slope method. The rats were maintained at normocapnia (Paco2 35–45 mm Hg) by adjustment of the ventilation volume. Body temperature was maintained at approximately 37° C with a heating lamp.

Following a 1-hour postoperative stabilization period, baseline CBF was measured three or four times at 10–15 minute intervals. MAP was then reduced in a stepwise fashion by controlled withdrawal of sufficient blood to lower it by approximately 30 mm Hg at each step. CBF was measured at each MAP range (145–135, 115–105, 85–75, 55–45, and 30–20 mm Hg). At each range, MAP was allowed to stabilize for at least 10 minutes before CBF was measured. In this way, the lower part of a CBF autoregulation curve based on 3–8 measurements was delineated for each rat. The lower blood pressure limit of CBF autoregulation was defined as the MAP at which CBF was equal to the baseline value (100%) in the regression equation calculated from the latter three CBF measurements after stepwise reduction of MAP.

Results are expressed as mean±SEM. The data were evaluated using Student’s unpaired t test, and p<0.05 was regarded as significant.

Results

In Experiment 1, there were no significant differences in the initial levels of systolic blood pressure among the three groups of SHR, although significant (p<0.01) differences were observed between SHR and WKY. In the low dose and high dose groups, systolic blood pressure was significantly (p<0.01) reduced within 1 week after treatment began and was kept constant at this level for 9 weeks (Figure 1). Treated and untreated SHR groups gained body weight at similar rates (data not shown). At the time of the CBF autoregulation study, base-
TABLE 1. Baseline CBF, MABP, Blood Gases, and Blood pH in SHR Treated With or Without Budralazine for 9 or 4 Weeks and in WKY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated SHR</th>
<th>Budralazine-treated SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=10)</td>
<td>19-27 mg/kg/day (n=9)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>CBF (ml/100 g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>57.3±3.7</td>
<td>55.2±5.5</td>
<td>48.2±3.9</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>57.0±1.8</td>
<td>54.4±5.8</td>
<td>53.7±4.7</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>166.1±2.6*</td>
<td>142.3±5.1*</td>
<td>195.3±6.0</td>
</tr>
<tr>
<td>Paco2 (mm Hg)</td>
<td>40.2±1.0</td>
<td>36.4±0.8</td>
<td>35.8±0.7</td>
</tr>
<tr>
<td>Pao2 (mm Hg)</td>
<td>111.3±5.0</td>
<td>123.3±13.1†</td>
<td>106.3±2.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.01</td>
<td>7.44±0.02</td>
<td>7.43±0.01</td>
</tr>
</tbody>
</table>

CBF, cerebral blood flow; MABP, mean arterial blood pressure; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.
Values are mean±SEM.
*tp<0.05, **tp<0.01, respectively, different from WKY by Student’s unpaired t test.
†p<0.05 different from untreated SHR by Student’s unpaired t test.

line MABP in the high dose and WKY groups were similar and MABP in the low dose group was intermediate between that in the untreated SHR and WKY groups (Table 1). Paco2 in the high dose and Pao2 in the low dose groups were slightly but significantly different from those in the WKY group. These values were within the range of normal, and during the CBF autoregulation study there were no significant differences in Paco2 among the four groups. In the untreated SHR group, the lower part of the CBF autoregulation curve was significantly shifted toward higher MABP in both cerebral regions compared with that in the WKY group (Figure 2). In the high dose group, the lower limit in the caudate nucleus was similar to that of the WKY group, and thus was normal (Table 2); in the parietal cortex, the lower limit was also significantly shifted toward normal. However, there was no significant difference between the high dose and the untreated SHR groups (Table 2) because CBF in the parietal cortex of the high dose group was slightly decreased from baseline at the MABP range 85–75 mm Hg. In the high dose group, although the lower limit in the parietal cortex was higher than that in the caudate nucleus, there were no significant differences between cerebral regions (Table 2). When MABP was lowered to approximately 50 mm Hg by controlled hemorrhage, CBF in the parietal cortex and caudate nucleus in the high dose group (40.4±1.8 and 46.4±3.0 ml/100 g/min, respectively) were significantly (p<0.01) greater than in the untreated SHR group (25.2±1.7 and 29.3±1.8 ml/100 g/min, respectively). In both cerebral regions, however, the lower part of the CBF autoregulation curve in the low dose group did not differ significantly from that in the untreated SHR group (Figure 2).

In Experiment 2, systolic blood pressure decreased to approximately normotension on Day 4 after the start of treatment with the high dose of budralazine and remained at this level for 4 weeks (data not shown). There was a tendency for baseline CBF in both cerebral regions to increase in the treated group relative to the untreated group (Table 1). CBF in both cerebral regions was significantly (p<0.05) greater at the MABP range 55–45 mm Hg in the treated than in the untreated group (Figure 3). Com-

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**Figure 2.** Lower part of cerebral blood flow autoregulation curve in two cerebral regions in spontaneously hypertensive rats (SHR) and in normotensive control Wistar-Kyoto rats (WKY; ○, n=10). SHR received low dose (19–27 mg/kg/day; △, n=9) or high dose (50–68 mg/kg/day; ▲, n=9) of budralazine or were untreated (●, n=10) for 9 weeks. Data are mean±SEM. *p<0.05, **p<0.01 different from untreated SHR by Student’s unpaired t test.
pared with the untreated group, the lower limit in both cerebral regions in the treated group was decreased by similar degrees (~21%), although the decrease was significant in the caudate nucleus but not in the parietal cortex (Table 2). The degree of restoration of the CBF autoregulation curves by treatment with the high dose of budralazine was smaller in Experiment 2 than in Experiment 1 for both cerebral regions studied.

In Experiment 3, baseline MABP at the time of the CBF autoregulation study in the budralazine-treated and control SHR groups were 111±8 and 191±9 mm Hg, respectively. On the other hand, baseline CBF was significantly greater in the treated than in the control group (90.1±9.2 and 58.6±5.2 ml/100 g/min, respectively, \( p<0.05 \) for parietal cortex; 73.0±3.5 and 48.2±1.2 ml/100 g/min, respectively, \( p<0.01 \) for caudate nucleus). There were no significant changes in arterial blood gases, blood pH, or body temperature between the two groups. The lower limit was not significantly different between the two groups (data not shown). Because of the greater baseline CBF in treated SHR, when MABP was lowered to approximately 50 mm Hg by controlled hemorrhage CBF in the parietal cortex and caudate nucleus were significantly \( (p<0.01) \) higher in the treated (38.9±2.3 and 46.3±3.1 ml/100 g/min, respectively) than in the control group (27.6±2.9 and 28.0±2.7 ml/100 g/min, respectively).

**Discussion**

Our results demonstrate that the calculated lower blood pressure limits of CBF autoregulation were significantly higher in SHR than in age-matched WKY and that adaptive changes in cerebral autoregulation in SHR were reversed by 9 weeks' treatment with a high dose of the antihypertensive drug budralazine. The impairment of CBF autoregulation in SHR that we observed is in good agreement with earlier findings\(^2,3\) and possibly reflects structural alteration in the cerebral resistance vessels, as has been reported by others.\(^5,9\)

Strandgaard\(^12\) found that CBF autoregulation is shifted upward in hypertensive patients and that long-term antihypertensive treatment causes their CBF autoregulation to readapt toward normal. The readaptation resulting from chronic antihypertensive therapy has also been observed in animal models of hypertension.\(^10,11\) These results suggest that chronic antihypertensive therapy can restore the hypertension-induced changes in CBF autoregulation in both animals and humans. In experimental studies,\(^10,11\) however, the relation between the degree of blood pressure reduction (as well as the duration of treatment) and the magnitude of the restoration is obscure because the therapy was...
performed as a regimen of only one dosage and one treatment period. In addition, since in the earlier studies\textsuperscript{10,11} antihypertensive treatment was conducted using a combination of three drugs, it is not clear whether CBF autoregulation can be restored with monotherapy.

In our study, therefore, two dosage and two treatment period regimens were used to elucidate the factors responsible for the improvement of altered CBF autoregulation. In Experiment 1, SHR were treated for 9 weeks with high and low doses of budralazine to produce different magnitudes of hypotensive responses. As a result, high-dose budralazine, which maintained normotension (approximately 140 mm Hg systolic blood pressure) during the treatment period, significantly reduced the upward-shifted lower limit of CBF autoregulation toward normal in both cerebral regions. However, low-dose budralazine, which maintained systolic blood pressure at levels (170–180 mm Hg) intermediate between those of untreated SHR and WKY during the treatment period, had no significant effect on the lower limit of CBF autoregulation. Our results suggest that the readaptation of the shifted CBF autoregulation is limited by a certain magnitude of hypotension induced by budralazine and that the threshold of systolic blood pressure to reverse the shift seems to be between approximately 140 and 180 mm Hg. Also, our data emphasize the ability of budralazine itself to reverse the shift in CBF autoregulation after long-term treatment. In Experiments 2 and 3, SHR were treated for 4 weeks with high-dose budralazine or with a bolus of the drug, respectively, to relate the duration of treatment to the degree of restoration. A slight but significant improvement in CBF autoregulation occurred after 4 weeks of budralazine treatment. In contrast, the drug had no beneficial effect on CBF autoregulation in SHR receiving a single dose that reduced systolic blood pressure to normotensive levels. Our observations may provide evidence that a certain period of antihypertensive treatment with budralazine is necessary for the restoration of shifted CBF autoregulation. At least under these experimental conditions, the period of drug treatment required to reverse the shift in CBF autoregulation seems to be ≤ 4 weeks.

Our data indicate that baseline CBF in the two cerebral regions were significantly greater in SHR treated with a single dose of budralazine than in control SHR. This is consistent with our previous observation that a single oral dose of budralazine produced a significant and dose-related increase in regional CBF concomitant with a significant decrease in the cerebral vascular resistance.\textsuperscript{19} The increase in baseline CBF in response to budralazine was less pronounced after 4 weeks of treatment (Experiment 2) and almost disappeared after 9 weeks of treatment (Experiment 1), indicating that the cerebral vasodilator response to this drug became smaller and negligible with prolonged therapy, despite the maintenance of systolic blood pressure at normotensive levels. Our results may provide indirect evidence that antihypertensive treatment with budralazine causes some structural and/or functional changes in cerebral vessels within 4 weeks. It has been shown that structural vascular adaptation, including vascular hypertrophy, is associated with changes in blood pressure in hypertensive animals.\textsuperscript{23–30} On the other hand, the cerebral vascular hypertrophy induced by hypertension could be reversed by chronic antihypertensive treatment.\textsuperscript{31} Recently, Harper\textsuperscript{32} reported that cerebral microvascular hypertrophy in SHR was improved by antihypertensive treatment for only 25 days. This observation may explain our view described above. In addition, the reduction in increased baseline CBF was associated with restoration of the increased lower limit in our studies. Therefore, we suggest that cerebrovascular changes are associated with the restoration of CBF autoregulation induced by long-term antihypertensive treatment with budralazine.

Under the condition of blood pressure below the lower limit of CBF autoregulation, absolute CBF was maintained at higher levels in SHR treated with a single dose of budralazine than in control SHR, resulting from increases in baseline CBF in response to the drug. Under similar conditions (in SHR treated with high-dose budralazine for 9 weeks), CBF was also maintained at levels higher than that in the untreated group, resulting from restoration of the shifted CBF autoregulation by the drug. Thus, budralazine may protect the brain from ischemia resulting from decreased CBF induced by further reduction of blood pressure below the lower limit of CBF autoregulation in SHR because of the drug’s ability to increase CBF.

In summary, we have shown that chronic antihypertensive treatment with budralazine in SHR reverses the upward shift in the lower limit of CBF autoregulation toward normal. Furthermore, our results provide substantial evidence that the restoration of CBF autoregulation induced by budralazine depends on the magnitude of the hypotensive response as well as on its duration.

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