SUMMARY Cerebrovascular responses to hemorrhage induced hypotension were tested in aged (24 month) spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto (WKY) after ten weeks of antihypertensive drug or sham treatment. Antihypertensive drug treatment consisted of 1 mg/kg/day minoxidil, 4 mg/kg/day hydralazine and 4 mg/kg/day propranolol given in the drinking water. Antihypertensive therapy produced a 30% decrease in systolic blood pressure in aged SHR to levels not significantly different from sham treated WKY. Cerebral blood flow (CBF), measured under control conditions using ketamine anesthesia, was not significantly different between sham and drug treated SHR and WKY. Cerebral oxygen metabolic rate (CMRO2) was significantly decreased in drug treated SHR and WKY compared to sham treated rats. Lowering blood pressure to a level between 80 and 95 mmHg produced no significant change in CBF or CMRO2 in sham or drug treated WKY or antihypertensive treated SHR, but a produced a significant decrease in both CBF and CMRO2 in sham treated SHR. Decreasing mean blood pressure to 50–60 mmHg produced a significant decrease in CBF but not CMRO2 in both WKY treatment groups and in antihypertensive treated SHR, but again in sham treated SHR both CBF and CMRO2 were significantly decreased. These results indicate that aged hypertensive rats are unable to maintain CBF or CMRO2 under even moderate hypotensive test conditions, but that cerebral autoregulation can be improved with antihypertensive therapy.

STUDIES HAVE SHOWN that chronic hypertension produces cerebrovascular changes which produce a shift in cerebral autoregulation and inhibit the ability of hypertensive subjects to maintain cerebral blood flow (CBF) and cerebral oxygen metabolism (CMRO2) during hypotensive stimuli.1-5 This problem may be increased in the aged hypertensive subject due to structural vascular changes such as atherosclerosis development, increased collagen formation, intimal fibrinoid deposition and loss of vascular elasticity.6-9 This is supported by reports that maintenance of CBF and CMRO2 during hypotension is complicated by aging and hypertension,5-10,11 Little is known concerning the effect of antihypertensive therapy on cerebral autoregulation in aged hypertensive subjects. It is important to determine whether antihypertensive drug treatment can improve cerebrovascular performance in aged subjects in which hypertension has been maintained chronically for prolonged periods. In these experiments we have tested the effect of ten weeks of antihypertensive therapy on cerebral autoregulation and the ability to maintain CMRO2 during hemorrhage induced hypotension in aged SHR and Wistar Kyoto rats (WKY).

Methods
Antihypertensive Drug Treatment
Twenty-four month old male retired breeder spontaneously hypertensive rats (SHR) and Wistar Kyoto controls (WKY) (Charles Rivers Inc.) were used in these experiments. Systolic blood pressure was measured indirectly 1–2 times per week in these rats using the tail cuff occlusion apparatus of Narco Biosystems. Rats were warmed to 32°C using a temperature controlled oven for pressure measurement. SHR and WKY receiving antihypertensive therapy were given the following drugs: minoxidil (1 mg/kg/day), hydralazine (4 mg/kg/day), propranolol (4 mg/kg/day). The drugs were added to the drinking water with the concentration adjusted to the daily intake of the rat. Four test groups were chosen with 15–20 rats per group. SHR and WKY received either ten weeks of antihypertensive drug or sham (water) treatment. Initiation of treatment was staggered so that an equal number of rats from each group completed the treatment regimen and could be tested each week.

Surgery
At the end of the ten week drug or sham treatment period, rats from each test group were prepared for microsphere test procedures. Rats received a constant iv infusion of 0.13 mg/kg/min ketamine during the surgical and test procedures using a Harvard infusion/withdrawal pump. Each rat received 0.1 mg/kg tubocurara every 30 minutes. A tracheostomy was performed and the rat was artificially ventilated with 30% oxygen and 70% room air using a Harvard small animal respirator. PE50 tubing catheters, filled with heparinized isotonic saline were inserted into both femoral arteries and both femoral veins and into the left ventricle via the right carotid artery. Pressure pulses were monitored to ensure proper placement of the ventricular catheter. Following the completion of this surgery all incisions were closed using wound clips and the rat was placed on a stereotaxic head holder. The skull was exposed, the bone over the sagittal sinus drilled away and a 23 gauge needle inserted into the sinus stereotactically to be used for drawing blood samples. Following the completion of all surgical procedures the rat was allowed 15 minutes to stabilize. During this time the arterial PCO2 was adjusted to approximately 35 mmHg. Rectal temperature was monitored using a Yellow Springs Inc. thermoprobe and body temperature was...
Laboratory co-oximeter. CMR02 was calculated by man et al.\(^1\) using an Inerdata computer. Oxygen content of each microsphere label in brain and blood samples was analyzed using a Nuclear Chicago 1035 gamma counter and a Nuclear Data 600 multi-channel analyzer. Before each microsphere injection the pressure pulses of the left ventricular catheter were monitored to ensure catheter placement. Three separate labelled microspheres were used, cobalt-57, ruthenium-103 and scandium-46 (New England Nuclear). Stock solutions containing 500,000 15\(\mu\) microspheres/ml were suspended in isotonic saline with 0.01% Tween-80. Microsphere samples were vortexed for one minute, an 0.2 ml sample withdrawn, injected into the left ventricle and flushed in with 0.2 ml saline over a 20 second period. Starting immediately before each injection and continuing 45 seconds after the end of the microsphere injection, blood was withdrawn from both femoral artery catheters at a rate of 0.4 ml/min using a 2 channel Harvard infusion/withdrawal pump. Arterial blood gas measurements were taken at the end of each microsphere test. Arterial and sagittal sinus blood samples were also taken after each microsphere test for measurement of cerebral arterial-venous oxygen content. Pressure was maintained at each respective pressure level throughout the testing procedure. At the end of the third or final microsphere test the rat was sacrificed, the brain removed, weighed and stored in 10% formalin overnight. The following day the brain was blotted dry and placed in counting vials. The activity of each microsphere label in brain and blood samples was analyzed using a Nuclear Chicago 1035 gamma counter and Nuclear Data 600 multi-channel analyzer. CBF was analyzed according to the methods of Heymann et al.\(^1\) using an Inerdata computer. Oxygen content of blood was analyzed using an Instrumentation Laboratory co-oximeter. CMRO2 was calculated by multiplying CBF, corrected for brain weight, by cerebral arterial-venous oxygen content. Preliminary tests indicated that 3 repeated control microsphere tests in the same rat produced reproducible measures of CBF and CMRO2 under the same experimental conditions as used here. All data are reported as mean ± se and statistical comparisons were made with unpaired t-tests and a treatment by groups linear model analysis of variance.

Results

The effect of antihypertensive drug treatment on systolic blood pressure is shown in figure 1. Sham treatment had no significant effect of blood pressure in either SHR or WKY over the ten week treatment period. Antihypertensive drug treatment produced a decrease in blood pressure in both SHR and WKY. Blood pressure decreased for approximately four weeks and was maintained at this level for the remainder of the ten week treatment period. Blood pressure decreased significantly more in SHR during antihypertensive drug treatment than in WKY (\(p < .05\)). Blood pressure, heart rate and blood gas changes under control anesthesia and during hemorrhage induced hypotension are shown in table 1. Sham treated SHR were significantly hypertensive under control conditions compared to WKY and to antihypertensive treated SHR (\(p < .01\)). Mean blood pressure under control conditions in antihypertensive treated SHR was significantly greater than antihypertensive treated WKY (\(p < .05\)) but not sham treated WKY. Arterial PCO2 did not vary significantly between treatment groups or over treatment conditions and average arterial PO2 was maintained above 100 torr for all tests. The effect of hemorrhage induced hypotension on CBF and CMRO2 is shown in figure 2. Under control conditions, CBF was not significantly different between any of the test groups. CMRO2 was not significantly different between sham treated SHR and WKY or between antihypertensive treated SHR and WKY, but CMRO2 was significantly decreased (\(p < .05\)) in antihypertensive compared to sham treated rats. Hemorrhage induced hypotension to a mid-pressure level of 80–95 torr significantly decreased CBF and CMRO2 only in sham treated SHR but not in other test groups. When blood pressure was decreased to 50–60 torr CBF

\[\text{FIGURE 1. Systolic blood pressure changes during antihypertensive or sham therapy in aged SHR and WKY (\(n = 15–20\) per group). Antihypertensive drug treatment consisted of 1 mg/kg/day minoxidil, 4 mg/kg/day hydralazine and 4 mg/kg/day propranolol given in the drinking water. Antihypertensive drug treatment produced a significant decrease in systolic blood pressure in both SHR and WKY compared to sham treated rats (\(p < .01\)). Systolic blood pressure decreased significantly more in SHR than in WKY during antihypertensive therapy (\(p < .01\)) and was not significantly different from sham treated WKY at the end of the ten week treatment period (\(p > .10\)).}\]
TABLE Arterial Blood Pressure, Heart Rate, Blood Gases and pH in Aged, Antihypertensive and Sham Treated SHR and WKY

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>Blood pressure (torr)</th>
<th>Heart rate (min⁻¹)</th>
<th>Arterial Pco₂ (torr)</th>
<th>Arterial Pco₂ (torr)</th>
<th>Arterial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham treated WKY</td>
<td>10</td>
<td>119 + 2 (control)</td>
<td>309 + 18</td>
<td>39 + 1</td>
<td>135 + 21</td>
<td>7.39 + 0.02</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>11 + 1* (mid)</td>
<td>358 + 11</td>
<td>38 + 1</td>
<td>119 + 20</td>
<td>7.38 + 0.02</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>56 + 2* (low)</td>
<td>300 + 24</td>
<td>33 + 2*</td>
<td>101 + 25</td>
<td>7.36 + 0.03</td>
</tr>
<tr>
<td>Antihypertensive treated WKY</td>
<td>14</td>
<td>112 + 3 (control)</td>
<td>326 + 11</td>
<td>39 + 1</td>
<td>139 + 8</td>
<td>7.39 + 0.01</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>83 + 1* (mid)</td>
<td>332 + 10</td>
<td>39 + 1</td>
<td>127 + 5</td>
<td>7.37 + 0.01</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>55 + 1* (low)</td>
<td>312 + 4</td>
<td>38 + 1</td>
<td>155 + 8</td>
<td>7.36 + 0.01</td>
</tr>
<tr>
<td>Sham treated SHR</td>
<td>10</td>
<td>173 + 3 (control)</td>
<td>353 + 15</td>
<td>38 + 1</td>
<td>132 + 16</td>
<td>7.38 + 0.02</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>94 + 2* (mid)</td>
<td>314 + 11</td>
<td>36 + 1</td>
<td>132 + 16</td>
<td>7.38 + 0.02</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>54 + 3* (low)</td>
<td>318 + 15</td>
<td>35 + 1</td>
<td>148 + 8</td>
<td>7.37 + 0.02</td>
</tr>
<tr>
<td>Antihypertensive treated SHR</td>
<td>10</td>
<td>129 + 6 (control)</td>
<td>277 + 10</td>
<td>37 + 1</td>
<td>164 + 14</td>
<td>7.38 + 0.02</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>84 + 2* (mid)</td>
<td>267 + 17</td>
<td>38 + 1</td>
<td>168 + 11</td>
<td>7.35 + 0.02</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>58 + 1* (low)</td>
<td>299 + 13</td>
<td>36 + 1</td>
<td>151 + 8</td>
<td>7.35 + 0.02</td>
</tr>
</tbody>
</table>

Values reported as mean ± SE. *p < 0.05 compared to control treatment in each group.

Discussion
Chronic hypertension produces a shift in cerebral autoregulation that impairs the ability of these subjects to maintain cerebral perfusion and oxygenation during hypotensive stimuli. In normotensive subjects the lower limit of cerebral autoregulation has been reported by Lassen and Olessen et al. to be between 60 and 70 torr. Below this limit oxygen extraction increases to maintain CMR02 constant to an hypoxic limit of 40 to 50 torr. In hypertensive subjects Stranggaard et al. reported a lower limit of cerebral autoregulation of approximately 120 torr and a hypoxic limit of 68 torr. Kety et al. found a similar shift in cerebral autoregulation with signs of oxygen lack occurring above 100 mm Hg. In aged hypertensive patients, signs of cerebral ischemia have been reported at normotensive levels. A shift in cerebral autoregulation also occurs in SHR in response to hypotensive stimuli such as hemorrhage, ganglionic blockade and sodium nitroprusside infusion. In addition, these changes are increased as a function of aging.

Vascular hypertrophy occurs in response to the onset of hypertension. These changes protect the vascular tissue from increased perfusion pressure but alter the ability of tissues such as the brain to maintain blood flow and oxygenation at low pressures. This problem may be increased in aged subjects as collagen formation increases and elasticity decreases in vascular tissue.

Figure 2. Mean blood pressure, CBF and CMR02 under control anesthetized conditions and during hemorrhage induced hypotension. The three bars for each test group represent CBF and CMR02 values obtained under control, mid pressure (85–90 torr) and low pressure (55–60) test conditions respectively. Asterisks indicate significant change from control treatment in each group. Under control conditions, sham treated SHR were significantly hypertensive compared to other treatment groups (p < .01) but antihypertensive treated SHR were not different from sham treated WKY (p > .05). CBF was not significantly between any treatment group but antihypertensive treated SHR and WKY had lower CMR02 compared to sham treated rats (p < .05). Hemorrhage induced hypotension produced significantly greater decreases in CBF in sham treated SHR compared to other treatment groups. CMR02 decreased significantly in sham treated SHR during hypotension but not in antihypertensive treated SHR or either WKY treatment group.
Lundgren et al. and Weiss reported that lowering the blood pressure of hypertensive rats will partially or totally reverse the hind limb vascular changes produced by the hypertension and improve the perfusion parameters of this tissue. Warshaw et al. reported that 23 weeks of antihypertensive drug therapy lowered resistance vessel smooth muscle cell content in SHR in direct proportion to the reduction in blood pressure. Strangaard indicated that hypertensive subjects receiving antihypertensive therapy had a significantly better cerebrovascular response to hypotensive challenges than untreated hypertensives. Data presented here agree with these previous results and indicate that cerebral autoregulation and maintenance of cerebral metabolism during hemorrhage induced hypotension can be improved by 10 weeks of antihypertensive therapy in aged SHR.

CBF was measured in these experiments for whole brain while brain venous samples were obtained from the sagittal sinus, which drains cerebral cortical regions primarily. CMRO2 values produced in these experiments are probably somewhat higher than whole brain CMRO2 because of the source of cerebral venous blood. However, since all treatment groups were tested under the same conditions, this would not affect observed differences between treatment groups or test conditions. Antihypertensive drug treatment, including the vasodilators minoxidil and hydralazine and the \( \beta \)-adrenergic antagonist, propranolol, produced a significant decrease in CMRO2 but not CBF in both SHR and WKY under control anesthetized conditions, compared to sham treated rats. We have observed similar results in young SHR and WKY using a similar drug treatment (unpublished results). Propranolol may be primarily responsible for these changes. Previously it has been reported that central \( \beta \)-adrenergic simulation, produced by the sympathetic nervous system, may produce an increase in CMRO2. This effect was blocked by propranolol pretreatment. In addition, propranolol produced an approximate 20% decrease in basal CMRO2. A similar effect of propranolol treatment on basal CMRO2 may have been present in these experiments. This effect apparently produced little change in cerebrovascular responsiveness since cerebrovascular autoregulation was similar between sham and drug treated WKY.

One question concerning these studies is the ability of aged SHR to model the cerebrovascular changes seen in man. Although several factors are similar with respect to aged SHR and man, including arterial intimal fibrinohyalin incorporation and decreased distensibility of the brain vasculature with aging, aged SHR do not develop arteriosclerosis. This factor may be of importance, depending on its development in the small resistance vessels of the brain which are primarily responsible for decreasing resistance during decreases in cerebral perfusion pressure. Other factors, however, such as a hypertension induced vascular lesions and decreased elasticity of cerebral resistance vessels are not a major hindrance to improvement of cerebrovascular performance following antihypertensive therapy, as shown by these results.

Acknowledgments

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