Cerebrovascular Response to Hypoxia in Young vs Aged Rats

William E. Hoffman, Ph.D., Ronald F. Albrecht, M.D., and David J. Miletich, Ph.D.

SUMMARY Cerebrovascular responses of young and aged rats were tested to graded levels of hypoxia using a modification of the Levine ischemic-hypoxic rat model in which one carotid artery was ligated. Rats were anesthetized with 70% N2O, 30% O2 and cortical and subcortical cerebral blood flow (CBF) were measured with radioactive microspheres. CBF and cerebral cortical oxygen consumption (CMRO2) were measured under control conditions and during hypoxia with arterial oxygen content maintained at approximately 9, 5 and 3 ml dl-1. CBF responses in cortical and subcortical tissues were similar between young and aged under control conditions and during moderate hypoxia (CaO2 = 9 ml dl-1). Maximum cerebrovascular responses to severe hypoxia were greater in young than in aged rats and these trends were significant in both ligated and unligated cortical tissue (p < 0.05). CMRO2 was maintained at control levels during moderate hypoxia but decreased significantly more in aged than in young rats when CaO2 was decreased to 3 ml dl-1. These results suggest that baseline CBF and the sensitivity of cerebrovascular receptors to moderate hypoxia are similar in young vs aged rats but that maximum reactivity to severe hypoxia is attenuated in aged subjects. CBF measured after one minute of hypoxia, before the induction of brain tissue acidosis, produced no significant change in the CBF response to hypoxia or in the difference between young and aged rats. Brain tissue pH changes do not appear to be the major factor for mediating CBF increases during hypoxia in young or aged rats, although it may interact with other mediators of the response.

Anesthetic Management of the aged patient is often complicated by decreased cardiovascular reserve and preoperative cardiorespiratory disease. This increases the risk of hypoxic-ischemic episodes during anesthetic procedures and produces an increased incidence of postoperative hypoxemia with advancing age. It is important to know whether changes occur in the aged cerebrovasculature may alter the sensitivity or reactivity of these vessels to hypoxic or metabolic stimuli and further increase the risk of hypoxic-ischemic brain damage. However, little is known about these possible changes. Several reports suggest that the cerebral blood flow (CBF) response to hypercapnic and hypocapnic stimuli are attenuated in aged compared to young subjects. On the other hand, Haining et al reported no difference in the initial CBF response to moderate hypoxia (PaO2 = 60-70 mm Hg) in young vs aged rats. These results suggest there may be differences in sensitivity and/or reactivity to various cerebral metabolic stimuli in young vs aged subjects. In these experiments we have tested cerebrovascular changes to graded levels of arterial hypoxia and evaluated the possibility that tissue pH changes may mediate these responses. The results indicate that cerebrovascular sensitivity and CBF responses to moderate hypoxic challenges are similar between young and aged rats but reactivity of aged vessels to severe hypoxic challenges is attenuated. Brain tissue pH changes do not play a major role in these responses.

Methods
Male F-344 rats, 6 months and 26-28 months old, were used in these experiments (Charles River Inc.). For surgery rats were anesthetized with halothane, tracheostomized and artificially ventilated with 1% halothane in 70% N2O and 30% oxygen using a Harvard small animal respirator. PE50 tubing catheters filled with heparinized saline were inserted into both femoral arteries and a femoral vein. A catheter was also inserted into the left ventricle via the right carotid artery. Pressure pulses were monitored using a Hewlett Pack-
ard pressure transducer and strip chart recorder to in-
sure proper placement of the ventricular catheter. Fol-
lowing the completion of this surgery, all incisions
were closed with wound clips and the rat placed in a
stereotaxic head holder. The skull was exposed, the
bone over the sagittal sinus drilled away and a 23
gauge needle inserted into the sagittal sinus stereotaxi-
cally. Following the completion of all surgical proce-
dures the halothane was removed from the inspired
gases and the rat allowed 30 minutes to stabilize. Arter-
ial PCO₂ was adjusted to 35–40 mm Hg. Rectal tem-
perature was measured with a Yellow Springs Inc.
thermistor probe and maintained at 37°C using over-
head heat lamps. Mean blood pressure was recorded
continuously from a femoral artery catheter.

Microspheres

Fifteen micron microspheres, labelled with cobalt-
57, ruthenium-103 or scandium-146 (New England
Nuclear) were used in these studies. Stock solutions
containing 500,000 microspheres/ml-l were suspend-
ed in isotonic saline with 0.01% Tween-80. Ventricu-
lar pressure pulses were monitored before each test.
Microspheres were vortexed for one minute, 0.2 ml
withdrawn (100,000 microspheres), injected into the
left ventricle via the ventricular catheter (dead space =
0.06 ml) and flushed in with 0.2 ml saline over 20
seconds. Starting immediately before each micro-
sphere test and continuing 45 seconds after the end of
each injection blood was withdrawn from a femoral
artery at a rate of 0.4 ml/min using a Harvard infusion-
withdrawal pump. Arterial and sagittal sinus blood
samples were taken at the end of each test for measure-
ment of blood gases and pH, using an IL 1303 blood
gas analyzer and oxygen content was measured using an
IL282 co-oximeter. Oxygen content was calculated as
the sum of the co-oximeter value plus 0.03 times the
PO₂ in each blood sample. Mean arterial blood pres-
sure was measured continuously throughout the micro-
sphere test from the second femoral artery to ensure
blood pressure did not change appreciably.

At the end of the last microsphere test the rat was
sacrificed, the brain removed and sectioned into left
and right cortical and subcortical samples and
weighed. The activity of each microsphere in brain and
blood samples was analyzed using a Nuclear Chicago
1035 gamma counter and a Nuclear Data 600 multi-
channel analyzer. CBF was analyzed according to the
methods of Heymann et al.⁹ CMRO₂ was calculated as
the average of left and right cortical CBF times arteri-
sagittal sinus oxygen content.

**Induction of Hypoxia**

A modification of Levine’s model of hypoxic-isch-
emia,¹⁰ consisting of unilateral carotid artery occlusion
was used in these experiments. Hypoxia was induced
by substituting oxygen with nitrogen in the inspired
gases. Arterial oxygen content (CaO₂) was decreased
to approximate levels of 9, 5 and 3.5 ml/dl-l in both
young and aged rats. CO₂ was added to the inspired
gases during hypoxia to maintain PaCO₂ at approxi-
mately 35 mm Hg. Hypoxia was maintained for 5–7
minutes before each microsphere test. Microsphere
tests were performed randomly in young and aged rats
under control and graded hypoxic conditions. Arterial
blood pressure was maintained above 100 mm Hg in
all tests. For one minute periods of hypoxia, methods
were similar to those described above except that one
level of hypoxia was induced (CaO₂ = 4.2-4.6 ml/dl-
1). Microsphere tests were performed 60 seconds after
the start of hypoxia. CO₂ was not added to the inspired
gases in these tests and CMRO₂ was not measured.

Data are reported as mean ± SE and statistics per-
formed included unpaired t-tests and two way analysis
of variance followed by Duncan’s multiple range test
to evaluate specific hypoxic levels when F values were
found to be significant.

**Results**

As shown in table 1, similar levels of graded hy-
poxia were produced in both young and aged rats.
Arterial PaCO₂ was maintained at control levels during
hypoxia by the addition of CO₂ to the inspired gases
and arterial blood pressure was maintained above 100

**Table 1 Blood Pressure, Heart Rate and Arterial Blood Gases in Young and Aged F-344 Rats during Normoxia and Hypoxia**

<table>
<thead>
<tr>
<th></th>
<th>Arterial blood pressure (mm Hg)</th>
<th>Heart rate (min-1)</th>
<th>PaO₂ (mm Hg)</th>
<th>CaO₂ (ml/dl-1)</th>
<th>PaCO₂ (mm Hg)</th>
<th>pH</th>
<th>Hemoglobin (g/dl-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>126 ± 6</td>
<td>425 ± 10</td>
<td>121 ± 9</td>
<td>15.1 ± 6</td>
<td>38 ± 1</td>
<td>7.37 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>126 ± 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>130 ± 4</td>
<td>447 ± 12</td>
<td></td>
<td>33 ± 1</td>
<td>5.4 ± 2</td>
<td>38 ± 1</td>
<td>7.26 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>114 ± 6</td>
<td>391 ± 23</td>
<td></td>
<td>25 ± 1</td>
<td>3.3 ± 1</td>
<td>38 ± 1</td>
<td>7.25 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>156 ± 4</td>
<td>423 ± 21</td>
<td></td>
<td>116 ± 7</td>
<td>17.6 ± 8</td>
<td>36 ± 1</td>
<td>7.40 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>416 ± 9</td>
<td></td>
<td>41 ± 2</td>
<td>9.0 ± 4</td>
<td>38 ± 1</td>
<td>7.23 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>370 ± 25</td>
<td></td>
<td>31 ± 2</td>
<td>5.1 ± 3</td>
<td>36 ± 1</td>
<td>7.25 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>313 ± 17</td>
<td></td>
<td>24 ± 1</td>
<td>3.2 ± 2</td>
<td>38 ± 1</td>
<td>7.22 ± 0.2</td>
</tr>
<tr>
<td><strong>Aged</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>143 ± 4</td>
<td>424 ± 14</td>
<td>25 ± 1</td>
<td>4.2 ± 3</td>
<td>27 ± 1</td>
<td>7.45 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>129 ± 4</td>
<td>385 ± 16</td>
<td>26 ± 1</td>
<td>4.6 ± 7</td>
<td>26 ± 1</td>
<td>7.44 ± 0.1</td>
</tr>
</tbody>
</table>

*One minute hypoxia*
mm Hg at all hypoxic levels, CBF changes during hypoxia are shown in table 2. The data are plotted as percent of control in figure 1. Decreasing arterial oxygen content produced progressive increases in CBF in both young and aged rats. These increases were significantly greater in the left (unligated) cortex and lower brain compared to the right side in both young and aged rats, as analyzed by analysis of variance (p < 0.01), but percentage increases were similar in the right vs the left side (fig. 1). There was no significant difference in the CBF response to hypoxia in subcortical tissue in young vs aged rats, although young rats had a higher maximum CBF response at the most hypoxic level (CaO₂ = 3 ml·dl⁻¹). In cortical tissue the CBF response was similar between young and aged during moderate hypoxia. Young rats showed increased CBF at CaO₂ values of 5 and 3 ml·dl⁻¹ and this trend was significantly greater compared to aged rats (p < 0.05). CMRO₂ changes during hypoxia are shown in figure 2. CMRO₂ was maintained at control levels in both young and aged rats until CaO₂ levels of approximately 3 ml·dl⁻¹ were reached. At that point CMRO₂ decreased significantly more in aged rats than in young (p < 0.05).

One minute periods of hypoxia performed without the addition of CO₂ to the inspired gases produced decreases in PaCO₂ and increased arterial pH compared to control values (table 1). As shown in table 2, CBF increases during this short period of hypoxia were consistent with changes seen in the first experiment in which arterial pH decreased. In addition, differences in CBF response to hypoxia between young and aged were still apparent.

### Table 2  Cerebral Blood Flow in Young and Aged F-344 Rats during Hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Arterial oxygen content (ml O₂/100 ml)</th>
<th>Cortex</th>
<th>Lower brain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Left (ml·100 g⁻¹)</td>
<td>Right (ml·100 g⁻¹)</td>
</tr>
<tr>
<td>Young</td>
<td>10</td>
<td>135 ± 10</td>
<td>96 ± 9</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>247 ± 27</td>
<td>138 ± 14</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>314 ± 35</td>
<td>186 ± 25</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>348 ± 23</td>
<td>228 ± 22</td>
</tr>
<tr>
<td>Aged</td>
<td>7</td>
<td>141 ± 18</td>
<td>101 ± 17</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>260 ± 20</td>
<td>158 ± 14</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>299 ± 31</td>
<td>183 ± 22</td>
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<tr>
<td></td>
<td>12</td>
<td>295 ± 25</td>
<td>175 ± 14</td>
</tr>
<tr>
<td></td>
<td>One minute hypoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>11</td>
<td>334 ± 20</td>
<td>216 ± 14</td>
</tr>
<tr>
<td>Aged</td>
<td>9</td>
<td>285 ± 29</td>
<td>159 ± 18</td>
</tr>
</tbody>
</table>

**Figure 1.** CBF changes in young and aged rats during hypoxia. Data are plotted as percent of control flow in each test group. Significance levels indicate difference between test groups at comparable CaO₂ value. Results indicate significant difference between young and aged rats which reaches a significant value at severe hypoxic levels in cortical tissue.

**Figure 2.** CMRO₂ changes in young and aged rats during arterial hypoxia. Data indicate maintenance of CMRO₂ not significantly different from control levels until severe hypoxic levels were reached (CaO₂ = 3.2–3.3 ml·100 ml⁻¹). At that point there was a significantly greater decrease in CMRO₂ in aged compared to young rats.
The relative stability of CMRO2 until severe hypoxic

tors. In addition, hypoxia (PaO2 = 22 mm Hg)

estration from arterial blood may be limited. Results

cortex, when relative ischemia is present and oxygen

3.3 ml-dl-1. At that point, significant decreases in

CORT, were observed in both young and aged rats.

levels are reached is in agreement with previous au-

poxic damage when the state of hypoxia or ischemia

hypoxia (CaO2 = 3.2-3.3 ml-dl-1) are attenuated in the

subjects. This would agree with Haining et al, who found no change in cerebrovascular responses to

moderate hypoxia in aged rats, but is also in agreement

with other reports describing increased signs of hy-

poxic damage when the state of hypoxia or ischemia

may be severe. Our results would indicate the cere-

brovascular response to severe hypoxia is attenuated in

aged subjects in cerebral cortical tissue both ipsilateral

and contralateral to carotid ligation, indicating an al-

tered response which is not dependent on the collateral

circulation.

Because the sagittal sinus blood samples taken in

these experiments reflect cerebral cortical drainage

from both right and left hemispheres, measured

CMRO2 may not reflect changes occurring in the ligat-

e vs the unligated brain hemisphere. This would be

tue particularly during severe hypoxia in the ligated
cortex, when relative ischemia is present and oxygen

extraction from arterial blood may be limited. Results

of these experiments indicate a stable CMRO2 in both

young and aged rats until CaO2 reached levels of 3.2–

3.3 ml-dl-1. At that point, significant decreases in

CMRO2 were observed in both young and aged rats.

The relative stability of CMRO2 until severe hypoxic

levels are reached is in agreement with previous au-

flow to the brain is decreased. This is consistent with

other studies that have shown that the cerebrovascu-

lar response to hypoxia is attenuated in aged rats.

Hypoxia is a common finding in aged animals and

humans, and it is thought that the aging process may

result in a decreased ability to tolerate hypoxic

conditions. In this study, the decrease in CBF in the

ipsilateral hemisphere was observed to be greater in

aged rats compared to young rats. This is consistent

with previous reports that have shown that the cere-

brovascular response to hypoxia is attenuated in aged

rats.

In conclusion, the results of this study suggest that

the cerebrovascular response to hypoxia is attenuated

in aged rats compared to young rats. This attenuation

may be due to a decrease in the ability of the aged

brain to tolerate hypoxic conditions. Further studies

are needed to determine the underlying mechanisms

responsible for this attenuated response.
HYPoxIA IN AGED Rats/Hoffman et al

...gy state depletion in young vs aged rats. We found no evidence that brain tissue pH may mediate CBF changes during hypoxia, although the possibility cannot be ruled out that tissue pH may interact with other mediators to influence CBF. The role of other potential mediators of the altered CBF response to hypoxia that occurs with aging remains to be tested.

References


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Stroke. 1984;15:129-133
doi: 10.1161/01.STR.15.1.129
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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