Effect of Chemodenervation on the Cerebral Vascular and Microvascular Response to Hypoxia

Mujahid Anwar, Inna Kissen, and Harvey R. Weiss

This study evaluated the effect of bilateral carotid chemodenervation on the cerebrovascular response to hypoxia in conscious rats. Cerebral blood flow was measured using 4-iodo-[N-methyl-14C]antipyrine, and the total and perfused microvasculature was studied by injection of fluorescein isothiocyanate dextran and alkaline phosphatase staining. To maintain constant Pco2, hypoxia was achieved in chemoreceptor-intact rats by the use of 4% CO2-8% O2-88% N2, and in chemodenervated rats by the administration of 8% O2-92% N2. Blood gas and hemodynamic parameters were similar in the two groups of rats. Chemodenervation had no significant effect on either resting blood flow or the perfused microvasculature during normoxia. A significant increase in cerebral blood flow (from 71±3 to 138±9 ml/min/100 g in control and from 91±5 to 127±7 ml/min/100 g in chemodenervated rats) and in the percent of cerebral arterioles and capillaries perfused occurred in both hypoxic control and chemodenervated rats. In chemoreceptor-intact rats, the greatest increase in blood flow and in perfused microvasculature occurred in caudal structures (medulla and pons) in comparison with rostral structures (cortex, thalamus, and hypothalamus). In chemodenervated rats, a similar increase in blood flow and perfused microvasculature occurred in all brain regions, with no regional differences. Thus, chemodenervation did not affect the overall cerebral blood flow or the microvascular response to hypoxia; however, rostral-to-caudal regional differences in the hypoxic response were lost after chemodenervation. (Circulation Research 1990;67:1365–1373)

There are two cardiovascular mechanisms that help to protect the brain from hypoxemia: increases in the cerebral blood flow and decreases in the diffusion distances for oxygen.1,2 It has been shown that hypoxia increases cerebral blood flow, especially to caudal structures of the brain.3 It is possible that the rostral-to-caudal gradient of the hypoxic blood flow is due to the sympathetic reflex restriction of the forebrain blood flow during hypoxia,4 which could be activated by peripheral chemoreceptors.

Peripheral arterial chemoreceptors have little influence on the cerebral circulation during normoxic steady-state conditions.5 Different results have been obtained regarding the role of the peripheral chemoreceptors in the regulation of the cerebral circulation during hypoxia. Some studies6–8 have shown that the peripheral chemoreflex does not play an important role in hypoxic cerebral vasodilation; others9,10 indicated that the peripheral chemoreceptors are primarily responsible for hypoxic vasodilation. In addition, there is evidence that the peripheral chemoreflex exerts effects on blood flow in some regions of the brain but that it does not affect blood flow in other brain areas.11,12

It has been suggested that the sympathetic nervous system limits cerebral vasodilation during hypoxia.3,13 After superior cervical ganglionectomy, the pattern of the cerebral blood flow response to hypoxia changes.3,14 Additionally, superior cervical ganglionectomy during hypoxia resulted in the significantly higher utilization of the arteriolar reserve, and it had a small effect on capillaries.14 One afferent limb of the neurogenic control of the peripheral sympathetic innervation originates, in part, from the carotid and aortic chemoreceptors and baroreceptors. In the present study, we have examined the role of the afferent limb of the neurogenic reflex originating from the carotid chemoreceptors on the hypoxic cerebrovascular responses. We hypothesized that the peripheral chemoreceptors limit the cerebral blood
flow and the number of perfused microvessels, especially in the rostral areas of the brain, during oxygen supply–deficit conditions. Regional cerebral blood flow was determined using 4-ido[14C]jodoantipyrine ([14C]jidoantipyrine), and the number of perfused microvessels was studied by fluorescein isothiocyanate dextran (FITC-dextran) and alkaline phosphatase staining, as described previously.15,16

Materials and Methods

Adult Long-Evans rats of either sex weighing 250–450 g were used. The rats were anesthetized with ether, and femoral arterial and venous catheters were inserted. The venous catheter was used for the administration of [14C]jidoantipyrine and FITC-dextran. The arterial catheter was used to measure heart rate, blood pressure, and blood gases. Chemodervation was performed through a midline incision in the neck. The bifurcation of the common carotid artery was isolated on both sides. The sinus nerve was traced to its origin from the glossopharyngeal nerve and was cut on both sides. The vagus nerves were left intact. Completion of nerve transection was tested by the administration of nitrogen. Rats with intact chemoreceptors initially responded with hyperventilation, whereas chemodenervated rats either had no response or became apneic. Control rats were sham-operated with visualization of the carotid bifurcation, but all nerves were left intact. All wounds were infiltrated with lidocaine. The rats were allowed to recover from anesthesia for 2 hours before any measurements were made.

Arterial blood pressure was continuously measured using a Statham P23Db transducer (Gould Instruments, Cleveland) coupled to the arterial catheter and recorded on an R-411 recorder (Beckman Instruments, Inc., Fullerton, Calif.). An arterial blood sample (0.2 ml) was withdrawn anaerobically and analyzed for PO2, PCO2, and pH on a blood gas analyzer (BMS model 3, Radiometer America, Westlake, Ohio) before the induction of hypoxia and determination of cerebral blood flow or the injection of FITC-dextran. Hypoxia was induced in the chemodenervated rats by the administration of 8% O2–92% N2. In chemoreceptor-intact rats, this gas mixture resulted in a fall in PCO2 to the low twenties.14 To control the PCO2, a gas mixture of 8% O2–4% CO2–88% N2 was used. The rats were allowed to breathe the hypoxic gas mixture for 15 minutes before cerebral blood flow or total and perfused capillary and arteriolar morphology was determined. Neither normoxic nor hypoxic rats appeared to be in visible distress, and all rats were quiescent at the time of the measurement.

We determined cerebral blood flow using a modified technique of Sakurada et al.,17 as described by Conway and Weiss.15 After final heart rate, blood pressure, and blood gas determination, [14C]jidoantipyrine (Amersham, Arlington Heights, Ill.) was infused through the venous catheter by means of an infusion pump (Sage Instruments, Cambridge, Mass.). At the time of the entry of the isotope into the venous circulation, the arterial catheter was cut to a length of 15–20 mm to minimize smearing in the sampling catheter. Timed blood samples were withdrawn from the arterial catheter and collected every 3 seconds in capillary tubes. These samples were collected over a period of 60 seconds, at which time the rat was decapitated to terminate perfusion at the moment the last sample was obtained. Approximately 1 ml blood was withdrawn from the rats over this period. Arterial bleeding was controlled with forceps. The brain was frozen in liquid nitrogen for later analysis. While frozen, the brain was cut in the midsagittal plane, and the following regions were dissected: cortex, thalamus, hypothalamus, pons, and medulla. Blood and tissue samples were then placed in Soluene (Packard Instrument Co., Inc., Downers Grove, Ill.) and 24 hours later in Dimiscent (National Diagnostics, Inc., Manville, N.J.) and agitated. These samples were counted on a Beckman LS-230 liquid scintillation counter. No appreciable quenching was found to occur.

Regional blood flow determinations were made using a computer program based on the equation 

$$Ci(T) = \lambda K \int_0^T CAe^{-K(T-t)} dt$$

where Ci(T) equals the tissue concentration of the [14C]jidoantipyrine at the time of decapitation (T), \(\lambda\) equals the tissue:blood partition coefficient, \(C_a\) is the arterial concentration of the tracer, and \(t\) equals time. \(K\) is defined as follows:

$$K = m/FW,$$

where \(m\) is a constant related to diffusion and \(F/W\) equals the blood flow per unit mass of tissue. The value of 0.80 calculated by Sakurada et al17 was used.

Regional perfused and total capillary and arteriolar morphology was determined in another group of rats. After the final determination of heart rate, blood pressure, and arterial blood gas, 150 mg/kg FITC-dextran (70,000 molecular weight, Sigma Chemical Co., St. Louis) was injected intravenously as a 0.5 ml bolus. Twenty seconds after the injection of FITC-dextran, the rats were killed, and the heads were quickly frozen in liquid nitrogen and stored at –70°C until analysis. Brains were exposed midsagittally, and the regions used for the flow determination were isolated and mounted on a microtome specimen holder and coated with embedding medium (O.C.T. Compound, Miles Laboratories, Inc., Elkhart, Ind.). Three-micrometer sections were cut on a microtome cryostat set at –35°C, transferred to glass slides, and allowed to dry. Approximately 12 sections were cut for capillaries and 15 for arterioles. Each section was cut 150–200 \(\mu\)m from the previous one.

The slides were photographed on a fluorescent microscope (Carl Zeiss, Inc., Thornwood, N.Y.) equipped for automated photography. A \(\times 40\) objective was used to photograph the capillaries, and a \(\times 10\) objective was used to photograph the arterioles. The slides were epi-illuminated with violet light from a 100-W halogen source to excite the fluorescence in the FITC-dextran. A barrier filter was placed in the viewing field to allow 495 nm or greater wavelength
light through. A second photograph of the same field was taken in normal light, which, together with the viewing coordinates obtained, helped relocate the field.

The slides were then stained for alkaline phosphatase as previously described. The slides were fixed in a sucrose-formalin buffer for 1 minute, were washed twice in distilled water, and were then placed in an incubation mixture for 30 minutes at 37°C. The incubation mixture consisted of 3.8 g/l fast blue RR, 0.5 g/l α-naphthyl phosphate, 3.8 g/l sodium metabolite, and 1.7 g/l magnesium sulfate. The slides were postfixed and dried. The field previously photographed for fluorescence was relocated.

Various stereological determinations were performed, counting each field twice, once for the total and once for the perfused microvasculature. These stereological principles have been reviewed and applied to the brain microvasculature. The system used was an image analyzing device (Dapple Systems, Sunnyvale, Calif.). We placed the slides that were stained for alkaline phosphatase under a microscope, relocating the field previously photographed for fluorescence. The image analyzer was used to evaluate the slides for the total morphometric indexes of the microvasculature. The image was obtained from a Panasonic TV camera attached to the microscope and digitized with the Dapple image analyzer. The programs digitize the raw image by measuring the brightness in 64 gray scale–level steps at each point in a 254×192 array in the picture. The image was automatically edited through the various subroutines in the Dapple system and manually edited with a light pen.

The volume fraction (Vv) (in cubic millimeters per cubic millimeter) of the microvasculature was determined by dividing the number of test points falling within the profile of a microvessel by the total number of points within the array. The total number of test points was selected so that probable error in Vv would be less than ±5%. The number of capillaries and arterioles (Nv) per square millimeter was determined from the number of vessels per unit test area. After the total microvasculature was studied, the perfused portion was obtained from photographs of the fluorescence within the microvasculature. The light pen was used to edit the digitized image for similar measurements of the perfused microvasculature. Vessels with an edge-to-edge diameter of less than 11 μm were considered capillaries, and all arterioles with diameters between 19 and 50 μm were counted. The diameters measured consisted of the lumen and the endothelial walls.

Three rats were used to compare our method of slide preparation with that of Gobel et al. FITC-dextran was allowed to circulate for 20 seconds, and the rats were prepared as described above. The cerebellum was used, and four different sets of slides were obtained. Fluorescent photographs were obtained from two sets of slides. One set was obtained by using the technique described above, “air drying”; the other set was obtained by using “alcohol impregnation.”

The alcohol-impregnated tissue sections were obtained from frozen sections placed on slides with frozen absolute alcohol, covered with alcohol, and then embedded with Entellan (EM Science, Cherry Hill, N.J.). Another set of slides was stained for alkaline phosphatase as described. In the final set of slides, basement membranes of the capillary walls were stained by a modified silver methenamine stain. Briefly, 8-μm sections were obtained. The sections were fixed in buffered sucrose–formalin, washed, placed in a 1% periodic acid solution, washed, and finally incubated at 60°C in a mixture of methenamine, borax, and silver nitrate. This procedure has been shown to stain the basement membrane of capillary endothelium.

Analysis of variance was used to determine the differences between the groups and regions for the various measurements performed. Post hoc multiple comparisons were made using Duncan’s procedure. Average values are the arithmetic mean of all examined regions. All values are expressed as mean±SEM unless otherwise specified. A value of p<0.05 was accepted as significant. This study followed the guidelines of the American Physiological Society and the Conference of Helsinki for the use of experimental animals and was approved by the Animal Care and Use Committee of the Robert Wood Johnson Medical School.

Results

Table 1 shows the hemodynamic and blood gas parameters of the rats studied. Mean blood pressure decreased in response to hypoxia in the chemodenervated rats, in comparison with the control period.
The decrease in pressure was not significant in the chemoreceptor-intact rats. Mean blood pressure was similar in hypoxia in both groups of rats. As expected, arterial Po2 was reduced to 46 mm Hg in chemodenervated rats and 49 mm Hg in rats with intact chemoreceptors in response to the hypoxic gas mixtures. Heart rate, arterial pH, and arterial PCO2 were not significantly different in any group either before or after the induction of hypoxia. It should be noted that the hypoxic rats with intact chemoreceptors received 4% CO2 in the gas mixture to achieve arterial PCO2 after hypoxia similar to that in chemodenervated rats.

Average cerebral blood flow is shown in Table 2. In normoxia, the cerebral blood flow was not significantly different in comparisons between the control and chemodenervated groups. Hypoxia resulted in a similar increase in cerebral blood flow in both the chemodenervated and chemoreceptor-intact rats. Regional cerebral blood flow is shown in Figure 1. During the normoxic condition, chemodenervation did not affect regional cerebral blood flow. The regional distribution of cerebral blood flow was similar in rats under normoxic conditions in both control and chemodenervated conditions. However, regional distribution of blood flow was different in the two groups of rats after induction of hypoxia. Hypoxia increased the control group cerebral blood flow in all regions, except in the cortex. After chemodenervation, hypoxic blood flow increased significantly only in the cortex and hypothalamus, and the increase in the thalamus, pons, and medulla was not significant compared with the normoxic chemodenervated group. Blood flow in the pons and medulla of the hypoxic chemodenervated rats was lower than in these regions of hypoxic control rats. In rats with intact chemoreceptors, the greatest increase in blood flow was observed in the pons and medulla. Whereas, in rats after chemodenervation, this regional difference in blood flow response was abolished.

Table 3 shows the average total arteriolar Vv and Nv in the different groups of rats. The total arteriolar Vv and Nv was similar in all groups of rats in all brain regions studied. Figure 2 shows the percent of arteriolar Vv and Nv perfused in different brain regions in the various groups of rats. Chemodenervation did not affect these parameters during normoxia, nor were regional differences found in either group during normoxia. The percent perfused arteriolar Vv and Nv

### Table 2. Average Cerebral Blood Flow in Normoxic and Hypoxic Rats With and Without Bilateral Chemodenervation

<table>
<thead>
<tr>
<th>Group</th>
<th>Cerebral blood flow (ml/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.1±2.9</td>
</tr>
<tr>
<td>Chemodenervated</td>
<td>91.4±5.4</td>
</tr>
<tr>
<td>Control hypoxia</td>
<td>138.2±9.1*</td>
</tr>
<tr>
<td>Chemodenervated+hypoxia</td>
<td>126.9±6.3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=5 rats per group. *Significantly different from the corresponding normoxic condition at p<0.05.

### Table 3. Total Arteriolar Volume Fraction and Number of Perfused Microvessels in Normoxic and Hypoxic Rats With and Without Chemodenervation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chemodenervated</th>
<th>Control hypoxia</th>
<th>Chemodenervated+hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vv ([mm³/mm³]×10³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>0.15±0.06</td>
<td>0.10±0.01</td>
<td>0.10±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.13±0.02</td>
<td>0.11±0.02</td>
<td>0.11±0.01</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.11±0.02</td>
<td>0.10±0.02</td>
<td>0.11±0.01</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td>Pons</td>
<td>0.12±0.02</td>
<td>0.11±0.02</td>
<td>0.12±0.02</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.10±0.01</td>
<td>0.10±0.02</td>
<td>0.13±0.01</td>
<td>0.10±0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Hypothalamus</th>
<th>Thalamus</th>
<th>Pons</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nv (number/mm³)</td>
<td>1.00±0.13</td>
<td>1.11±0.10</td>
<td>1.05±0.11</td>
<td>1.20±0.11</td>
<td>1.21±0.18</td>
</tr>
<tr>
<td>Cortex</td>
<td>1.21±0.18</td>
<td>1.10±0.11</td>
<td>1.00±0.16</td>
<td>1.00±0.31</td>
<td>1.00±0.19</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.10±0.15</td>
<td>1.03±0.11</td>
<td>1.08±0.06</td>
<td>1.18±0.36</td>
<td>1.18±0.19</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.01±0.23</td>
<td>1.14±0.23</td>
<td>1.15±0.15</td>
<td>1.00±0.19</td>
<td></td>
</tr>
<tr>
<td>Pons</td>
<td>1.00±0.09</td>
<td>1.02±0.16</td>
<td>1.26±0.15</td>
<td>1.18±0.19</td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=5 rats per group. Vv, arteriolar volume fraction; Nv, number of perfused microvessels per square millimeter.
increased in all brain regions studied in response to hypoxia in both the chemoreceptor-intact rats and chemodenervated rats. This increase was significant in all regions of control hypoxic rats except in the cortex. The percent perfused arteriolar Vv and Na was significantly higher in the hypothalamus of chemodenervated hypoxic rats than in this region of control hypoxic rats. In addition, the greatest increase in arteriolar Vv and Na occurred in the medulla and the pons in chemoreceptor-intact rats, whereas these regional differences were abolished in chemodenervated rats.

Table 4 shows the total capillary Vv and Na in various brain regions in the four groups of rats. Total capillary Vv and Na was similar in all groups of rats for all the brain regions studied. The percent of capillary Vv and Na perfused is shown in Figure 3 for the various brain regions in the four examined groups. Chemodenervation did not affect the percent perfused capillary Vv and Na during normoxia nor were any regional differences observed in normoxia in either group. The percent perfused capillary Vv increased significantly in all examined regions of control hypoxic and chemodenervated hypoxic rats except in the medulla of chemodenervated hypoxic rats. The percent perfused capillary Na increased significantly in all examined regions of control hypoxic rats compared with control normoxic conditions except for the cortex. After chemodenervation, the percent perfused capillary Na was significantly higher in all examined regions of hypoxic rats compared with chemodenervated normoxic rats. In the medulla of the chemodenervated hypoxic rats, this parameter was significantly lower than in the control hypoxic group.

Na was determined by four independent methods in the cerebellum of three rats in which FITC-dextran was allowed to circulate for 20 seconds. Two fluorescent methods, “air-drying” and “alcohol impregnation,” were used. In addition, two anatomic capillary staining methods were employed, alkaline phosphatase and silver methenamine stains. The results are shown in Table 5. There were no significant differences in Na between the alkaline phospha-

**Table 4. Total Capillary Volume Fraction and Number of Perfused Microvessels in Normoxic and Hypoxic Rats With and Without Chemodenervation**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Chemodenervated</th>
<th>Control hypoxia</th>
<th>Chemodenervated + hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vv [(mm³/mm³) x 10⁷]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>3.56±0.74</td>
<td>3.03±0.10</td>
<td>3.10±0.17</td>
<td>3.03±0.22</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>3.88±0.82</td>
<td>3.11±0.24</td>
<td>3.25±0.05</td>
<td>3.35±0.20</td>
</tr>
<tr>
<td>Thalamus</td>
<td>3.69±0.32</td>
<td>2.96±0.17</td>
<td>3.54±0.20</td>
<td>3.38±0.14</td>
</tr>
<tr>
<td>Pons</td>
<td>3.58±0.47</td>
<td>3.30±0.13</td>
<td>3.06±0.17</td>
<td>3.11±0.20</td>
</tr>
<tr>
<td>Medulla</td>
<td>4.31±0.61</td>
<td>3.08±0.23</td>
<td>3.18±0.17</td>
<td>3.40±0.33</td>
</tr>
<tr>
<td>Na (number/mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>395±77</td>
<td>278±12</td>
<td>292±16</td>
<td>282±15</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>336±35</td>
<td>289±18</td>
<td>252±9</td>
<td>313±27</td>
</tr>
<tr>
<td>Thalamus</td>
<td>431±64</td>
<td>262±13</td>
<td>274±16</td>
<td>316±36</td>
</tr>
<tr>
<td>Pons</td>
<td>283±47</td>
<td>290±12</td>
<td>291±25</td>
<td>290±17</td>
</tr>
<tr>
<td>Medulla</td>
<td>315±66</td>
<td>309±20</td>
<td>319±24</td>
<td>322±10</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=5 rats per group. Vv, arteriolar volume fraction; Na, number of perfused microvessels per square millimeter.
tase and silver methenamine stains. Our air drying method showed significantly less capillaries labeled than either staining technique, 20 seconds after FITC-dextran injection. The alcohol impregnation technique had a significantly higher Ns than any other technique. Figure 4 shows cerebellar sections prepared using the four techniques.

**Discussion**

In this study, we determined the effects of the peripheral chemoreceptors on regional cerebral blood flow and the number of perfused microvessels during normoxia and hypoxia. It was hypothesized that peripheral chemoreceptors limit the cerebral blood flow response and the utilization of microvascular reserve during hypoxia. The major findings of this study were that chemodenervation in rats did not abolish the increase in brain blood flow or perfused microvessels in response to hypoxia; however, the regional distribution of blood flow and perfused microvessels was affected by chemoreceptors during hypoxia. The rostral to caudal flow and microvascular perfusion gradient seen in chemoreceptor-intact rats during hypoxia were lost in hypoxic chemodenervated rats. We studied the effect of peripheral carotid chemoreceptors since it has been suggested that functional aortic chemoreceptors are absent in the rat. In addition, there is histological evidence that the chemoreceptor tissue in the aortic arch of the rat is poorly defined.

The present study reports a reserve of cerebral capillaries that can be used to reduce diffusion distance. A similar reserve of cerebral capillaries has been reported using other techniques. Recently, there have been reports that there is no cerebral capillary reserve using a technique similar to ours. There have been reports comparing the two techniques. The major difference appears to be in the method of slide preparation. Using our technique, we are able to show that approximately 50% of the capillaries contain fluorescent label 20 seconds after FITC-dextran injection. In previous studies, we have shown correspondence between our fluorescent technique and alkaline phosphatase stain when a long time interval after injection (6 minutes) was allowed or when the animal was asphyxiated. In addition, we showed correspondence between India ink injections and alkaline phosphatase stain. In the current report, we show that a silver methenamine stain for the capillary basement membrane also produces similar data. Thus, three visible light techniques (alkaline phosphatase, India ink, and silver methenamine) and our air-dried fluorescent photographs, when the FITC-dextran is allowed to circulate for 6 minutes or when the animal is asphyxiated, produce roughly identical counts of capillaries per square millimeter. In this and previous studies, the technique of Gobel et al produces counts per square millimeter that are approximately one third higher than either visible light staining technique. The comparison in the recent Gobel et al publication between their technique and a fluorescent fibronectin stain may present difficulties, since one cannot determine whether all capillaries (and only capillaries) were stained. According to the one published report using this stain, “some vessels were reactive” to their antifibronectin antibody. Further, Gobel et al study their sections 1 minute after

**Figure 3.** Bar graphs showing the percent of regional cerebral capillary volume fraction (Vv) (left panel) and arteriolar number (Na) per square millimeter (right panel) perfused in normoxic and hypoxic rats with and without bilateral peripheral chemodenervation (n=5 rats per group). *Significantly different from control normoxic group. **Significantly different from control hypoxic group.

**Table 5. Comparison of Methods of Slide Preparation on the Number of Capillaries per Square Millimeter Labeled in the Cerebellum of Three Rats**

<table>
<thead>
<tr>
<th>Method</th>
<th>Ns (number/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent labels</td>
<td></td>
</tr>
<tr>
<td>Air-dried</td>
<td>150±13*†</td>
</tr>
<tr>
<td>Alcohol-impregnated</td>
<td>357±23*</td>
</tr>
<tr>
<td>Anatomic capillary stains</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>258±17†</td>
</tr>
<tr>
<td>Silver methenamine</td>
<td>278±24†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Ns, number of capillaries per square millimeter.

*Significantly different from alkaline phosphatase and silver methenamine methods.

†Significantly different from alcohol-impregnated method.
Figure 4. Photograph of 20 seconds of fluorescein isothiocyanate dextran labeling of cerebellar capillaries obtained by "alcohol impregnation" (top left panel) and "air drying" (top right panel). A silver methenamine (bottom left panel) and an alkaline phosphatase (bottom right panel) stain of the capillary bed are also shown. Bar, 20 μm.
dye injection at a time when, even with our technique, most of the vessels would be marked. It appears that the absolute alcohol technique significantly overestimates the number of capillaries. Thus, there appears to be a capillary reserve that can be used in hypoxic conditions. Figure 4 shows a comparison between the various techniques.

Chemodenervation had little effect on blood flow or the number of perfused microvessels in different brain regions during normoxia. The average blood flow and the number of perfused microvessels in the normoxic control and chemodenervated rats in this study were similar to those values previously reported from this laboratory in control animals. The values for average cerebral blood flow and perfused microvasculature in hypoxic chemoreceptor-intact rats were also similar to those reported previously. It has been shown that cervical sympathectomy increased the blood flow and the utilization of microvascular reserve during hypoxia in comparison with control hypoxic rats. In addition, regional differences in blood flow and perfused microvasculature were abolished in ganglionectomized hypoxic rats. In our present study, the average blood flow and the number of perfused microvessels were similar in hypoxic chemoreceptor-intact and chemodenervated rats. However, the regional differences in the blood flow and the number of perfused microvessels during hypoxia were lost after chemodenervation.

Our results can be explained by a loss of both sympathetic and possibly parasympathetic influence on cerebral blood vessels. Brain blood vessels are extensively supplied with noradrenergic fibers. The extraparenchymal blood vessels are supplied by noradrenergic fibers that originate in the superior cervical ganglion. The innervation and functional effects appear more pronounced in rostral brain regions. The noradrenergic innervation of intraparenchymal vessels is primarily from the locus ceruleus and other brain stem centers. In addition, the extraparenchymal blood vessels are supplied by the cholinergic fibers that originate in the facial nerve and are then carried via the greater superficial petrosal nerve to the carotid plexus. The extraparenchymal vessels have not been shown to receive cholinergic innervation. One afferent arch for the neurogenic control of the cerebral vasculature by the peripheral sympathetic and parasympathetic systems originates from the peripheral chemoreceptors. Sympathetic fibers are primarily vasoconstrictor, whereas parasympathetic fibers are vasodilator. Thus, sympathectomy with intact chemoreceptors may result in a greater than usual increase in the blood flow response to hypoxia. However, when the vasodilatory parasympathetic influence is also lost after chemodenervation, the intrinsic vascular response to hypoxia, which is similar in all brain regions, can be unmasked.

Our cerebral blood flow results are at variance with those of James et al., Ponte and Purves, and James and MacDonell. These authors showed an important role of chemoreceptors in the hypoxic cerebrovascular response. Hypoxic cerebral vasodilation was lost after chemodenervation. However, these authors measured cerebral blood flow by or clearance techniques, in which there is a possibility of contamination with extracranial blood. Our results are in agreement with those of Frayman et al., Heistad et al., and Bates and Sundt, who showed no effect of chemodenervation on cerebral blood flow response to hypoxia. Regional distribution of cerebral blood flow during hypoxia in animals with intact chemoreceptors may be different in different species depending on the degree of peripheral sympathetic and parasympathetic innervation of the blood vessels in various regions of the brain.

The effect of chemoreceptors on the microvascular perfusion of the brain has not been reported previously. We have previously shown that approximately 50% of the capillaries and arterioles are perfused in both awake and anesthetized rats under resting condition during normoxia. This microvascular reserve can be mobilized during hypoxia. Chemodenervation had little effect on the utilization of microvascular reserve during hypoxia. However, as noted previously for cerebral blood flow, the regional differences in the utilization of arteriolar reserve during hypoxia was abolished after chemodenervation, whereas the utilization of the capillary reserve did not differ from the control hypoxic group. This finding suggests that the effect of chemodenervation during hypoxia is at the arteriolar level and contributes to the reduction of the rostral-to-caudal gradient in the hypoxic cerebral blood flow increase.

We conclude that peripheral chemoreceptors do not appear to play an important role in the regulation of the cerebral circulation during normoxia. During hypoxia the chemoreceptors appear to contribute to forebrain blood flow restriction through their effect on the utilization of the arteriolar reserve and have little effect on capillaries. Chemodenervation had little effect on either the resting blood flow or the perfused microvasculature during normoxia. Similarly, increases in average blood flow or the utilization of microvascular reserve during hypoxia were not affected by chemodenervation. However, the regional differences in cerebral blood flow and the number of perfused arterioles during hypoxia were lost after chemodenervation.

References

Key words: peripheral chemoreceptors • cerebral blood flow • cerebral capillaries • carotid body • cerebral arterioles • hypoxia • rats
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