
Cerebral Circulatory Responses to Arterial Hypoxia in Normal and Chemodenervated Dogs

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SUMMARY Cerebral hemodynamic responses to arterial hypoxia were studied in 13 normal and 9 chemodenervated anesthetized, paralyzed dogs. Arterial O2 content was lowered from control (18.0 vol%) to 14.0, 8.0, and 4.0 vol%, respectively, by either decreasing arterial PO2 (hypoxic hypoxia) or increasing carboxyhemoglobin saturation (CO hypoxia) at normal PO2. Both hypoxic hypoxia and CO hypoxia at each value of the lowered arterial O2 content resulted in progressive significant increases in cerebral blood flow (134, 169, 276, and 146, 206, 244% of control, respectively). Before chemoreceptor denervation, arterial blood pressure increased with hypoxic hypoxia but decreased with CO hypoxia. After chemodenervation, hypoxic hypoxia and CO hypoxia at each value of lowered arterial O2 content resulted in similar significant increases in cerebral blood flow. These increases were not significantly different from those observed prior to chemodenervation. After chemodenervation, hypoxic hypoxia and CO hypoxia both resulted in similar decreases in arterial blood pressure and cerebral vascular resistance, whereas, before chemodenervation, cerebral vascular resistance decreased more with CO hypoxia than with hypoxic hypoxia. These data show that cerebral vasodilation induced by both forms of hypoxia in chemodenervated dogs resembles that in animals with CO hypoxia and intact chemoreceptors in which PO2 is high and the carotid chemoreceptors may not be activated. We also have shown that the transient responses to both types of hypoxia are not altered by carotid chemodenervation, and conclude that the carotid chemoreceptors do not play a role in the mechanism by which cerebral blood flow increases during decreased blood O2 content.

IN THE PAST several years, there has been renewed interest in the possible role of the autonomic nervous system in the regulation of cerebral blood flow. Despite much evidence supporting an autonomic nerve supply to parts of the cerebral vasculature, the functional significance of these nerves remains uncertain. Neural regulatory mechanisms for the cerebral circulation have been assumed to be negligible or nonexistent because of the failure of autonomic stimulation and denervation to affect cerebral blood flow significantly. The effect of the sympathetic nervous system on cerebral blood flow remains especially controversial. Many investigators have failed to show any significant effects of sympathetic stimulation on cerebral blood flow, whereas others using similar techniques have demonstrated pronounced cerebral vasodilation.6-9

The effect of arterial hypoxia on cerebral blood vessels and flow has been studied for many years, and there can be no doubt that hypoxia produces cerebral vasodilation and an increase in cerebral blood flow. However, the precise mechanism by which hypoxia produces this vasodilation is unclear.7 There is evidence that oxygen can act directly on the smooth muscle of the cerebral vessels, with a low PO2 resulting in vasodilation.8, 9 It also has been suggested that local factors, such as cerebral parenchymal acidosis secondary to anaerobic metabolism caused by hypoxia, may be responsible for cerebral vasodilation.10, 11

Finally, Sokoloff12 pointed out that neurogenic mechanisms may be involved in the cerebral vasodilator response to hypoxia, and most recently Ponte and Purves13 have suggested that the carotid chemoreceptors acting through neurogenic mechanisms are responsible for virtually all of the cerebral vasodilation in response to hypoxia. Their conclusion indicates that the neurogenic (chemoreflex) control of cerebral blood flow in response to hypoxia is the most important mechanism responsible for cerebral vasodilation.

In this investigation, we studied cerebral hemodynamic
responses (steady state and transient) to two different types of arterial hypoxia in normal and carotid chemodener- nated dogs. The purpose of these studies was to test the hypothesis that carotid chemoreceptor stimulation produces the cerebral vasodilation which occurs with hypoxia. The types of hypoxia studied were: (1) hypoxic hypoxia (decreased partial pressure and content) which stimulates the carotid chemoreceptor, and (2) carbon monoxide hypoxia (normal partial pressure but decreased content) which would not be expected to stimulate the carotid chemoreceptor.

Methods

General Procedures

Experiments were done on 13 normal and 9 chemode- nerved adult, mongrel dogs of either sex (16-23 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv). Heparin (500 units/kg, iv) was used as the anticoagulant, with additional doses given every 90 minutes. Dogs were paralyzed with succinylcholine (Sucostrin) (40 mg) and ventilated with a positive pressure respirator (Harvard respiration pump 607) connected to a tracheal cannula. Tidal volume and respiratory rate were adjusted to give an alveolar (end-expiratory) carbon dioxide of 4.0%, as monitored by a CO₂ gas analyzer (Godart Capnograph). The CO₂ analyzer was calibrated regularly with mixtures of CO₂ in air analyzed to a precision of 0.01%. Dissection to expose the femoral artery and vein, carotid arteries, and cranium was done with an electric cautery. Arterial blood pressure (iliac arterial pressure) was measured via a cannula advanced from the femoral artery. To prevent cooling, the dogs were covered with a plastic sheet and all surgical areas, where possible, were sutured with skin clips. Rectal temperature was maintained around 38°C throughout the experiment by a heat lamp. All pressures were measured with Statham P-23 transducers, and all data were recorded on an Electronics for Medicine recorder.

Measurement of Blood Flows

The technique used to measure cerebral venous blood outflow has been described by Rapela and Green. The confluence of the cerebral sinuses was cannulated, and the lateral sinuses and occipital emissary veins were occluded with bone wax to prevent communication between the intracranial and extracranial venous circulations. From the confluence of the sinuses the blood then passed through a previously calibrated electromagnetic flow probe, before returning to the dog via the femoral vein (Fig. 1). With this technique, approximately 50-70% of the mass of the brain is drained at the confluence of the sagittal and straight sinuses. Blood flow measured at the confluence of the sinuses before and after occlusion of the lateral sinuses will be designated, respectively, as "venous outflow" and "cerebral venous outflow." Blood outflow from the confluence of the sinuses, regardless of the condition of the lateral sinuses, will be referred to as "cerebral venous outflow." Venous and cerebral venous outflow pressures were measured upstream from the flowmeter. This pressure merely measures the resistance to the flow of blood induced by the flow transducer, since the outflow cannula was set at the level of the right atrium and all pressures were referred to this common zero reference plane for all pressure measurements which was at the level of the right atrium.

Figure 1  Preparation used to measure cerebral venous outflow, common carotid blood flow, and blood pressures. Right: Blood from the confluence of the sinuses was diverted through an electromagnetic flowmeter into a 20-ml reservoir open to atmospheric pressure and returned to the dog through a femoral vein by a system composed of an electronic level detector, switch, and a pump which maintained constant the level of blood in the reservoir. Cerebral venous outflow pressure was measured upstream to the flowmeter; the tip of the outflow tubing was maintained at a height corresponding to the common zero reference plane for all pressure measurements which was at the level of the right atrium. Collateral communications between intracranial and extracranial venous circulations were effectively occluded by injecting bone wax into both lateral sinuses (marked with ×). Left: Common carotid blood flow (left, right, or both) was measured with an electromagnetic flow probe placed around the artery.
reference plane. Brain perfusion pressure was estimated as systemic arterial pressure minus cerebral venous outflow pressure. Intracranial vascular resistance was calculated by dividing brain perfusion pressure by cerebral venous outflow.

Extracranial blood flow was estimated by means of a noncannulating electromagnetic flow probe placed around the left, right, or both common carotid arteries (Fig. 1). Systemic arterial pressure was taken as extracranial perfusion pressure and used to calculate extracranial vascular resistance.

**Denervation of Carotid Bifurcations**

Chemodenervation was accomplished by cutting the carotid sinus nerves bilaterally. Both carotid bifurcations were exposed before the surgical approach to the confluence of the cerebral venous sinuses. After identification of the external carotid artery, the internal carotid artery, carotid sinus, and the occipital artery, the mass of nerves and fascia, including the carotid sinus nerve, was exposed in each side for subsequent ligation and section. This procedure denervated not only the carotid bodies but also the carotid sinuses. The integrity of the receptors of the carotid body and carotid sinus was tested in each dog by observing respiratory changes which occurred when 5 µg of NaCN were injected into the common carotid arteries. This test was performed before the dogs were paralyzed with succinylcholine.

**Administration of Hypoxia and Blood Gas Analysis**

Arterial O2 content was lowered by one of two methods: (1) by inhalation of various O2 mixtures in nitrogen at constant ventilation (hypoxic hypoxia), or (2) by inhalation of various levels of carbon monoxide, also at constant ventilation, to produce equivalent reductions in arterial O2 content (CO hypoxia). Oxygen content for both types of hypoxia was reduced from 18.0 vol% (control) to approximately 14.0, 8.0, and 4.0 vol% (random order). An important point to emphasize is that, although arterial O2 content is reduced with both types of hypoxia, with CO hypoxia there is no reduction in the arterial O2 tension. For steady state measurements, dogs were maintained at a given level of hypoxic hypoxia for 15-20 minutes and CO hypoxia for 35-40 minutes to allow equilibration of ventilatory and blood gases before final gas samples were taken, and to allow time for hemodynamic responses to occur and to be maintained. Arterial and cerebral venous blood samples were taken from the femoral artery and cerebral venous outflow cannulas, respectively. The experimental protocol was such that each dog acted as its own control for different levels of both hypoxic hypoxia and CO hypoxia. Oxygen tension (PO2), carbon dioxide tension (PCO2), and pH were measured at 37°C immediately after the samples were obtained, using Instrumentation Laboratories electrodes and analyzer (IL-113). The electrodes were calibrated with air (20.98 O2), and mixtures of oxygen in nitrogen (around 8%) and carbon dioxide in air (around 5 and 10% CO2) were analyzed to a precision of 0.01%. The pH electrode was calibrated with standard phosphate buffers (6.840; 7.381). Oxygen and carboxyhemoglobin saturation and hemoglobin also were measured immediately after samples were taken with an IL CO-oximeter (model 182). Electrodes were calibrated before and after each set of samples was taken. End-expiratory CO2 was maintained constant throughout the experiment.

**Verification of the Measurement of Cerebral Blood Flow**

The verification procedure for this technique has been described in detail elsewhere. However, because of its importance, it will be briefly described here. In each dog, a hemodynamic test was carried out to verify that intracranial venous outflow was not contaminated with venous blood from extracranial sources. This test consists of occluding the venous outflow tube and observing the response of venous outflow pressure before and after occlusion of the lateral sinuses. Two factors should be considered in the interpretation of this test: one involves the rate of rise of venous outflow pressure and the other the highest level that venous outflow pressure reaches following occlusion of the venous outflow tubing. Prior to occlusion of the lateral sinuses, occlusion of the venous outflow tube results in only a small rise in venous outflow pressure (10 mm Hg). When the venous outflow tube was occluded following the occlusion of the lateral sinuses, venous outflow pressure increases immediately to very high levels. In every case, venous outflow pressure rises to values above 50 mm Hg. This hemodynamic test supports the concept that, prior to occlusion of the lateral sinuses, numerous anastomotic channels exist between the intracranial and extracranial venous circulations. These extracranial drainage routes, open to the venous outflow when the outflow tube is occluded prior to occlusion of the lateral sinuses, account for the slight increase in venous outflow pressure observed under this condition. After occlusion of the lateral sinuses, the major proportion of these anastomotic channels is eliminated, and thus, on outflow tube occlusion, there is a considerable rise in cerebral venous outflow pressure.

A second verification procedure involves the effect of occlusion of both jugular veins (increased extracranial venous pressure) on venous outflow from the confluence of the sinuses before and after occlusion of the lateral sinuses. Occlusion of both jugular veins results in a significant increase in venous outflow only when performed prior to occlusion of the lateral sinuses. Under these conditions, jugular vein occlusion increases resistance to blood flow via normal channels draining extracranial venous blood which in part is diverted via patent cranial drainage routes, open to the venous outflow when the outflow tube is occluded following the occlusion of the lateral sinuses, account for the slight increase in venous outflow pressure observed under this condition. After occlusion of the lateral sinuses, the major proportion of these anastomotic channels is eliminated, and thus, on outflow tube occlusion, there is a considerable rise in cerebral venous outflow pressure.
Results

The effects of hypoxic hypoxia and CO hypoxia on cerebral blood flow (intracranial) in control and carotid chemodenervated conditions is shown in Figure 2. In all cases, cerebral blood flow was significantly increased above control as arterial O₂ content was reduced from control (18.0 vol%) to 14.0, 8.0, and 4.0 vol%. Hypoxic hypoxia and CO hypoxia in control dogs increased cerebral blood flow to 134, 169, 276 and 146, 206, 244% of control, respectively, with each lowering of arterial O₂ content. After chemodenervation, with each successive reduction of arterial O₂ content, hypoxic hypoxia and CO hypoxia increased cerebral blood flow to 136, 148, 222 and 141, 205, 220% of control, respectively. These increases in flow were not significantly different from those observed in dogs with intact chemoreceptors.

Figure 3 shows the effects of both types of hypoxia on arterial blood pressure in control and chemodenervated conditions. Blood pressure with hypoxic hypoxia in control dogs progressively increased to a maximum of 116% of control as arterial O₂ content was lowered to 4 vol%. CO hypoxia in either control or chemodenervated dogs decreased systemic blood pressure to a maximum of about 70% of control at 4 vol%. In chemodenervated dogs given hypoxic hypoxia, blood pressure decreased to 76% of control at 4 vol%. Thus, blood pressures after carotid chemodenervation with hypoxic hypoxia or CO hypoxia or with CO hypoxia in control dogs all resemble one another and are not significantly different. Only in the case of hypoxic hypoxia in control dogs does blood pressure increase, making it significantly different from the other three conditions. Since cerebral blood flow increased approximately equally with all conditions (Fig. 2), and considering the changes in systemic arterial blood pressure (Fig. 3), it is inescapable that cerebral vascular resistance decreases to a lesser extent with hypoxic hypoxia in control dogs than in all other conditions. Cerebral vascular resistance decreased equally with hypoxic hypoxia or CO hypoxia after chemodenervation, or with carbon monoxide in control dogs. This is seen in Figure 4.

The effects of both types of hypoxia, in control and chemodenervated conditions on cerebral O₂ consumption, are shown in Figure 5. These curves are not significantly different from one another, and it can be seen that O₂ consumption actually is fairly well maintained even at the very low levels of O₂ content. Hypoxic hypoxia in control or chemodenervated dogs initially increases O₂ consumption by 30-40% at the milder hypoxia range (14 vol%). This increase possibly may be attributed to the release of 

![Figure 2](image_url)

**Figure 2** Effect of hypoxic hypoxia and CO hypoxia on cerebral blood flow in 13 control and 9 chemodenervated dogs. Each point represents the mean ± se. Analysis of variance showed that the four slopes were not significantly different from each other. Point-by-point analysis using Student's t-test showed that the minimum difference that was significant was between chemodenervated hypoxic hypoxia and chemodenervated CO hypoxia at 8 vol% (P < 0.05).

![Figure 3](image_url)

**Figure 3** Effect of hypoxic hypoxia and CO hypoxia on arterial blood pressure in control and chemodenervated dogs. Analysis of variance showed that control hypoxic hypoxia was significantly different from the other three slopes. Point-by-point analysis, using Student's t-test, showed that the minimum difference that was significant was between control hypoxic hypoxia and chemodenervated hypoxic hypoxia at 4 vol% (P = 0.001).

![Figure 4](image_url)

**Figure 4** Effect of hypoxic hypoxia and CO hypoxia on cerebral vascular resistance in control and chemodenervated dogs. Analysis of variance showed that control hypoxic hypoxia was significantly different from the other three slopes. Point-by-point analysis showed that the minimum difference that was significant was between control hypoxic hypoxia and chemodenervated CO hypoxia at 14 vol% (P = 0.01).
catecholamines with hypoxia which has been shown by other investigators to increase cerebral O$_2$ consumption.$^{12-15}$

Figure 6 shows the effects of both types of hypoxia in control and chemodenervated conditions on common carotid blood flow. Common carotid blood flow with hypoxic hypoxia in control dogs progressively increased to a maximum of 120% of control as arterial O$_2$ content was lowered to 4 vol%. CO hypoxia in either control or chemodenervated dogs reduced common carotid blood flow to 77% and 66% of control, respectively, at 4 vol%. In chemodenervated dogs subjected to hypoxic hypoxia, carotid blood flow decreased to 74% of control at 4 vol%. Common carotid resistance showed no change in any of the four conditions of decreased oxygen content. The same point is to be made here about carotid blood flow that was made in Figure 3 about arterial blood pressure, which is that carotid blood flow measurements after chemodenervation, with hypoxic hypoxia or CO hypoxia, or with CO hypoxia in control dogs, all resemble one another and are not significantly different. Only in the case of hypoxic hypoxia in control dogs does carotid blood flow increase, and it is only this condition which is significantly different from the other three conditions.

The effects of hypoxic hypoxia and CO hypoxia on blood gases and pH in control and chemodenervated dogs are shown in Table 1.

Figure 7 shows the transient responses of cerebral blood flow (% of control) in 12 control and carotid chemodenervated dogs exposed to two different levels of hypoxic hypoxia (6 dogs at each hypoxic level). The left side of the figure shows the transient cerebral blood flow responses when arterial O$_2$ content was reduced from control (around 18.0 vol%) to about 8.8 vol%, and the right side shows these responses when arterial O$_2$ content was reduced from control (around 18.5 vol%) to around 13.7 vol%. Cerebral blood flow increased progressively and equally with time, as arterial O$_2$ content decreased with hypoxic hypoxia, in control and chemodenervated dogs. It should be noted that the percentage increases in cerebral blood flow observed during the transient responses to hypoxic hypoxia at given arterial O$_2$ content were not significantly different from those cerebral blood flow increases observed under steady state conditions at equivalent levels of arterial O$_2$ content (Fig. 2) for either control or chemodenervated dogs. Figure 8 shows the transient responses of cerebral vascular resistance in control and chemodenervated dogs exposed to two levels of hypoxic hypoxia. At both content levels, the control graph differs significantly from the chemodenervated graph.

Figure 9 shows the transient responses of cerebral blood flow in 12 control and carotid chemodenervated dogs exposed to two different levels of CO hypoxia (6 dogs at each level). The left side of the figure shows the transient cerebral blood flow responses when arterial O$_2$ content was reduced from control (around 18.0 vol%) to about 8.0 vol%, while the right side shows these responses when arterial O$_2$ content was reduced from control (19.6 vol%) to around 14.3 vol%. Arterial O$_2$ contents are shown as the numbers in parentheses and are not statistically different at any given time. Cerebral blood flow increased progressively and equally with time, as arterial O$_2$ content decreased with CO hypoxia in control and chemodenervated dogs. As before, the percentage increases in cerebral blood flow observed during the transient responses to CO hypoxia at given arterial O$_2$ content (Fig. 9) were not significantly different from those cerebral blood flow increases observed under steady state conditions at equivalent levels of arterial O$_2$ content (Fig. 2) for either control or chemodenervated dogs. Figure 10 shows the transient responses of cerebral vascular resistance in control and chemodenervated dogs exposed to two levels of CO hypoxia. The two curves for both medium and high O$_2$ content are not significantly different, nor do these two
Discussion

Our data support the hypothesis that the brain increases its blood flow in response to its O₂ needs with both hypoxic hypoxia and CO hypoxia in either control or carotid chemodenervated dogs in order to maintain O₂ consumption constant. Our data also show that before carotid sinus nerve section the decrease in cerebral vascular resistance, perhaps via the nervous system. However, this influence is in the reverse direction of what earlier investigators have suggested. In fact, stimulation of the carotid and aortic chemoreceptors produces increased sympathetic tone in most vascular beds. There has been no evidence for activation of vasodilator nerves or for inhibition of vasoconstrictor nerves in any other vascular bed. On the other hand, the smaller decrease in cerebral vascular resistance during hypoxic hypoxia might be due to the increase in arterial blood pressure sensed by the carotid sinus. An increase in cerebral vascular resistance with an increase in systemic blood pressure (cerebral autoregulation) has been reported previously. However, the increase in cerebral blood flow during all four challenges is virtually identical, even though carotid blood flow decreases in three of the four challenges, as does systemic blood pressure. This does not occur in the control dog during hypoxic hypoxia. Carotid body stimulation appears to influence carotid blood flow indirectly through changes in systemic blood pressure. Since carotid vascular resistance remains unchanged in all conditions while ca-

### TABLE 1 Effect of Hypoxic Hypoxia (HH) and CO Hypoxia (COH) on Blood Gases and pH in Control and Chemodenervated Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control (18 vol%)</th>
<th>Chemodenervated</th>
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<tbody>
<tr>
<td><strong>pH</strong></td>
<td>7.419 ± 0.010</td>
<td>7.421 ± 0.014</td>
</tr>
<tr>
<td><strong>Paco₂</strong></td>
<td>31.31 ± 1.25</td>
<td>31.61 ± 1.75</td>
</tr>
<tr>
<td><strong>Pao₂</strong></td>
<td>108.42 ± 1.10</td>
<td>104.50 ± 1.71</td>
</tr>
<tr>
<td><strong>Pvo₂</strong></td>
<td>30.77 ± 1.66</td>
<td>31.00 ± 1.87</td>
</tr>
<tr>
<td><strong>COH (4 vol%)</strong></td>
<td>7.440 ± 0.020</td>
<td>7.449 ± 0.022</td>
</tr>
<tr>
<td><strong>HH (8 vol%)</strong></td>
<td>29.21 ± 0.21</td>
<td>29.71 ± 0.22</td>
</tr>
<tr>
<td><strong>HH (14 vol%)</strong></td>
<td>36.41 ± 0.36</td>
<td>40.50 ± 0.36</td>
</tr>
<tr>
<td><strong>HH (4 vol%)</strong></td>
<td>18.93 ± 0.89</td>
<td>21.17 ± 0.89</td>
</tr>
<tr>
<td><strong>Control (18 vol%)</strong></td>
<td>7.438 ± 0.024</td>
<td>7.440 ± 0.014</td>
</tr>
<tr>
<td><strong>COH (14 vol%)</strong></td>
<td>28.72 ± 0.25</td>
<td>29.33 ± 0.27</td>
</tr>
<tr>
<td><strong>COH (8 vol%)</strong></td>
<td>7.46 ± 0.53</td>
<td>15.83 ± 1.54</td>
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<tr>
<td><strong>COH (4 vol%)</strong></td>
<td>7.48 ± 0.36</td>
<td>7.50 ± 1.15</td>
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</table>

Each value represents the mean ± SE of 13 control and 9 chemodenervated dogs. pH, Paco₂, Pao₂ = arterial blood gases and pH. Pvo₂ = cerebral venous oxygen tension.

Curves differ significantly from the curves for the chemodenervated dogs during hypoxic hypoxia.

### Figure 7

**Transient responses of cerebral blood flow in control and chemodenervated dogs exposed to hypoxic hypoxia.** Each point represents the mean ± SE of six dogs. The values in parentheses are the arterial O₂ content measured at that time period.

### Figure 8

**Transient responses of cerebral vascular resistance in control and chemodenervated dogs exposed to hypoxic hypoxia.** Analysis of variance showed a significant difference between control and chemodenervated hypoxic hypoxia for both medium and high O₂ content.
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those of Ponte and Purves\textsuperscript{13} who reported that arterial
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endothelium. While the precise mechanism by which either form of
hypoxia produces a decrease in cerebral vascular resistance
is still questionable, our data clearly show that the
carotid arterial chemoreceptors are not necessary for this
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drawn concerning the regulation of cerebral blood flow when the responses of two distinctly different vascular systems, intracranial and extracranial, are not carefully
and completely separated have been discussed previously.\textsuperscript{2}
Specific reference was made in that report to experimental
FIGURE 9  Transient responses of cerebral blood flow in control
and chemodenervated dogs exposed to CO hypoxia. Each point
represents the mean \( \pm \) SE of six dogs. The values in parentheses
represent the arterial \( O_2 \) content measured at that time period.

rotid blood flow changes in accordance with arterial blood
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procedures in which extracranial contamination may have
been facilitated by surgical techniques which include liga-
tion, or blockage of numerous vessels either on the arterial
or venous side of the circulation. In the study of Ponte and
Purves,\textsuperscript{13} in addition to the complex and extensive
surgery to the head and neck region, numerous arterial
vessels to the head were ligated, thus leading to consid-
orable extracranial contamination of their measured blood
flow. They report that hypoxia produced an increase in
the measured blood flow in their animals. However, the
increase in blood flow could just as well have been produced
by an increase in extracranial blood flow. We
show that hypoxic hypoxia increases both cerebral (Fig. 2)
and extracranial (Fig. 6) blood flow, while arterial blood
pressure increased (Fig. 3). After carotid chemodenerva-
tion, hypoxic hypoxia resulted in the same increase in
cerebral blood flow, but decreased both extracranial blood
flow and arterial blood pressure. Depending upon the
extent of extracranial contamination, Ponte and Purves
easily could have observed no change in blood flow, the
observation they actually reported, or even a decrease in the
measured blood flow.

Two other differences between our work and Ponte and
Purves' study\textsuperscript{13} also could account for the opposite find-
ings: (1) we used a venous outflow technique to measure
cerebral blood flow and they used a \( ^{133} \text{Xe} \) clearance technique; and (2) our studies were done with dogs;
theirs, with baboons. We used a venous outflow technique
which averages the blood flow from several regions of the
brain. It is possible that chemoreceptor denervation may
eliminate the cerebral vasodilator response to hypoxia in
specific regional areas of the brain which are not reflected
in the mean outflow measurement. Thus, the vasodilator
response to hypoxia and the subsequent elimination of
that response in certain regions by chemodenervation may
be overlooked with the technique we used. In addition,
this technique has been estimated to measure approxi-
mately 50-70\% of total brain venous outflow. Therefore,
if the response to hypoxia and chemodenervation occurred
primarily in areas that are not represented using this
technique, then we could not observe any change in total
venous outflow. This, however, does not appear to be a
plausible explanation for the difference between our work
and that of Ponte and Purves,\textsuperscript{13} Heistad et al.\textsuperscript{17} used
radioactive-labeled microspheres to measure total and
regional cerebral blood flow and demonstrated that the
cerebral vasodilator response to hypoxia was unaffected
by carotid chemodenervation. In these experiments, both
dogs and baboons were used and the results for each were
the same; i.e., chemodenervation did not alter the cere-
bral vasodilator response to hypoxia. This would also
appear to rule out the second possible difference, the
species difference, as a reason for the two opposing
viewpoints.

In trying to account for our results, we have proposed that
the cerebral hemodynamic response during hypoxia is
due to the local effects of hypoxia on cerebral tissue, and
that the carotid chemoreceptor-initiated reflexes were
unnecessary for this response. This proposition is based
on two assumptions: (1) that carbon monoxide does not

Figure 9  Transient responses of cerebral blood flow in control
and chemodenervated dogs exposed to CO hypoxia. Each point
represents the mean \( \pm \) SE of six dogs. The values in parentheses
represent the arterial \( O_2 \) content measured at that time period.

FIGURE 10  Transient responses of cerebral vascular resistance in
control and chemodenervated dogs exposed to CO hypoxia.
stimulate carotid arterial chemoreceptors to any appreciable degree if $P_{\text{ao}_2}$ is maintained at a normal level, and (2) that the aortic chemoreceptors do not play a major role in the cerebrovascular response to both types of hypoxia.

The fact that the cerebrovascular responses to carbon monoxide in the control dogs resembled the responses to both types of hypoxia in the carotid body denervated dogs suggested the possibility that carbon monoxide does not stimulate the carotid body. The question of whether carbon monoxide stimulates the carotid chemoreceptors has been debated for close to 40 years. This field of investigation is still controversial. Some investigators have suggested that carbon monoxide does not stimulate the carotid bodies, although Paintal has demonstrated that the aortic bodies are responsive to carbon monoxide. The study of Mills and Edwards gives quantitative results from only one carotid fiber and four aortic fibers responding to carbon monoxide in cats. They concluded that both chemoreceptors are sensitive to carbon monoxide. However, Meyer et al. showed that the neural output from carotid chemoreceptors did not respond even when carboxyhemoglobin levels reached 50% as long as $P_{\text{ao}_2}$ remained normal. Lahiri and Delaney showed essentially the same result. Recently, Dehghani and Fitzgerald reported for cats that the aortic bodies respond as much to carbon monoxide hypoxia as to hypoxic hypoxia, whereas the carotid body shows no response to carbon monoxide hypoxia but does respond to hypoxic hypoxia. The observations of Mitchell on the effect of anemia on chemoreceptor output support the possibility that the aortic body is sensitive to decreases in oxygen content, whereas the carotid body does not respond to a decrease in content if the partial pressure of oxygen is normal.

In the present study during all four hypoxic challenges, the aortic bodies were intact. Inasmuch as leaving the carotid chemoreceptor area intact or removing it had no effect on the responses of the cerebral circulation to hypoxia or CO, we conclude that if peripheral arterial chemoreceptors were responsible for these responses, then the whole effect must be due to the aortic chemoreceptors. In view of the fact that in these studies and elsewhere we have observed significant differences in other cardiovascular variables before and after carotid body denervation, it seemed unlikely to us that the aortic bodies, unable to influence these changes, would be totally responsible for an identical increase in cerebral blood flow in the four experimental conditions.

Data supporting our suspicions are provided by the recent study of Bates and Sundt in cats. Their study shows that the increase in cerebral blood flow in response to decreases in $P_{\text{ao}_2}$ is the same before and after sectioning of the IX and X cranial nerves. Therefore, since hypoxic hypoxia certainly stimulates both carotid and aortic chemoreceptors and since the presence or absence of these receptors made no difference to cerebral blood flow in the cat during hypoxic hypoxia, we do not think the aortic bodies play a significant role in controlling cerebral blood flow in response to either hypoxic hypoxia or CO hypoxia in anesthetized, paralyzed, ventilated dogs. We feel further that the analysis of the time course of the cerebral blood flow and resistance responses to hypoxic hypoxia and CO hypoxia confirms the lack of any significant but transitory influence of the peripheral arterial chemoreceptors on cerebral circulation.

Our data support the concept that cerebral vessels are relatively unresponsive to reflex stimuli and that the physiological role of the cerebral innervation is of only minor significance. Although the precise mechanism of action of hypoxic hypoxia and CO hypoxia to increase cerebral blood flow and to maintain oxygen consumption relatively constant has not been established in this paper, it is clear that information traveling via the carotid sinus nerve is not involved in the mechanism.

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References


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Regional Choline Acetyltransferase Activity in the Guinea Pig Heart

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SUMMARY Choline acetyltransferase is the enzyme that catalyzes the biosynthesis of acetylcholine, the neurotransmitter of the pre- and postganglionic parasympathetic system. To assess the extent of parasympathetic innervation, enzyme activity was measured in specialized and contractile regions throughout the guinea pig heart. Enzyme activity in the right atrial appendage was 137 nmol g⁻¹ hr⁻¹. Activity was greatest in the region of the sinoatrial node (187 nmol g⁻¹ hr⁻¹). Also, enzyme activity was high in the regions of the atrioventricular node (153 nmol g⁻¹ hr⁻¹), the proximal conduction bundles (133 nmol g⁻¹ hr⁻¹), and the base of the anterior papillary muscle of the right ventricle (179 nmol g⁻¹ hr⁻¹), which contains the moderator band and Purkinje fibers. In contrast, the enzyme activity in the inferior interventricular septum and the free walls of the right and left ventricles, which are more predominantly contractile tissue, was 67 ± 6, 108 ± 14, and 56 ± 11 nmol g⁻¹ hr⁻¹, respectively. This activity is significantly lower than in the right atrial appendage. These results suggest that the density of parasympathetic innervation is similar in all the components of the conduction system, from the sinoatrial node to Purkinje tissues. Furthermore, the parasympathetic innervation of regions specialized for conduction is up to four times more dense than that of contractile regions.

ACETYLCHOLINESTERASE activity and the effects of efferent vagal nerve stimulation vary considerably throughout the heart. Therefore, it is inferred that the parasympathetic innervation of the heart is non-uniform. However, these variations are difficult to quantitate. For example, acetylcholinesterase activity is identified histochemically and variations cannot be readily quantitated; furthermore, acetylcholinesterase activity may be non-specific, since it is found in red blood cells and other non-neuronal tissues. In a similar context, the magnitude of chronotropic, dromotropic, and inotropic effects of efferent vagal nerve stimulation may not be representative solely of the density of parasympathetic innervation.

These responses also could be affected by regional variability in cholinergic receptors and sympathetic neural influences. In view of these considerations, it seemed appropriate to examine another index of the parasympathetic innervation that might be more specific and also quantifiable.

Accordingly, in guinea pig heart, we have investigated the activity of choline acetyltransferase, the enzyme that catalyzes the biosynthesis of acetylcholine in neural tissue. The in vitro determination of choline acetyltransferase activity minimizes the possible influence of modulating factors and uses optimal concentrations of substrates. Thus, the enzyme activity quantitatively represents the parasympathetic innervation in discrete specimens of the heart. In previous studies of the guinea pig heart, we determined the choline acetyltransferase activity in contractile tissues, the atria, and ventricles. The present study was designed to assess choline acetyltransferase activity in specialized regions containing pacemaking and conducting tissues and to compare the activity in specialized and contractile regions.
Cerebral circulatory responses to arterial hypoxia in normal and chemodenervated dogs.
R J Traystman, R S Fitzgerald and S C Loscutoff

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