The Effect of Sympathetic Denervation on Cerebral CO₂ Sensitivity

BY H. L. STONE, PH.D.,* M. E. RAICHLE, M.D.,† AND M. HERNANDEZ, PH.D.‡

Abstract: The responsiveness of cerebral blood flow to changes in arterial carbon dioxide tension was determined in six monkeys following bilateral superior cervical ganglionection. Experiments were conducted 10 to 14 days following the removal of both ganglions using phencyclidine hydrochloride as the anesthetic agent. Following the initial acute experiments, the animals were placed in a sealed environmental chamber for five days at an inspired carbon dioxide level of 6%. The responsiveness to carbon dioxide was repeated following the chronic exposure to carbon dioxide. The animal was killed immediately and the brain removed. The major vessels of the circle of Willis were examined histochemically for the presence of sympathetic nerve fibers. The results of the study demonstrated that: (1) autoregulation was still present, (2) acute exposure to increased levels of carbon dioxide increased flow, and (3) some adaptation of flow occurred following a chronic exposure to 6% carbon dioxide. The carbon dioxide sensitivity of this group of animals was found to be 0.37 cm per second mm Hg as compared to a value of 0.94 cm per second mm Hg for normal animals. The difference in these two values was significant. It is concluded that the sympathetic nervous system is necessary for the normal responsiveness to changes in arterial carbon dioxide.

Additional Key Words
autonomic nervous system
superior cervical ganglion
chronic carbon dioxide
bicarbonate

The responsiveness of the cerebral vessels to changes in inspired carbon dioxide has been well documented. Reivich found a relatively linear relationship between the change in the arterial level of carbon dioxide and cerebral blood flow over a range of arterial carbon dioxide levels from about 30 to 90 mm Hg. The change in cerebral blood flow per change in arterial carbon dioxide in this range was found to be about one. In a previous work, we have demonstrated that the carbon dioxide responsiveness of the cerebral vascular bed in the monkey was nearly the same as shown by Reivich over this range of values, and further that upon chronic exposure to elevated carbon dioxide levels, an adaptation of the flow would occur. Cerebral flow decreased at all levels of inspired carbon dioxide tested following chronic exposure to an environment containing 6% carbon dioxide. The adaptation was found to be associated with normalization of the cerebrospinal fluid hydrogen ion concentration and an increase in bicarbonate levels.

The rich innervation of the cerebral vessels with adrenergic nerve fibers led us to question the role played by these nerves in the response of the cerebral vessels to carbon dioxide and adaptation to chronic carbon dioxide exposure. The results of this study indicate that the sympathetic nerves are necessary for the normal responsiveness of the cerebral vessels to carbon dioxide. Removal of the superior cervical ganglion in the monkey reduced the carbon dioxide sensitivity when compared to a group of normal animals. Exposure to a chronic carbon dioxide environment led to some adaptation of the carbon dioxide response but the sensitivity did not change a great deal.

Methods

The experiments were conducted on six adult monkeys (Macaca mulatta) weighing 4 to 6 kg. The animals were anesthetized with phencyclidine hydrochloride (0.5 mg per kilogram) and maintained on halothane and oxygen for sterile surgery. Incisions were made bilaterally from the angle of the mandible down the neck to approximately the midcervical region. The superior cervical ganglion was isolated and removed on each side. Careful inspection was made at this time to ensure that all of the ganglion was removed. The external carotid was dissected and ligated on
both sides of the neck. The left common carotid was dissected in the cervical region and a Doppler flow probe placed around it and anchored in place. At this juncture, a careful inspection was made of the artery between the flow probe implantation and the base of the cranium to ensure that all branches had been tied. The lead wires from the flow probe were passed ventrally under the skin and buried for future use.

After a two-week recovery period, the animals were used in the following experimental protocol. Anesthesia was accomplished with phencyclidine hydrochloride and the animal was placed in a supine position. An endotracheal tube was positioned. The wires from the Doppler flow probe were exposed and connected to appropriate electronics to record the velocity of blood flow in the carotid artery. The left femoral artery and vein were exposed and cannulated. The arterial catheter was attached to a Statham P23Db pressure transducer zeroed to the midchest level of the animal to measure arterial pressure. The endotracheal tube was connected to a Harvard respirator with the frequency and tidal volume adjusted for each animal. Arterial blood samples were taken before and after the animal was placed on the respirator to ensure that arterial blood gases were very near the spontaneous breathing values. Arterial blood gases were measured on an Instrument Laboratories Model 313 blood gas analyzer, and cerebrospinal fluid values were determined using a Radiometer gas analyzer.

Cerebral blood flow autoregulation was determined by changing mean arterial pressure. Pressure was lowered by the withdrawal of blood very rapidly from the femoral vein catheter and increased by the infusion of metaraminol. Arterial pressure and the Doppler flow signal were recorded on magnetic tape and an X-Y plotter as well as an oscillographic recorder. After obtaining an autoregulatory curve with the animal breathing room air, the respirator intake was successively connected to a cylinder containing 6%, 9% and 12% carbon dioxide in air. At each level another autoregulatory curve was obtained. The animal was respired on each mixture for ten minutes before an arterial blood sample was taken and the autoregulatory curve determined. Upon completion of the experiment, a 1-cc sample of cerebrospinal fluid was obtained from each animal and the wires from the flow probe were placed beneath the skin again.

The animals were placed immediately in a sealed environmental chamber for five days. The inspired carbon dioxide partial pressure was maintained at 42 mm Hg and the alveolar oxygen pressure was maintained at 100 mm Hg. At the end of the five-day period, the experimental protocol given earlier for the acute phase of the study was repeated. The animals were removed from the chamber breathing from a bottle containing 6% carbon dioxide and were maintained on this mixture when connected to the respirator. At the end of this portion of the experiment, a second cerebrospinal fluid sample was taken and the animal immediately killed. The brain was removed and the major vessels composing the circle of Willis were tied. The lead wires from the flow probe were passed ventrally under the skin and buried for future use.

The data analysis was accomplished from the magnetic tape record through the use of an analog computer. Statistical analysis was accomplished on the carbon dioxide sensitivity data using an unpaired t-test for small sample sizes.

Results
The carotid flow response to changes in mean arterial pressure and inspired carbon dioxide levels for a representative animal used in this study can be seen in figure 1. When the animal was respired on room air (RA), there was a small change in flow as mean arterial pressure was changing over a large range. As the inspired level of carbon dioxide was increased, the resting flow increased. This can be noticed by following the change in flow at a pressure of 100 or 120 mm Hg. At each successive level of inspired carbon dioxide, flow changed more with changes in arterial pressure. At 12% inspired carbon dioxide, flow was almost a linear function of arterial pressure.

The average response of all six animals used in the study to change in mean arterial pressure and inspired carbon dioxide can be seen in figure 2. The average response of the animals demonstrates the same as that shown for the individual animals. Constancy of flow was found to be almost lost at the highest level of inspired carbon dioxide (12%) in each experiment in what would be termed the physiological pressure range. At 100 mm Hg mean arterial pressure, the flow with the animal breathing room air averaged 25 ± 2 cm per second and while breathing 12% carbon dioxide averaged 38 ± 4 cm per second. More variability in the response was noted in the animals being respired on 12% carbon dioxide. The changes in arterial carbon dioxide partial pressure with the various gas mixtures can be found in table 1. The average arterial carbon dioxide tension was 41.2 ± 1.9 mm Hg breathing room air, which increased to

![CAROTID BLOOD FLOW VELOCITY PLOTTED AGAINST CHANGES IN MEAN ARTERIAL PRESSURE](image_url)
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Average carotid blood flow velocity plotted against mean arterial pressure obtained from six monkeys. The symbols at the right of the figure are the same as given in figure 1. The bars through the points represent one standard error of the mean.

69.6 ± 2.2 mm Hg while breathing 12% carbon dioxide.

Following the chronic exposure to 6% inspired carbon dioxide for five days, the above experiments were repeated. The average response of flow to change in mean arterial pressure can be seen in figure 3. Each animal in this group showed the same response as the average depicts. The main noticeable feature was the presence of a range of pressure over which flow remained fairly constant while breathing 12% carbon dioxide. Some adaptation of the flow response had occurred in this group of animals. The difference between the acute and chronic exposure to levels of carbon dioxide in this group of animals can be seen better in figure 4. With the chronic exposure to carbon dioxide, the 6% CO2 curve appeared as the room air curve before the chronic exposure. This same trend was true for the remaining levels of carbon dioxide studied. A comparison between the cerebrospinal fluid values for pH and bicarbonate before and after the chronic exposure can be found in table 1. The increase in bicarbonate from 22 mEq per liter to 32 mEq per liter was significant (P < 0.02). It should be noted that there was not any change in the cerebrospinal fluid pH value.

The histochemical examination of the major cerebral vessels revealed that in most of the animals very few sympathetic fibers could be found when compared to normal animals. It should be emphasized that in only two of the six animals used in this study was there a complete lack of any sympathetic fibers. In the remaining animals very little innervation by the sympathetic system could be found; when it was observed, it consisted of thin-appearing fibers running in the adventitia with no varicosities along the length of the fiber. We have termed this latter appearance as dener-

<table>
<thead>
<tr>
<th></th>
<th>Room air</th>
<th>6% CO2</th>
<th>9% CO2</th>
<th>12% CO2</th>
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<tr>
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<td>7.31 ± 0.01</td>
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<tr>
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<td>-</td>
<td>7.30 ± 0.01</td>
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<tr>
<td>CSF - HCO3⁻ (mEq/liter)</td>
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<tr>
<td>Acute</td>
<td>22.0 ± 1.0</td>
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<tr>
<td>Chronic</td>
<td>-</td>
<td>32.0 ± 1.0</td>
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Values are given with the standard error of the mean. The chronic values represent an exposure to 6% carbon dioxide for five days. The average cerebrospinal fluid (CSF) values are for pH and HCO3⁻ before and after the chronic exposure.
Average carotid blood flow velocity plotted against mean arterial pressure in all six monkeys. The heavy dark lines represent the average control data obtained in the animal as given in figure 2. The lower dark curve represents the room air (RAj control while the other heavy curves are the 6%, 9%, and 12% carbon dioxide exposures. The symbols at the right of the figure are the same as shown in figure 3.

Average carotid blood flow velocity plotted against mean arterial pressure in all six monkeys. The heavy dark lines represent the average control data obtained in the animal as given in figure 2. The lower dark curve represents the room air (RAj control while the other heavy curves are the 6%, 9%, and 12% carbon dioxide exposures. The symbols at the right of the figure are the same as shown in figure 3.

FIGURE 4

Average carotid blood flow velocity plotted against mean arterial pressure in all six monkeys. The heavy dark lines represent the average control data obtained in the animal as given in figure 2. The lower dark curve represents the room air (RAj control while the other heavy curves are the 6%, 9%, and 12% carbon dioxide exposures. The symbols at the right of the figure are the same as shown in figure 3.

The responsiveness of the cerebral vasculature to change in arterial carbon dioxide levels was determined by taking the flow at 100 mm Hg mean arterial pressure and plotting this versus the change in arterial carbon dioxide. The results of this can be observed in figure 5. The curve labeled control was obtained from a group of normal animals, while the denervated curve represents the results from the current group of experimental animals. It can be readily observed that cerebral blood flow increased less with an equivalent increase in arterial carbon dioxide in the denervated group as compared to the control group. The slope of these two curves can be termed the sensitivity of cerebral blood flow to carbon dioxide. The slope of the control curve was 0.94 cm per second mm Hg⁻¹ while that from the denervated group was 0.37 cm per second mm Hg⁻¹. The difference between the slope of these two curves was found to be significantly different (P < 0.05). The cerebral bed in the denervated group was less sensitive to arterial carbon dioxide levels than the cerebral bed of the control group.

DISCUSSION

The major question posed in this study was the role of the sympathetic nervous system in the cerebral vascular response to carbon dioxide. Referral to figure 5 clearly demonstrates that the carbon dioxide sensitivity is reduced in the absence of the sympathetic innervation. However, there was still a response to changes in carbon dioxide. How can this be reconciled with the other factors known to affect cerebral flow?

Prior to a discussion of the results of this study one must be sure that the technique used to measure cerebral flow is valid and measures very little extracranial flow, if any at all. In several animals, cerebral angiograms were performed to ascertain the route of any extracranial flow. It was found that the contrast material visualized the ophthalmic artery which comprised less than 10% of the estimated total flow. Acrylic casts have been made in several animals to anatomically determine the extent of any extracranial contamination. The casts demonstrated the same results as that found with the angiograms. We have diligently searched for extracranial flow beyond the site of our flow probe implantation and have not been able to find any except a small perforating branch from the internal carotid in some animals. Another validation for this inflow technique is the very close similarity with other data collected using a different technique.

Extracranial contamination would have made the difference extreme because the extracranial bed does not respond as does the intracranial bed to carbon dioxide. In other experiments in the monkey we compared the response of a carotid artery approach for measuring cerebral flow to an intracranial flow probe around the middle cerebral artery. In these animals, we could not find any difference in the direction of the response to several stimuli when a comparison was made between the two probes. For these reasons, the method used in the current study does measure primarily cerebral blood flow. Another potential problem area encompasses the
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tissue encapsulation of the flow probe in the animal. How would this affect the results? In many animals we have found that the tissue encapsulation process is just about complete in 10 to 14 days. This means that the fibrotic tissue reaction to the flow probe has reached a maximum level and will remain stable for the remainder of the experimental period. Any change in the cross-sectional area of the vessel will be reflected in a change in the measured velocity up until this time. We have not experienced any additional change in measured velocity or cross-sectional area as determined from angiograms after approximately ten days and usually not after seven days. Therefore, the change in the responsiveness of the cerebral vessels reflects a real change from the resting measurement at the time of the experiment.

The data obtained in this study clearly indicate that autoregulation of cerebral flow remains intact but that the responsiveness of the vessels to carbon dioxide is reduced. Others have demonstrated similar results concerning the intact nature of the autoregulatory response following denervation in the monkey. Stimulation of cervical sympathetic nerves has been demonstrated to produce a vasoconstriction in several experiments. In the latter series of experiments the sensitivity to changes in carbon dioxide following acute denervation was determined and found to increase at arterial carbon dioxide levels greater than 45 mm Hg, which is in contrast to the data presented in this paper. This difference may be explained by the lingering presence of norepinephrine in the vessel walls following the acute sympathectomy. Edvinsson and co-workers have shown that within the first six hours following sympathectomy, there was no measureable change in the cerebral blood volume of mice, implying that the quantity of norepinephrine present in the vessel walls was not changing greatly. A small increase in flow has been observed with acute sympathectomy, implying possible release of small amount of neurogenic tone. A reduction in the amount of norepinephrine at smooth muscle receptor terminals would imply that a greater dilation might occur when a stimulus was applied that would normally interfere with the action of norepinephrine. The difficulty with acute denervation was circumvented in the present study by using chronic denervation.

Ten days were used as the period between denervation and the initial experiment. The ability to show some adaptation to chronic carbon dioxide exposure might indicate a dual effect of carbon dioxide or hydrogenion. This could be directly on the vascular smooth muscle of the cerebral vessels.

To explain the results obtained in this study a scheme for the local control of cerebral blood flow is proposed as shown in figure 6. Assuming adequate mean arterial pressure, the flow (Q) to any area of the brain will be determined by the radius of the vessels in the vicinity. The vessel radius will be acted upon by endogenous change in norepinephrine level from sympathetic nerves and by an inherent ability to make small changes in radius in response to changes in mean arterial pressure. The local level of norepinephrine will depend on the degree of activation of the sympathetic nerves in the locality which in turn will be dependent on some area of the brain stem. The assumption is made that there is some tonic level of sympathetic activity to the cerebral vessels. Some evidence for this has been obtained in previous studies. With unilateral superior cervical sympathectomy, it was shown that the denervated cerebral vasculature received more flow than the opposite innervated vessels. A local factor that will modulate the radius of the vessel could be the extracellular fluid hydrogen ion concentration (BECF- H+) as demonstrated by Wahl and co-workers. As the hydrogen ion concentration increases, it blocks the release of norepinephrine (minus sign in fig. 6) and thus the radius would increase to some new level. If the hydrogen ion decreases in the extracellular fluid, then the radius would tend to decrease since more norepinephrine would be present (plus sign in fig. 6). Carbon dioxide as used in this study would act through this proposed mechanism by adding to the extracellular fluid hydrogen ion concentration. Brain metabolism also would change the extracellular fluid hydrogen ion concentration locally.

The carbon dioxide responsiveness would be dependent on the hydrogen ion concentration or something directly related to it such as the number of smooth muscle receptors for norepinephrine or the liberation of calcium inside the smooth muscle cell. This might explain a dual effect of carbon dioxide as seen in this study. It would appear that the dominant effect involves an interaction with the sympathetic nervous system with a secondary direct effect on the vessels themselves. As suggested by the work of Stromberg, the contribution of various segments of the cerebral vasculature may change as the
physiological condition changes. The role of the hypothalamic centers in this relationship cannot be separated since they are part of the system under investigation. Direct stimulation of areas of the hypothalamus has been shown to affect cerebral flow, but how this would affect carbon dioxide responsiveness directly is not known at the present time.

In summary, the sympathetic nervous system is directly involved in determining the cerebral vascular responsiveness to carbon dioxide. Any change in the system should affect this responsiveness as shown with cervical sympathectomy. Response to alteration in mean arterial pressure did not change. We could not determine if any change in resting flow had occurred in comparison with a group of control animals. Loss of carbon dioxide sensitivity would appear to be related to the loss of some brain center’s effect on sympathetic tone in the cerebral vessels or the quantity of norepinephrine present in the wall of the cerebral vessels.

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References

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