Responses of the Cerebral Circulation to Hypercapnia and Hypoxia after 7th Cranial Nerve Transection in Baboons

JULIAN T. HOFF, ERIC T. MACKENZIE, AND A. MURRAY HARPER

SUMMARY

It has been proposed that the responses of the cerebral circulation to hypoxia, hypercapnia and hypotension may be partially mediated by an autonomic reflex with receptors in the carotid body or sinus serving as sensors and the efferent limbs being the 7th cranial nerves. Transection of the 7th cranial nerve has been reported to impair the cerebral circulatory response to isolated chemoreceptor stimulation by hypoxia and hypercapnia. To test this hypothesis we measured cerebral blood flow (CBF) by an intra-arterial $^{133}$Xe technique in 10 baboons during periods of induced hypoxia and hypercapnia, both before and after transection of the 7th cranial nerve. We found that the responses of CBF were unaltered by either unilateral or bilateral section of the nerve. Our results showing the preservation of normal CBF responses, following transection, suggest that neurogenic control of the cerebral circulation by an autonomic reflex involving the 7th nerve is unlikely.

THE SIGNIFICANCE of neurogenic mechanisms in the regulation of the cerebral circulation remains uncertain.1,2 There is some evidence that the sympathetic nervous system modulates cerebral blood flow (CBF) under certain conditions.3-4 Also, findings from light and electron microscopy and histochemical studies15-16 indicate that parasympathetic nerves innervate the cerebral vessels. However, the role of the parasympathetic nervous system in controlling CBF is less clear, chiefly because the few studies reported have described conflicting results.5-16

Autonomic reflexes were described recently which may contribute to the control of the cerebral circulation during hypoxia, hypercapnia, and hypotension.17 Ponte and Purves17 suggested that peripheral chemo- and baroreceptors and the sinus nerve compose the afferent limbs of the reflex arcs, and the efferent limb is found within the 7th cranial nerve. This hypothesis was based on the cerebrovascular responses obtained from studies in anesthetized baboons following chemoreceptor stimulation of one vascularly isolated carotid sinus region. After the contralateral sinus nerve and the aortic nerves were sectioned, CBF was increased when the isolated carotid sinus was perfused with venous blood; this response was almost completely abolished when the 7th cranial nerves were cut intracranially.

The current experiments, also performed on anesthetized baboons, tested CBF responses to hypercapnia and hypoxia following 7th nerve section.

**Methods**

Ten adult baboons (9-14 kg) were sedated initially with phencyclidine (12 mg, im) and anesthesia then was induced with thiopental sodium (7.5 mg/kg, iv). After endotracheal intubation the baboons were paralyzed (succinylcholine, 100 mg, im) to control the arterial CO$_2$ tension, and then ventilated with 75% N$_2$O-25% O$_2$ by a constant volume respirator in open circuit. After surgical preparation had been completed, anesthesia was maintained with nitrous oxide supplemented at 1/2-hour intervals by succinylcholine (50 mg, im) and phencyclidine (5 mg, im). Body temperature was controlled (36–38°C) by a heating lamp. Cannulas were placed in the femoral artery and vein to record arterial blood pressure and heart rate continuously, to sample arterial blood for P$\text{CO}_2$, pH, and P$\text{O}_2$, and to administer fluids (approximately 500 ml of normal saline) throughout each experiment. After the scalp and temporal muscles had been excised, the superior sagittal sinus was cannulated to measure pressure and to sample cerebral venous blood. Blood gases were measured by the micro-Astrup system. The right linguofacial artery was cannulated retrograde to the carotid bifurcation to inject $^{133}$Xe, dissolved in saline (approximately 0.5 ml), into the internal carotid artery. The remaining branches of the external carotid artery then were ligated. The scalp and temporalis muscle were excised. The baboons were placed in the left semiprone position. CBF was measured after intracarotid injection of $^{133}$Xe and calculated from the 10-minute height/area equation.18 The scintillation crystal was 2.5 cm in diameter and was shielded by a thick lead collimator. Values were analyzed by pulse height: the detectors were peaked to a value of 81 keV with a gate of ±10%. Thus, only emission energies within the range of 73–89 keV were detectable. The crystal was placed over the denuded calvarium in the right parietal region. The surgical preparation combined with pulse-height analysis minimized the effect of extracranial contamination by $^{133}$Xe or compton scatter.

**FACIAL NERVE SECTION**

The occipital bone was removed down to the foramen magnum after it had been exposed through a suboccipital midline incision. The dura was incised from the cisterna magna to the lateral sinus, allowing CSF to egress and the cerebellar hemisphere to relax. The 7th and 8th nerves...
were exposed and divided under the operating microscope. The cerebellar hemisphere was barely retracted. At their entrance to the auditory foramen, both nerves were divided sharply, but no attempt was made to separate them. When the nerves were transected bilaterally (experiments 9 and 10), bony exposure was wider and, again, cerebellar retraction was minimal. It was not necessary to resect cerebellar tissue in any experiment. Head position was not changed once CBF measurements began.

**CO2 RESPONSES**

After a suitable control period inhaled CO2 was administered and the concentration increased in steps to a maximum arterial Pco2 of 53–63 mm Hg. Four to ten CBF measurements were made at various values of arterial Pco2 in five baboons used for the CO2 response group. Arterial Pco2 was returned to normal values between individual CO2 responses.

In three baboons (nos. 1–3) the CBF response to inhaled CO2 was measured before the dura was incised, after the 7th nerve was exposed, and after the nerve was transected unilaterally at the internal auditory meatus. In the remaining two experiments (nos. 9 and 10) CBF responses to inhaled CO2 were tested only before the dura was opened and after the nerves had been divided bilaterally.

Arterial Pco2 was plotted against CBF and calculated as a linear regression by the least squares method with a standard computer program. The slope of the line, i.e., the CO2 response, and the correlation coefficient of the regression were determined.

**RESPONSES TO HYPOXIA**

After a base line period with normoxia, the oxygen content of the inspired gas was reduced and CBF values were determined. CBF responses to hypoxia were tested once before and then after the 7th nerve was transected unilaterally in five baboons (experiments 4–8). When the 7th and 8th nerves were transected bilaterally (experiments 9 and 10), arterial Po2 was lowered after, but not before, transection.

Arterial Po2 was plotted against the percent change of CBF from the mean normoxic value for each baboon. The result of CBF-hypoxia curve from seven experiments (nos. 4–10) was compared with that derived by McDowall from normal dogs.

Oxygen saturation of both arterial and cerebral venous blood taken from the sagittal sinus was determined during each CBF measurement. Hemoglobin concentration was measured several times throughout the experiment. Cerebral metabolic rate for oxygen (CMRO2) was calculated from the product of CBF, the appropriate arteriovenous oxygen saturation differences, and the hemoglobin concentration.

**Results**

**CBF RESPONSE TO HYPERCAPNIA**

The three baboons (experiments 1–3) with unilateral transection responded to stepwise increases in arterial Pco2 by increasing CBF under all conditions studied: before dural incision, after unilateral exposure of the 7th and 8th nerves, and after their transection at the internal auditory meatus.

In two baboons (nos. 9 and 10) arterial Pco2 was varied before and after bilateral division of the 7th nerve. No overall reduction in the CO2 sensitivity of the cerebral circulation was noted following transection (Fig. 1). The responses to hypercapnia before and after 7th nerve transection in three baboons (experiments 1, 2, and 10) did not differ significantly. CBF responses to hypercapnia did differ in two animals (experiments 3 and 9) before and after 7th nerve transection; in both, responses were greater after transection than before.

Blood pressure rose slightly (88 ± 1 vs. 90 ± 5 mm Hg) and arterial pH fell (7.36 ± 0.01 vs. 7.31 ± 0.03) during hypercapnia (arterial Pco2 = 51 ± 0.6 mm Hg) before and after nerve section but differences between responses were not significant (paired t-test). When arterial Pco2 was 60 ± 1 mm Hg, blood pressure did not change significantly (84 ± 3 vs. 83 ± 3 mm Hg) before and after nerve section, but arterial pH did fall after transection (7.30 ± 0.01 vs. 7.19 ± 0.02, P < 0.005).

**RESPONSES TO HYPOXIA**

When arterial Po2 fell before unilateral 7th nerve transection, CBF rose in five baboons; in four of these, CBF rose when arterial Po2 fell after nerve division. When the 7th nerve was divided bilaterally in two baboons, both responded to hypoxia by elevation of CBF (Table 1).

The response of mean arterial pressure to the hypoxic episode was variable. On the five occasions (experiments 4–8) when hypoxia was induced prior to 7th nerve section, the MABP was 88 ± 4 mm Hg during normoxia and fell to 87 ± 5 mm Hg during hypoxia. Following 7th cranial nerve section the arterial pressure during normoxia was 86 ± 2 mm Hg (experiments 4–10); arterial pressure rose to 98 ± 5 mm Hg during hypoxia; this was a significant change (P < 0.005) (Table 2).

**FIGURE 1** Gradients of CO2 responses before and after division of the 7th cranial nerve in five baboons. The values of the CO2 reactivity (ml/100 g per min per mm Hg) are shown against the gradients obtained prior to (solid line) and following (dashed line) division of the 7th nerve. Animals 1–3 underwent unilateral division, and animals 9 and 10 underwent bilateral division of the 7th nerve. In animals 1–3 the mean CO2 response (in ml/100 g per min per mm Hg) before the transection was 1.72 ± 0.41 (mean ± SE) and following the transection was 1.93 ± 1.18. This was not significantly different by the analysis of covariance. In animals 9 and 10 before the bilateral transection the mean CO2 response was 2.10 ± 0.32 and after transection was 3.26 ± 0.39. This was significant (P < 0.03). In the individual responses animals 3 and 9 were significantly different (P < 0.005 and P < 0.025, respectively). CBF = cerebral blood flow.
The CBF following the transection was 54 ± 3 per min. The changes in CBF and CMRO$_2$ before and after 7th nerve section were not significant by the t-test.

Under conditions of normoxia and normocapnia was 47 ± 3 ml/100 g per min; the CMRO$_2$ was 3.08 ± 0.13 ml/100 g per min. The cerebral metabolic rate for oxygen (CMRO$_2$) was severely reduced by hypoxia in two baboons (experiment 4, before transection; experiment 7, after transection). Severe depression of the CMRO$_2$ in those two instances (56% and 65% reduction from baseline values, respectively) may have accounted for the poor CBF response in one and an absence of response in the other. CMRO$_2$ was affected by hypoxia less severely in the remainder of the experiments. The current data fit a curve found previously for normal

TABLE 2

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>7th nerve section</th>
<th>CBF (ml/100 g/min)</th>
<th>CMRO$_2$ (ml/100 g/min)</th>
<th>Δ% (from normoxia)</th>
<th>CBF</th>
<th>CMRO$_2$</th>
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<td>65 ± 4.45</td>
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<td>Before</td>
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<td>2.68 ± 0.12</td>
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<td>Before</td>
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<td>9</td>
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<td>Before</td>
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<td>45 ± 4.36</td>
<td>2.23 ± 0.10</td>
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Results are expressed as mean ± SEM.
The mean arterial Pco$_2$ at normoxia was 97 ± 2 mm Hg and at hypoxia, 41 ± 2 mm Hg. The mean arterial Pco$_2$ was maintained at 40 ± 0.3 mm Hg throughout the entire study. Experiments 4-8 were unilateral 7th nerve transections; experiments 9 and 10 were bilateral transections.

Dashes indicate that the cerebral metabolic rate for oxygen (CMRO$_2$) was omitted because sagittal sinus venous blood was not sampled.

The resting CBF before transection of the 7th nerve under conditions of normoxia and normocapnia was 47 ± 3 ml/100 g per min; the CMRO$_2$ was 3.16 ± 0.16 ml/100 g per min. The CBF following the transection was 54 ± 3 ml/100 g per min; the CMRO$_2$ was 3.08 ± 0.13 ml/100 g per min. The changes in CBF and CMRO$_2$ before and after 7th nerve section were not significant by the t-test.

CMRO$_2$ was severely reduced by hypoxia in two baboons (experiment 4, before transection; experiment 7, after transection). Severe depression of the CMRO$_2$ in those two instances (56% and 65% reduction from baseline values, respectively) may have accounted for the poor CBF response in one and an absence of response in the other. CMRO$_2$ was affected by hypoxia less severely in the remainder of the experiments.

The current data fit a curve found previously for normal

TABLE 1

<table>
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<tr>
<th>Expt. no.</th>
<th>7th nerve section</th>
<th>MABP (mm Hg)</th>
<th>Arterial Pco$_2$ (mm Hg)</th>
<th>pH</th>
<th>Arterial Pco$_2$ (mm Hg)</th>
<th>pH</th>
<th>MABP (mm Hg)</th>
<th>Arterial Pco$_2$ (mm Hg)</th>
<th>pH</th>
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<td>After</td>
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<td>7.36 ± 0.01</td>
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<td>125</td>
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<td>38 ± 0</td>
<td>7.52 ± 0.00</td>
<td>94 ± 1</td>
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<td>87 ± 2</td>
<td>41 ± 2</td>
<td>7.47 ± 0.01</td>
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<td>90</td>
<td>41</td>
<td>7.50</td>
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Results are expressed as mean ± SEM.

Blood gases, pH, and MABP values were obtained before and after transection for seven baboons at normoxia and for five at hypoxia.

* These two animals underwent bilateral transection of the 7th cranial nerve.
responses of CBF to hypoxia\textsuperscript{10} with two possible exceptions (Fig. 2). In both exceptions to predicted responses, cerebral metabolic rate was depressed severely and in one, this depression occurred before (experiment 4) transection of the 7th nerve.

Discussion

In our study the baboon brain responded normally to hypoxia and to hypercapnia both before and after unilateral or bilateral transection of the 7th cranial nerve. Thus, we found no evidence to suggest that the parasympathetic fibers of the 7th cranial nerve play a significant role in the cerebral vasodilation that occurs during hypoxia and hypercapnia.

These findings differ from those reported by James et al.\textsuperscript{3} and Ponte and Purves\textsuperscript{17} in which the CBF response to CO\textsubscript{2} was reduced and the CBF response to hypoxia was abolished after 7th nerve section. We are unable to explain the discrepancy between these results and ours except by differences of surgical technique. Neurovascular structures were dissected extensively in the earlier studies.\textsuperscript{3,17} We avoided neck dissection except to cannulate one branch of the external carotid artery and ligate the others. Thus, CBF changes induced by manipulation of neck structures were minimized.\textsuperscript{29} Brain tissue was not removed in our study. The 7th and 8th cranial nerves were exposed and divided by microsurgical techniques under the operating microscope, and thus we hoped to avoid potential artifacts such as postoperative subarachnoid hemorrhage.\textsuperscript{21}

The use of contrast agents to determine the diameter of larger cerebral arteries was excluded from our study, because these agents not only damage the blood-brain barrier,\textsuperscript{22} but also can disturb fundamental physiological responses of the cerebral circulation, including autoregulation.\textsuperscript{23}

The CMRO\textsubscript{2} remained within physiological limits during the experiments, except for two determinations during hypoxia. In each instance CMRO\textsubscript{2} was depressed severely, once before and once after transection of the 7th nerve. The failure of CBF to rise, despite an hypoxic stimulus, may have resulted from impaired cerebral tissue metabolism in both instances.

Chorobski and Penfield\textsuperscript{9} were among the first to describe how parasympathetic fibers innervated the pial vessels via the greater superficial petrosal branch of the 7th cranial nerve. Histochemical techniques for localizing acetylcholinesterase have confirmed the findings of earlier light microscopic studies,\textsuperscript{8} as have recent electron microscopic investigations.\textsuperscript{6,7,9}

The postulated role of parasympathetic nerves in the control of cerebral circulatory responses has a strong morphological basis. Confirmation of parasympathetic denervation of cerebral vessels necessarily requires chronic experiments after facial nerve transection.

Cerebral vessels in vitro react to acetylcholine by constricting\textsuperscript{6} or by dilating,\textsuperscript{12} depending on the resting tone of the vessel. The micropuncture experiments in vivo of Kuschinsky et al.\textsuperscript{12} show that pial vessels have cholinergic receptors that mediate dilation. The authors of the latter paper conclude, in their report on the cerebrovascular effects of atropine, that no significant cholinergic component influences resting arterial tone. Systemic administration of cholinomimetic or cholinergic blocking agents\textsuperscript{13,14,16} is unlikely to contribute further to an understanding of any cerebrovascular parasympathetic influences, because all these drugs probably disturb underlying neuronal metabolism. This metabolic disturbance alone could affect the integrity of the cerebral circulation.

The weak muscarinic and nicotinic actions of succinylcholine may provoke objections to its use as a muscle relaxant in this study. However, its systemic muscarinic effects are minimal\textsuperscript{26} and, more importantly, it does not cross the blood-brain barrier.\textsuperscript{23} Since the parasympathetic nerves that supply the pial vessels are protected by this barrier,\textsuperscript{18} we consider the effects of succinylcholine on our results to be negligible.

The current study did not include any investigation of the afferent limb of the postulated reflex arc.\textsuperscript{17} CBF was examined only before and after transection of the postganglionic, cholinergic, efferent limb. CBF responses to hypercapnia and hypoxia initially were normal, and remained so after the parasympathetic fibers of the 7th cranial nerve were divided. Since normal responses to physiological stimuli were intact after 7th nerve transection, there is little rationale for contending that a neurogenic reflex is essential to these aspects of the cerebral circulation.

We conclude, therefore, that the 7th cranial nerve is not integral to CBF regulation. Although parasympathetic nerves may affect CBF control, their location, functions, and relative importance remain to be determined.

Acknowledgments

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References

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