Increased Cerebral Blood Flow During Hypercapnia Is Not Affected by Lesion of the Nucleus Locus Ceruleus

SAMI I. HARIK, M.D., RICARDO PRADO, M.D.,* RAUL BUSTO, B.S.* AND MYRON D. GINSBERG, M.D.*

SUMMARY To test the hypothesis that the putative noradrenergic innervation of intraparenchymal cerebral blood vessels from the nucleus locus ceruleus mediates the vasodilatory response to hypercapnia, regional cerebral blood flow was measured by iodo-[14C]antipyrine autoradiography in awake and restrained rats with unilateral 6-hydroxydopamine lesion of the nucleus locus ceruleus and in unlesioned control rats. Hypercapnia, induced by the inhalation of 5% or 8% CO₂ in air for 8 minutes caused a 2 to 5-fold increase in regional cerebral blood flow. However, despite a marked reduction of about 90% in cortical norepinephrine levels ipsilateral to the lesion, blood flow to the frontal and parietal cortex, striatum and cerebellum increased to the same extent in ipsilateral and contralateral regions. Thus, lesion of the locus ceruleus and the resultant depletion of endogenous cortical and hippocampal norepinephrine, does not influence the cerebrovascular response to hypercapnia.

ARTERIAL CARBON DIOXIDE TENSION (PaCO₂) exerts a potent influence on cerebral blood flow (CBF), but the exact mechanisms which underly its effects remain controversial.1-6 There are conflicting reports as to whether selective lesions of the central and peripheral nervous systems alter the effects of PaCO₂ on CBF.4,6-11 Two lines of evidence derived from our work have led us to investigate the role of the putative central noradrenergic innervation of cerebral microvessels12-14 from the nucleus locus ceruleus (LC) in mediating increased CBF during hypercapnia in awake rats. First, the increased cerebral blood volume that is consistently induced by brief trains of electrical impulses applied to the cerebral cortex is markedly decreased or abolished after norepinephrine (NE) depletion of the cerebral cortex by LC lesion.15,16 Second, we and others have reported the existence of β₂-adrenergic receptors, which typically mediate relaxation of contractile elements, in cerebral microvesSEL membranes from a variety of mammalian species including man.15-20

The objective of this study is to test whether endogenous cerebral NE from the LC influences the increase in regional CBF produced by hypercapnia. To test this, we assessed the effect of unilateral LC lesion on the regional CBF response to hypercapnia in awake rats. Regional CBF was determined by iodo-[14C]antipyrine autoradiography. Advantage was taken of the fact that most of the noradrenergic innervation of the cerebral cortex and hippocampus originates in the ipsilateral LC; thus unilateral LC lesion causes marked depletion of NE in the ipsilateral cerebral cortex and hippocampus without affecting contralateral forebrain regions, which serve as an internal control. Because LC neuronal activity is inhibited by general anesthetics,21 and also because anesthesia alters the cerebrovascular reactivity to hypercapnia,1,22 we considered that it would be important to examine the effect of unilateral LC lesion on regional CBF during hypercapnia in awake but restrained animals.

Methods

Adult male Wistar rats (250-350 g) were used in these experiments. Rats were housed under diurnal light conditions with unlimited access to laboratory food and water. Unilateral LC lesion was produced under chloral hydrate anesthesia and pargyline pretreatment by the local stereotaxic microinfusion of 2.5 μl of 6-hydroxydopamine solution containing 5 μg of 6-hydroxydopamine base. The details of the procedure have been described previously.23,24 The LC lesions were equally distributed between the right and left sides. Rats were returned to their cages after recovery from anesthesia and allowed to recover for 2 weeks before further experimental procedures. The control group of rats were not lesioned.

Two weeks after LC lesion, the rats were anesthetized by inhalation of a gaseous mixture containing 3% halothane, 30% oxygen and 67% nitrogen oxide. Both femoral arteries and one femoral vein were cannulated for continuous monitoring of mean arterial blood pressure (MABP), for obtaining samples of arterial blood, and for the injection of iodo-[14C]antipyrine. After completion of the surgical procedures, the skin was infiltrated with local anesthetic solution, all wounds were closed, and general anesthesia was discontinued. Upon awakening, the rats were restrained in loose-fitting plaster casts and allowed to recover for 1-2 hours. Their temperature was maintained at 37°C by a rectal thermocouple attached to a heating lamp. Arterial blood gases and pH were measured, and MABP was monitored during the period of stabilization.

Hypercapnia was produced by surrounding the rat's head with a plastic bag connected to a gas reservoir containing either 5% (8 rats), or 8% (7 rats) CO₂ bal-
Inhalation of high CO₂ gas mixture was continued for 9 min. Eight min after the start of hypercapnia, an arterial blood sample was drawn to determine PaO₂, PaCO₂ and pH, and 30 μCi of iodo-[¹⁴C]antipyrine was infused intravenously at a constant rate over 45 sec while sequential arterial blood samples were drawn. The rats were decapitated at the end of iodo-[¹⁴C]antipyrine infusion and their brains were quickly removed and frozen. Coronal brain sections (20 μm thick) were cut in a cryostat at −20°C and exposed to Kodak SB-5 film for 10 days. The resulting autoradiograms were digitized on a rotating drum scanning densitometer interfaced to a minicomputer, and optical density information was transformed to color-coded quantitative representations of regional CBF by means of the usual operational equation. CBF was measured interactively from multiple regions of interest. Three standard coronal brain levels were selected for analysis, corresponding to the levels of the caudate, thalamus and cerebellum (fig. 1). Measurements were made bilaterally from the frontal and parietal cerebral cortex, anterior hippocampus, striatum and cerebellar cortex. A minimum of 3 serial sections was used for replicate measurements at each level.

Control CBF values were derived from a group of 4 unlesioned, awake, restrained rats breathing room air. Samples were taken from the frontal cortex for NE assays to ascertain the efficacy of the LC lesion. Tissue samples were homogenized in 0.1 M perchloric acid and portions of the supernatants were assayed for NE by high performance liquid chromatography with electrochemical detection. LC lesion was considered successful if the NE content of the ipsilateral cortex was decreased to 25% or less of the contralateral cortex. Only 1 rat was inadequately lesioned by this criterion and was excluded from data analysis.

In rats with LC lesions, regional CBF and NE data from regions ipsilateral and contralateral to the LC lesion were compared by the paired Student t test (2-tailed). Significance was considered at p < 0.05.

**Results**

Inhalation of gas mixtures containing 5% and 8% CO₂ resulted in reproducible elevation of PaCO₂ and depression of arterial pH, but did not significantly alter PaO₂ or MABP (table 1). Hypercapnia caused generalized increase in regional CBF compared to normocapnic controls; but, despite the marked depletion of NE.

![Figure 1](image1.png)

**Figure 1.** Schematic representation of the anatomical regions (shaded areas) that were assessed for cerebral blood flow determinations. The regions of interest in the frontal cerebral cortex and striatum are shown in the upper section; those of the parietal cerebral cortex and anterior hippocampus are shown in the middle section; and those of the cerebellum are shown in the lower section.

![Figure 2](image2.png)

**Figure 2.** An iodo-[¹⁴C]antipyrine autoradiogram at the level of the anterior hippocampus, representing regional brain blood flow in a rat with right locus ceruleus lesion, taken at the end of 8 min of hypercapnia induced by inhalation of 5% CO₂ in air. Norepinephrine content of the left frontal cortex was 272 ng/g, while that of the right frontal cortex was 9 ng/g. Despite the marked depletion of norepinephrine in the cerebral cortex ipsilateral to the lesion, there were no side-to-side differences in cerebral blood flow.
in the ipsilateral cerebral cortex by about 90%, there were no significant differences in regional CBF between ipsilateral and contralateral brain structures (fig. 2 and table 2). The frontal cortex, parietal cortex and hippocampus were chosen for analysis because they derive the vast majority of their NE content from the ipsilateral LC. 23, 26, 27 On the other hand, the striatum receives only about 30% of its NE content from the ipsilateral LC. 23, 26, 27 The search for neural reflex pathways that mediate the effect of hypercapnia on cerebral vessels has been the subject of much research. Wolff and Cattell (quoted by Wolff¹), found that the effect of hypercapnia on CBF persists in decerebrate animals, in animals with spinal transection, in animals in which the sixth, seventh and eighth cranial nerves have been transected bilaterally, and after the removal of the carotid sinus and vagus nerves, and the cervical sympa-

Discussion

These results, showing lack of effect of unilateral LC lesion on regional CBF in hypercapnia, in addition to previous results showing lack of effect of such lesions on regional CBF in awake rats at normocapnia²⁴ and during bicuculline-induced status epilepticus²⁵, ²⁶ constitute further evidence that the putative noradrenergic innervation of cerebral blood vessels from the LC does not contribute much to the control of CBF during physiological and pathological conditions associated with a marked increase in CBF. These conclusions are consistent with the opinion expressed at a recent conference summarizing the effect of neurotransmitters and nerves on the cerebral circulation. ²⁷ The lack of effect of noradrenergic denervation from the LC on CBF, however, does not exclude other important functions that this innervation may subserve, such as modulation of the permeability and transport properties of the blood brain barrier.

Our findings are consistent with those of Dahlgren et al., ²⁸ who also found no effect of dorsal noradrenergic tract ablations on CBF. They, however, performed their studies under anesthesia, which is less satisfactory in that depression of the contralateral LC by general anesthetics may obliterate differences between the two sides in rats with unilateral lesions. Because hypercapnia increases the firing rate of LC neurons, ²³ our experimental paradigm would maximize differences between ipsilateral and contralateral regions in rats with unilateral LC lesion.

However, our results are in disagreement with those of Bates et al., ⁷ who showed that bilateral electrolytic LC lesions caused increased resting CBF in anesthetized cats and also abolished the hypercapnia-induced increase in CBF without interfering with autoregulation in response to arterial blood pressure fluctuations; and with the results of Mendelow et al., ¹¹ who found that intracisternal 6-hydroxydopamine, which destroys several central monoaminergic systems, attenuated the cerebrovascular reactivity to hypercapnia in baboons. In contrast, Edvinsson et al. ¹⁰ found increased cerebrovascular reactivity to hypercapnia in rats given 6-hydroxydopamine by intracerebroventricular injections. Multiple methodological differences, which include animal species, lesion procedures, anesthetics, and the method of CBF determination probably account for these widely conflicting results.

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### Table 1. Effects of Hypercapnia on Arterial Blood Pressure, Arterial Blood Gases and pH in Awake Rats

<table>
<thead>
<tr>
<th>Region</th>
<th>Control</th>
<th>Before</th>
<th>After</th>
<th>5% CO₂ (n=8)</th>
<th>8% CO₂ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>54.6 ± 1.7</td>
<td>36.2 ± 1.8</td>
<td>49.5 ± 1.0</td>
<td>40.1 ± 3.7</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td></td>
<td>94.7 ± 6.2</td>
<td>83.1 ± 7.6</td>
<td>107.6 ± 7.3</td>
<td>93.0 ± 21.3</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td></td>
<td>7.382 ± 0.025</td>
<td>7.428 ± 0.027</td>
<td>7.281 ± 0.031</td>
<td>7.364 ± 0.055</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>126 ± 12</td>
<td>114 ± 8</td>
<td>118 ± 6</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td></td>
<td>129 ± 1</td>
<td>129 ± 1</td>
<td>129 ± 1</td>
<td>129 ± 1</td>
</tr>
</tbody>
</table>

Values denote means ± SD for 4 control rats, 8 rats in the 5% CO₂ group, and 7 rats in the 8% CO₂ group.

Measurements were made immediately before CO₂ inhalation and 8 min after the start of hypercapnia.

### Table 2. Regional CBF during Normocapnia and Hypercapnia in Awake Control Rats and in Rats with Unilateral Locus Ceruleus Lesions

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (n=4)</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
<th>Ratio (ipsi/contra)</th>
<th>Ipsilateral</th>
<th>Contralateral</th>
<th>Ratio (ipsi/contra)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>1.09 ± 0.19</td>
<td>2.90 ± 0.33</td>
<td>3.11 ± 0.75</td>
<td>1.12 ± 0.13</td>
<td>5.16 ± 1.43</td>
<td>5.81 ± 1.35</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>1.18 ± 0.29</td>
<td>2.97 ± 0.52</td>
<td>3.27 ± 0.63</td>
<td>1.09 ± 0.04</td>
<td>3.45 ± 0.73</td>
<td>3.74 ± 0.86</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.62 ± 0.10</td>
<td>1.63 ± 0.36</td>
<td>1.46 ± 0.25</td>
<td>1.08 ± 0.06</td>
<td>1.58 ± 0.25</td>
<td>1.46 ± 0.11</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.89 ± 0.09</td>
<td>2.13 ± 0.37</td>
<td>2.14 ± 0.38</td>
<td>1.02 ± 0.06</td>
<td>2.83 ± 0.50</td>
<td>3.04 ± 0.47</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>0.67 ± 0.07</td>
<td>1.32 ± 0.17</td>
<td>1.40 ± 0.23</td>
<td>0.96 ± 0.04</td>
<td>1.40 ± 0.06</td>
<td>1.34 ± 0.10</td>
<td>1.03 ± 0.05</td>
</tr>
</tbody>
</table>

The blood flow values, in ml/g/min, are means ± SEM of the number of observations (n). There were no significant differences between ipsilateral and contralateral blood flow in any of the regions. The ratio values are the means ± SEM of individual ratios. The mean NE content (± SEM) of the cerebral cortex ipsilateral and contralateral to LC lesion was 13 ± 4 and 215 ± 65 ng/g in the 5% CO₂ group, and 31 ± 10 and 267 ± 61 ng/g in the 8% CO₂ group.
thetic ganglia.” On the other hand, Shalit et al reported that relatively large pontine lesions render the brain circulation resistant to hypercapnia. It is unlikely that these observations were due to destruction of the LC or its efferent rostral pathways since many of the lesions involved the ventral brain stem. Whatever the mechanism by which pontine lesions can conceivably affect the reactivity of the brain vasculature to hypercapnia, our results indicate that such effects are not mediated by noradrenergic pathways that emanate from the LC.

Acknowledgments

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