Caudoputamen Is Damaged by Hypocapnia During Mechanical Ventilation in a Rat Model of Chronic Cerebral Hypoperfusion

Etsuko Miyamoto, MD; Hidekazu Tomimoto, MD; Shin-ichi Nakao, MD; Hideaki Wakita, MD; Ichiro Akiuchi, MD; Katsuichi Miyamoto, MD; Koh Shingu, MD

Background and Purpose—Postoperative brain dysfunction, such as delirium, is a common complication of anesthesia and is sometimes prolonged, especially in patients with cerebrovascular disease. In the present study we investigated the effect of hypocapnia during anesthesia on neuronal damage using a rat model of chronic cerebral hypoperfusion.

Methods—Chronic cerebral hypoperfusion was induced by clipping the bilateral common carotid arteries in male Wistar rats. Fourteen days after the operation, these animals were mechanically ventilated for 2 hours and then kept in suitable conditions for an additional 14 days. Twenty-four rats were assigned to 4 groups: those with chronic cerebral hypoperfusion with either hypocapnia or normocapnia during anesthesia, and those given sham operation with either hypocapnia or normocapnia. White matter lesions in the brain sections were evaluated with Klüver-Barrera staining. Proliferation of glial cells was estimated with the use of immunohistochemistry of glial fibrillary acidic protein, a marker for astroglia, and CD11b, a marker for microglia. Computer-assisted morphometry was applied to the immunohistochemical results of microtubule-associated protein 2 to evaluate the loss of neurons.

Results—The histological damage was localized almost exclusively in the white matter in the rats subjected to chronic cerebral hypoperfusion but without hypocapnia. Neuronal damage and astroglial proliferation occurred with aggravated white matter lesions in the caudoputamen in the rats with chronic cerebral hypoperfusion and hypocapnia. No lesions were observed in sham-operated rats with either hypocapnia or normocapnia.

Conclusions—These results indicate that hypocapnia during anesthesia causes tissue damage in the caudoputamen, which may be responsible for long-lasting postoperative delirium in patients with stroke and/or dementia. (Stroke. 2001;32:2920-2925.)

Key Words: caudate nucleus ■ cerebral hypoperfusion ■ delirium ■ hypocapnia ■ postoperative complications ■ putamen ■ rats

Brain dysfunction is a major postoperative complication. In most cases, however, obvious structural damage, such as cerebral infarction or bleeding, is rarely encountered. Delirium or acute confusional state is a common postoperative complication and is characterized by a disturbance of consciousness and a cognitive impairment that persist for only a short period.1 A high incidence of postoperative delirium, ranging from 10% to 60% in the elderly, has been reported.2 Although delirium is usually a benign condition and <20% of episodes persist for >1 week,3,4 it occasionally develops to long-lasting or permanent cognitive impairment. Levkoff et al5 reported that only 4% of elderly patients with delirium had recovered completely at discharge, and 80% had residual impairment 6 months later. Thus, delirium is not necessarily a transient disorder, and in some cases there may be subtle structural brain damage leading to permanent cognitive impairment.

Many etiologic factors may influence postoperative delirium, such as aging, preoperative brain disease (including stroke and dementia), the type of surgery or anesthesia, hypoxia, and hypocapnia.2,6 In aged subjects and patients with vascular dementia, the cerebral blood flow (CBF) is inevitably reduced.7,8 In addition, hypocapnia induces a further reduction in CBF, eg, resulting in a 43% CBF decrease at a Paco2 level of 19 mm Hg.9 Patients under mechanical ventilation during anesthesia are likely to experience hypocapnic conditions, and deliberate hyperventilation to reduce the intracranial pressure (ICP) is common in neuroanesthesia for patients with a brain tumor or cerebral hemorrhage. Therefore, we hypothesized that such a decrease in Paco2 may be critical, especially in the elderly and/or in stroke patients.

A rat model of chronic cerebral hypoperfusion is well established as a model for vascular dementia and is charac-
terized by cognitive impairment and white matter (WM) lesions,10–15 which occur frequently in advancing age and in patients with previous episodes of stroke and cognitive impairment of presumed vascular origin.16 In the present study we investigated the effect of hypocapnia during mechanical ventilation on regional neuronal damage using this model.

**Materials and Methods**

**Experimentation Models**

Twenty-four male Wistar rats weighing 250 to 350 g were randomly assigned to 4 groups: chronic cerebral hypoperfusion with hypocapnia (group 1; n = 6); chronic cerebral hypoperfusion with normocapnia (group 2; n = 6); sham operation with hypocapnia (group 3; n = 7); and sham operation with normocapnia (group 4; n = 5). The procedures for the chronic cerebral hypoperfusion were approved by the animal research committee of Kansai Medical University.

The rats were anesthetized with 3% halothane in 50% N2O and 50% O2 and were left to respiration spontaneously. The bilateral common carotid arteries were exposed through a midline cervical incision and double ligated with silk sutures. Sham-operated animals underwent the same surgical procedure but without bilateral carotid ligation. The rectal temperature was monitored and maintained between 36.5°C and 37.5°C with the use of a warm water mattress and a heating lamp during the surgical procedure. After the operation, the rats were kept under controlled environmental conditions (ambient temperature 23°C to 26°C, 12/12-hour light/dark cycle, lights on at 7 AM), and food and water were allowed ad libitum.

At 14 days after the ligation procedure, the rats were anesthetized and mechanically ventilated as follows. In group 1, anesthesia was induced by inhalation of a mixture of 4% halothane in 50% N2O and 50% O2 as a mask. After the tracheal intubation, anesthesia was maintained with 2% halothane in 50% N2O and 50% O2, and the rats were mechanically ventilated for 2 hours. The right femoral artery was cannulated for the measurement of arterial blood pressure and sampling of blood for gas analysis. The PacO2 was maintained at 20 to 25 mm Hg in this group. In group 2, the experimental conditions were the same as for group 1, but the PacO2 was maintained at 35 to 45 mm Hg. In group 3, the experimental conditions were the same as for group 1, but sham-operated rats were used instead of rats with chronic cerebral hypoperfusion. In group 4, the experimental conditions were the same as for group 2, but sham-operated rats were used instead of rats with chronic cerebral hypoperfusion. The rectal temperature was monitored and maintained between 36.5°C and 37.5°C with the use of a warm water mattress and a heating lamp during the procedure (this range of the rectal temperature corresponded to that of the cranial temperature between 36.4°C and 36.9°C in animals treated with either sham operation or chronic cerebral hypoperfusion). The rectal temperature was monitored and maintained between 36.5°C and 37.5°C with the use of a warm water mattress and a heating lamp during the surgical procedure. After the operation, the rats were kept under controlled environmental conditions (ambient temperature 23°C to 26°C, 12/12-hour light/dark cycle, lights on at 7 AM), and food and water were allowed ad libitum.

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At 14 days after the mechanical ventilation, the rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.01 mol/L PBS and then with a fixative solution containing 4% paraformaldehyde and 0.2% picric acid in 0.1 mol/L phosphate buffer (pH 7.4). The brain was cut into coronal blocks, postfixed for 24 hours in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4), and stored in 20% sucrose in 0.1 mol/L phosphate buffer (pH 7.4). After they were sectioned in a cryostat (20 μm thick), the rest of the brain blocks were embedded in paraffin and cut on a microtome (6 μm thick) for histological and immunohistochemical investigations. With the use of Klüver-Barrera staining, the severity of the WM lesions was graded as normal (grade 0), disarrangement of the nerve fibers (grade 1), formation of marked vacuoles (grade 2), and disappearance of myelinated fibers (grade 3).

**CBF Measurement**

In a separate set of experiments, the relative cortical CBF was continuously measured with the use of a laser-Doppler flowmeter (TBF-LN1, Unique Medical) in at least 2 to 3 rats from each group. The rats were anesthetized with halothane, intubated, mechanically ventilated, and placed in a stereotaxic frame. A burr hole (3 mm in diameter) was made on the right frontotemporal region to allow placement of a photodetector. The contact probe (2.5 mm in diameter) was stereotaxically placed on the cortex (1.5 mm posterior and 2.8 mm lateral to bregma). The rectal temperature was monitored and maintained between 36.5°C and 37.5°C with a warm water mattress and a heating lamp during the procedure.

**Immunohistochemistry**

Unless otherwise stated, all incubations were performed at room temperature. For immunohistochemistry, paraffin sections were incubated overnight at 4°C with mouse monoclonal antibodies (dilutions in parentheses) against microtubule-associated protein 2 (MAP2; Sigma; 1:20) and glial fibrillary acidic protein (GFAP; DAKO; 1:100) as markers for neurons and astroglia, respectively. Cryosections were incubated overnight at 4°C with a mouse monoclonal antibody against CD11b (Sera Laboratory; 1:100) and a rabbit polyclonal antibody against the recombinant N-terminal 92 residues of the amyloid precursor protein (APP) fusion protein (APP 592; a generous gift from Dr Y. Tokushima, Asahi Chemical Industry Co Ltd, Tokyo, Japan; 1:10 000) to label microglia and damaged axons, respectively.

The sections were subsequently incubated with biotinylated antimouse IgG for monoclonal antibodies or anti-rabbit IgG for polyclonal antibodies (Vector Laboratories; 1:200) for 1 hour and then incubated with an avidin-biotin peroxidase complex solution (Vector Laboratories; 1:100) for 1 hour. Between each incubation, the sections were rinsed for 15 minutes with 0.1 mol/L PBS. The immunoreaction products were visualized with a solution of 0.02% 3, 3′-diaminobenzidine tetrahydrochloride and 0.005% H2O2 in 0.05 mol/L Tris buffer (pH 7.6). Then the sections immunostained for GFAP and CD11b were counterstained by hematoxylin.

**Morphometry and Statistical Analysis**

The MAP2 immunoreactive area in a unit area of 0.09 mm2 was determined in the caudoputamen and the cerebral cortex with the use of a computer-assisted image analysis system (Mac ASPECT/PPC, Mitani Coop) attached to a light microscope at ×250 magnification and a high-resolution color video camera.17 The numerical density of GFAP immunopositive astrocytes in a unit area of 0.3 mm2 was also counted in the caudoputamen, optic tract, and corpus callosum with the use of the computer-assisted image analysis system. The WM lesions were graded by an investigator blind to the experimental protocol in the following brain regions: the optic tract, the fiber bundle in the caudoputamen, and the corpus callosum. Physiological data were analyzed by 1-way ANOVA. Post hoc differences between groups were identified by Bonferroni’s t test. All data were expressed as mean±SEM. Statistical comparisons among groups were determined by a 2-factor factorial ANOVA followed by Bonferroni’s modification of the t test. A P value <0.05 was considered statistically significant.

**Results**

**Physiological Variables**

Of the animals that were subjected to chronic cerebral hypoperfusion, 2 of 8 died in group 1, 2 of 8 in group 2, 2 of 9 in group 3, and 1 of 6 in group 4. There were no significant differences in the mortality rate between groups. The PaCO2 levels were significantly lower and the values of pH significantly higher in the hypocapnia groups (groups 1 and 3) than

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*Note: The image contains a watermark and a citation that reads: "Miyamoto et al. Neuronal Damage by Hypocapnia During Anesthesia" 2921."*
in the normocapnia groups (groups 2 and 4). The values of PaO2 and body temperature were not significantly different among groups (Table 1).

**CBF Measurement**

The physiological variables (mean arterial pressure, heart rate, blood gases, and rectal temperature) during CBF measurement were similar to those in Table 1 in groups 1 and 3. The CBF values decreased significantly after the hyperventilation and subsequent hypocapnia in both sham-operated rats and rats with chronic cerebral hypoperfusion (Figure 1). These values gradually returned to baseline level when hyperventilation was discontinued. The CBF values were reduced by 25% after hyperventilation was started in the sham-operated rats (Figure 1A). In the rats with chronic cerebral hypoperfusion, the CBF values were reduced by 12% after hyperventilation (Figure 1B).

**WM Rarefaction Grading**

In the gray matter regions, scattered foci of microinfarcts were occasionally observed in the cerebral cortex, but the pyramidal neurons in the hippocampus showed no definite morphological changes in rats with chronic cerebral hypoperfusion, either with or without hypocapnia. As reported previously, chronic cerebral hypoperfusion alone caused an intense number of WM lesions in the optic tract and a moderate number in the corpus callosum and the fiber bundles of the caudoputamen. In addition, hypocapnia aggravated WM lesions exclusively in the caudoputamen in rats with chronic cerebral hypoperfusion (Table 2). There were no apparent gray matter and WM lesions in the sham-operated rats (groups 3 and 4), even with hypocapnia.

**Immunohistochemistry**

Neither chronic cerebral hypoperfusion nor hypocapnia independently reduced the MAP2 immunoreactive area in the

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### TABLE 1. Physiological Variables During Anesthesia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>95±3</td>
<td>93±3</td>
<td>92±2</td>
<td>96±5</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>347±6</td>
<td>340±9</td>
<td>345±8</td>
<td>353±4</td>
</tr>
<tr>
<td>pH</td>
<td>7.61±0.02*</td>
<td>7.41±0.01</td>
<td>7.57±0.01*</td>
<td>7.39±0.01</td>
</tr>
<tr>
<td>PaCO2, mm Hg</td>
<td>22.6±1.2*</td>
<td>39.6±2.1</td>
<td>23.3±0.4*</td>
<td>38.5±2.0</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>185.0±12.6</td>
<td>191.4±23.0</td>
<td>212.7±20.4</td>
<td>180.7±39.9</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>37.4±0.2</td>
<td>37.3±0.3</td>
<td>37.2±0.3</td>
<td>37.3±0.2</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>128±4</td>
<td>132±4</td>
<td>131±5</td>
<td>125±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.01 vs group 2, 4.

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**Figure 1.** Left, Representative tracings of CBF profile after hyperventilation in a rat given a sham operation and a rat with chronic cerebral hypoperfusion. The figures on the y axis indicate the relative CBF value in arbitrary units. Right, CBF values before hyperventilation have been shown as 100% (white columns); black columns show CBF values after hyperventilation in the sham-operated rats (A) and in rats with chronic cerebral hypoperfusion (B). The mean results of 2 to 3 independent experiments are shown in each column.
brain region examined. However, hypocapnia induced a significant decrease in the MAP2 immunoreactive area in the cerebral cortex and the caudoputamen in group 1 (25.5 ± 2.2% and 14.6 ± 0.2%, respectively) compared with those in group 2 (48.2 ± 1.8% and 41.2 ± 0.7%, respectively) (Figure 2).

Chronic cerebral hypoperfusion alone increased the number of GFAP immunoreactive astroglia in the WM regions including the optic tract, corpus callosum, and caudoputamen. With hypocapnia, the GFAP immunoreactive astroglia increased in number exclusively in the caudoputamen (411 ± 40/0.3 mm²; mean ± SD, group 1) compared with those in group 2 (244 ± 18/0.3 mm²) (Figure 3).

Immunohistochemistry of CD11b showed that microglia were activated in the fiber bundle of the caudoputamen in rats with chronic cerebral hypoperfusion either with or without hypocapnia (Figure 4D and 4E, respectively) but not in sham-operated animals (Figure 4F). Similarly, APP immunoreactive axons appeared in the fiber bundles of the caudoputamen in rats with chronic cerebral hypoperfusion either with or without hypocapnia (Figure 4G and 4H, respectively). There were no APP immunoreactive axons in sham-operated animals (Figure 4I).

There were no significant differences in the immunohistochemistry of CD11b and APP between groups 3 and 4.

**Discussion**

The present study revealed that damage to the caudoputamen was increased by hypocapnia in rats with chronic cerebral hypoperfusion, whereas no brain regions were injured by hypocapnia in the sham-operated rats. WM lesions are frequently present in ischemic cerebrovascular disease and Alzheimer’s disease and constitute the core pathology inBinswanger’s disease, a form of vascular dementia.18–20 These WM lesions are responsible for cognitive impairment and are thought to result from chronic cerebral hypoperfusion.16 They can be experimentally induced in rat brains by permanent occlusion of both of the common carotid arteries.

**TABLE 2.** Grading Scores for WM Lesions in Each Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic tract</td>
<td>2.8 ± 0.2*</td>
<td>3.0 ± 0.0*</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>1.4 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Caudoputamen</td>
<td>2.0 ± 0.0*†</td>
<td>1.0 ± 0.0*</td>
<td>0.3 ± 0.2</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*Significant at P < 0.05 vs group 3, 4.
†Significant at P < 0.05 vs group 2.
Use of this procedure invariably decreases the CBF to 40% to 82% of normal values over a prolonged period. 14–21,22 Our previous studies revealed that the WM is preferentially damaged in these rats with an increase in reactive astroglia and activated microglia and that WM lesions are found mostly in the optic nerve and optic tract and to a lesser extent in the medial part of the corpus callosum, anterior commissure, internal capsule, and caudoputamen. 10–14 Furthermore, these animals are cognitively impaired in the Morris water maze and radial maze tasks. 23,24 All of these features are characteristic of patients with cerebrovascular insufficiency, but this model also has a few drawbacks, such as the abrupt reduction in CBF and the absence of hypertensive small-vessel disease. In the present study we confirmed that chronic cerebral hypoperfusion induced WM lesions with only slight damage in the gray matter. We also demonstrated that hypocapnia aggravates rarefaction and astrogliosis proliferation exclusively in the caudoputamen (Table 2, Figures 3 and 4).

APP, an axonally transported protein, accumulates in regions with disturbed axonal transport and can be used as a marker for WM lesions. 25–28 Accumulation of APP immunoreactive fibers in the caudoputamen seems to be indicative of the rarefaction and astrogliosis in this region. In the present experimental conditions, microglial activation was not enhanced by hypocapnia. However, microglial cells, already activated in chronic hypoperfusion alone, may play a role in the pathogenesis of WM lesions, since they are a key source of cytokines and cytokotaxis, such as proteases, reactive oxygen radicals, and nitrogen intermediates. 29–34

MAP2, which stabilizes microtubules and helps to regulate microtubule spacing, 35 is located almost exclusively in the neuronal perikarya and dendrites. Therefore, a decrease in MAP2 staining in the caudoputamen and the cerebral cortex clearly indicated that the brain damage had expanded from the WM to the gray matter (Figure 2). WM is usually spared from insults in transient global ischemia models, in which CBF is reduced to 5% of normal values. The neuronal damage occurs in the gray matter, including the hippocampus, the cerebral cortex, the caudoputamen, and the ventrolateral part of the thalamus in association with reactive astrogliosis and activation of microglia in the same regions. 36–40 Conversely, the gray matter lesions in chronic cerebral hypoperfusion are mild or scarce, 10,21,41 suggesting that regional tissue vulnerability depends on the mode of ischemic insult. Therefore, additional gray matter damage after hypocapnia, as evidenced by the loss of MAP2 staining, was not detected in the caudoputamen and the cerebral cortex, may indicate an overlay of acute ischemic insult onto chronic ischemic damage.

The caudoputamen receives input from the frontal and temporoparietal cortices and sends output fibers to the globus pallidus and the other regions of the basal ganglia. 32,42 Recent studies have revealed that the caudoputamen plays an essential role in the acquisition of motor, perceptual, and cognitive skills and in the spatial working memory. 44–49 Thus, the ischemic damage in the caudoputamen as a result of hypocapnia may impair striatocortical neural networks and may be responsible for the postoperative prolonged delirium.

Conclusions

The present study demonstrated that hypocapnia induced WM rarefaction and astrogliosis proliferation in the caudoputamen and that neuronal damage also occurred in the cerebral cortex in a rat model of chronic cerebral hypoperfusion. These observations indicate that in the elderly and/or those with cerebrovascular disease, hyperventilation may cause brain damage and may result in irreversible cognitive impairment.

Acknowledgments

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References


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