Difference between Halothane and Barbiturate Anesthesia in the Influence of Cerebral Ischemia on the Vagal Baroreflex in Dogs

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ABSTRACT—In halothane-anesthetized dogs, a decrease in baroreflex sensitivity (BRS) of approximately 20% was observed during the reperfusion period following 5-min global cerebral ischemia. When compared with our previous study on animals under pentobarbital anesthesia, the extent of the decrease in BRS was smaller and apparently more severe ischemia was necessary to damage the vagal component of the baroreflex. Substitution of halothane with pentobarbital during the reperfusion period failed to affect either BRS or the ratio of the vagal component. In another group, pre- and post-ischemic measurements of BRS was performed under halothane anesthesia, but the ischemic insult was given under thiopental anesthesia. In these animals, the extent of decrease in BRS (about 50%) was greater than that in animals subjected to ischemia under halothane anesthesia. The present results suggest that the anesthetics used during the ischemic insult may affect the extent of post-ischemic dysfunction of the baroreflex. The vagal component of the baroreflex may be more resistant to ischemia under halothane anesthesia than under barbiturate anesthesia.

We have reported that transient global cerebral ischemia of longer than 5 min produced a marked dysfunction of the central pathway of the vagal baroreflex in dogs (1, 2). Since these experiments have been performed under pentobarbital anesthesia, a possibility exists that the influence of ischemia might be underestimated due to a cerebroprotective effect of barbiturates (3). In the present study, therefore, the influence of 5-min global cerebral ischemia on the baroreflex was investigated in dogs anesthetized with halothane, which has been reported to be less cerebroprotective than barbiturates against ischemic injury (4–7). The results were compared with those from our previous experiments on animals under pentobarbital anesthesia; and unexpectedly, we found that the vagal baroreflex system may be more resistant to ischemia under halothane anesthesia in comparison with barbiturate anesthesia. Part of this study was presented at the XIth International Congress of Pharmacology (8).

MATERIALS AND METHODS

Animals

Thirty mongrel dogs of either sex weighing 7 to 15 kg were anesthetized with sodium thiopental, 20 to 30 mg/kg, i.v. Trachea, right and left cephalic veins, left femoral artery and right saphenous artery were cannulated. The
cortical EEG was recorded by means of stainless steel electrodes screwed into the parietal skull, and the depth of anesthesia was monitored by a frequency analyzer (Nihon Kohden, OEE-7102, Japan). After an appropriate recovery period, 2% halothane in room air was given for about 10 min through an artificial ventilator (a tidal volume of 20 ml/kg at a rate of 20 breaths/min). Thereafter, anesthesia was maintained by halothane, 0.75 to 1.25% in room air. The animals were immobilized with 2 mg/kg suxamethonium chloride, followed by an infusion of 1 mg/kg/hr into the right cephalic vein. Blood was withdrawn periodically from the right saphenous artery for blood gas analysis, and appropriate volumes of O_2 and CO_2 were provided to maintain arterial PO_2 and PCO_2 at about 100 mmHg and 35 mmHg, respectively. Rectal temperature was maintained at about 38°C using a heating pad and lamp.

Measurement of baroreflex sensitivity (BRS)

Arterial blood pressure was measured from the left femoral artery by means of a pressure transducer (Nihon Kohden, TP-200T), and heart rate was measured by a heart rate counter (Nihon Kohden, AT-600G) triggered by the lead II ECG. Baroreflex was assessed by bolus injections into the left cephalic vein of 4 to 5 doses of l-phenylephrine hydrochloride within the dose range of 0.3 to 10 μg/kg, i.v. The phenylephrine-induced increase in pulse interval (msec) was correlated with the increase in mean arterial blood pressure (mmHg) by the method of least squares. The slope of the regression line (msec/mmHg) was used as a measure of BRS, as ischemia did not displace the regression line, but altered the slope.

Production of cerebral ischemia

Thoracotomy was performed at the fifth intercostal space. Approximately 14 arteries descending along the thoracic aorta were ligated to obstruct severely the collateral blood flow to the brain. Transient global cerebral ischemia was produced by a 5-min occlusion with clamps of the left subclavian artery and the brachiocephalic artery as close as possible to the aortic arch. The extent of the reduction of cerebral blood flow during ischemia was monitored by the thermocouple electrode (see below).

Measurement of cerebral blood flow

The animals were placed in a prone position, and the head was fixed in a stereotaxic frame. The head was tilted down at an angle of about 30 degrees to help the manipulation of the medulla oblongata. The foramen magnum was enlarged by removal of the caudal edge of the occipital bone. Regional cerebral blood flow (rCBF) was continuously measured by a tissue flow monitor (Unique Medical, UMW-101, Japan) using the plate type thermocouple electrode placed on the left fasciculus cuneatus in the dorsal medulla oblongata proximal to the obex. The blood flow level immediately prior to ischemia was regarded as 100%, and zero flow was obtained by killing the animal with saturated KCl at the end of each experiment. Mean residual blood flow during ischemia was calculated as described in the previous report (2) to assess the severity of ischemia. Furthermore, a platinum electrode was inserted contralaterally to the thermocouple electrode to a depth of 1 to 2 mm, and the absolute value of rCBF was measured by the hydrogen clearance method (9). rCBF was calculated from the clearance curve during the period of 1 to 7 min after the peak concentration. When the clearance curve was biexponential, mean flow for the two components was calculated according to Lassen’s weighing method (10).

Experiment 1

Influence of 5-min global cerebral ischemia was investigated in 10 halothane-anesthetized animals. BRS was measured before ischemia and during the periods of 60 to 90, 120 to 150 and 180 to 210 min of reperfusion. rCBF in the medulla oblongata was measured just before each BRS measurement by the hydrogen clearance method in 8 animals. Thereafter,
influence of the bilateral section of the cervical vagosympathetic trunk (vagotomy) on BRS was examined. Additionally, the influence of methylatropine, 0.1 mg/kg, i.v. administered 10 min prior to the BRS measurement, on BRS was investigated in 6 sham-operated animals.

**Experiment 2**

In 6 animals, halothane was withdrawn just after the BRS measurement during the period of 60 to 90 min of reperfusion following 5-min global cerebral ischemia. After the recovery period of 15 to 20 min, pentobarbital, 10 mg/kg, i.v. followed by an infusion of 3.2 mg/kg/hr, i.v., was administered, and BRS was measured. Then, the influence of vagotomy on BRS was examined under pentobarbital anesthesia. rCBF in the medulla oblongata was measured just before each BRS measurement.

**Experiment 3**

In 6 animals, halothane was withdrawn just after the BRS measurement before ischemia. After the recovery period of 15 to 20 min, thiopental at 10 mg/kg, i.v. was administered for over a 3-min period. The animals received the 5-min ischemic insult 10 min after thiopental administration, when EEG frequency analysis revealed the same pattern as under halothane anesthesia. After a recovery period of 1 to 2 hr (91 ± 10 min in average) during the reperfusion period, the animals were reanesthetized with halothane, and BRS was measured during the period of 120 to 150 min of reperfusion. Then, the influence of vagotomy on BRS was examined. rCBF in the medulla oblongata was measured just before each BRS measurement.

**Statistics**

The correlation between the severity of ischemia and the extent of the influence of vagotomy on BRS (see Fig. 1) was assessed by a non-linear regression technique with a modified Marquardt algorithm, which fitted the data on a sigmoid logistic curve (11). Multiple comparisons between mean values before and after ischemia were performed using one-way analysis of variance followed by a control-significant-difference method (Dunnett’s t-test). Single comparisons between paired values were performed with the paired Student’s t-test. Differences giving P < 0.05 were judged to be statistically significant.

**RESULTS**

**Experiment 1**

**Blood pressure, heart rate, BRS, rCBF and EEG:** During the period before ischemia, blood pressure and heart rate in the present study (Table 1) were apparently lower than those in our previous study on animals under pentobarbital anesthesia. On the other hand, pressor responses to phenylephrine, the correlation coefficient between phenylephrine-induced increases in mean blood pressure and pulse interval, BRS and rCBF were within the range of the values under pentobarbital anesthesia. The percent ratios of delta (1 to 3.5 Hz), theta (4 to 7.5 Hz), alpha (8 to 12.5 Hz) and beta (13 to 32 Hz) components of the EEG power spectrum to the total power were 24.2 ± 2.0, 40.7 ± 1.5, 26.3 ± 1.5 and 8.8 ± 1.5% (n = 10), respectively. This pattern of the EEG power spectrum was similar to that obtained under pentobarbital anesthesia (1, 2).

The ischemic insult reduced rCBF in the medulla oblongata and produced a flattening of the cortical EEG. The calculated mean residual blood flow during 5-min ischemia was 36.5 ± 5.9% (n = 10), and this value was similar to that reported under pentobarbital anesthesia (2).

During the reperfusion period, as shown in Table 1, a significant decrease in BRS was observed. However, the extent of the decrease in BRS in the present study (about 20% decrease) was less than that under pentobarbital anesthesia (about 50% decrease, see references 1 and 2), and the change in the absolute value of BRS was not statistically significant. Furthermore, in contrast to the results under
Pentobarbital anesthesia (2), no significant reduction of rCBF in the medulla oblongata was observed during the period of 60 to 180 min of reperfusion (Table 1). Additionally, there was no significant change in EEG pattern during the reperfusion period.

**Table 1. Influence of 5-min global cerebral ischemia on mean blood pressure (MBP), heart rate (HR), baroreflex sensitivity (BRS) and regional cerebral blood flow (rCBF) in halothane-anesthetized dogs**

<table>
<thead>
<tr>
<th></th>
<th>Before ischemia</th>
<th>60–90</th>
<th>120–150</th>
<th>180–210</th>
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</thead>
<tbody>
<tr>
<td><strong>MBP (mmHg)</strong></td>
<td>100 ± 4</td>
<td>94 ± 3</td>
<td>97 ± 4</td>
<td>98 ± 4</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>113 ± 3</td>
<td>123 ± 4</td>
<td>125 ± 5</td>
<td>127 ± 5</td>
</tr>
<tr>
<td><strong>BRS (msec/mmHg)</strong></td>
<td>2.74 ± 0.33</td>
<td>2.31 ± 0.38</td>
<td>2.19 ± 0.29</td>
<td>2.12 ± 0.24</td>
</tr>
<tr>
<td>(%</td>
<td>100</td>
<td>82.6 ± 5.9*</td>
<td>80.3 ± 4.8**</td>
<td>78.9 ± 3.5**</td>
</tr>
<tr>
<td><strong>rCBF (ml/min/100 g)</strong></td>
<td>31.8 ± 2.8</td>
<td>28.1 ± 3.5</td>
<td>27.9 ± 3.5</td>
<td>31.1 ± 4.2</td>
</tr>
<tr>
<td>(%)</td>
<td>100</td>
<td>88.4 ± 8.0</td>
<td>87.8 ± 7.8</td>
<td>98.2 ± 10.0</td>
</tr>
</tbody>
</table>

Data are the means ± S.E.M. from 10 animals except for rCBF (8 animals). *P < 0.05, **P < 0.01: significantly different from the value before ischemia. MBP and HR values are those at the time of BRS measurement. rCBF was measured just before each BRS measurement. Percent changes in rCBF are based on the absolute values.

Vagal component of BRS: In sham-operated animals, methylatropine at 0.1 mg/kg, i.v. decreased BRS from 2.66 ± 0.51 to 1.25 ± 0.26 msec/mmHg (n = 6). The extent of the decrease in BRS (49.6 ± 7.1%) was similar to that under pentobarbital anesthesia (49.3 ± 7.6%, n = 6; see reference 12), indicating that there was no difference between halothane and pentobarbital anesthesia in the extent of participation of the vagal component in the reflex bradycardia.

Since our previous study under pentobarbital anesthesia revealed that a 5-min period of cerebral ischemia produced a selective dysfunction of the vagal component of the baroreflex (12), the correlation between the severity of ischemia and the extent of the decrease in the vagal component was investigated, as shown in Fig. 1. When the mean residual blood flow during ischemia was greater than approximately 30%, BRS during the reperfusion period was decreased by vagotomy to the same extent as that by methylatropine in sham-operated animals, indicating that the pentobarbital anesthesia (2), no significant reduction of rCBF in the medulla oblongata was observed during the period of 60 to 180 min of reperfusion (Table 1). Additionally, there was no significant change in EEG pattern during the reperfusion period.

**Fig. 1.** Correlation between the severity of ischemia and the extent of the influence of vagotomy on the baroreflex sensitivity during the reperfusion period. Abscissa scale: severity of ischemia is indicated as the mean residual blood flow in the dorsal medulla oblongata during ischemia. Ordinate scale: The extent of the influence of vagotomy is indicated as the ratio of the baroreflex sensitivity after vagotomy to that before vagotomy. Two animals that received the ischemic procedure without preceding ligation of the intercostal artery (○) were also investigated in addition to the animals in Table 1 (●). The solid line H is a logistic curve obtained by a non-linear regression technique. The broken line P is a superimposition of a logistic regression curve obtained in the previous study under pentobarbital anesthesia (reference 12).
vagal component of BRS survived an ischemic insult of such severity. As the severity of ischemia further increased, the influence of vagotomy was attenuated; the ratio of BRS after vagotomy to that before vagotomy was nearly 1. The apparent threshold mean residual blood flow during ischemia for the dysfunction of the vagal baroreflex in the present study was clearly lower than that under pentobarbital anesthesia (see solid and broken lines in Fig. 1).

Experiment 2
To investigate the possibility that halothane might facilitate the baroreflex and mask the dysfunction of the baroreflex system during the reperfusion period, the influence of substituting halothane by pentobarbital was examined. The mean residual blood flow during ischemia in this experiment was 41.0 ± 8.0% (n = 6). As shown in Table 2, BRS and rCBF during the reperfusion period under halothane anesthesia was not significantly different from the values before ischemia under halothane anesthesia. Substitution of halothane by pentobarbital increased the mean blood pressure and heart rate and decreased rCBF; the regional resistance of the cerebral vascular bed was increased by 65.1 ± 12.5%. In spite of these changes, BRS was not affected by the substitution of anesthetics. Furthermore, as shown in Fig. 2, the correlation between the severity of ischemia and the extent of decrease in the vagal component of BRS fitted well the regression curve obtained in experiment 1.

Experiment 3
This part of study was conducted to examine whether the anesthetics utilized during ischemia is primarily responsible for the difference between the results in experiment 1 and those in our previous study with pentobarbital-anesthetized animals. Mean residual blood flow during ischemia in this experiment was 38.1 ± 5.8% (n = 6). Although pre- and post-ischemic BRS and rCBF were measured under halothane anesthesia, a marked decrease in BRS was observed during the reperfusion period following the 5-min ischemic insult under thiopental anesthesia (Table 3), which was in contrast to the results in experiment 1. The extent of the decrease in BRS was similar to that observed in our previous study under pentobarbital anesthesia. Furthermore, the correlation between the severity of ischemia and the extent of decrease in the vagal component of BRS fitted well the regression curve obtained previously in pentobarbital-anesthetized animals (Fig. 3). On the other hand,

| Table 2. Influence of substitution of halothane by pentobarbital on baroreflex sensitivity (BRS) and regional cerebral blood flow (rCBF) during the reperfusion period following 5-min global cerebral ischemia |
|-------------------------------------------------|--------------------------|--------------------------|
|                                                  | Before ischemia          | Reperfusion period        |
|                                                  | Halothane                | Pentobarbital             |
| MBP (mmHg)                                       | 100 ± 7                  | 88 ± 4                   | 116 ± 7**                |
| HR (beats/min)                                   | 138 ± 8                  | 136 ± 8                  | 160 ± 6*                 |
| BRS (mmHg)                                       | 2.41 ± 0.43              | 2.06 ± 0.33              | 2.09 ± 0.35              |
| (%)                                              | 100                      | 87.7 ± 5.4               | 89.5 ± 7.1               |
| rCBF (ml/min/100 g) (%)                          | 36.0 ± 5.2               | 34.2 ± 6.4               | 28.1 ± 5.8*              |
|                                                  | 100                      | 92.9 ± 7.0               | 75.8 ± 7.6*              |

Data are the means ± S.E.M. from 6 animals. *p < 0.05, **p < 0.01; significantly different from the value during the reperfusion period under halothane anesthesia. MBP and HR values are those at the time of BRS measurement. rCBF was measured just before each BRS measurement. Percent changes in rCBF are based on the absolute values.
there was no significant reduction of rCBF during the reperfusion period (Table 3), which was consistent with the results in experiment 1 (Table 1).

DISCUSSION

There have been numerous studies concerning the influence of anesthetic on ischemic injury of the brain, because the general depression of cerebral functions is expected to attenuate the energy imbalance during ischemia, leading to the preservation of the cellular integrity. However, since effects other than the neuronal depressive effects of anesthetics may either facilitate or mask the potentially beneficial effect, the degree of cerebral protection will not necessarily be equal between the anesthetic agents.

For example, Smith et al. (4) showed that pretreatment of dogs with barbiturates, but not with halothane, reduced the neurological or histopathological deficits following permanent ligation of both the unilateral middle cerebral artery and the internal carotid artery. In this case, the authors speculated that the difference in the cerebrovascular effect of

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**Table 3. Influence of 5-min global cerebral ischemia under thiopental anesthesia on baroreflex sensitivity (BRS) and regional cerebral blood flow (rCBF)**

<table>
<thead>
<tr>
<th></th>
<th>Before ischemia</th>
<th>Reperfusion period</th>
</tr>
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<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>104 ± 4</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>131 ± 15</td>
<td>143 ± 14</td>
</tr>
<tr>
<td>BRS (msec/mmHg)</td>
<td>2.91 ± 0.09</td>
<td>1.46 ± 0.04 **</td>
</tr>
<tr>
<td>(%)</td>
<td>100</td>
<td>50.6 ± 2.2 **</td>
</tr>
<tr>
<td>rCBF (ml/min/100 g)</td>
<td>30.7 ± 4.7</td>
<td>26.8 ± 5.3</td>
</tr>
<tr>
<td>(%)</td>
<td>100</td>
<td>86.8 ± 6.9</td>
</tr>
</tbody>
</table>

Data are the means ± S.E.M. from 6 animals. All values were obtained under halothane anesthesia. **p < 0.01: significantly different from the value before ischemia. MBP and HR values are those at the time of BRS measurement. rCBF was measured just before each BRS measurement. Percent changes in rCBF are based on the absolute values.
anesthetics might be responsible for the difference in cerebral protection. That is, barbiturates may reduce cerebral blood flow and intracranial pressure, and redistribute the blood to the ischemic region through the inverse steal phenomenon, all of which may be favorable for cerebral protection. On the other hand, cerebral vasodilation by halothane may increase the intracranial pressure, predispose the animal to cerebral edema, or produce the steal phenomenon to exaggerate the ischemia; these effects may overwhelm the beneficial effect of halothane. Other studies with animal models of transient focal cerebral ischemia also revealed that the extent of ischemic injury of the brain may be less under barbiturate anesthesia than under halothane anesthesia (5-7). However, the results from the present study were surprisingly opposite to these studies, suggesting that the central baroreflex system may be more resistant to ischemia under halothane anesthesia than under barbiturate anesthesia.

It should be noted that the animal models of cerebral ischemia as well as the method used to evaluate the ischemic injury of the brain in the present study are considerably different from those in the previous studies cited above. First, the model used in the present study was that of transient global ischemia of 5-min duration, which is in contrast to the focal ischemia of longer than 2 hr in the previous studies. The differential influence of barbiturates and halothane on the distribution of cerebral blood flow, as mentioned above, will be restricted to the focal ischemia model. Secondly, the present study utilized BRS to evaluate the ischemic dysfunction of the brainstem, whilst the previous studies examined the changes in the neurological or histological scores and some biochemical parameters such as the concentrations of high energy phosphates, lactate, pyruvate, K⁺ or H⁺ in the cerebral cortex. All these differences may be responsible for the apparent contradiction between the present study and the previous ones. Furthermore, since the present study dealt with only the early phase of reperfusion, up to few hours, differences in the long term recovery of BRS up to several days are unknown.

The present results resemble the recent observation by Salzman et al. (13), who showed that the outcome following the traumatic injury of the rat spinal cord was better under halothane anesthesia than under pentobarbital anesthesia. They excluded the possibility that changes in the respiratory condition or body temperature might be responsible for the different outcome. This is also the case in the present study, because the arterial Pₒ₂ and Pₐ₆ as well as rectal temperature were maintained artificially within the physiological ranges. Furthermore, since the mean residual blood flow during ischemia and the ratio of the vagal component of BRS, in the present study, were within the ranges observed in the previous studies under pentobarbital anesthesia (2, 12), these variables can be excluded as the reasons for the different outcome.

Since anesthetic doses of halothane have been shown to sensitize baroreceptors in dogs (14), one possible hypothesis was that such an effect of halothane might mask the dysfunction of the central baroreflex system during the reperfusion period. However, such was not the case in the present study, because the substitution of halothane by pentobarbital during the reperfusion period failed to affect BRS and the ratio of the vagal component of BRS (see experiment 2).

There were some cardiovascular parameters other than BRS which differed between halothane anesthesia and pentobarbital anesthesia. That is, the basal arterial blood pressure and heart rate were lower, and no significant secondary reduction of rCBF was observed during the reperfusion period in halothane-anesthetized animals (Table 1). The latter observation was consistent with that by Urbanics et al. (7). However, it is unlikely that these differences might contribute to the difference in the extent of ischemic dysfunction of the baroreflex system, judging from the results in experiment 3, where pre- and
post-ischemic measurements of BRS and rCBF were carried out under halothane anesthesia, but the ischemic insult was performed under thiopental anesthesia. In this experiment, the extent of post-ischemic decrease in BRS and the correlation between the extent of the damage of the vagal component of BRS and the severity of ischemia were quite similar to those observed in pentobarbital-anesthetized animals despite the absence of the secondary reduction of rCBF. These results suggest that the anesthetic utilized during the ischemic insult may be the primary determinant of the extent of the post-ischemic dysfunction of the baroreflex system.

In the present study, the severity of ischemic insult was assessed by the mean residual blood flow in the dorsal medulla oblongata during ischemia, which has been shown to be a good determinant of the post-ischemic decrease in BRS (2). Although the apparent threshold blood flow for the baroreflex dysfunction was shown to be lower under halothane anesthesia than under barbiturates anesthesia, it should be noted that the practical extent of energy imbalance is determined by the combination of the rate of metabolic supply and demand. In this context, Cohen and Britt (15) found no difference in the ability of anesthetic doses of halothane and pentobarbital to influence brainstem auditory evoked responses, which may imply that the metabolic demand may be decreased equally by both anesthetics. However, this might be the case only under physiological conditions, but not during ischemia. A characteristic of the medulla oblongata is that functional excitation occurs in response to ischemia (16). Hence, the possibility remains that different degrees of ischemic excitation might be produced by the same degree of reduction of rCBF under different anesthetics, resulting in a different degree of energy imbalance.

In summary, the present study demonstrated that the anesthetics used during the ischemic insult may affect the extent of the post-ischemic dysfunction of the baroreflex. The vagal component of the baroreflex may be more resistant to 5-min global cerebral ischemia under halothane anesthesia than under barbiturates anesthesia.

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