Effects of Blood Glucose Changes and Physostigmine on Anesthetic Requirements of Halothane in Rats

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Background: Although hyper- and hypoglycemia induce neurophysiologic changes, there have been no reports on the effects of blood glucose changes on anesthetic requirements. This study examined the effects of hyper- and hypoglycemia on the minimum alveolar concentration (MAC) of halothane in rats. In addition, based on a previous finding that the level of brain acetylcholine was reduced during mild hypoglycemia, the authors examined the influence of physostigmine on MAC during hypoglycemia.

Methods: In Sprague-Dawley rats, anesthesia was induced and maintained with halothane in oxygen and air. The MAC was determined by observing the response to tail clamping and tested during mild hypoglycemia (blood glucose level, 60 mg/dl) and hyperglycemia (blood glucose level, 300 and 500 mg/dl) induced by insulin and glucose infusion, respectively (experiment 1). The effects of 0.3 and 1.0 mg/kg physostigmine given intraperitoneally on MAC were examined in rats with mild and severe hypoglycemia (blood glucose level, 60 and 30 mg/dl; experiment 2).

Results: In experiment 1, mild hypoglycemia significantly reduced the MAC of halothane (0.76 ± 0.03%) compared with the control value (0.92 ± 0.04%), but hyperglycemia did not change MAC. In experiment 2, mild and severe hypoglycemia reduced MAC of halothane in a degree-dependent manner. Physostigmine (1 mg/kg) had no effect on MAC regardless of blood glucose level, but 0.3 mg/kg reduced MAC.

Conclusions: Hypoglycemia reduced anesthetic requirements in a degree-dependent manner, whereas hyperglycemia had no effects. Although the mechanism of hypoglycemia MAC reduction needs further investigations, physostigmine studies suggest that this may not be related to inhibition of cholinergic transmission. (Key words: Anesthetics, volatile; halothane. Hyperglycemia. Hypoglycemia. Physostigmine. Potency: anesthetic; minimum alveolar concentration.)

CHANGES in blood and brain glucose concentration induce neurophysiologic and neurochemical changes in the brain.1-5 During hypoglycemia, these changes are clinically shown as somnolence and lethargy, slow waves on the electroencephalogram (EEG), and eventual unconsciousness.1-5 Thus hypoglycemia could affect anesthetic requirements. Hyperglycemia by itself7 and diabetes-induced hyperglycemia6,7 have also been reported to affect brain function. The anesthetic requirement of inhaled anesthetics was reported to decrease in streptozotocin-induced diabetic rats.8 However, neither the effect of hypoglycemia nor hyperglycemia on the minimum alveolar concentration (MAC) have been investigated.

Under hypoglycemia of 30-45 mg/dl blood glucose, in which slow waves in EEG are observed, acetylcholine in the brain has been reported to be significantly reduced, probably due to impaired incorporation from choline to acetylcholine.1-2,9 At this level of moderate hypoglycemia, decreases in amino acid neurotransmitters or failure of ion transport have not developed.1,2 Those events are appreciable when energy failure ensues with blood glucose levels less than 20 mg/dl.1,2,10 Acetylcholine thus might play an important role in changes in the brain function during hypoglycemia, particularly mild to moderate hypoglycemia.

The present studies were designed to determine (1) whether hyperglycemia and hypoglycemia affect MAC and (2) whether physostigmine, which has been confirmed to increase brain acetylcholine in experimental animals,11-15 alters the effects of hypoglycemia on MAC if hypoglycemia changes MAC. The experiments were performed in rats anesthetized with halothane.
EFFECTS OF BLOOD GLUCOSE CHANGES AND PHYSOSTIGMINE ON MAC

Materials and Methods

The protocol was approved by our animal investigation committee.

Animals

Male Sprague-Dawley rats of similar age (9–11 weeks) and weight (300–350 g) were maintained with free access to food and water until the day of the study. All experiments were done between 12 and 6 PM.

Anesthetic Management and Preparation

Anesthesia was induced by inhalation of halothane in a transparent container. After induction, the animal’s airway was secured by a tracheostomy. The endotracheal catheter was connected to a Mapleson D circuit, and the lungs were mechanically ventilated with 1.0–1.5% halothane in oxygen and air (FiO2 0.25−0.5). The femoral artery and vein were cannulated to allow for arterial blood gas sampling and hydration with lactated Ringer’s solution. Rectal temperature was continuously monitored and maintained at 37°C with a heating pad.

Two-channel EEGs were obtained from needles inserted into the midline of the scalp and a reference electrode inserted into the frontalis during the experiments (Neuropack 8, Nihon Koden, Tokyo, Japan). Changes in EEG were observed in simultaneous recordings of the waves and in a compressed spectral array with 95% edge frequency.

Minimum Alveolar Concentration with Changing Blood Glucose (Experiment 1)

Forty-eight rats were randomly assigned to one of five groups. Figure 1A shows the experimental protocol for experiment 1. The control group was infused with 10 ml·kg⁻¹·h⁻¹ lactated Ringer’s solution. Mild hypoglycemia (blood glucose level, 60 mg/dl) and normoglycemia (blood glucose level, 80–100 mg/dl) were induced by insulin in lactated Ringer’s solution (Actrapid, Novo Nordisk, Denmark). An intravenous bolus of 0.1 U was given to the animals followed by intravenous infusion of 0.1–0.2 U/h and 0.05–0.1 U/h in mild hypoglycemia and normoglycemia groups, respectively. Desired blood glucose concentrations were subsequently achieved by adjusting the concentration of insulin in solution. Mild and severe hyperglycemia (blood glucose level, 300 mg/dl and 500 mg/dl, respectively) were induced by infusing lactated Ringer’s solution containing 20% and 30–50% glucose, respectively. The infusions containing insulin or glucose were initiated at the completion of surgical preparation. End-tidal gas samples were obtained with an air-tight glass syringe through a 26-gauge needle inserted into a tracheal tube during 15–20 expired breaths.14 Halothane concentrations were analyzed using an infrared analyzer (Normac; Datex, Helsinki, Finland). A two-point calibration was performed before the study.

Sixty minutes after the start of lactated Ringer’s solution infusion with insulin or glucose, MAC was determined using the method described previously.14,15 Briefly, administration of halothane was adjusted in steps of 0.1%, and a stable end-tidal concentration for 15–30 min was obtained before stimulation. Noxious stimulation was applied with a 6-inch hemostat to the middle third of the tail for 60 s at the full ratchet position. The criteria for positive movements included purposeful movements of either the head or the four extremities. When the animal had a positive response, the halothane concentration was increased; when there was no response, the concentration was decreased until movement was observed. When the interval was bracketed by positive and negative responses, the midpoint of the interval was tested. The MAC of halothane for each rat was defined as being midway between the highest end-tidal concentration with a positive response.
Table 1. Blood Glucose, MAC of Halothane, and 95% Confidence Interval (CI) for MAC in Experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Blood Glucose (mg·dl⁻¹)</th>
<th>MAC of Halothane (vol %)</th>
<th>95% CI for MAC (vol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>125 ± 4</td>
<td>0.92 ± 0.04</td>
<td>0.82–1.01</td>
</tr>
<tr>
<td>Mild hypoglycemia</td>
<td>9</td>
<td>59 ± 2</td>
<td>0.76 ± 0.03</td>
<td>0.70–0.82</td>
</tr>
<tr>
<td>Normoglycemia</td>
<td>9</td>
<td>91 ± 2</td>
<td>0.88 ± 0.03</td>
<td>0.81–0.96</td>
</tr>
<tr>
<td>Mild hyperglycemia</td>
<td>11</td>
<td>295 ± 6</td>
<td>0.90 ± 0.02</td>
<td>0.85–0.95</td>
</tr>
<tr>
<td>Severe hyperglycemia</td>
<td>9</td>
<td>500 ± 36</td>
<td>0.84 ± 0.04</td>
<td>0.74–0.94</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

*P < 0.05 versus control using one-way ANOVA followed by Student-Neuman-Keuls test.

and the lowest end-tidal concentration with a negative response.

Blood glucose was measured with the glucose oxidase-peroxidase method (Reflolux II M; Boehringer-Mannheim, Tokyo, Japan) three times during the measurements of MAC (fig. 1). Arterial blood gas tensions and pH were measured at the completion of the MAC measurements.

Minimum Alveolar Concentration with Physostigmine and Hypoglycemia (Experiment 2)

Eighty rats were randomly assigned to one of nine groups according to blood glucose (control, mild hypoglycemia, severe hypoglycemia) and physostigmine dosage (0.0, 0.3, 1.0 mg/kg). Figure 1B shows the protocol for experiment 2. Physostigmine salicylate (Sigma Chemical Co., St. Louis; 0.3 or 1.0 mg/kg) was administered intraperitoneally after 30 min of lactated Ringer’s infusion with or without insulin. Mild hypoglycemia (blood glucose, 60 mg/dl) was induced as described in experiment 1, and severe hypoglycemia (blood glucose, 30 mg/dl) was induced by an insulin infusion of 0.1–0.2 U/h after a 0.2-U bolus injection. The MAC was determined 30 min after physostigmine administration.

Statistical Analysis

Data are presented as means ± SE. Differences in MAC in experiment 1 and the other physiologic variables in both experiments 1 and 2 were compared using one-way analysis of variance. Differences in MAC in experiment 2 were compared using two-way analysis of variance. The Student-Neuman-Keuls procedure was used for post hoc comparisons. The significance of the interaction between blood glucose level and physostigmine dosage was examined in a general linear model for two-way analysis of variance (SAS Proc GLM; SAS Institute, Cary, NC). Probability levels less than 0.05 were considered significant.

Results

In experiment 1, the partial pressure of oxygen ranged from 113–193 mmHg, the partial pressure of carbon dioxide ranged from 36–59 mmHg, arterial pH was 7.39–7.46, and rectal temperatures were 36.8–37.2°C. In experiment 2, partial pressure of oxygen values were 101–146 mmHg, partial pressure of carbon dioxide values were 33–40 mmHg, arterial pH values were 7.41–7.48, and rectal temperatures were 36.8–37.5°C. No two groups in each experiment were significantly different for any of these values.

Effects of Hyperglycemia and Hypoglycemia on Minimum Alveolar Concentration

In experiment 1, the MAC of halothane was reduced by approximately 20% in the rats with mild hypoglycemia compared with the control value (P < 0.05, table 1). The MAC during normoglycemia with insulin infusion was not statistically different from the control value. There were no significant differences in MAC between the control and mild or severe hyperglycemia.

Effects of Hypoglycemia and Physostigmine on Minimum Alveolar Concentration

Table 2 shows the data for blood glucose and MAC in each group in experiment 2. There were statistically significant effects of blood glucose and physostigmine on MAC (P = 0.0001 and P = 0.026, respectively, by two-way analysis of variance). However, there was no apparent overall interaction between hypoglycemia and physostigmine. In the groups with physostigmine levels of 0.0 and 1.0 mg/kg, there were significant degree-dependent effects of hypoglycemia on MAC. However, the degree-dependent effect of hypoglycemia was not found in the groups with 0.3 mg/kg physostigmine. The control MAC with 0.3 mg/kg physostigmine was significantly lower than the control with 0.0 mg/dl physostigmine.
Table 2. Blood Glucose, MAC of Halothane, and 95% Confidence Interval (CI) for MAC in Experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Glucose (mg·dl⁻¹)</th>
<th>N</th>
<th>MAC of Halothane (vol %)</th>
<th>95% CI for MAC (vol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 Control</td>
<td>122 ± 5</td>
<td>11</td>
<td>0.93 ± 0.04</td>
<td>0.85–1.00</td>
</tr>
<tr>
<td>Mild hypoglycemia</td>
<td>59 ± 2</td>
<td>8</td>
<td>0.77 ± 0.03*</td>
<td>0.71–0.84</td>
</tr>
<tr>
<td>Severe hypoglycemia</td>
<td>32 ± 2</td>
<td>8</td>
<td>0.51 ± 0.04†</td>
<td>0.40–0.61</td>
</tr>
<tr>
<td>0.3 Control</td>
<td>133 ± 6</td>
<td>8</td>
<td>0.74 ± 0.04‡</td>
<td>0.65–0.84</td>
</tr>
<tr>
<td>Mild hypoglycemia</td>
<td>49 ± 3</td>
<td>8</td>
<td>0.66 ± 0.03</td>
<td>0.59–0.73</td>
</tr>
<tr>
<td>Severe hypoglycemia</td>
<td>29 ± 1</td>
<td>9</td>
<td>0.51 ± 0.06</td>
<td>0.38–0.64</td>
</tr>
<tr>
<td>1.0 Control</td>
<td>145 ± 4</td>
<td>11</td>
<td>0.88 ± 0.03</td>
<td>0.81–0.94</td>
</tr>
<tr>
<td>Mild hypoglycemia</td>
<td>55 ± 2</td>
<td>9</td>
<td>0.72 ± 0.05*</td>
<td>0.61–0.83</td>
</tr>
<tr>
<td>Severe hypoglycemia</td>
<td>30 ± 2</td>
<td>8</td>
<td>0.53 ± 0.07†</td>
<td>0.36–0.71</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
*P < 0.05 versus control in the same physostigmine dose group.
†P < 0.05 versus mild hypoglycemia in the same physostigmine dose group.
‡P < 0.05 versus control of physostigmine 0.0 mg·kg⁻¹ using two-way ANOVA followed by Student-Neuman-Keuls test.

Figure 2 summarizes the data obtained from experiments 1 and 2 and shows the relation of MAC and blood glucose.

Effects of Hypoglycemia and Physostigmine on the Electric Activity of the Brain

Hypoglycemia increased low frequency and high amplitude components in two-channel EEG recording under end-tidal halothane 1.0%. Physostigmine at 0.3 and 1.0 mg/kg doses was accompanied by increases in high-frequency and low-amplitude components in EEG during mild and severe hypoglycemia (fig. 3). Clinical symptoms of awaking, such as chewing and increasing spontaneous breathing, were observed after physostigmine doses of 0.3 and 1.0 mg/kg during hypoglycemia. In the control rats, similar EEG changes and clinical symptoms were also observed after physostigmine.

Discussion

Minimum Alveolar Concentration and Blood Glucose Changes

We have shown that insulin-induced acute hypoglycemia reduced the MAC of halothane in rats in a degree-

![Graph](image)

Fig. 2. The MAC of halothane and blood glucose in the rats receiving 0.0, 0.3, and 1.0 mg/kg physostigmine. The data were combined for experiments 1 and 2.

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dependent manner. The MAC of halothane was decreased by 45% in the rats with severe hypoglycemia (blood glucose, 32 mg/dl; experiment 2) and by approximately 20% in rats with mild hypoglycemia (blood glucose, 59 mg/dl; experiments 1 and 2). However, lowering the blood glucose value to normoglycemic levels (91 ± 2 mg/dl) by insulin infusion did not affect the MAC of halothane. Mild and severe hyperglycemia induced by glucose infusion did not alter the MAC of halothane.

Because the brain normally metabolizes glucose exclusively, decreases in the cerebral glucose level result in cerebral dysfunction, which is clinically observed as somnolence and coma. In the rats with mild hypoglycemia (experiments 1 and 2), blood glucose was mildly decreased and ranged from 51 - 69 mg/dl. At this level of mild hypoglycemia, energy state, neurotransmitter metabolism, ion balance in the brain, and electric activity of the brain have been reported to remain normal. However, hypoglycemic symptoms, such as somnolence and lethargy, are recognized in humans. Our present finding that the MAC was significantly reduced in rats during mild hypoglycemia is consistent with the clinical symptoms, but there have been no reported neurological changes in the brain suggesting decreases in MAC at this level of mild hypoglycemia.

Effects of acute hyperglycemia on nociceptive responses remain controversial. Glucose-induced hyperglycemic rats had a significant increase in pain threshold using hot plate testing, but a decrease in pain threshold assessed with tail flick latency was reported in another study. The effect of acute hyperglycemia on a nociceptive threshold is controversial in humans as well. In streptozotocin-induced diabetic rats, acetylcholine content and synthesis in striatum have been reported to decrease at 7 weeks of diabetes with blood glucose levels of approximately 650 mg/dl. Brian et al. reported that insulin-reversable MAC reduction in streptozotocin-induced diabetic rats with blood glucose levels of 449 mg/dl. However, a similar level of acute hyperglycemia did not change the MAC of halothane in this study. Chronic hyperglycemia, diabetes-associated changes, or both may be necessary to alter nociceptive responses.

Insulin has been considered not to affect central nervous system function. However, several lines of evidence have indicated that insulin enters the cerebrospinal fluid rapidly and may modulate neurotransmission in the brain. Probably insulin inhibits neural electric activities by altering membrane potentials. Although the present study was not designed to investigate the effects of insulin on MAC, the MAC of halothane did not change in the rats during normoglycemia (91 ± 2 mg/dl) induced by insulin infusion. In streptozotocin-induced diabetic rats, insulin treatment restored the MAC-reducing effects of diabetes. The effects of insulin on MAC, if any, might not be as significant as those of changing blood glucose.

Effects of Hypoglycemia and Physostigmine on Minimum Alveolar Concentration

In the present study, we hypothesized that physostigmine reversed hypoglycemic MAC reduction by increasing brain acetylcholine based on the following previous findings. First, when blood glucose was decreased progressively to 30 - 45 mg/dl, acetylcholine concentrations in the cortex and striatum were significantly diminished in rats. One of the mechanisms suggested for the decrease was impaired incorporation of choline into acetylcholine. At this level of hypoglycemia, however, other neurotransmitters in the brain, such as glutamate and GABA, have been reported to be normally metabolized. Thus it is conceivable that the reduction of MAC during mild to moderate hypoglycemia might attribute to decreases in acetylcholine or to increases in choline in the brain.

Second, several studies have confirmed that physostigmine, an acetylcholinesterase inhibitor, increased acetylcholine in the brain. Romano and Shih reported that 0.65 mg/kg physostigmine given intraperitoneally increased acetylcholine in the cortex, hippocampus, and striatum in rats. Saclens et al. and Kato et al. also showed that 0.5 mg/kg physostigmine increased acetylcholine in the brain stem and cortex, respectively. Although it is not known to what extent physostigmine increases acetylcholine in the brain during hypoglycemia, observed awakening patterns in the EEG after physostigmine injection during hypoglycemia indicated that physostigmine altered brain activities during hypoglycemia.

In this study, a low dose of physostigmine reduced MAC of halothane, and a higher dose did not change the MAC. A systemically administered small dose of physostigmine, such as 0.1 - 0.17 mg/kg in rats and 0.1 mg/kg in mice, has been shown to have antinoceptive activities, which is consistent with the MAC-reducing effects of low-dose physostigmine in this study. On the other hand, 1 mg/kg physostigmine has been reported to increase plasma and brain catecholamines in

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The observed difference in the effects on MAC between low-dose and high-dose physostigmine thus might be attributed to the dose-dependent changes in its actions at multiple sites.21-24 Because physostigmine recently was reported to bind to neuronal nicotinic acet-
choline receptors,25,26 the effects of physostigmine on the central nervous system and on anesthetic re-
quirements need to be further elucidated.

Cholinergic neurotransmission in the brain has been suggested to play an important role in the medi-
ation of general anesthesia.27 The effect of physostig-
ine per se on anesthetic requirement was tested in the previous studies.27,28 Physostigmine at a dose of 0.25 mg/kg given intraperitoneally was reported to increase MAC of isoflurane by 14% in rats.27 In dogs, Horrigan29 reported that 0.004 - 4.0 mg/kg physostigmine given intravenously first increased and then decreased anesthetic requirements of halothane. However, Horrigan29 determined the acute increase in anesthetic requirements only by observing signs of arousal, but not by measuring MAC. In our rat model, there was a significant difference between MAC of halothane determined 15 - 45 min after physostigmine injection and that determined later on (unpublished data), suggesting physostigmine does not have a biphasic effect on MAC. Our present findings imply that clinical arousa responses and awake patterns in EEG after administration of physostigmine do not indicate that animals need more anesthetics in terms of mobile responses, such as MAC.

In summary, we found that insulin-induced acute hypoglycemia significantly reduced the MAC of halothane in a degree-dependent manner, but acute hyperglycemia did not affect the MAC. Physostigmine appeared not to have an interaction with hypoglycemia on MAC, suggesting hypoglycemic MAC reduction may not be related to inhibition of cholinergic transmission.

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