Comparative Effects of Propofol and Halothane on Outcome from Temporary Middle Cerebral Artery Occlusion in the Rat

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Because propofol has cerebral effects similar to those observed for barbiturates, we postulated that it too might offer protection against a focal cerebral ischemic insult. Spontaneously hypertensive male rats were anesthetized with halothane (50% O2/balance N2), and their tracheas were intubated and their lungs mechanically ventilated. A right subtemporal craniectomy was performed and a 10-0 suture placed around the middle cerebral artery. Rats were then randomly assigned to one of two anesthetic groups. In one half of the rats (n = 14), the inspired halothane concentration was reduced to 0.5–0.7%. In the remainder (n = 14), halothane was discontinued, and an intravenous infusion of 1% propofol was given in doses sufficient to produce and maintain electroencephalographic burst suppression. The middle cerebral artery was then reversibly ligated for 2 h while pericranial temperature was maintained at 37.0 ± 0.1°C (mean ± SD). After the ligation was removed and reperfusion confirmed, all rats were allowed to recover for 96 h. Neurologic evaluations were performed at both 24 and 96 h posts ischemia. The rats were then killed and the brains removed, frozen, sectioned, and stained with nitro blue tetrazolium. Infarct volume was determined by computerized planimetry. Physiologic values were similar between anesthetic groups, although plasma glucose was significantly greater during ischemia in the halothane group (125 ± 25 vs. 83 ± 8 mg/dl, P < 0.001). At both 24 and 96 h posts ischemia, neurologic deficits were mild but without a difference between groups. Neurologic scores at 96 h posts ischemia correlated with cerebral infarct volume (r = 0.49, P < 0.02). There was no statistically significant difference in infarct volume between groups. Our results indicate no protective difference during focal cerebral ischemia between these two agents at the anesthetic levels studied. (Key words: Anesthetics, intravenous: propofol. Anesthesiology, volatile: halothane. Brain: infarction; ischemia; neurology. Animal: rat.)

WIDE CLINICAL APPLICATION has been found for propofol, an intravenous anesthetic. Besides allowing prompt emergence from anesthesia and a reduced incidence of postoperative nausea and vomiting,1 propofol has several properties that historically appealed to anesthesiologists. For example, propofol reduces cerebral metabolic rate (CMR) in a dose-dependent manner.2 Maximal reduction of CMR coincides with the appearance of an electroencephalographic (EEG) pattern of burst suppression.3 Such properties would presumably allow EEG monitoring to guide dosage regimens intended to achieve maximal benefit from CMR reduction. Propofol also reduces CBF in a dose-dependent manner (i.e., parallel to the reduction in CMR) while apparently leaving autoregulation intact.4,5 Finally, the administration of propofol to humans with intracranial hypertension has been associated with a reduction in intracranial pressure.6–8

Despite this knowledge, little is known regarding the effect of propofol on outcome from cerebral ischemic events. Because propofol may appropriately find use as an anesthetic agent for patients undergoing some neurosurgical procedures, the following study was designed to compare the effects of propofol anesthesia with those of halothane anesthesia on the neurologic and neuropathologic outcome from temporary middle cerebral artery (MCA) occlusion in the rat.

Materials and Methods

This study was approved by the University of Iowa Animal Care and Use Committee. At 13–14 weeks of age, spontaneously hypertensive male rats (Harlan, Indianapolis, IN) were fasted for 12–16 h prior to the experiment but allowed free access to water. All animals were weighted and then anesthetized with 3–4% halothane in 50% O2/balance N2. After tracheal intubation, the lungs were mechanically ventilated to achieve normocapnia. For surgical procedures, the delivered halothane concentration was adjusted to 1.2–1.5% in 50% O2/balance N2. The tail artery was catheterized for measurement of blood pressure and blood gases. Via a transverse neck incision, the left internal jugular vein was cannulated with a Silastic catheter to provide venous access. A right subtemporal craniectomy was then performed. Aided by an operating microscope, the dura and arachnoid were opened, and the right MCA was identified. The MCA was loosely encircled with a 10-0 suture (Ethilon monofilament nylon taper 2870 G 3/8 Circle BV75-8 needle, Ethicon, Inc., Somerville, NJ) just distal to the lenticulostriate artery.9,10 Bilateral EEG needle electrodes were percutaneously placed adjacent to the parietal bone bilaterally and a reference electrode was placed subcutaneously over the right shoulder. Pancuronium was given as a single 0.4-mg intravenous bolus for control of ventilation. Following preparation, all rats received 50 IU heparin intravenously. During surgical preparation, oropharyngeal temperature was regulated by servomecha-
nism at 37.0 ± 0.1°C with a heat lamp. One hour was allowed to complete the above tasks.

Rats were then randomly assigned to one of two experimental groups. In the first group (propofol, n = 14), halothane was discontinued, and an intravenous infusion of 1% propofol (Diprivan™, Stuart Pharmaceuticals, Wilmington, Delaware) was begun. The initial infusion rate (≈ 2 mg·kg⁻¹·min⁻¹) was adjusted to produce an EEG pattern of burst suppression (interburst interval 15–30 s) within 20 min. This EEG endpoint was maintained throughout the remainder of the experiment (until emergence from anesthesia was begun), requiring an infusion rate of ≈ 1.0–1.5 mg·kg⁻¹·min⁻¹. In the second group (halothane, n = 14), halothane anesthesia was continued. Pilot studies had indicated that deep propofol anesthesia would result in a mean arterial pressure (MAP) of 140–160 mmHg in this strain of rat. Accordingly, to maintain MAP comparable between the two anesthetic groups, the halothane concentration was reduced to the range of 0.5–0.7% inspired, after MCA occlusion.

Thirty minutes after onset of propofol infusion (or an identical waiting interval in the halothane group), the MCA was occluded and a timer begun. A single-pass instrument tie of the 10-0 suture provided occlusion but allowed later release and reperfusion of the MCA. Occlusion was verified by a Blanching of the vessel distal to the ligature, as observed through the operating microscope. A 23-G needle thermistor (model 524, Yellow Springs Instruments) was placed immediately adjacent to the craniectomy defect. The wound was then loosely closed with suture, and pericranial temperature thenceforth was regulated by servomechanism with a heat lamp at 37.0 ± 0.1°C. Arterial blood gases were measured immediately before and at 30-min intervals after MCA ligation. Hematocrit and plasma glucose concentration were measured after 60 min of ischemia.

After 2 h, the wound was reopened, the thermistor removed, and the MCA ligature released. The MCA could be seen to reexpand its distal portion and regain a normal contour. The temporals and skin overlying the craniectomy were closed in separate layers after hemostasis was assured. During surgical closure and emergence from anesthesia, oropharyngeal temperature was regulated by servomechanism to 37.0 ± 0.1°C. In the halothane group, the inspired halothane concentration was increased to 0.8–1.0% during surgical closure. The tail artery and central venous catheters were removed. In the propofol group, propofol infusion was discontinued after release of the ligature. In the halothane group, halothane anesthesia was maintained for an additional 30 min, to produce similar intervals from reperfusion to awakening in the two groups. In both groups, the trachea was extubated when spontaneous ventilation resumed. Animals were placed in a room-temperature, Plexiglas chamber containing 50% N₂/50% O₂ for 1 h, after which they were returned to their cages for 4 days. Free access to moistened pellet food and water was allowed.

All animals were neurologically evaluated 24 h prior to surgery and at 24 and 96 h afterward on a scale of 0–3, where 0 = no observable deficit; 1 = forelimb flexion; 2 = decreased resistance to lateral push without circling; and 3 = same behavior as 2, with circling.¹¹ Neurologic tests were performed in a dimly lit, quiet room by a single observer who was blinded to the experimental condition of the animal.

After the 96-h neurologic evaluation, the animals were weighed and anesthetized with 4% halothane in O₂. They were then decapitated and the brains removed and frozen at −20°C in 2-methylbutane. With the use of a cryotome, 20-μm-thick coronal sections were taken at 360-μm intervals over the rostral-caudal extent of the infarct. The sections were dried and stained for 30 min with 4.052 g sodium succinate and 0.135 g nitro blue tetrazolium (Sigma) dissolved in 300 ml buffered distilled water (pH = 7.2, 50°C). The slides were then rinsed with saline, dehydrated in graded strengths of ethanol, and cleared with xylene. This process caused normal tissue to stain blue while infarcted tissue remained unstained.¹²

Infarct volume was measured by placing each section under a television camera interfaced with a Digital Microvax II computer and a Gould image analyzer. The image of each section was digitized according to optical density (reflectance) and the data stored as a matrix of pixel units. For each tissue section, the pixel units were calibrated to give values as squared millimeters. The digitized image was then displayed on a video terminal. With the observer blinded to experimental condition, the infarct border was outlined using an operator-controlled cursor, and total infarct area within the ipsilateral hemisphere was determined. Infarct volume (cubic millimeters) was computed as the sum of infarct area multiplied by the 360-μm interval between sections over the extent of the infarct.

Physiologic values as well as infarct volumes were compared between groups at each measurement interval by one-way analysis of variance. Nonparametric neurologic scores were compared between groups using the Mann Whitney U test. Associations between infarct size versus neurologic deficit score were analyzed with the Spearman rank correlation coefficient. Parametric values are presented as mean ± standard deviation. Significance was assumed if P < 0.05.

Results

Physiologic data are presented in table 1. PaO₂, PaCO₂, arterial pH, and MAP were similar between groups prior to MCA ligation. After 60 min of ischemia, MAP,
Table 1. Physiologic Values for the Two Anesthetic Groups

<table>
<thead>
<tr>
<th></th>
<th>Halothane (n = 14)</th>
<th>Propofol (n = 14)</th>
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<tbody>
<tr>
<td>5 min before ischemia onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>140 ± 12</td>
<td>146 ± 9</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.41 ± 0.03</td>
<td>7.39 ± 0.05</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>37.3 ± 2.5</td>
<td>38.5 ± 4.1</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>188 ± 47</td>
<td>187 ± 26</td>
</tr>
<tr>
<td>60 min after ischemia onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>146 ± 13</td>
<td>153 ± 5</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.37 ± 0.04</td>
<td>7.35 ± 0.04</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>40.5 ± 4.2</td>
<td>39.4 ± 4.1</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>161 ± 31</td>
<td>183 ± 21*</td>
</tr>
<tr>
<td>Pericranial temperature (°C)</td>
<td>37.0 ± 0.1</td>
<td>37.0 ± 0.1</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>125 ± 25</td>
<td>85 ± 8*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44 ± 3</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Body weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia (g)</td>
<td>287 ± 11</td>
<td>282 ± 20</td>
</tr>
<tr>
<td>96 h postischemia (g)</td>
<td>277 ± 13</td>
<td>262 ± 23*</td>
</tr>
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Values = mean ± standard deviation.
* P < 0.05.
† P < 0.001.

PaO2, arterial pH, pericranial temperature, and hematocrit were similar between groups. Plasma glucose was greater in the halothane group (125 ± 25 vs. 83 ± 8 mg/dl; P < 0.001), whereas PaO2 was greater in the propofol group (183 ± 21 vs. 161 ± 31 mmHg; P < 0.05). The total dose of propofol required to produce and maintain EEG burst suppression during ischemia was 123 ± 11 mg/kg.

There were no preischemic body weight differences between groups. It is noteworthy that propofol-treated rats consistently resumed feeding during the first few hours of recovery, whereas while rats in the halothane group did not. Nevertheless, at 96 h postischemia, rats in the the propofol group weighed less than those in the halothane group (262 ± 23 vs. 277 ± 13 g; P < 0.05).

One rat in the halothane group and no rats in the propofol group died during the recovery interval. Infarcted tissue extended over the rostral-caudal aspect of the neocortex. Infarcted tissue was also present in the more rostral and lateral aspects of the basal ganglia. Infarct volumes for individual animals are presented in figure 1. Although mean infarct volume was numerically smaller (14%) in the propofol group, there was no significant difference between anesthetic regimens for infarct volume (154 ± 31 mm³ for halothane and 133 ± 65 mm³ for propofol; P = 0.30).

Twenty-four hours before ischemia, rats in both groups were neurologically normal. Neurologic deficit scores assigned at the 24- and 96-h recovery intervals are given in figure 2. No differences for neurologic deficit score were observed between groups at either interval. No significant correlation between neurologic deficit score and infarct volume was seen in either group at 24 h postischemia. At 96 h postischemia, a correlation between these variables was present in the propofol (r = 0.62, P < 0.03) group but not in the halothane (r = 0.25, P = 0.38) group. When values from both groups were pooled, a significant correlation between infarct volume and neurologic deficit score was present at both 24 h (r = 0.46, P < 0.02) and 96 h postischemia (r = 0.49, P < 0.02).

Discussion

In this model of temporary focal cerebral ischemia, propofol failed to significantly alter both neurologic and neuropathologic outcome as compared to halothane anesthesia. The reversible focal ischemia model used in this experiment was chosen for several reasons. First, in a similar experiment using 2 h of MCA occlusion, we documented a 33% reduction in infarct volume in rats anesthetized with methohexital as opposed to halothane.10 Therefore, this model is sensitive enough to detect differences in outcome that are attributable to specific anesthetic agents when halothane anesthesia is used as a control state. Second, halothane, at concentrations of < 1%, causes only modest changes in CMR.13,14 In contrast, deep propofol anesthesia, as used in this experiment, has been reported to reduce CMR by 50–60%.2,3,15 If propofol was to provide cerebral protection by virtue of its ability to substantially reduce CMR, the presumed difference in metabolic rates between these two anesthetic states should have resulted in a better outcome for the

![FIG. 1. Cerebral infarct volumes, for individual rats, as determined 96 h after reperfusion for the two anesthetic groups. There was no statistical difference between groups.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931329/ on 04/01/2017)
propofol group. The absence of such a protective effect yields several questions.

Most relevant physiologic values were similar between groups, with the exception of intrainfarct plasma glucose, which was less in propofol-anesthetized rats. Plasma glucose has consistently been observed to affect outcome from global cerebral ischemia adversely. The relationship between plasma glucose and outcome from focal ischemia is not as clear. Preischemic hyperglycemia has been demonstrated to increase, reduce, or have no effect on infarct size resulting from focal ischemic insults. We do not believe that the difference observed in this experiment was important. In studies in which a glucose effect was observed, differences in plasma glucose were of a considerably greater magnitude than those reported here. Furthermore, the plasma glucose was greater in the halothane-anesthetized rats, which would, if anything, have favored a reduced infarct volume in the propofol group.

Another question concerns the use of halothane anesthesia as the "control" group. Because neurosurgical procedures involving temporary intracranial vascular occlusion (e.g., aneurysm clipping) are performed during general anesthesia, it seems important to determine if commonly administered anesthetic agents pose either a potential benefit or a threat to patients sustaining an ischemic insult. Since some anesthetic must be given, anesthetic agents must be compared against one another to identify relevant differences. Comparison with the awake state seems largely irrelevant. Such reasoning has undoubtedly influenced the design of many experiments, including this one. Furthermore, most laboratory preparations used for the study of cerebral ischemia are surgically invasive and require continued presence of an anesthetic agent throughout the ischemic protocol for humane reasons. The limitation of these arguments is that although little or no outcome difference between propofol and halothane anesthesia was observed in this experiment, it remains a possibility that both agents equally altered outcome as compared to no anesthetic at all. Different, less invasive, models of focal cerebral ischemia, which would allow comparison with the awake state, will need to be used to discount this possibility.

There is reason to believe that "anesthesia" protects. Such evidence comes from a series of studies performed in a rat recovery model of unilateral carotid artery occlusion combined with systemic hypotension. Such an insult produces hemispheric forebrain ischemia. If the "control" group receives 70% N2O/02 only, virtually all anesthetic agents evaluated produce a substantial and essentially equivalent improvement in outcome (e.g., halothane, isoflurane, methohexital, etomidate, and midazolam). Because 70% N2O/02 has only minimal sedative properties in the rat (unpublished personal observation), the "control" groups were essentially awake. The mechanistic basis for these observations remains speculative. However, both increased circulating catecholamines and central sympathetic activity have been associated with worsened ischemic outcome in this model during N2O sedation. It is possible that an increased depth of anesthesia attenuates this response and thus minimizes injury. The relationship between these observations and our experiment is currently speculative. By design, the propofol and halothane groups were hemodynamically similar. We are unaware of a direct comparison between these two agents with respect to adrenergic activity. To the extent that MAP reflects adrenergic stimulation, however, one would predict similar effects on ischemic outcome for the two anesthetic states evaluated.

Several statistical issues also need to be raised regarding the observed infarct volumes. First, the coefficients of variation were considerably different for the two groups (propofol = 0.49; halothane = 0.20). In fact, three of the propofol anesthetized animals had very small infarcts (< 50 mm3), whereas no such cases were observed in the halothane group. With this information, we carefully re-
viewed the experimental data for all animals to verify satisfaction of the experimental protocol. With respect to the physiologic parameters that were monitored and controlled (e.g., $\text{PaO}_2$, MAP, and pericranial temperature), none of the rats could be excluded. In addition, the use of analysis of variance can be questioned if there is an inhomogeneity of variance between comparator groups. A test of homogeneity of variance was performed (Bartlett's test) and indicated that variances for the two infarct groups were not homogeneous ($P < 0.001$). We then performed a natural logarithmic transformation of individual infarct volumes so as to render the variances more similar between groups and then repeated the analysis of variance. Again, no significant differences were seen ($P = 0.30$).

Another statistical question involves the power of this experiment. A 14% reduction of infarct volume was seen in the propofol group, but this failed to reach statistical significance. Given our previous observation that an anesthetic-mediated 33% reduction of infarct volume could be detected by this model using even smaller sample sizes, a power of 0.63 (at $P < 0.05$) was calculated for this experiment, allowing a 37% chance of committing of a type II error. To determine if larger sample sizes might have detected a difference, a computer-simulated increase in sample size was performed by reiterating individual infarct volumes determined in this experiment, thus yielding the same mean $\pm$ SD but a larger sample size. By this method, significance might have been achieved with a sample size of 60 rats per group. Hence, it is possible that some small reduction in infarct volume might have been produced by propofol that was not detected by the sample sizes used in this experiment.

The above considerations are consistent with the following interpretation. First, in this model propofol clearly does not worsen outcome from focal cerebral ischemia when compared to halothane anesthesia. Second, if any added brain protection is present (relative to halothane), the effect is small and would require a much larger study to be identified. Since this model has been shown to be responsive to numerous manipulations influencing infarct volume, including mild hypothermia, methohexitol anesthesia, and duration of MCA occlusion, it would seem reasonable to conclude that propofol has little protective value, even given the limited statistical power calculated. However, because this drug is in widespread use and has other important properties of potential benefit to the neurosurgical patient, the evaluation of propofol as a cerebral protectant against focal ischemia should still be continued in other species and laboratories.

In conclusion, spontaneously hypertensive rats underwent 2 h of reversible MCA occlusion while anesthetized with either propofol or halothane. After 96 h of recovery, no differences in neurologic deficit scores or cerebral infarct volume were observed. This experiment thus failed to identify a significant protective effect of propofol versus halothane. Because CMR presumably was less in the propofol group, this experiment raises further questions concerning the significance of CMR depression as a component of anesthetic-mediated brain protection.

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