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Exaggerated Anesthetic Requirements in the Preferentially Anesthetized Brain

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Background: The brain is assumed to be the site of anesthetic action, but anesthetics have effects elsewhere, such as the spinal cord. A preferentially anesthetized goat brain model was used to determine the importance of anesthetic action in the brain.

Methods: Six goats were anesthetized with isoflurane; after tracheal intubation and insertion of a femoral arterial catheter, bilateral neck dissections were performed to isolate the external carotid arteries and external jugular veins. The ocular arteries were ligated to prevent vertebral blood from entering the carotid system. (Goats do not have direct, significant vertebral artery contributions to the brain, and they lack internal jugular veins.) Control isoflurane minimum alveolar concentration (MAC) was determined using a dew-claw clamp as the painful stimulus. Following this, cranial venous blood was drained into a bubble oxygenator in which an isoflurane vaporizer was placed in line with the gas flow. Oxygenator arterial isoflurane concentration was estimated from the isoflurane partial pressure in the oxygenator exhaust. Isoflurane administration via the lungs was discontinued and the isoflurane partial pressure in the blood delivered via the carotid artery was increased by an amount required to bracket the partial pressures permitting and preventing movement in response to dew-claw stimulation. The native circulation was reestablished and MAC determined again.

Results: Cerebral isoflurane requirements were 1.2 ± 0.3% (mean ± SD) before bypass, increased to 2.9 ± 0.7% during bypass when the brain was preferentially anesthetized, and decreased to 1.3 ± 0.1% after bypass.

Conclusions: The results support the importance of subcortical structures, such as the spinal cord, in the generation of purposeful movement in response to a painful stimulus under general anesthesia. (Key words: Anesthesia: mechanisms. Anesthetics, volatile: isoflurane. Brain isolation: nociception. Potency: minimum alveolar concentration.)

Despite the widespread use of inhaled anesthetics since the mid 19th century, the mechanism and sites of anesthetic action remain unknown. Classically, we have thought of the brain, specifically the cerebral cortex, as the site of anesthetic action, because anesthetics acting there might result in several components of general anesthesia: unconsciousness, amnesia, and immobility in response to a painful stimulus. We know, however, that inhalational anesthetics have significant effects elsewhere, such as the spinal cord.¹,² Inhalational anesthetics have little effect on peripheral nerves³,⁴ but, paradoxically, may sensitize peripheral nociceptors.⁵,⁶ It is unknown whether these effects in the brain, spinal cord, and periphery interact or whether action in the brain is sufficient to produce general anesthesia.

Goats have a unique cerebral circulation that makes them useful for investigations requiring cerebral vascular isolation. The goat's cerebral blood flow arises solely from the external carotid arteries with no contribution from the vertebral arteries, which perfuse the spinal cord no higher than the caudal medulla.⁷-¹⁰ The internal carotid artery is present only as a small intracranial branch of the retrol aminale. Brain venous blood drains via the external jugular vein; the internal jugular vein is absent.¹¹ By draining cranial venous blood into a bubble oxygenator and, via a roller pump, infusing the oxygenated blood into an external carotid artery, the brain and head can be preferentially perfused and anesthetized exclusive of the remainder of the body. The purpose of this study was to determine anesthetic requirements in this model, where we can preferentially anesthetize the intact brain.
Methods and Materials

This study was approved by the Animal Care and Use Committee. Six goats aged 2–4 yr and weighing 48 ± 17 kg (mean ± SD), were anesthetized with isoflurane in oxygen via mask, and the tracheas were intubated. The animals were mechanically ventilated. A stomach tube was placed to drain rumen contents. A catheter was inserted into the femoral artery for measurement of mean arterial pressure (MAP) and determination of arterial blood gases and hematocrit. Thermistors were placed into the cranial vena cava and the nasopharynx for measurement of core and head temperature; these were matched to within approximately 1°C and maintained at 38.1 ± 1.5°C with a heating lamp and, during bypass, with the oxygenator heat exchanger.

Bilateral neck dissections were performed to isolate the carotid and occipital arteries and the external jugular veins. After anticoagulation with heparin (4 mg/kg), a Y cannula was placed in an external jugular vein to permit alternation of cranial venous blood flow between the body and bypass unit (fig. 1). A cannula was placed in the remaining jugular vein for additional venous return to the oxygenator during bypass. A small, superiorly directed catheter was placed in the remaining carotid artery to measure cranial blood pressures. The electroencephalogram (EEG) (model 8-10E, Grass, Quincy, MA) was monitored with bipolar and bifrontal electrodes in three animals.

Following the surgical procedures, the isoflurane minimum alveolar concentration (MAC) was determined. A catheter was placed in the endotracheal tube with the tip near the carina, and alveolar samples were measured with a calibrated Datex 254 agent analyzer (Tewksbury, MA). The end-tidal isoflurane concentration was held constant for 15–20 min, and a 10-inch clamp was applied to a dew-claw and moved vigorously for 1 min. (We previously had determined that this was more painful than a tail clamp.) We specifically excluded a simple reflex withdrawal of the extremity as a positive response; only gross, purposeful movement of the head or another extremity was considered positive. Coughing, straining, chewing, and swallowing were ignored. Depending on the response, the end-tidal isoflurane concentration was increased or decreased 0.2%, was held constant for 15–20 min, and the dew-claw clamp was reapplied. This process was continued until two concentrations were found that just permitted and just prevented movement, respectively. The average of these was MAC.

After control MAC determination, additional isotonic crystalloid was administered (500–1,000 ml) and the bubble oxygenator (B-10, Baxter, Irvine, CA) was primed with 300–500 ml of blood from the goat. Gas flows (95% O₂/5% CO₂) were 3–4 l/min, and an isoflurane vaporizer was placed in line. The oxygenator exhaust was sampled by a calibrated Datex 254 agent analyzer in an air-tight fashion, and the isoflurane partial pressure was used as the measure of the isoflurane arterial concentration in the oxygenator blood. Bypass was initiated with flows of 5–10 ml·kg⁻¹·min⁻¹ after the remaining carotid artery and the occipital arteries had been ligated and the venous blood shunted to the oxygenator. (The occipital arteries anastomose with the vertebral arteries and can maintain cerebral blood flow if not ligated.) Once the reservoir level had stabilized, isoflurane administration was discontinued to the lungs and was maintained only in the head and brain. The lung end-tidal isoflurane concentration decreased to 0.2%; further decreases were not sought because of the concomitant increased waiting time while on bypass. Glucose was infused into the bypass unit to maintain levels >50–60 mg/dl. At this point, isoflurane requirements were measured from the oxygenator exhaust, with a clamp applied to the dew-claw for 1 min as before. Depending on the response, the exhaust isoflurane partial pressure was increased or decreased approximately 10–15% of its value before the clamping, was stabilized for 15–20 min, and the clamp was reapplied. This process was continued until two concentrations were found that just permitted and just prevented movement, respectively. Once isoflurane requirements were determined, isoflurane was reintroduced to the lungs, the native circulation was reestablished, and bypass was terminated. Isoflurane MAC was then redetermined to detect any deterioration.

Arterial blood gases and hematocrit were determined before, during, and after bypass. During bypass, electrolytes (Na⁺, K⁺, Cl⁻) and glucose were measured systematically and from the bypass unit. Additionally, blood gas analysis (arterial and venous) and hematocrit (arterial) were determined on the oxygenator blood.

Data are presented as the mean ± SD. Changes in MAC were evaluated with a repeated measures analysis of variance. P < 0.05 was considered significant.

Results

Control isoflurane requirements were 1.2 ± 0.3%, and increased to 2.9 ± 0.7% when isoflurane was iso-
lated to the head and brain ($P < 0.0001$). With return to the native circulation, isoflurane requirements decreased to $1.3 \pm 0.1\%$.

The animals developed a mild metabolic acidosis that was not progressive (table 1). The hematocrit did not change significantly, and the electrolytes were in the normal range, as was the glucose (table 2).

In one pilot goat and two study goats, after the post-bypass MAC determination, bypass was reinitiated, and the roller pump was stopped; there was abrupt EEG slowing and isoelectricity. In another goat, during bypass conditions, methylene blue was injected systemically; after the animal was killed, the heart and brain were removed. Gross visual inspection revealed intense staining of the heart and barely detectable patchy staining of the cerebral cortex. During bypass, the high isoflurane concentrations in the brain resulted in marked EEG suppression (fig. 2).

Systemic MAP was $88 \pm 31$, $83 \pm 20$, and $64 \pm 15$ mmHg, for the prebypass, bypass, and postbypass periods, respectively; for these periods, cranial MAP was $76 \pm 25$, $48 \pm 14$, and $52 \pm 16$, respectively. Fluids were $2.7 \pm 1.3\text{ l}$, experimental time was $10 \pm 1\text{ h}$, and bypass time was $2.5 \pm 0.4\text{ h}$.
Table 1. Blood Gases and Hematocrit in Six Goats

<table>
<thead>
<tr>
<th></th>
<th>P&lt;sub&gt;0&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt; (mmHg)</th>
<th>P&lt;sub&gt;co2&lt;/sub&gt; (mmHg)</th>
<th>pH</th>
<th>BE (mm)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>354 ± 126</td>
<td>35 ± 8</td>
<td>7.42 ± 0.11</td>
<td>-1 ± 5</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Bypass</td>
<td>310 ± 112</td>
<td>35 ± 7</td>
<td>7.34 ± 0.08</td>
<td>-6 ± 3</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>Post</td>
<td>383 ± 141</td>
<td>36 ± 7</td>
<td>7.32 ± 0.11</td>
<td>-7 ± 4</td>
<td>25 ± 6</td>
</tr>
<tr>
<td><strong>Cranial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bypass (arterial)</td>
<td>506 ± 44</td>
<td>38 ± 4</td>
<td>7.27 ± 0.03</td>
<td>-10 ± 2</td>
<td>25 ± 7</td>
</tr>
<tr>
<td>Bypass (venous)</td>
<td>184 ± 123</td>
<td>45 ± 4</td>
<td>7.21 ± 0.04</td>
<td>-10 ± 2</td>
<td></td>
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</tbody>
</table>

Data are mean ± SD.
BE = base excess; Hct = hematocrit; Systemic = obtained from systemic artery; Pre = value obtained before cranial bypass; Bypass = value obtained during cranial bypass; Post = value obtained after cranial bypass; Cranial = obtained from cranial bypass unit.

**Discussion**

The standard for determining anesthetic requirements is the MAC of an inhaled agent that prevents gross purposeful movement in response to a supramaximal painful stimulus. Because gross purposeful movement is the endpoint, a behavioral response is examined. Because of its simplicity and reproducibility, the MAC concept has remained an important tool for studying anesthetic action.

The brain has been assumed to be the site of anesthetic action, however, our results suggest that subcortical structures are important in the behavioral response to a painful stimulus under general anesthesia. The complexity of spinal cord reflexes is consistent with these results. For example, the wiping reflex in the decerebrate frog demonstrates that the spinal cord can modulate complex purposeful movements in the absence of the forebrain. Likewise, scratching and walking reflexes are preserved in dogs and cats with transected spinal cords. Some brain-dead humans have spontaneous movements that appear purposeful. Further evidence supporting the role of the spinal cord in anesthetic action is that rats with transected spinal cords require a significant amount of anesthesia to suppress purposeful movement. Also, precocilicular decerebration in rats does not alter anesthetic requirements. Although our model is not able to separate the roles of the spinal cord and periphery in the anesthetic process, the paradoxical sensitizing effect of anesthetics on peripheral nociceptors and the lack of effect on peripheral nerves suggest that the spinal cord is involved in the anesthetic process.

Table 2. Electrolytes and Glucose in Five Goats during Cranial Bypass

<table>
<thead>
<tr>
<th></th>
<th>Na&lt;sup&gt;+&lt;/sup&gt; (mm)</th>
<th>K&lt;sup&gt;+&lt;/sup&gt; (mm)</th>
<th>Cl&lt;sup&gt;-&lt;/sup&gt; (mm)</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic</strong></td>
<td>143 ± 2</td>
<td>4.0 ± 0.7</td>
<td>114 ± 2</td>
<td>86 ± 38</td>
</tr>
<tr>
<td><strong>Cranial</strong></td>
<td>144 ± 2</td>
<td>3.8 ± 0.8</td>
<td>116 ± 4</td>
<td>126 ± 80</td>
</tr>
</tbody>
</table>

Data are mean ± SD.
Systemic = obtained from systemic artery; Cranial = obtained from bypass unit.

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Fig. 2. Biparietal electroencephalogram (EEG) in one goat. (A) Prebypass control; isoflurane = 1.4%. The EEG is active. (B) EEG during bypass; isoflurane = 3.7% in brain and 0.2% in body. There are periods of isoelectricity with burst suppression. (C) Return to native circulation, after bypass; isoflurane = 1.4%. The EEG is active, but there is some loss of high-frequency power. Control isoflurane requirement in this animal was 1.2%, and during bypass, brain isoflurane requirement was 3.2%.
cord (or another subcortical structure) is an important site of anesthetic action. Further support for this is the analgesic effect of inhalational anesthetics on the spinal cord. Our findings have important implications because the MAC concept has been used extensively as a tool to investigate mechanisms of anesthesia. Many of the factors that alter anesthetic requirements and MAC have been assumed to have their action in the brain; however, our results suggest these factors may be operant in subcortical structures.

In addition, our results may explain why monitoring cortical function (EEG) fails to reliably predict anesthetic depth. The EEG in isoflurane anesthetized rats may be isoelectric, or nearly so, and yet purposeful movement may occur following a painful stimulus. Hypothermia eliminates anesthetic requirements at about 20°C, a temperature at which marked EEG slowing occurs, indicating that purposeful movement (in response to pain) can occur when the EEG is profoundly depressed. In thiorpental-anesthetized humans, purposeful movement may occur despite an isoelectric EEG. Thus, these studies corroborate our results, indicating that subcortical structures are important in the generation of purposeful movement in response to a painful stimulus under general anesthesia.

It is possible that our results can be explained by inadequate isolation, with subsequent mixing of systemic, isoflurane-poor blood with cranial blood. This is unlikely for the following reasons:

1. The marked EEG suppression and isoelectricity associated with zero pump flow indicate that there is insufficient cerebral blood flow from collaterals to maintain cell function. There may be some blood flow despite an isoelectric EEG, but not enough to greatly decrease the cranial isoflurane concentration, based on normal 60–80 ml·100 g⁻¹·min⁻¹ goat cerebral blood flow and the <10–15 ml·100 g⁻¹·min⁻¹ blood flow required to alter the EEG during isoflurane anesthesia.

2. Methylene blue injected into one goat demonstrated virtually no cerebral staining.

3. Mixing of venous blood (i.e., drainage of cranial blood into the systemic circulation, or vice versa) seems unlikely because the reservoir level remained stable. Also, in a pilot study, we stopped the heart while maintaining cerebral blood flow for 1.5 h, and the reservoir level was stable, indicating that blood was not leaving or entering the cranial circulation via collateral channels.

We used the partial pressure of isoflurane in the oxygenator exhaust as a representation of isoflurane partial pressure in the oxygenator arterial blood. Nussmeier et al. found that these correlate reasonably well, although they did not achieve partial pressures as high as those in our study; however, the EEG during bypass was clearly depressed, consistent with high isoflurane concentrations. We cannot exclude the possibility that we measured concentrations that were higher than those in the arterial blood, but any difference is likely to be much smaller than the change in isoflurane requirements. Because the gas flow to the oxygenator greatly exceeded the blood flow, we would expect the blood to equilibrate quickly.

The nonpulsatile, nonphysiologic nature of bypass itself would not explain our results. We previously have shown that bypass does not alter MAC. Also, in one goat, we isolated the cerebral circulation and initiated bypass but maintained isoflurane in both torso and head. Under these circumstances, MAC did not change (data not reported).

Obviously, the brain is important in the production of anesthesia. We presume that amnesia and unconsciousness occur as a result of anesthetic action in the brain. The concentration needed for amnesia and unconsciousness is approximately 25–40% of that needed to suppress purposeful movement. This suggests that areas of the brain associated with memory and unconsciousness are more sensitive to inhaled anesthetics than areas associated with purposeful movement, that anesthetic action in the spinal cord is important to suppression of purposeful movement during pain, or both. Further, although the amount of anesthesia needed in the brain is increased when there is little anesthetic in the body, nonetheless, this amount suppressed purposeful movement. This is perhaps related to supraspinal pain inhibition, as demonstrated by Komatsu et al. In their study, nitrous oxide exerted its analgesic effect by activating an inhibitory system. Alternatively, the high isoflurane concentration may depress the brain to an extent seen in deep coma (e.g., functional decerebration) and thereby suppress purposeful movement following a painful stimulus. This is akin to severe brain damage or high spinal cord section, with subsequent "spinal shock."

In summary, our results indicate that subcortical structures modulate movement in response to painful stimuli during general anesthesia. Different endpoints (unconsciousness vs. movement during anesthesia) may be associated with different sites of anesthetic ac-
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tion. Therefore, investigations into mechanisms of anesthesia should consider the sites of anesthetic action as well as the endpoints to determine anesthetic effect.

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