Effects of isoflurane anesthesia on cerebrovascular autoregulation in horses

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Objective—To test a hypothesis predicting that isoflurane would interfere with cerebrovascular autoregulation in horses and to evaluate whether increased mean arterial blood pressure (MAP) would increase cerebral blood flow and intracranial pressure (ICP) during isoflurane anesthesia.

Animals—6 healthy adult horses.

Procedures—Horses were anesthetized with isoflurane at a constant end-tidal concentration sufficient to maintain MAP at 60 mm Hg. The facial, carotid, and dorsal metatarsal arteries were catheterized for blood sample collection and pressure measurements. A subarachnoid transducer was used to measure ICP. Fluorescent microspheres were injected through a left ventricular catheter during MAP conditions of 60 mm Hg, and blood samples were collected. This process was repeated with different-colored microspheres at the same isoflurane concentration during MAP conditions of 80 and 100 mm Hg achieved with IV administration of dobutamine. Central nervous system tissue samples were obtained after euthanasia to quantify fluorescence and calculate blood flow.

Results—Increased MAP did not increase ICP or blood flow in any of the brain tissues examined. However, values for blood flow were low for all tested brain regions except the pons and cerebellum. Spinal cord blood flow was significantly decreased at the highest MAP.

In an earlier study, limited data suggested that severe hypotension markedly reduces cerebral perfusion in horses anesthetized with isoflurane. Dobutamine hydrochloride, a β-adrenergic receptor agonist, is routinely used during equine anesthesia to manage hypotension and maintain organ perfusion. However, it is unknown whether dobutamine effectively increases CBF in anesthetized horses. If autoregulation is intact, then CVR varies inversely with CPP to keep CBF constant. If autoregulation is not intact, it is possible that increased MAP may cause increased CBF velocity without increasing CBV (thereby without increasing ICP). Alternatively, increased MAP may increase CBV, and increased ICP may lessen the effects of dobutamine on CPP and CBF.

We hypothesized that isoflurane anesthesia would interfere with cerebrovascular autoregulation in horses and that increased cardiac output and MAP would result in increased perfusion throughout the brain and spinal cord of isoflurane-anesthetized horses.

Materials and Methods

Animals—Six horses (4 mares and 2 geldings; 4 Thoroughbreds, 1 Quarter Horse, and 1 Standardbred) were included in the study. Mean ± SD age of the horses was 10 ± 8 years, and mean ± SD weight was 478 ± 18 kg. All horses were determined to be healthy on the basis of a physical examination by a veterinarian and had no evidence of cardiovascular, respiratory, or neurologic disease. The study protocol was approved by the Institutional Animal Care and Use Committee at the University of California-Davis.

Anesthetic protocol—Food was withheld for 12 hours prior to anesthesia, but water was available ad libitum. Horses were restrained on a hydraulic tilt table without preanesthetic medication. Anesthesia was induced with isoflurane in oxygen delivered via face mask. A cuffed 30-mm endotracheal tube was placed and connected to a large-animal anesthetic circuit with an ascending bellows ventilator. The anesthetic gas mixture was delivered by use of an agent-specific, out-of-circuit vaporizer. Horses were positioned in right lateral recumbency, horizontal to the ground on the padded table. After hair was clipped and the skin was aseptically prepared, a 14-gauge IV catheter was placed in the right jugular vein, and lactated Ringer's solution was administered at a rate of 3 mL/kg/h to replace insensible and urinary fluid losses.

Instrumentation and response measurement—Sites for the percutaneous placement of remaining catheters were prepared as described for the fluid administration site. For arterial pressure measurements and sample collection, a 20-gauge, 5-cm catheter was percutaneously placed in the left ascending facial artery and connected via low-compliance, saline-filled tubing to a transducer that was calibrated by use of a water manometer and checked for linear responsiveness over the measurement range. A strain-gauge tip, ICP transducer was surgically placed via a left parietal craniotomy into the subarachnoid space by use of a published technique and was connected to a digital display. The jugular veins were briefly occluded bilaterally to effect a 4- to 5-mm Hg increase in ICP within 15 to 20 seconds as a test of transducer responsiveness. Transducer calibration was evaluated after each experiment by measurement of pressure within a graduated cylinder filled with distilled water.

Blood was collected anaerobically from the facial artery and was immediately analyzed by use of an automated blood gas machine that was calibrated prior to and during each experiment. End-tidal isoflurane and CO₂ concentrations were measured by use of dedicated infrared analyzers that were calibrated with multiple certified gas standards encompassing the range of measured values.

Data collection—Instrumentation was completed within 1 hour after anesthetic induction. Horses were then mechanically ventilated to a peak inspiratory pressure of 20 cm H₂O at a frequency sufficient to maintain PaCO₂ between 40 and 50 mm Hg. The end-tidal isoflurane concentration was adjusted to maintain a constant MAP of 60 mm Hg. This end-tidal concentration was then kept constant for the remainder of the experiment.

After 30 minutes of anesthesia at the constant end-tidal concentration of isoflurane sufficient to maintain a MAP of 60 mm Hg, arterial blood samples were obtained and blood pressures and ICP were recorded. A single color of fluorescent microspheres (designated as lemon, orange, or persimmon) was prepared for use in each of 3 blood flow determinations (blood samples and pressures obtained during MAP conditions of 60, 80, and 100 mm Hg [low, medium, and high MAPs, respectively]). Microspheres were prepared by discarding 6.3 mL of supernatant from each of three 20-mL vials that contained microspheres of the same color. The remaining contents were then combined in a sterile
The facial, carotid, and both dorsal metatarsal arterial catheters were connected by extension tubing to matched glass syringes in calibrated withdrawal syringe pumps. After an initial 10-second simultaneous sample collection from each artery, the microsphere suspension in the glass syringe was delivered via constant infusion pump through the left ventricular catheter over a 60-second time period; the catheter was then flushed with heparinized physiologic saline solution (12 mL) over an additional 10 seconds. Reference blood withdrawal samples (50 to 60 mL) were collected at the calibrated rate throughout the microsphere injection period and for 60 seconds following injection. Syringe contents were transferred to separate heparin-containing glass Erlenmeyer flasks, and the syringes were rinsed with copious volumes of physiologic saline solution into the corresponding flasks. Sample flasks were covered and stored in the dark for later processing.

Dobutamine was then administered at a rate sufficient to maintain a medium MAP of 80 mm Hg or a high MAP of 100 mm Hg. The order in which medium and high MAPs were studied was randomized among horses. Once the infusion rate that resulted in the desired MAP was established, a 30-minute equilibration time was allowed before pressures were recorded and arterial blood samples were collected. Fluorescent microspheres of a color different than that used for the low MAP evaluation were prepared, and microsphere injection and reference blood sample collection were performed in a manner identical to that described for the low MAP measurements. The dobutamine infusion rate was then changed to target the third MAP of the study (either medium or high MAP), and physiologic responses were remeasured after a 30-minute equilibration period at that MAP. Upon completion of the experiment, anesthetized horses were euthanatized via IV injection of a saturated KCl solution (30 mL).

**Tissue sample collection and processing**—After each horse was euthanatized, the calvarium was opened and the brain was removed en bloc. The entire thoracolumbar spinal cord was also removed after severing of connective tissue and nerve root attachments.

The cerebrum was digitally separated from the thalamus at the region that grossly corresponded to the internal capsule. A 1.4-cm-thick, transverse slice through the cerebrum at the center of the brain (equivalent to the fifth of 10 sections described in a previous study) was collected and divided into right and left halves for separate analysis. Other cerebral slices were not created for analysis because results of an earlier study indicated that blood flow is not significantly different between rostral and caudal transverse brain slices in isoflurane-anesthetized horses. Gray matter was collected from superficial scrapings of the gyri of both remaining cerebral hemispheres. White matter was collected from the corona radiata of both cerebral hemispheres. The cerebellar peduncles were cut to separate the cerebellum from the brainstem, and the cerebellar vermis was cut from the hemispheres and saved for analysis. The thalamus was severed from the midbrain immediately rostral to the rostral colliculus and cut into right and left halves. The brainstem was divided into 3 regions: the colliculi region comprised transverse midbrain sections bounded rostrally by the rostral colliculi and caudally by the caudal colliculi, the pontine region continued distally to the caudal margin of the cerebellum, and the medulla comprised the remainder of the brainstem to the first cervical segment. All brainstem regions were divided into right and left halves, which were analyzed separately. Thoracolumbar segments from approximately T17 to L2 were used for spinal cord measurements.

Sections of brain and spinal cord tissues were sliced, weighed on a calibrated scale, and placed into a commercially available sample-processing unit that contained a filter unit and reservoir; microspheres were extracted by use of a validated technique. Tissue samples were digested within the filter reservoir by use of 4M KOH containing 2% polysorbate 80 (ie, Tween-80) and 2 mL of isopropyl alcohol. Tissues were incubated in a 60°C water bath for 48 hours, and then the filter chambers were rinsed with PBSS (pH, 7.4) with suction and centrifuged dry. Reference blood samples were similarly digested and processed. Each sample tube was then attached to a different filter unit, 2 mL of 2-ethoxyethyl acetate was added, tubes were capped and centrifuged at 4°C, and the extracted fluorescent dye was collected and sealed in the sample tubes. A control microsphere sample containing approximately 500 microspheres of each color/mL of 2-ethoxyethyl acetate was also prepared.

Samples were analyzed by use of a fluorometer and automated data-acquisition software. The lemon-, orange-, and persimmon-colored fluorophores excited at 390-, 495-, and 540-nm wavelengths and emitted at 445-, 525-, and 560-nm wavelengths, respectively. Samples were diluted to concentrations within a predetermined linear response range for each fluorophore and were analyzed in triplicate in matched quartz cuvettes. Blank samples of 2-ethoxyethyl acetate were used to establish zero fluorescence, and the control microsphere samples were used to measure and correct for temporal drift of the instrument.

**Calculated values**—The fluorescence signal detected for each reference blood sample was divided by the syringe withdrawal flow rate to yield an Fref value. Because homogeneous microsphere mixing is essential for accurate blood flow measurements, values obtained from reference blood samples collected at the 4 cannulated arteries (left ascending facial, left carotid, and right and left dorsal metatarsal) were compared. These were expected to yield similar values for Fref if mixing was adequate. If for any color, the Fref for any arterial sample differed by >10% from the mean value for all arterial samples, sample collection error or sample-processing error for that arterial sample was assumed. However, if for any color, the Fref for 2 or more arterial samples differed by >10% from the mean value for all arterial samples or if the Fref for samples obtained from the metatarsal arteries differed by >10% from the mean value for samples obtained from the facial and carotid arteries, then microsphere homogeneity could not be assumed. For the horses in the study reported here, all criteria for reference blood sample agreement were satisfied.
Fluorescence signal intensities from sample-processing units that contained the same tissue were added together and then divided by the sample tissue weight to calculate a mass-specific signal intensity. The $Q_{tis}$ (mL/min/g of tissue) for brain and spinal cord samples was calculated via division of the mass-specific signal intensity by the mean $F_{ref}$.

The CPP was calculated as the difference between MAP and ICP. Mass-specific resistance for brain tissues was calculated as CPP divided by $Q_{tis}$: the result-

**Table 1**—Mean ± SD physiologic responses and end-tidal isoflurane concentrations (\(P_{\text{aIso}}\)) determined at 3 targeted MAPs in 6 healthy isoflurane-anesthetized horses.

<table>
<thead>
<tr>
<th>Targeted MAP</th>
<th>Low (60 mm Hg)</th>
<th>Medium (80 mm Hg)</th>
<th>High (100 mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>58.2 ± 2.7</td>
<td>60.5 ± 3.0</td>
<td>102.9 ± 2.0</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>16.2 ± 5.9</td>
<td>13.7 ± 7.2</td>
<td>10.2 ± 10.8</td>
</tr>
<tr>
<td>ICP (mm Hg)</td>
<td>24 ± 6</td>
<td>27 ± 4 (2 ± 6)</td>
<td>29 ± 4 (5 ± 4)</td>
</tr>
<tr>
<td>CPP (mm Hg)</td>
<td>34 ± 8</td>
<td>54 ± 4 (20 ± 8)</td>
<td>74 ± 5 (40 ± 4)</td>
</tr>
</tbody>
</table>

**Table 2**—Mean ± SD $Q_{tis}$ (mL/min/g) and mass-specific tissue resistance (R [Mdyne*s*cm²]) determined at 3 targeted MAPs in the 6 isoflurane-anesthetized horses in Table 1.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Variable</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum (left hemisphere)</td>
<td>$Q_{tis}$</td>
<td>0.330 ± 0.111</td>
<td>0.340 ± 0.098</td>
<td>0.322 ± 0.092</td>
</tr>
<tr>
<td>Gray matter</td>
<td>$Q_{tis}$</td>
<td>0.458 ± 0.126</td>
<td>0.441 ± 0.122</td>
<td>0.454 ± 0.133</td>
</tr>
<tr>
<td>White matter</td>
<td>$Q_{tis}$</td>
<td>0.102 ± 0.047</td>
<td>0.125 ± 0.060</td>
<td>0.127 ± 0.056</td>
</tr>
</tbody>
</table>

**ND** = Not determined; See Table 1 for remainder of key.
ing values were divided by 12.5 to convert Wood units (mm Hg·min·g/mL) to SI units (Mdyne·s·g/cm²). Because CSF pressure around the spinal cord was not determined, spinal perfusion pressure could not be evaluated, and tissue resistance for the thoracolumbar spinal cord was not calculated.

**Statistical analysis**—Data were summarized as mean ± SD and were analyzed by use of a commercially available software package. Normality of each variable was evaluated by use of a Wilk-Shapiro test with a Dunn-Sidak adjustment because values for 3 targeted MAPs were obtained for each tissue sample. Blood pressures, blood flow rates, and tissue resistance values were related by use of an ANOVA with a Dunn-Sidak correction for multiple post hoc comparisons among targeted MAP. Differences were accepted as significant when \( P < 0.05 \).

**Results**

Mean blood gas values and blood pressures were evaluated for all horses in the study, and values obtained at each of the 3 targeted MAPs were compared (Table 1). The end-tidal isoflurane concentration required to reduce MAP in horses of the present study to 60 mm Hg ranged from 1.70% to 2.55% (approx 1.3 to 2.0 times the reported minimum alveolar concentration for isoflurane in horses19). No difference was detected in blood pH or PaCO\(_2\) values among the 3 targeted MAP conditions. Although a statistically significant difference in PaO\(_2\) was detected between the low and high MAP conditions, this difference was considered to be of little physiologic importance because PaO\(_2\) remained > 100 mm Hg in all horses throughout the study.

A power analysis suggested that a significant difference in CPP without any effect on ICP started to become evident between anesthetic depth and ICP. Increased MAP resulted in increased CPP with a Dunn-Sidak correction for multiple post hoc comparisons among targeted MAP. Differences were accepted as significant when \( P < 0.05 \).

**Cerebrovascular autoregulation** provides for the matching of oxygen delivery to metabolic oxygen consumption in the brain. The metabolic rate of CNS tissues is assumed to be constant during normothermic constant-dose isoflurane anesthesia. Hence, the finding that blood flow in brain tissues remained constant in anesthetized horses of the present study, despite more than doubling of the CPP, suggests that cerebrovascular autoregulation is preserved in healthy horses even at isoflurane doses consistent with a moderate-to-deep anesthetic plane. In contrast, blood flow to the spinal cord actually decreased during dobutamine infusions that increased MAP.

Isoflurane administration causes a dose-dependent decrease in cardiac output and MAP in mammals. Isoflurane also decreases matching of cerebral metabolic rate with CBF, particularly at concentrations above the minimum alveolar concentration in rats, rabbits, cats, dogs, pigs, and humans. Although the \( \beta\)-adrenoceptor agonist dobutamine does not directly affect cerebral hemodynamics, increased cardiac output and MAP during anesthetic-mediated cerebral vasodilation should cause increases in CBF, CBV, and ICP.

Results from the study reported here suggest that clinically relevant inhalation anesthetic dose effects on cerebrovascular autoregulation in the horse are fundamentally different than in other species for which data have been reported. However, physiologic response measurements are qualitatively similar to available values in equids. In previous studies of isoflurane-anesthetized horses, no significant dose effect was shown for ICP or regional CBF. Quantitatively, ICP and blood flow values for brain tissues in horses of the present study were up to 20% and 40% lower, respectively, than those reported in other studies. This finding may be a consequence of differences in horses versus ponies or of the anesthetic protocols selected (differences in PaCO\(_2\) and co-administration of vasoactive drugs).

The question arises as to how cerebrovascular autoregulation could be preserved in isoflurane-anesthetized horses and how ICP and CBF could remain constant in the face of variations in CPP. Isoflurane could spare myogenic or active cerebral vasoconstrictor responses in horses, as reflected by an increase in brain tissue resistance during administration of dobutamine at high infusion rates (Table 2). Inhalation anesthetics act through modulation of 1 or more ion channels or cell receptors to decrease net cellular excitability. The relative potency and efficacy at various anesthetic-sensitive targets varies by agent. Isoflurane may be less efficacious or potent a modulator of arterial receptors that contribute to vascular tone in horses than in other species. In contrast, the inhalation anesthetic halothane decreases CBF in a dose-dependent manner in ponies; this suggests that cerebrovascular autoregulation may not be preserved as well with other anesthetics as it is with isoflurane.

It is also possible that active vasoconstriction in the present study was not better preserved, but rather more intensely stimulated. Normal resting CBF is approximately 50 mL/min/100 g of tissue and may increase during isoflurane anesthesia even though ce-
Cerebral metabolism is reduced. In the study reported here, blood flow to the cerebrum was approximately 33 mL/min/100 g of tissue, regardless of MAP (Table 2). With such low blood flow rates, cerebral oxygen delivery may be regulated to maintain a marginally adequate tissue PaO2. Further reduction in oxygen delivery would then result in the release of local vasodilators to maintain CBF and prevent critical tissue hypoxia, similar to the process by which hypoxemia that results from decreased inspired oxygen fractions increases CBF to maintain cerebral oxygen delivery.

Apparent cerebrovascular autoregulation in horses may also, in part, reflect a reduction in arterial compliance that counteracts the effects of increased CPP on CBF. Horses have higher ICP and lower intracranial elastance than do smaller species under similar anesthetic conditions. Because brain tissues and fluids are enclosed within a rigid cranial vault, increased ICP adds to the extramural vascular pressure of cerebral arteries and veins and thereby decreases their compliance; this may potentially oppose further increases in intravascular volume. Inhalation anesthetics also increase vasopressin and norepinephrine concentrations in equine CSF, either of which could increase basal arterial tone in the brain.

Variations in anatomy may contribute to variations in arterial compliance. Rabbits, dogs, sheep, pigs, and humans all have fenestrations within the tunica intima of cerebral arteries, but horses and cattle do not. Absence of these fenestrations may reduce arterial compliance and allow large animals to better withstand large changes in hydrostatic gradients and transmural pressure associated with changes in head position. Reduced arterial compliance may also obviate the changes to cerebral arterial blood flow and volume otherwise expected from a dobutamine-mediated increase in CPP during anesthesia. If these mechanisms are valid, then cerebrovascular responses to inhalation anesthetics in other large animals such as cattle and giraffes may resemble those of horses.

In contrast to results of blood flow measurements for the brain, spinal cord blood flow appeared to decrease with dobutamine infusions that increased MAP (Table 2). This seems unlikely to be the result of selectivity increased spinal cord vasoconstriction because vascular resistance in all other tissue beds outside the CNS in horses is either unchanged or decreased by isoflurane administration. Vascular resistance within the brain does increase during dobutamine infusion, but this serves to prevent a change in CBF from increased CPP, not to reduce CBF. Results of another study indicated that spinal cord blood flow was autoregulated in barbiturate-anesthetized rats over a pressure range similar to that in the brain and at blood flow rates that were 3 to 5 times those measured in horses of the present study. In contrast, isoflurane anesthesia interfered with spinal cord autoregulation in a dose-dependent manner in rats, which resulted in increased spinal blood flow during increased MAP conditions. Decreased spinal cord perfusion pressure could passively reduce blood flow; however, this would require an improbably large increase in CSF pressure to counter the increase in MAP that results from dobutamine administration. A much more likely explanation is that blood traveling through the spinal cord may pass through vessels that are too large to trap the 15-µm fluorescent microspheres used in the present study. The meninges and radicular circulation of healthy dogs contain many large arteriovenous anastomoses that allow bypass of spinal tissue capillaries. It is unknown whether similar vascular communications are present in horses, but should they exist, increased MAP might disproportionately increase shunt vessel flow within the spinal cord while at the same time reducing nutrient delivery through the small capillaries.

The implications of these findings for the anesthetic management of equine patients are cause for concern. In this study, spinal cord blood flow was only 11 to 15 mL/min/100 g of tissue, a very low value that is similar to other measurements in horses reported by our group. Marginal spinal cord perfusion may increase the risk for hemorrhagic myelopathy, especially in large draft-type horses anesthetized in dorsal recumbency, when increased CSF and venous pressure around the spinal cord could further decrease perfusion pressure and tissue blood flow. However, increasing cardiac output and blood pressure via dobutamine administration might not improve tissue oxygen delivery to the spinal cord, since increased MAP may simply increase the spinal shunt fraction.

Our hypothesis that isoflurane anesthesia would interfere with cerebrovascular autoregulation in horses was incorrect. However, although cerebrovascular autoregulation was preserved in the brains of isoflurane-anesthetized horses of the present study, the set point for this autoregulation during isoflurane anesthesia was at a relatively low flow, compared with that of smaller animals. Consequently, horses as a species may be particularly susceptible to regional ischemia caused by small variations in cerebral metabolic rate or tissue blood flow. Severe ischemic injury will undoubtedly cause overt signs of neurologic injury; less severe injuries to the brain or spinal cord might contribute to violent emergence delirium and uncoordinated movements that contribute substantially to postanesthetic morbidity and mortality rates for horses. As previously mentioned, routine management techniques that rely on dobutamine administration to improve regionally compromised CBF may be rendered ineffective by a low global autoregulation set point.

Results of the present study revealed new information regarding the potential risk for insufficient blood flow in tissues of the CNS during anesthesia in horses. New anesthetic management techniques are needed that can adequately address these risks in horses.

a. Attane, Minrad, Orchard Park, NY.
b. Model 2800C, Mallard Medical, Redding, Calif.
c. Ponemah, Data Sciences International, Saint Paul, Minn.
e. ICP Express, Codman & Shurtleff Inc, Raynham, Mass.
f. ABL700, Radiometer America, Westlake, Ohio.
g. LB2, Sensormedics, Aneheim, Calif.
h. Dye-Trak F, Triton Technology, San Diego, Calif.
i. Dobutrex, Eli Lilly, Indianapolis, Ind.
j. Kunststoff- und Metallprodukte GmbH, Kappel-Grafenhausen, Germany.
k. Fluoromax-3, Jobin Yvon Inc, Edison, NJ.
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