Evaluation of administration of isoflurane at approximately the minimum alveolar concentration on depression of a nociceptive withdrawal reflex evoked by transcutaneous electrical stimulation in ponies

Claudia Spadavecchia, DVM, PhD; Olivier Levionnois, DVM; Peter W. Kronen, DVM; Massimo Leandri, DM, PhD; Luciano Spadavecchia, DP, PhD; Urs Schatzmann, DVM, PhD

Objective—To investigate effects of isoflurane at approximately the minimum alveolar concentration (MAC) on the nociceptive withdrawal reflex (NWR) of the forelimb of ponies as a method for quantifying anesthetic potency.

Animals—7 healthy adult Shetland ponies.

Procedure—Individual MAC (iMAC) for isoflurane was determined for each pony. Then, effects of isoflurane administered at 0.85, 0.95, and 1.05 iMAC on the NWR were assessed. At each concentration, the NWR threshold was defined electromyographically for the common digital extensor and deltoid muscles by stimulating the digital nerve; additional electrical stimulations (3, 5, 10, 20, 30, and 40 mA) were delivered, and the evoked activity was recorded and analyzed. After the end of anesthesia, the NWR threshold was assessed in standing ponies.

Results—Mean ± SD MAC of isoflurane was 1.0 ± 0.2%. The NWR thresholds for both muscles increased significantly in a concentration-dependent manner during anesthesia, whereas they decreased in awake ponies. Significantly higher thresholds were found for the deltoid muscle, compared with thresholds for the common digital extensor muscle, in anesthetized ponies. At each iMAC tested, amplitudes of the reflex responses from both muscles increased as stimulus intensities increased from 3 to 40 mA. A concentration-dependent depression of evoked reflexes with reduction in slopes of the stimulus-response functions was detected.

Conclusions and Clinical Relevance—Anesthetic-induced changes in sensory-motor processing in ponies anesthetized with isoflurane at concentrations of approximately 1.0 MAC can be detected by assessment of NWR. This method will permit comparison of effects of inhaled anesthetics or anesthetic combinations on spinal processing in equids. (Am J Vet Res 2006;67:762–769)

The mechanisms by which anesthetics act on sensory-motor processing to cause immobility are poorly understood. Analysis of results of studies15 in which investigators used the MAC technique of evaluating anesthetic potency through observation of gross purposeful movement in response to noxious stimuli suggests that volatile anesthetics block movement largely by means of action at the spinal cord. However, MAC is a nonquantitative, all-or-none measure of motor output that does not allow for investigation of subtle, graded anesthetic effects on depression of motor function. The MAC is assessed by use of a widely used but arbitrary criterion to check for evidence of complex movements after supramaximal noxious stimulation, whereas simpler and potentially more quantitative variables, such as the reflex withdrawal of a stimulated extremity, are usually neglected.

Evidence is lacking to support the contention that a supramaximal noxious stimulation of a limb systematically induces flexion in animals anesthetized with approximately the MAC of anesthetic agents. The motor response evoked during MAC assessment consists of 2 patterns (flexion withdrawal vs complex limb movements) that use differing neural circuits, which possibly undergo differing modulation for volatile anesthetics.5

Studies7,8 in human volunteers revealed that the electrically induced NWR of the lower limb disappears at sub-MAC end-tidal concentrations of isoflurane. Analysis of results of these studies indicates an early depression of simple reflexes, whereas complex movements can still be elicited. Conversely, rats anesthetized with approximately the MAC of volatile agents still have limb withdrawal reflexes.10 On the basis of the aforementioned evidence, important interspecific differences in the depressive action of anesthetics on nociceptive reflexes can be hypothesized.

Limb NWRs evoked by transcutaneous electrical stimulation have been described11,12 in conscious horses. The stimulation and quantification protocols applied in these studies were extremely similar to those
described for humans.\textsuperscript{7} Evaluating the effects of approximately the MAC of isoflurane on the NWR in equids could provide a quantitative method for assessment of anesthetic-induced sensory-motor depression in these animals and provide interesting data for interspecific comparisons.

The objective of the study reported here was to assess the effects of isoflurane administered at concentrations of approximately 1.0 MAC on the NWR. The working hypothesis was that isoflurane would begin to depress the NWR at the same concentrations that inhibited gross purposeful movements of pain avoidance.

**Materials and Methods**

**Animals**—Experiments were conducted on 7 gelding Shetland ponies. The ponies were 4 years old with a mean ± SD body weight of 121 ± 25 kg. Ponies were judged to be healthy on the basis of results of physical, biochemical, and hematologic examinations. Food was withheld from the ponies for 24 hours before the experiments, but they had ad libitum access to water. Two years before the study reported here was conducted, the left carotid artery of each pony was surgically translocated to a subcutaneous position. The Committee for Animal Experimentation, County of Berne, Switzerland, approved the study, which was part of a larger investigation on determination of isoflurane MAC in ponies.

**Induction and monitoring of anesthesia**—Oxygen was administered to each pony. Anesthesia was then induced by administration of isoflurane\textsuperscript{1} in oxygen via a face mask by use of a conventional circle anesthetic system.\textsuperscript{3} The isoflurane vaporizer setting was 1\% for 1 minute and then increased to 3\% until endotracheal intubation could be performed. For the first 30 minutes, the end-tidal concentration of isoflurane was set at approximately 1.3\%, which is the established MAC for equids,\textsuperscript{13} to permit instrumentation. Mechanical ventilation (ie, intermittent positive-pressure ventilation) was started, with initial settings of 8 breaths/min and a tidal volume of 12 mL/kg.

Catheters were inserted into a saphenous vein and carotid artery; these catheters were used for administration of lactated Ringer’s solution and monitoring of arterial blood pressure. Esophageal body temperature, ECG, pulse oximetry, arterial blood pressure, inspired and end-tidal oxygen concentration, and end-tidal anesthetic concentration were continuously monitored by use of a calibrated unit.\textsuperscript{12} The EMG signals were amplified with an overall gain of 5,000 and band-pass filtered (7 to 200 Hz; first-order filter)\textsuperscript{11} where.\textsuperscript{12} The EMG signals were amplified with an overall gain of 1 Hz. The EMG responses obtained were averaged and formed by use of a computerized system, as described elsewhere.\textsuperscript{12} Stimulation and recordings were performed at a fixed intensity of 40 mA and frequency of 1 Hz. To be considered a reflex response, the EMG burst following stimulation had to be at least 3 times the amplitude of the background activity with a duration of at least 10 milliseconds after stimulation had to be at least 3 times the amplitude of the background activity with a duration of at least 10 milliseconds after stimulation onset.\textsuperscript{11,12}

At each MAC, the lowest stimulation intensity able to evoke 2 consecutive EMG reflex responses was defined as the reflex threshold. Intensity of the current was initially set at 3 mA and increased in increments of 1 mA until a reflex response could be detected for each muscle. Additional electrical stimulations at 3, 5, 10, 20, 30, and 40 mA were delivered in ascending order at 1-minute intervals to assess the intensity–response function. Finally, 60 consecutive stimuli were administered at a fixed intensity of 40 mA and frequency of 1 Hz. The EMG responses obtained were averaged and quantified.

Latency of the reflex response was defined as the amount of time that elapsed between the onset of the stimulus and onset of the EMG reflex (deflection from baseline). To quantify the muscular response, the RMS value for reflex amplitude was calculated for the period from 20 to 70 milliseconds after stimulation. The background EMG amplitude was calculated as the RMS amplitude during the 100-millisecond interval before stimulation. To minimize the influence of possible variability among ponies, the relative amplitude of the reflex in the period from 20 to 70 milliseconds after stimulation was calculated as the ratio between the RMS amplitude detected...
during such periods and the RMS of the background EMG in the 100-millisecond period before stimulation. During recordings performed as part of preliminary experiments, it was observed that a burst of EMG reflex activity in response to electrical nerve stimulation could appear within the period from 20 to 70 milliseconds after stimulation in ponies at a light plane of isoflurane-induced anesthesia.

Recovery and reflex recordings in standing ponies—After completion of MAC determination and administration of the series of electrical stimulations, ponies were allowed to breathe pure oxygen until the return of the swallowing reflex was detected. They were then assisted for recovery. Stimulation and recording electrodes applied during anesthesia were left in place throughout the recovery period. One hour after disconnection from the anesthetic circuit, with the ponies in standing position and residual ataxia resolved, the reflex threshold was again assessed. Intensity of electrical stimulation was initially set at 1 mA and gradually increased in increments of 0.5 mA until a reflex response was detected during the period from 20 to 70 milliseconds after stimulation, as described previously.

Statistical analysis—Nonparametric analysis of data was chosen on the basis of tests for normal distribution. Group MAC was expressed as mean ± SD. Other results were reported as median and IQR (25% to 75%) values.

Relative amplitudes, latencies, and durations of the reflexes at various stimulus intensities and MAC concentrations were analyzed by use of Friedman repeated-measures ANOVA on ranks, with post hoc Tukey tests for multiple comparisons. Values obtained for the 2 muscles were compared by use of the Mann-Whitney rank sum test. Values of P < 0.05 were considered significant. Statistical analyses were performed by use of commercially available software.5

Results
Anesthetic period—Mean ± SD isoflurane MAC was 1.0 ± 0.2%, with values of iMACs < 1.2% (corrected for a barometric pressure of 760 mm Hg). Mean total duration of anesthesia was 471 ± 37 minutes. Normocapnia and normotension were maintained throughout anesthesia in all ponies, with mean endtidal carbon dioxide concentration of 33.9 ± 1.6 mm Hg and mean arterial blood pressure of 92.3 ± 6.8 mm Hg. Mean PaCO2 was 37.2 ± 1.5 mm Hg. All ponies recovered uneventfully from anesthesia.

Table 1—Median (IQR) values of relative amplitude, latency, and duration of the NWR obtained during EMG recordings for the common digital extensor (CDE) and deltoid muscles in 7 ponies when stimulated at NWR threshold intensities during anesthesia induced by administration of isoflurane at concentrations of 0.85, 0.95, and 1.05 iMAC and in standing ponies after recovery from anesthesia (awake).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Muscle</th>
<th>0.85 iMAC</th>
<th>0.95 iMAC</th>
<th>1.05 iMAC</th>
<th>Awake</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWR threshold intensity (mA)</td>
<td>CDE</td>
<td>6 (5.0–8.0)</td>
<td>8 (7.2–9.5)</td>
<td>8 (7.2–9.5)</td>
<td>3 (3.0–4.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Deltoid</td>
<td>15 (8.5–19.5)</td>
<td>18 (11.2–21.5)</td>
<td>24 (19.0–41.2)</td>
<td>4.5 (4.0–6.5)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Relative amplitude†</td>
<td>CDE</td>
<td>4.4 (3.4–7.3)</td>
<td>8.4 (4.5–9.2)</td>
<td>7.7 (5.1–8.6)</td>
<td>6.4 (3.4–6.5)</td>
<td>0.400</td>
</tr>
<tr>
<td>Deltoid</td>
<td>4.3 (3.7–5.3)</td>
<td>4.4 (3.4–11)</td>
<td>3.8 (2.4–11.9)</td>
<td>12.7 (3.8–26.8)</td>
<td>0.610</td>
<td></td>
</tr>
<tr>
<td>NWR latency (ms)</td>
<td>CDE</td>
<td>20 (19.2–21.7)</td>
<td>20 (19.2–21.7)</td>
<td>20 (17.7–21.7)</td>
<td>20 (17.7–21.7)</td>
<td>0.600</td>
</tr>
<tr>
<td>Deltoid</td>
<td>32 (27.2–33)</td>
<td>32 (27.2–33)</td>
<td>32 (27.2–33)</td>
<td>32 (27.2–33)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>NWR duration (ms)</td>
<td>CDE</td>
<td>25 (20.5–25.0)</td>
<td>22 (20.2–30.2)</td>
<td>23 (20.5–31.0)</td>
<td>24 (20.7–32.5)</td>
<td>0.310</td>
</tr>
<tr>
<td>Deltoid</td>
<td>25 (21.7–28.7)</td>
<td>28 (24.2–30.5)</td>
<td>25 (21.2–25.7)</td>
<td>30 (30.5–45)</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent results for the Friedman test. Ratios between the RMS amplitude of the EMG activity in the period 20 to 70 milliseconds after stimulus and the RMS amplitude of the EMG activity in the 100-millisecond interval before stimulus.
†Within a row, values with different superscript letters differ significantly (P < 0.05; Tukey test for multiple comparisons).
‡Within a variable within a column, values with different superscript letters differ significantly (P < 0.05; Mann-Whitney rank sum test).
tions at intensities that would barely evoke an EMG reflex response from the common digital extensor muscle were not accompanied by any visible movement of the stimulated limb, whereas stimulations that evoked reflexes from the deltoid muscle were always accompanied by a visible flexion-protraction movement of the stimulated limb. In 2 ponies at 1.05 iMAC, the maximal stimulation intensity of 44 mA was not sufficient to evoke a reflex response and the reflex threshold intensities could not be determined.

At threshold intensities, relative amplitudes of the reflex responses were similar for both muscles during and after anesthesia and no concentration-related changes were observed (Figure 2). Latency of the reflex responses remained stable and did not change significantly (P = 0.600) for the common digital extensor muscle, with a median overall value of 20 milliseconds (IQR, 19 to 22 milliseconds). Conversely, latency of the reflex response for the deltoid muscle had significant changes, decreasing from a median value of 33 milliseconds (IQR, 31 to 36 milliseconds) during anesthesia to 19 milliseconds (IQR, 16 to 32 milliseconds) after anesthesia. In awake ponies, no significant difference was found for latency of the reflex response between the 2 muscles. Duration of the reflex response did not change during and after anesthesia for the common digital extensor muscle, with a median overall value of 23 milliseconds (IQR, 20 to 25 milliseconds). Conversely, duration of the reflex response for the deltoid muscle increased significantly from a median value of 25 milliseconds (IQR, 22 to 29 milliseconds) during anesthesia to a median value of 38 milliseconds (IQR, 30 to 45 milliseconds) in awake ponies (Table 1).

Reflex responses to stimulations of increasing intensity—During anesthesia and at each MAC tested, amplitudes of the reflex responses from both muscles increased significantly (P < 0.001) as stimulus intensity increased from 3 to 40 mA (Figure 3). Reflex responses were consistently recorded for the common digital extensor muscle but not for the deltoid muscle when stimulations of 5 mA were applied (Table 2). The reflex responses usually consisted of a burst of EMG activity during the period from 20 to 70 milliseconds after stimulation. Graded electrical-evoked reflexes during isoflurane-induced anesthesia were detected at 0.85, 0.95, and 1.05 iMAC (Figure 4). Between 0.85 and 1.05 iMAC, there was concentration-dependent depression of electrically evoked reflexes with a reduction in the slopes
of stimulus-response functions. For the common digital extensor muscle, amplitudes of the reflex response decreased significantly for increasing anesthetic concentrations when stimuli were administered at 30 mA. Similarly, a concentration-dependent decrease was observed for stimulations at 20, 30, and 40 mA for the deltoid muscle. Significant differences were always detected between the lowest and the highest isoflurane concentrations.

In general, latencies of the reflex responses during isoflurane-induced anesthesia decreased with increasing stimulation intensities (Table 2). Significance of this pattern was detected at each isoflurane concentration for the reflex response of the common digital extensor muscle. Duration of the reflex responses tended to increase with increasing stimulation intensities, with significant differences between responses for the common digital extensor muscle at 0.85 and 0.95 iMAC. At 1.05 iMAC, duration of the reflex responses remained stable regardless of the stimulation intensity.

Averaged reflex responses—The averaged reflex responses to 60 stimuli administered at 40 mA and 1 Hz during isoflurane-induced anesthesia permitted visual interpretation of 2 reflex components with differing latencies. Median latencies of the 2 reflex components were 19 milliseconds (IQR, 17 to 20 milliseconds) and 39 milliseconds (IQR, 39 to 40 milliseconds) for the common digital extensor muscle and 19 milliseconds (IQR, 17 to 21 milliseconds) and 36 milliseconds (IQR, 36 to 40 milliseconds) for the deltoid muscle. Averaged reflex responses appeared to be unaffected by isoflurane concentration because there was no change in latency, amplitude, or duration. Conversely, purposeful movement during the series of 60 stimuli was a concentration-dependent event. At 0.85 iMAC, 4 of 5 ponies had purposeful movement (the other 2 ponies were judged to be at too light of a plane of anesthesia to tolerate stimulation), whereas at 0.95 iMAC, 2 ponies had purposeful movements and 5 did not. None of the ponies had purposeful movement at 1.05 iMAC. In contrast, reflex limb withdrawals in

<table>
<thead>
<tr>
<th>Variable</th>
<th>Muscle</th>
<th>iMAC</th>
<th>3 mA</th>
<th>5 mA</th>
<th>10 mA</th>
<th>20 mA</th>
<th>30 mA</th>
<th>40 mA</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative amplitude†</td>
<td>Deltoid</td>
<td>0.85</td>
<td>1.1 (0.9–1.2)</td>
<td>1.2 (0.9–1.4)</td>
<td>1.7 (1.2–3.0)</td>
<td>10.9 (3.1–45.9)†</td>
<td>57.7 (17.3–89.0)†</td>
<td>43.7 (19.7–74.0)†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>1.1 (0.8–1.0)</td>
<td>1.1 (0.8–1.1)</td>
<td>1.6 (1.4–15.6)</td>
<td>2.9 (2.0–48.4)‡</td>
<td>9.1 (3.2–53.3)‡</td>
<td>31.0 (4.0–50.9)‡</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>1.1 (0.8–1.5)</td>
<td>0.9 (0.6–1.1)</td>
<td>1.0 (0.8–1.5)</td>
<td>1.7 (1.5–3.7)‡</td>
<td>3.1 (1.8–4.0)‡</td>
<td>13.0 (3.8–21.0)‡</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CDE</td>
<td>0.85</td>
<td>1.3 (1.0–1.7)</td>
<td>4.4 (1.4–7.3)</td>
<td>9.3 (5.1–13.6)</td>
<td>21.1 (10.0–28.6)</td>
<td>21.9 (13.8–28.9)‡</td>
<td>25.0 (12.5–37.8)‡</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>1.1 (1.0–1.4)</td>
<td>3.2 (2.1–4.1)</td>
<td>8.9 (5.1–14.1)</td>
<td>9.2 (5.4–19.6)</td>
<td>11.9 (6.0–20.1)‡</td>
<td>13.3 (10.4–16.9)‡</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>1.2 (1.0–1.4)</td>
<td>2.4 (1.1–4.6)</td>
<td>8.4 (3.7–15.1)</td>
<td>7.8 (5.7–17.0)</td>
<td>7.2 (5.4–23.5)‡</td>
<td>8.2 (6.8–31.5)‡</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NWR latency (ms)</td>
<td>Deltoid</td>
<td>0.85</td>
<td>NN</td>
<td>NN</td>
<td>33 (27–37)</td>
<td>22 (21–32)</td>
<td>21 (21–25)‡</td>
<td>21 (21–26)‡</td>
<td>0.430</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>NN</td>
<td>NN</td>
<td>31 (27–35)</td>
<td>26 (23–31)</td>
<td>24 (22–28)‡</td>
<td>23 (21–28)‡</td>
<td>0.370</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>NN</td>
<td>NN</td>
<td>28 (27–30)</td>
<td>31 (28–33)</td>
<td>33 (29–38)‡</td>
<td>31 (29–35)‡</td>
<td>0.210</td>
</tr>
<tr>
<td>CDE</td>
<td>0.85</td>
<td>NN</td>
<td>23 (20–23)</td>
<td>21 (20–22)‡</td>
<td>20 (19–22)‡</td>
<td>19 (19–21)‡</td>
<td>19 (19–21)‡</td>
<td>19 (19–20)‡</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>NN</td>
<td>22 (19–23)</td>
<td>20 (19–23)‡</td>
<td>19 (19–21)‡</td>
<td>19 (19–21)‡</td>
<td>19 (19–20)‡</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>NN</td>
<td>24 (21–27)</td>
<td>22 (21–25)‡</td>
<td>21 (21–22)‡</td>
<td>21 (21–22)‡</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>NWR duration (ms)</td>
<td>Deltoid</td>
<td>0.85</td>
<td>NN</td>
<td>NN</td>
<td>24 (21–31)</td>
<td>34 (23–38)</td>
<td>40 (36–42)‡</td>
<td>41 (38–45)‡</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>NN</td>
<td>NN</td>
<td>29 (28–30)</td>
<td>34 (25–40)</td>
<td>36 (27–38)‡</td>
<td>41 (31–43)‡</td>
<td>0.370</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>NN</td>
<td>NN</td>
<td>20 (11–26)</td>
<td>25 (22–26)</td>
<td>20 (18–28)‡</td>
<td>22 (20–28)‡</td>
<td>0.950</td>
</tr>
<tr>
<td>CDE</td>
<td>0.85</td>
<td>NN</td>
<td>23 (19–24)</td>
<td>24 (21–32)</td>
<td>29 (25–37)</td>
<td>35 (32–38)</td>
<td>33 (32–35)</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>NN</td>
<td>20 (11–24)</td>
<td>23 (20–27)</td>
<td>25 (22–30)</td>
<td>32 (28–39)</td>
<td>32 (26–40)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.05</td>
<td>NN</td>
<td>20 (16–25)</td>
<td>22 (15–30)</td>
<td>23 (22–34)</td>
<td>29 (23–35)</td>
<td>29 (22–34)</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Table 2—Median (IQR) values for relative amplitude, latency, and duration of the NWR obtained during EMG recordings for the CDE and deltoid muscles in 7 ponies when stimulated at 3, 5, 10, 20, 30, and 40 mA during anesthesia achieved by administration of isoflurane at concentrations of 0.85, 0.95, and 1.05 iMAC.

*Within a variable within a column, values with different superscript letters differ significantly (P < 0.05; Mann-Whitney rank sum test).

NN = No NWR evoked.
See Table 1 for remainder of key.

Figure 4—Representative EMG recordings obtained from the deltoid muscle of a pony during anesthesia at isoflurane concentrations of 0.85, 0.95, and 1.05 iMAC after stimulus with 3, 5, 10, 20, 30, and 40 mA. The onset of stimulus is indicated for each iMAC value (vertical dotted lines).
response to stimulation were always evident, beginning with the second or third stimulus.

Discussion
The study reported here revealed that subtle anesthetic-induced changes in sensory-motor processing in ponies anesthetized with isoflurane at concentrations of approximately 1.0 MAC can be detected by assessment of NWR. In fact, in ponies anesthetized at a light plane of anesthesia, NWR thresholds increased with increasing isoflurane concentrations. Furthermore, reflex activities in response to single electrical stimuli were largely depressed at isoflurane concentrations able to prevent purposeful movements in response to supramaximal noxious stimulations, which indicated parallel inhibition of the 2 evoked motor patterns (flexion withdrawal and complex limb movements) in ponies.

Although isoflurane abolishes the electrically induced NWR in humans at concentrations substantially <1.0 MAC, it did not do so in our ponies, even at approximately MAC, which suggested species-specific anesthetic modulation of spinal withdrawal reflexes despite homogeneous interspecific MAC values. At all tested MAC multiples, amplitude of the reflex response increased with stimulus intensity, whereas the slope of the stimulus-response function was reduced with increasing isoflurane concentrations. Similarly, concentrations of isoflurane at approximately 1.0 MAC decreased the force of reflexive limb withdrawals in response to noxious thermal stimuli in a concentration-dependent manner in rats, with the largest reduction between 0.9 and 1.1 MAC. It can be concluded that abolishment of gross purposeful movement is paralleled by inhibition of withdrawal reflexes.

Determination of MAC depends on detection of complex gross purposeful movements, which typically involve the limbs and head. These complex movements are probably dependent on the central pattern generator, which is the neural network involved in processing this behavior. The central pattern generator receives afferent inputs from the spinal cord and brain and acts to initiate and terminate complex movements. Isoflurane-induced disruption of activity and coordination of the central pattern generator has been described in spinal cords isolated from lampreys. Fortunately, only scarce data are available about interspecies differences in organization and control of the central pattern generator and its sensitivity to anesthetics.

Withdrawal reflexes in awake animals incorporate flexion of the stimulated limb with extension of the contralateral limb. It is not currently known whether this reflex remains intact during inhalant anesthesia. The study reported here provides evidence of reflex activity in ponies anesthetized with isoflurane concentrations of approximately 1.0 MAC that is consistent with the withdrawal reflex pattern described in conscious horses. When single stimuli of increasing intensity were applied during anesthesia, latency of the NWR decreased but duration of the NWR increased, confirming that the reflex undergoes intensity-dependent modulation.

The concentration-dependent increase of the NWR threshold was a muscle-specific phenomenon, with lowest values found for the common digital extensor muscle (a muscle of the forelimb), compared with values for the deltoid muscle (a muscle of the shoulder). Visible flexion of the stimulated limb accompanied NWRs of the deltoid muscle, whereas limb flexion was mostly lacking when NWRs were recorded for the common digital extensor muscle. The common digital extensor muscle consistently was activated at lower stimulation intensities, compared with values for the deltoid muscle. Reflex activity of the common digital extensor muscle had only a moderate increase in threshold, even for the highest isoflurane concentrations, which indicated a low sensitivity to the depressant effects of the anesthetic. Muscle-specific NWR thresholds were not observed in conscious horses in another study, in which other muscles of the forelimb were examined, nor were they observed after recovery from anesthesia in the ponies of the study reported here, in which results suggested that isoflurane, lateral recumbency, or both must account for the difference in patterns of reflexive muscle activation during anesthesia.

The reflex response recorded from both forelimb muscles was detected during the period from 20 to 70 milliseconds after stimulus. The early part of the reflex was probably attributable to activation of Aβ fibers. With a mean distance of 85 cm between the stimulation electrodes and dorsal point of the shoulders, and considering a peripheral conduction velocity of 75 m/s for ponies, an afferent time of 11 milliseconds was expected. Adding an approximate central delay of 5 milliseconds and neglecting the amount of time needed for the motor component of the reflex, the observed minimal latency of 16 milliseconds appears reasonable. The late part of the reflex, which always terminated before 70 milliseconds after the stimulus, could have reflected activation of Aδ fibers, with a conduction velocity between 15 and 35 m/s, which corresponds to an afferent time between 25 and 55 milliseconds. In the study reported here, it was not possible to separate reflex components originating from Aβ fibers from those originating from Aδ fibers when examining a single reflex. When 60 consecutive stimuli at the intensity of 40 mA were applied during 1 minute and the reflex responses were averaged, 2 peaks of differing latencies appeared for both muscles, which confirmed that the 2 expected components exist in ponies (similar to results reported for humans and horses but probably have more intrasubject variation).

The method applied to determine MAC for each pony in the study reported here is considered the standard for equids and was described for the first time in 1977. It consists of supramaximal electrical stimulation of the oral mucous membranes; the stimulation is applied at a fixed voltage and frequency during 1 minute or until a gross purposeful movement is observed. Repetition of the stimulus causes a temporal summation of the nociceptive input, which influences MAC values of the volatile agent. A similar temporal summation effect was evident when 60 transcutaneous stimuli at 40 mA were applied for 1 minute over the
digital nerve and was responsible for the purposeful movements observed during the stimulation series in ponies receiving isoflurane at concentrations < 1.0 MAC. As expected, none of the ponies had purposeful movements when anesthetized at 1.05 IMAC, whereas NWRs still were facilitated as a result of stimulus repetition. The MAC values determined for each of the Shetland ponies of the study were less than the MAC values reported for horses. Similarly, lower MAC values for ponies, compared with MAC values for horses, have been found for desflurane, and large differences in halothane MAC have been reported for horses even when the same standard stimulation method was applied. Among the possible causes for MAC variations, it is likely that variation in sensitivity among subjects, genetic influences, failure of the noxious stimulus to generate a supramaximal effect, and the selective effects of inhaled anesthetics on afferent and efferent reflex pathways. An invasive approach in laboratory animals has been used to examine the selective effects of inhaled anesthetics on neurons in the dorsal horn and motoneurons. Depression of neurons in the dorsal horn of rats is mainly at concentrations of isoflurane < 1.0 MAC. Analysis of such findings suggests that the immobilizing action of isoflurane is not mediated by depression of nociceptive transmission through the dorsal horn, but is mediated instead by depression at a more ventral location. Isoflurane modulation of the F wave and H reflex as noninvasive measures of motoneuron excitability has been investigated in humans and rats. These methods have revealed that a prevalent inhibition of the efferent nociceptive pathways is probably responsible for isoflurane-induced immobility evident at concentrations of approximately 1.0 MAC. In addition to theoretic considerations on the mechanisms of action of isoflurane on the motor system, results of the study reported here provide a practical tool to be applied in veterinary anesthesia for research purposes that will complement studies of MAC. Analysis of our results indicates that a quantitative noninvasive assessment of sensory-motor depression of a simple NWR of a limb at isoflurane concentrations of approximately 1.0 MAC is feasible in ponies. In the future, it will be possible to apply this method to compare the effects of various inhalant agents or anesthetic combinations on spinal processing at concentrations related to MAC in equines and other species, thus improving our knowledge of agent-specific and species-specific mechanisms of anesthetic action.

References


a.Isoflor, Abbott AG, Baar, Switzerland.
b.Roche electronic respirator 3100, F. Hoffmann-La Roche, Basel, Switzerland.
c.S/5 compac, Datex-Ohmeda, Helsinki, Finland.
e.Grass S88, Grass Instruments, Quincy, Mass.
32. Antognini JF, Carstens E. Increasing isoflurane from 0.9 to 1.1 minimum alveolar concentration minimally affects dorsal horn cell responses to noxious stimulation. Anesthesiology 1999;90:208–214.