Median effective dose of isoflurane, sevoflurane, and desflurane in green iguanas

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Objective—To determine the median effective dose (ED50; equivalent to the minimum alveolar concentration [MAC]) of isoflurane, sevoflurane, and desflurane for anesthesia in iguanas.

Animals—6 healthy adult green iguanas.

Procedure—in unmedicated iguanas, anesthesia was induced and maintained with each of the 3 volatile drugs administered on separate days according to a Latin square design. Iguanas were endotracheally intubated, mechanically ventilated, and instrumented for cardiovascular and respiratory measurements. During each period of anesthesia, MAC was determined in triplicate. The mean value of 2 consecutive expired anesthetic concentrations, 1 that just permitted and 1 that just prevented gross purposeful movement in response to supramaximal electrical stimulus, and that were not different by more than 15%, was deemed the MAC.

Results—Mean ± SD values for the third MAC determination for isoflurane, sevoflurane, and desflurane were 1.8 ± 0.3%, 3.1 ± 1.0%, and 8.9 ± 2.1% of atmospheric pressure, respectively. The MAC for all inhaled agents was, on average, 22% greater for the first measurement than for the third measurement.

Conclusions and Clinical Relevance—Over time, MACs decreased for all 3 agents. Final MAC measurements were similar to values reported for other species. The decrease in MACs over time may be at least partly explained by limitations of anesthetic uptake from the lungs, the plane of anesthesia may deepen over time, which could contribute to increased morbidity during prolonged procedures.

Although pharmacologic data for most drugs in iguanas are lacking,1 inhaled agents are favored for general anesthesia because similar potencies and kinetics across a wide range of animal species facilitate extrapolation of such parameters to reptiles. However, results of a recent study2 revealed that the ED50 of isoflurane for anesthesia in green iguanas is 30% to 80% higher than the MAC measured in other species.4-6 The MAC is a commonly used indicator of inhaled anesthetic potency and represents an ED50 to suppress movement in response to supramaximal noxious stimulation.7 In addition to reports of high MACs, measurements of isoflurane potency in 2 trials varied by as much as 111% within a single iguana.8 In another study,9 an LD50 for isoflurane could not be demonstrated at a dose in excess of 4 times the ED50, even though cardiovascular collapse in mammals usually occurs at 2 to 3 times the MAC for volatile anesthetics.10-13

Reptilian respiratory gas exchange units have different morphologic and structural characteristics, compared with mammalian alveoli, and nomenclature in the literature varies.10 In our study, the term alveoli will be used to describe gas exchange units of the reptilian lung and the term MAC used to designate an end-tidal anesthetic ED50 for mammals and reptiles. To our knowledge, no published reports on the MAC for sevoflurane or desflurane in green iguanas exist.

Differences in MAC between iguanas and other species could be a result of a pharmacodynamic effect, meaning that the potency of isoflurane is much less in iguanas than in other species. Because inhaled anesthetics are currently postulated to act via modulation of neurotransmitter receptors and ion channels,11 such differences in potency would likely reflect species variability in membrane receptors. If correct, the study of inhalant anesthesia in iguanas might be useful for elucidation of inhaled anesthetic mechanisms. Alternatively, high MAC measurements, or an apparent lack of anesthetic effect, could instead be the result of a pharmacokinetic effect, reflecting delayed anesthetic equilibration between the CNS and alveoli.

Measurement of MAC necessitates equilibration of partial pressures between the alveoli and effect site (ie, the CNS). Anesthetic uptake from the lungs is described by the following equation:

\[
\dot{V}_x = \lambda \times \dot{Q} \times P_v - P_a
\]

where \(\dot{V}_x\) is the rate of uptake of agent x, \(\lambda\) is the blood-gas partition coefficient, \(\dot{Q}\) is the cardiac output.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>BT</td>
<td>Body temperature</td>
</tr>
<tr>
<td>ED50</td>
<td>Median effective dose (produces desired effect in 50% of population)</td>
</tr>
<tr>
<td>MAC</td>
<td>Minimum alveolar concentration</td>
</tr>
<tr>
<td>LD50</td>
<td>Median lethal dose</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
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Received June 20, 2005.
Accepted August 15, 2005.
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put, $P$ is the partial pressure of the agent in pulmonary venous ($P_v$) and pulmonary arterial ($P_a$) blood, and $P_b$ is barometric pressure. Reptiles have a much lower cardiac output per gram of tissue than similarly sized mammals. Moreover, some reptile species have considerable right-to-left shunting as a result of systemic blood washout from the cavum venosum. Shunts of up to 80% of cardiac output have been reported, and the shunt fraction can vary greatly with BT, cardiac preload, respiratory cycle, and vagal tone. Intracardiac shunting results in venous admixture, thus decreasing blood flow ($Q$) available for anesthetic uptake.

We hypothesized that low specific cardiac output coupled with high intracardiac shunting would delay equilibration of anesthetic concentrations between alveoli and the CNS, and as such, measured expired anesthetic partial pressures would exceed actual CNS arterial anesthetic partial pressures. Hence, MAC measurements early in an anesthesia period may not represent a true anesthetic ED$_{50}$ in iguanas. If this pharmacokinetic mechanism is correct, then MAC measurements should decrease over time. If iguanas instead develop anesthetic resistance from a pharmacodynamic effect, then MACs for the inhaled anesthetics isoflurane, sevoflurane, and desflurane should all be proportionally high and values should not change with time.

### Materials and Methods

**Animals**—Three male and 3 female adult green iguanas (Iguana iguana) weighing a mean $±$ SD of 1.2 $±$ 0.7 kg were used in our study with the approval of the Animal Care and Use Committee of the University of California, Davis. Health of the iguanas was assessed on the Animal Care and Use Committee of the University of California, Davis. Health of the iguanas was assessed on the basis of findings on physical examination, CBC determination, and serum biochemical analysis and the absence of fecal parasites. Iguanas were housed individually in a heated and humidified (> 60%) room. A temperature gradient from 27 to 37°C was provided in each cage by use of infrared lamps. Overhead and cage lights provided UV B during brief cessation of fresh gas delivery. CO$_2$ was measured continuously by use of a Raman spectrometer with the sample collection line placed at the level of the thoracic inlet. A constant-flush device was connected to the arteriocal catheter infusing approximately 3 mL/h of normal saline (0.9% NaCl) solution. This fluid volume was considered as part of each iguana's fluid requirements, and the volume of supplementary crystalloid solution administered was reduced accordingly.

Blood samples (700 µL) were taken at the time of each MAC determination (via refractometer) concentration, and arterial blood gas analysis for a total of 3 samples/anesthetic study, in which an arterial catheter was in place. Inspired and expired oxygen and carbon dioxide concentrations were measured continuously by use of Raman spectrometry with the sample collection line connected to the endotracheal tube connector. Anesthetic concentrations were measured by an infrared gas analyser and values were corrected according to linear regression curves obtained from measurements of 3 calibration gas standards and room air before each experiment. Values for blood pressure, heart rate, respiratory rate, BT, partial pressure of inspired oxygen, and as expired anesthetic gas concentration, were recorded every 15 minutes; however, only values taken at the time of MAC determination are presented in our study.

**MAC determination**—Minimum alveolar concentration was determined in triplicate for each iguana. After maintenance of constant end-tidal anesthetic concentration for 30 minutes, physiologic variables were measured. A 20-V, 50-Hz electrical stimulation was then applied via two 23-gauge needles, one placed each side 5 mm caudolateral to the cloaca. This stimulus is equivalent to the supramaximal tail clasp stimulus for MAC determination in rats and has been used in a previous determination of MAC in iguanas. If gross purposeful movement was observed during the stimulation, the end-tidal anesthetic concentration was increased by 10% to 15% and maintained for a 30-minute equilibration period before restimulation. If no movement was observed, then the end-tidal concentration was decreased by 10% to 15% and maintained for 30 minutes before restimulation. The MAC value was defined by the mean value of 2 successive concentrations that allowed and prevented gross purposeful movement. This procedure was repeated 2 to 3 additional times to yield 3 separate determinations of MAC.

After obtaining MACs, instruments were removed. Digital pressure was applied for 10 minutes to the removal site of the carotid catheters to achieve hemostasis, followed
Chewing. Thermal support was maintained and the iguana were removed once the iguana had righted itself and was chewing. Endotracheal tubes were removed once the iguana resumed spontaneous ventilation. Manual ventilation was provided until the triceps after extubation to those iguanas that had undergone carotid catheterization. Iguanas were returned to their cages and observed twice daily for signs of discomfort or reduction in appetite and were given additional doses of butorphanol the following day if necessary. Sutures were removed 4 weeks after placement.

Data analysis—Data are presented as mean ± SD values. Physiologic variables were log transformed to fit normality as assessed by the Shapiro-Wilk test. Comparisons between agent, time, and order of treatment were made by use of a repeated-measures ANOVA. Differences were significant at values of P < 0.05.

Results

First, second, and third values for MAC were 2.4 ± 0.7%, 2.1 ± 0.5%, and 1.8 ± 0.3% for isoflurane, respectively; 4.1 ± 1.4%, 3.1 ± 1.0%, and 3.1 ± 1.0% for sevoflurane, respectively; and 10.3 ± 3.0%, 9.3 ± 2.3%, 8.9 ± 2.1% for desflurane, respectively (Table 1). Time to first, second, and third MAC determinations was similar for all 3 agents (Figure 1).

Values for PaO₂ ranged from 41 to 366 mm Hg. Iguanas with lower blood oxygen tensions often had greater changes in MAC during the first 2 measurements. No significant differences in measurements of PaCO₂, PaO₂, Doppler ultrasonographic blood pressure, direct MAP, or BT were detected among the 3 agents or 3 MAC measurements.

Table 1—Mean ± SD measurements at each of 3 determinations of MAC for isoflurane (Iso), sevoflurane (Sevo), and desflurane (Des) in 6 adult green iguanas.

<table>
<thead>
<tr>
<th>Variables</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iso</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAC (%)</td>
<td>2.4±0.7</td>
<td>2.1±0.5</td>
<td>1.8±0.3</td>
<td>2.1±0.5</td>
</tr>
<tr>
<td>Time (min)*</td>
<td>277±149</td>
<td>509±118</td>
<td>631±98</td>
<td>NA</td>
</tr>
<tr>
<td>Doppler (mm Hg)</td>
<td>43±10</td>
<td>47±10</td>
<td>49±9</td>
<td>46±10</td>
</tr>
<tr>
<td>MAP (mm Hg)*</td>
<td>38±8</td>
<td>37±7</td>
<td>38±12</td>
<td>37±9</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)*</td>
<td>193±134</td>
<td>132±86</td>
<td>136±109</td>
<td>153±105</td>
</tr>
<tr>
<td>BT (°C)</td>
<td>35.6±0.3</td>
<td>35.4±0.3</td>
<td>35.5±0.4</td>
<td>35.5±0.3</td>
</tr>
</tbody>
</table>

Sevo       |     |     |     |         |
| MAC (%)   | 4.1±1.4 | 3.1±1.0 | 3.1±1.0 | 3.4±1.2 |
| Time (min)* | 277±53 | 468±61 | 620±100 | NA |
| Doppler (mm Hg) | 40±9 | 46±12 | 49±7 | 45±10 |
| MAP (mm Hg)* | 46±8 | 46±7 | 52±12 | 48±9 |
| PaO₂ (mm Hg)* | 94±46 | 108±95 | 74±23 | 92±58 |
| BT (°C) | 35.5±0.2 | 35.5±0.3 | 35.5±0.3 | 35.5±0.2 |

Des        |     |     |     |         |
| MAC (%)   | 10.3±3.0 | 9.3±2.3 | 8.9±2.1 | 9.5±2.5 |
| Time (min)* | 262±80 | 460±117 | 583±138 | NA |
| Doppler (mm Hg) | 46±17 | 46±18 | 43±12 | 45±15 |
| MAP (mm Hg)* | 150±144 | 171±111 | 144±99 | 155±109 |
| PaO₂ (mm Hg)* | 33±14 | 31±13 | 32±15 | 32±13 |
| BT (°C) | 35.6±0.3 | 35.6±0.3 | 35.5±0.2 | 35.5±0.3 |

NA = Not available.

*Anesthesia time represents time from initial administration of anesthetic agent to determination of that MAC value. †Data for MAP, PaO₂, and PaCO₂ obtained from 4 iguanas.

No significant differences were found among agents or MAC determinations for any reported variable.

Figure 1—Minimum alveolar concentration of isoflurane, sevoflurane, and desflurane versus time from initial administration of anesthetic agent. The MACs were determined in triplicate for 6 adult green iguanas anesthetized at least 7 days apart according to a Latin square design. The same symbol represents the same iguana in each of the 3 graphs.

by placement of nonabsorbable skin sutures. Once anesthetic delivery ceased, manual ventilation was provided until the iguana ambulated well and alert. Butorphanol (0.5 to 1.0 mg/kg) was administered IM in the triceps after extubation to those iguanas that had undergone carotid catheterization. Iguanas were returned to their cages and observed twice daily for signs of discomfort or reduction in appetite and were given additional doses of butorphanol the following day if necessary. Sutures were removed 4 weeks after placement.

Discussion

To our knowledge, our study provides the first potency comparison of 3 inhaled anesthetic agents in reptiles. By the third determination, MACs for isoflurane, sevoflurane, and desflurane were 1.8%, 3.1%, and 8.9% of atmospheric pressure, respectively. These values are modestly higher than anesthetic potencies given for most mammalian species, although the potencies of these 3 anesthetic agents closely observed until it was ambulating well and alert.
relative to each other are similar to those in other species. Despite maintenance of higher cloacal temperatures in our study, compared with that in a previous report of isoflurane MAC in iguanas, which should have resulted in higher MAC measurements, the final isoflurane MAC in our study is lower than that in the previous report. This discrepancy is most likely a function of total anesthesia duration because MAC in our study decreased as a function of time.

Three possible explanations exist for a temporal MAC decrease in our study. First, repeated electrical stimulation might cause desensitization at the tissue stimulation site or at the level of the spinal cord, thereby reducing the concentration of anesthetic required to prevent movement. Electrical pulses applied to rat tails at > 15 V can cause desensitization over time, with an apparent decrease in MAC. It is unknown whether similar events can occur when stimuli are applied axially, as in our study. However, high-frequency electrical stimulation promotes neuronal windup and after-discharges, which facilitate withdrawal reflexes that would oppose a centrally mediated decrease in response sensitivity or anesthetic requirement.

Second, it is possible that anesthetic potency itself increases with time. Although a single report exists in which the MAC decreased by 24% during 3 hours of isoflurane anesthesia, results of most studies reveal no temporal change in MAC. It would seem unlikely that iguanas uniquely develop increasing anesthetic sensitivity, as this would suggest that there must be species-specific receptor interactions for inhaled agents. Such a hypothesis is inconsistent with the otherwise similar pharmacodynamic effects of volatile anesthetics across disparate phyla. Nonetheless, insufficient data are available to totally exclude a possible time-dependent drug effect.

Finally, MAC could decrease with time if anesthetic partial-pressure equilibration between the alveoli and brain is delayed as a result of large anatomic shunts, low specific cardiac output, and diffusion limitations present in reptiles. The validity of MAC as a measure of anesthetic potency relies on the assumption that the alveolar anesthetic partial pressure is equal to the anesthetic partial pressure at the effect site, the CNS. Because blood flow to the nervous system in mammals is high, the rate of rise of anesthetic concentration at the effect site has a short time constant, so that CNS partial pressure nearly equals alveolar partial pressure by 15 minutes. In the presence of a large right-to-left shunt, systemic arterial and CNS anesthetic partial pressures would be less than the alveolar anesthetic partial pressure. Moreover, if cerebral blood flow is sufficiently low, redistribution of anesthetic gas to fat depots within the CNS may further decrease the active site anesthetic concentration. In this case, anesthetic partial pressure in the alveoli (ie, MAC) would overestimate the anesthetic partial pressure in the CNS. With increasing duration of anesthesia, anesthetic partial pressure in highly perfused tissues would gradually increase and the alveolar-to-tissue anesthetic partial-pressure gradient would decrease. Hence, MAC would appear to decrease in the face of a constant effect site anesthetic concentration until the alveolar partial pressure reached equilibrium with the effect site partial pressure.

Right-to-left shunts in dogs and children can produce alveolar-to-arterial anesthetic gradients for at least 15 to 20 minutes. If the temporal decrease in MAC in iguanas is a result of delayed equilibration, results of our study would suggest that an alveolar-arterial gradient exists for hours. To test this hypothesis, it would be necessary to compare the arterial and end-tidal anesthetic partial pressures at each MAC determination. Thus, at present, whether the extremes of pulmonary and cardiovascular physiologic characteristics of iguanas can completely account for this difference remains unresolved.

Although intracardiac shunting produced profound hypoxemia, PaO2 was probably not low enough in these iguanas to substantively affect MAC. In addition, a lower metabolic rate and right-shifted, oxygen-hemoglobin dissociation curve in reptiles would mitigate the negative effects of hypoxemia. Although blood pressure was low, compared with anesthetized mammals, values of our study are similar to those obtained in unanesthetized turtles and snakes and only 10% to 20% less than pressures in manually restrained iguanas. Temporal changes in MAC present important considerations for the clinical management of anesthetized iguanas and, possibly, other reptiles. End-tidal anesthetic concentrations needed to prevent movement of healthy iguanas during the beginning of surgery may actually produce deep anesthesia in mammalian and avian patients. In fact, during this early period, cardiac arrest in iguanas does not occur even with end-tidal isoflurane concentrations of 9.2%, a lethal dose in other animals. However, as MAC decreases during the course of anesthesia, a constant end-tidal concentration will produce a deepening plane of anesthesia. On the basis of findings in our study, we predict that the LD50 for the inhaled agents, as measured by the end-tidal concentration, would similarly decrease over time. It also appears that this time dependence may account for the apparent lack of anesthetic effect of sevolurane in reptiles, as suggested in some anecdotal reports. High concentrations may indeed be needed initially, concentrations a clinician may be reluctant to deliver. In our study, purposeful movement following electrical stimuli in 1 iguana could not initially be suppressed at the maximum sevolurane vaporizer setting (7%). However, higher concentrations (achieved by adding a second vaporizer in series) did suppress responses to noxious electrical stimulation, and the concentration needed to achieve immobility during noxious stimulation decreased over time to values comparable to those required in other species.

To summarize, results of our study indicate that MACs for isoflurane, sevolurane, and desflurane in iguanas decrease with time over several hours of anes-
thiesia. Rather than a pharmacodynamic effect, this decrease in anesthetic requirement in this species may, in part, reflect profound pharmacokinetic limitations to anesthetic uptake and distribution. We suggest that early in the anesthesia period in reptiles, expired gas concentrations overestimate CNS anesthetic partial pressures. Design and interpretation of volatile anesthetic potency studies in reptiles should incorporate these limitations. However, after a prolonged period of anesthesia, MACs in iguanas are only modestly higher than values reported in mammals.

b. Attane, provided by Minrad Inc, Philadelphia, Penn.
c. SevoFlo, Abbott Laboratories, North Chicago, Ill.
d. Suprana, provided by Baxter Healthcare Corp, Deerfield, Ill.
e. Fortec, Ohio Medical, Cincinnati, Ohio.
g. Tec 6, Ohmeda Inc, Madison, Wis.
h. Bain breathing circuit, Hudson Respiratory Care Inc, Temecula, Calif.
i. Bird Mark 8 respirator, Bird Corp, Palm Springs, Calif.
j. Wright's respiriometer, Ferraris Medical Ltd, London, UK.
k. BD Insyte, Becton Dickinson Infusion Therapy Systems Inc, Sandy, Utah.
l. Medfusion 2010 syringe pump, Medex Inc, Carlsbad, Calif.
m. Bair Hugger model 505, Arizant Healthcare Inc, Eden Prairie, Minn.

References
