At a minimum, anesthesia consists of at least 2 distinct pharmacologic end points: centrally acting immobilization and amnesia. This list may be expanded to include 3 additional objectives traditionally considered part of anesthesia: analgesia, unconsciousness, and autonomic quiescence. However, it is not known whether these effects are independent of each other or whether changes in 1 end point can be used to predict changes in other end points.

The MAC is an index of the immobilizing potency for inhalation anesthetics. Reductions in MAC have been proposed as a means by which the relative potencies of coadministered analgesic agents may be compared. However, to our knowledge, the assumption that analgesic drugs should decrease inhalation anesthetic requirements has not been validated. From the viewpoint of anesthetic mechanisms, there are several reasons why this assumption may not, in fact, be true. First, pain is not simply a sensory-discriminative phenomenon; rather, it is also a supraspinal phenomenon that encompasses motivational-affective and cognitive-evaluative components. However, immobilization with inhalation anesthetics, as measured by use of MAC, is a spinaly mediated phenomenon that is independent of the cerebral cortex and dorsal horn of the spinal cord. Second, there is no evidence that contemporary volatile agents have analgesic effects; rather, these agents cause hyperalgesia at low MAC fractions that may antagonize analgesic drug actions. Third, some drugs exhibit

### Effects of remifentanil on measures of anesthetic immobility and analgesia in cats

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**Objective**—To evaluate effects of various doses of remifentanil on measures of analgesia in anesthetized cats.

**Animals**—6 healthy adult cats.

**Procedures**—Minimum alveolar concentration (MAC) for isoflurane and thermal threshold responses were evaluated in anesthetized cats. Remifentanil infusions of 0 (baseline), 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 μg/kg/min were administered; after a 45-minute equilibration period, isoflurane MAC and responses were determined. Isoflurane MAC was determined in anesthetized cats once for each remifentanil infusion rate by use of a standard tail clamp technique. Thermal threshold was measured in awake cats by use of a commercially available analgesiometric probe placed on the lateral portion of the thorax; remifentanil infusions were administered in randomized order to anesthetized cats, and thermal threshold determinations were made by an investigator who was unaware of the infusion rate.

**Results**—Mean ± SEM median effective concentration (EC₅₀) for remifentanil and its active metabolite, GR90291, for the thermal threshold test was 1.00 ± 0.35 ng/mL and 307 ± 28 ng/mL of blood, respectively. Dysphoria was detected in all awake cats at the 2 highest remifentanil infusion rates. However, isoflurane MAC during remifentanil infusions was unchanged from baseline values, even at blood opioid concentrations approximately 75 times the analgesic EC₅₀.

**Conclusions and Clinical Relevance**—Immobility and analgesia as reflected by thermal threshold testing were independent anesthetic end points in the cats. Results of MAC-sparing evaluations should not be used to infer analgesic potency without prior validation of an MAC-analgesia relationship for specific drugs and species. (Am J Vet Res 2009;70:1065–1071)

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%TE</td>
<td>Percentage thermal threshold excursion</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>Median effective concentration</td>
</tr>
<tr>
<td>ED₅₀</td>
<td>Median effective dose</td>
</tr>
<tr>
<td>ERC</td>
<td>Equivalent remifentanil concentration</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>MAC</td>
<td>Minimum alveolar concentration</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>PETCO₂</td>
<td>End-tidal partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>SWG</td>
<td>Standard wire gauge</td>
</tr>
<tr>
<td>WDR</td>
<td>Wide dynamic range</td>
</tr>
</tbody>
</table>
Effects that are irreconcilable with an MAC-analgesia relationship. For example, acepromazine lacks analgesic efficacy, yet it decreases halothane MAC in dogs by approximately 40%.16 In contrast, administration of the opioid-antagonist naloxone has no effect on halothane MAC in cats.17 Fourth, many opioid agonists have a variable capacity, depending on species, to cause central excitation. If these drugs are antagonistic to anesthetic immobilizing effects, excitation could mask an MAC-analgesia relationship.

We hypothesized that dose-effect curves for measures of anesthetic immobility (as measured via MAC) and analgesia (as measured via thermal threshold) would not be collinear in cats administered the phenylpiperadine opioid, remifentanil. We further hypothesized that remifentanil doses that induce dysphoria in awake cats would increase isoﬂurane MAC in anesthetized cats. Therefore, the study reported here was conducted to evaluate the effects of remifentanil administration on measures of anesthesia and analgesia in cats.

Materials and Methods

Animals—Six healthy 1.5-year-old spayed female cats were used in the study. Mean ± SEM body weight of the cats was 4.2 ± 0.2 kg. Cats were used in a randomized crossover design to evaluate the effects of remifentanil on isoﬂurane MAC and thermal threshold analgesia; there was at least a 1-week interval between paired evaluations for any cat. Remifentanil pharmacokinetics were measured in this same group of cats approximately 1 month prior to the study reported here; those measurements were conducted while the cats were awake and again while they were anesthetized.13 Cats were maintained in a vivarium on a cycle of 12 hours of light and 12 hours of darkness, and food and water were available ad libitum. Food was withheld from cats overnight prior to either the MAC or thermal threshold evaluations. The Institutional Animal Care and Use Committee at the University of California, Davis, approved this project.

Remifentanil and isoﬂurane MAC—Anesthesia was induced in unmedicated cats by use of isoﬂurane in oxygen delivered in an acrylic glass chamber. Each cat was then intubated with a 4.5-mm cuffed endotracheal tube that had a sampling port near the distal tip; endotracheal tubes were then connected to a coaxial Mapleson F breathing circuit with an oxygen flow rate of 1 L/min and a temperature-compensated out-of-circuit isoﬂurane vaporizer setting of approximately 2%. A 20-SWG, 2.2-cm polytetrafluoroethylene infusion catheter was percutaneously placed in a medial saphenous vein, and a 22-SWG, 10-cm central venous polyurethane sampling catheter was placed in a jugular vein by use of a modified Seldinger technique. Aseptic procedures were used for catheter insertion.

Cats were mechanically ventilated with a pneumatically powered, pressure-controlled ventilator that delivered 10 cm H2O during peak inspiration at a rate sufficient to maintain PETCO2 between 35 and 45 mm Hg, as measured by use of a Raman scatter analyzer.4 No attempt was made to prevent cats from spontaneous respiration during ventilator expiratory pauses, which at times resulted in a PETCO2 < 35 mm Hg. The same analyzer was also used to obtain pulse oximeter measurements from the tongue of each cat.

Alveolar gases were collected by use of 3-mL glass syringes from 3 or 4 end-expiratory breaths, and samples were analyzed by use of an infrared gas analyzer.5 Values were corrected by use of a least-squares calibration curve generated from multiple standard mixtures of isoﬂurane of certified accuracy1 that encompassed the range of isoﬂurane concentrations measured in this study. At each gas collection, systolic blood pressure and heart rate were measured by use of a Doppler probe placed over a radial artery and a sphygmomanometer with a cuff width equal to 40% of the limb circumference. Body temperature was measured with an esophageal thermistor probe calibrated against a thermometer of certified accuracy. Normothermia was maintained by use of recirculating warm water blankets and forced-air warmers as needed.

After an initial equilibration of 1 hour, cardiopulmonary measurements were collected, and movement in response to application of a tail clamp was assessed. When a cat moved, the inspired isoﬂurane concentration was increased by 10% to 15% for 20 minutes. When a cat did not move, the inspired isoﬂurane concentration was decreased by 10% to 15% for 20 minutes. Then, cardiopulmonary responses and gross movement (or lack of gross movement) following application of a tail clamp were recorded. This process was repeated until there were 2 end-tidal isoﬂurane concentrations that differed by ≤ 15% and that permitted and prevented movement in response to application of the tail clamp; the arithmetic mean of these concentrations was defined as the MAC.2

Remifentanil was then administered via a syringe pump through the catheter in the saphenous vein. Remifentanil was infused at rates of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 µg/kg/min. Lyophilized remifentanil powder was constituted with sterile, preservative-free saline (0.9% NaCl) solution to create a solution of 5 µg/mL for infusion rates ≤ 1 µg/kg/min or a solution of 80 µg/mL for infusion rates ≥ 2 µg/kg/min. Cats were allowed an equilibration period of at least 45 minutes at the start of the remifentanil infusions or after each change in remifentanil infusion rate, which was equal to approximately 3 terminal half-lives for remifentanil in isoﬂurane-anesthetized cats.3 Cardiopulmonary measurements and MAC determinations were performed following the equilibration period for each infusion rate. At the end of each MAC determination, 1 mL of blood was collected from the jugular vein via the catheter, added to a cryogenic tube containing 20 µL of a 50% citric acid solution, flash-frozen in liquid nitrogen, and stored at ~80°C until analysis. Cats recovered from anesthesia at the end of the experiment; once they appeared clinically normal, they were returned to the vivarium.

Remifentanil and analgesia—On the day prior to an experiment, cats were briefly anesthetized with isoﬂurane. A central venous catheter was inserted for collection of samples, and the lateral portion of the thorax was shaved. On the day of an experiment, a catheter...
was inserted in a medial saphenous vein for infusion of solutions; a nonrestrictive cohesive bandage was then wrapped around both catheters. A 10-mm² thermal threshold testing probe, which consisted of a heater element and adjacent temperature sensor, was held in constant contact with the shaved thoracic skin by use of an air bladder inflated to 100 mm Hg beneath a circumferential elastic band. The temperature sensor was calibrated each day with a digital reference thermometer. This remote thermal threshold testing device has been specifically developed and validated for use in cats. Instrumented cats were allowed to move freely within an 80 × 80 × 65-cm mirrored metabolic cage with an acrylic glass front. Cables for the testing device were passed through a hole in the top of the cage. After an acclimation period of at least 30 minutes, skin temperature was measured and then the temperature of the heater element was increased at a rate of 0.6°C/s until the cat responded to the noxious stimulus (ie, jumping, biting, scratching, licking, or turning toward the probe) or until a thermal cutoff of 59°C was reached. After determining the first threshold value, 1 mL of blood was collected from a jugular vein into citrated cysogen tubes, flash-frozen, and stored at −80°C. A second 1-mL sample was collected from the jugular vein and used immediately for blood gas analysis with an automated gas analyzer and cooximeter. Rectal temperature was measured by use of a digital thermometer. Heart and respiratory rates were also recorded. Cats were subjectively evaluated for behavior (defined as frenetic jumping, twisting, or rolling) characteristic of dysphoria. Baseline thermal threshold (remifentanil infusion rate, 0 µg/kg/min) was retested 15 minutes later.

Remifentanil solutions were prepared as previously described and then infused by use of a syringe pump at a rate of 0.0625, 0.125, 0.25, 0.5, 1.2, 4, 8, or 16 µg/kg/min. The infusion order was assigned by use of a randomization procedure, and the investigator who performed all thermal threshold assessments was unaware of the rate and concentration of administered drug. After an equilibration period of 45 minutes at each infusion rate, which exceeded the time equal to 2.5 terminal half-lives for remifentanil in awake cats, thermal threshold assessments, blood sample collections and analyses, and physiologic response measurements were conducted as described for the baseline evaluations. When a cat became severely dysphoric, remifentanil infusion was stopped for 10 minutes and administration was then continued at the subsequent infusion rate. In the event of hyperthermia (body temperature ≥ 40°C), cats were cooled with fans and bags of ice placed in the cage. After data for all 9 remifentanil infusions were collected, drug administration ceased for 45 minutes and all data were collected as described for the initial baseline measurements. Cats appeared clinically normal at the end of the experiment and were returned to the vivarium.

Remifentanil analysis—Concentrations of remifentanil and its active metabolite, GR90291, were measured in blood samples by use of LC-MS via modification of a method published elsewhere. Acetic acid, acetonitrile, and water were high-performance liquid chromatography grade, and formic acid was spectrophotometric grade. Fentanyl was used as an analytic internal standard.

Remifentanil and GR90291 were quantitated by use of LC-MS analysis of extracted blood samples. The calibration standards were prepared from stock solutions made by dissolving 10.0 mg of remifentanil standard in 10.0 mL of acetonitrile. Working solutions were prepared by dilution of the remifentanil stock solution with acetonitrile to achieve concentrations of 100 and 500 ng/mL. Blood calibrators were prepared by dilution of the working remifentanil solutions with drug-free blood to achieve concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10 ng/mL. Blood calibrators were prepared by dilution of the working GR90291 solutions with drug-free blood to achieve concentrations of 0.5, 1.0, 5.0, 10, 50, 100, 500, and 1,000 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality-control samples (blood fortified with analytes at concentrations at the midpoint of the standard curve) were routinely included as an additional evaluation of accuracy. The concentration of remifentanil in each sample was determined by use of the internal standard method via peak area ratio and linear regression analysis. Fentanyl was used as an internal standard. Limits of quantification were 0.05 and 0.5 ng/mL for remifentanil and GR90291, respectively.

Quantitative analyses were performed on a mass spectrometer coupled to a liquid chromatography system. High-performance liquid chromatography was performed with a 5-cm × 2.1-mm, 3-µm column and a linear gradient of acetonitrile in water with 0.2% formic acid at a flow rate of 0.4 mL/min. The acetone concentration was maintained at 10% for 0.4 minutes and then incrementally increased to 90% during the next 7 minutes. Prior to analysis, blood samples, standard solutions, and quality-control samples were allowed to thaw at 20°C. Plasma proteins were extracted by precipitation via the addition of 0.5 mL of acetonitrile containing internal standard (10 ng/mL). All samples were vortexed for 1.5 minutes, which was followed by centrifugation (2,000 × g for 5 minutes at 4°C). Injection volume was 10.0 µL.

Detection and quantification were performed via full-scan LC-MS transitions of initial protonated precursor ions for remifentanil and GR90291 (m/z, 377 and 363, respectively). The response for the major product ions for remifentanil (m/z, 317 and 285) and GR90291 (m/z, 331, 303, and 259) was plotted, and peaks at the proper retention time were integrated by use of a computer program. That program was also used to generate calibration curves and quantitate analytes in all samples.

To evaluate the accuracy and precision of the analytic method, quality-control samples were prepared by spiking blank blood samples with remifentanil to achieve concentrations of 0.1, 1.0, and 10 ng/mL and with GR90291 to achieve concentrations of 150, 450, and 750 ng/mL. Standard solutions and quality-control samples were treated in the same manner as samples obtained from the cats. The method yielded linear results between 0.05 and 50 ng/mL (R² ≥ 0.998) for remifentanil and between 5.0 and 1,000 ng/mL for GR90291 (R² ≥ 0.998). Interassay and intra-assay coefficients of variation were < 10%.
Data analysis—Data were reported as mean ± SEM. Physiologic responses as a function of dose were analyzed by use of a repeated-measures ANOVA with Dunn-Sidak corrections for multiple post hoc comparisons (when appropriate) by use of commercially available statistical software. Results were considered significant at values of *P* < 0.05.

For analgesia assessments, the %TE was calculated by use of the following equation:

\[
\text{%TE} = 100 \times \left(\frac{T_s - T_i}{T_s - T_c}\right)
\]

where *T*<sub>s</sub> is the thermal threshold temperature, *T*<sub>i</sub> is the original skin temperature, and *T*<sub>c</sub> is the thermal cutoff temperature. Because the active metabolite, GR90291, increased the apparent analgesic efficacy of low blood concentrations of remifentanil, effects of remifentanil could not be directly modeled and analyzed without first correcting the data for these metabolite effects. The %TE was plotted as a function of the GR90291 concentration measured at the end of each analgesic experiment (when the blood remifentanil concentration was less than the limit of quantitation), and an EC<sub>50</sub> for GR90291 was calculated from a linear regression of the rectilinear response. A subset of thermal threshold test data for which blood GR90291 concentration was < 170 ng/mL (which corresponded to < 20% analgesic effect) was plotted as a function of blood remifentanil concentration. The sigmoid curve was fit to the Hill equation by use of nonlinear regression, and the ED<sub>50</sub> of remifentanil was computed. Remifentanil-versus-metabolite potency was then calculated as the ratio of the ED<sub>50</sub> for GR90291 to the ED<sub>50</sub> for remifentanil. The ERC was defined as the sum of the blood remifentanil concentration plus the quotient of the blood GR90291 concentration divided by the remifentanil-versus-metabolite potency. The full data set of %TE was then graphed and modeled as a sigmoidal regression function of the ERC by use of the Hill equation.

**Results**

Mean ± SEM isoflurane MAC in cats was 1.94 ± 0.08% and was unchanged by infusion of remifentanil (Figure 1). Variations in P<sub>E</sub>TCO<sub>2</sub> and body temperature, although at times significant, were small in magnitude and not likely to be of physiologic importance (Table 1).

Remifentanil caused a dose-dependent increase in thermal threshold. The thermal cutoff (35°C) was reached with a remifentanil infusion of 1.58 ± 0.52 µg/kg/min. Infusions ≥ 1 µg/kg/min were also associated with dysphoric behavior, as characterized by increased and sometimes frenetic locomotor activity that precluded evaluation of the highest infusion rate in 2 cats and the second-highest rate in 1 cat. Physiologic changes were consistent with increases in metabolic activity: namely, an increase in P<sub>V</sub>CO<sub>2</sub>, a decrease in venous pH, and an increase in heart rate (Table 2). Although not significant, there was also a slight increase in respiratory rate and body temperature. The P<sub>V</sub>CO<sub>2</sub> was 13 mm Hg at the highest infusion rate evaluated in 1 cat, which suggested high blood oxygen extraction by peripheral tissues.

![Figure 1](image-url)

**Figure 1**—Change in isoflurane MAC as a function of the remifentanil concentration for 6 cats administered infusions of remifentanil at rates of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, and 16 µg/kg/min. Each symbol represents results for 1 cat. Mean ± SEM change in isoflurane MAC for each of the 9 remifentanil infusion rates is identified (mean is the horizontal marker on the vertical bars, and SEM is the height of the bars). Remifentanil did not have a significant (*P* < 0.05) effect on isoflurane MAC.

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**Table 1**—Mean ± SEM results for physiologic variables and blood concentrations of remifentanil and GR90291 on the basis of the remifentanil infusion rate in 6 cats anesthetized with isoflurane at 1.0 MAC.

<table>
<thead>
<tr>
<th>Infusion rate (µg/kg/min)</th>
<th>Remifentanil concentration (ng/mL)</th>
<th>GR90291 concentration (ng/mL)</th>
<th>P&lt;sub&gt;E&lt;/sub&gt;TCO&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Respiratory rate (breaths/min)</th>
<th>SpO&lt;sub&gt;2&lt;/sub&gt; (%)</th>
<th>Body temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>102 ± 3</td>
<td>174 ± 5</td>
<td>9 ± 1</td>
<td>98 ± 1</td>
<td>38.3 ± 0.1</td>
<td>38.9 ± 0.1</td>
</tr>
<tr>
<td>0.0625</td>
<td>0.45 ± 0.08</td>
<td>148 ± 70</td>
<td>31 ± 1</td>
<td>167 ± 5</td>
<td>9 ± 1</td>
<td>98 ± 0</td>
<td>38.7 ± 0.1*</td>
<td>38.8 ± 0.1</td>
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<tr>
<td>0.125</td>
<td>0.76 ± 0.10</td>
<td>175 ± 79</td>
<td>31 ± 1</td>
<td>179 ± 8</td>
<td>10 ± 1</td>
<td>98 ± 0</td>
<td>38.6 ± 0.1</td>
<td>38.9 ± 0.1</td>
</tr>
<tr>
<td>0.25</td>
<td>1.41 ± 0.29</td>
<td>268 ± 121</td>
<td>34 ± 1*</td>
<td>185 ± 2</td>
<td>11 ± 2</td>
<td>98 ± 0</td>
<td>38.8 ± 0.1*</td>
<td>38.8 ± 0.1</td>
</tr>
<tr>
<td>0.5</td>
<td>2.02 ± 0.48</td>
<td>280 ± 110</td>
<td>32 ± 1</td>
<td>199 ± 8</td>
<td>13 ± 3</td>
<td>98 ± 0</td>
<td>39.0 ± 0.1</td>
<td>39.4 ± 0.1</td>
</tr>
<tr>
<td>1</td>
<td>4.35 ± 1.36</td>
<td>399 ± 148</td>
<td>33 ± 1</td>
<td>198 ± 8</td>
<td>16 ± 5</td>
<td>98 ± 0</td>
<td>38.9 ± 0.1</td>
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</tr>
<tr>
<td>2</td>
<td>7.01 ± 1.67</td>
<td>649 ± 258</td>
<td>35 ± 2</td>
<td>193 ± 6</td>
<td>11 ± 2</td>
<td>98 ± 0</td>
<td>39.1 ± 0.2</td>
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<tr>
<td>4</td>
<td>18.78 ± 3.36</td>
<td>860 ± 542</td>
<td>35 ± 3</td>
<td>177 ± 12</td>
<td>11 ± 2</td>
<td>99 ± 0</td>
<td>38.8 ± 0.1</td>
<td>38.9 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>27.70 ± 8.42</td>
<td>1,038 ± 327</td>
<td>33 ± 1</td>
<td>183 ± 12</td>
<td>10 ± 1</td>
<td>98 ± 0</td>
<td>38.8 ± 0.1*</td>
<td>38.8 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>74.78 ± 8.18</td>
<td>405 ± 137</td>
<td>33 ± 0*</td>
<td>182 ± 10</td>
<td>11 ± 1</td>
<td>99 ± 0</td>
<td>38.6 ± 0.1</td>
<td>38.6 ± 0.1</td>
</tr>
</tbody>
</table>

*Within a column, value differs significantly (*P* < 0.05; Dunn-Sidak correction for multiple paired comparisons) from the value for the infusion rate of 0 µg/kg/min (baseline).

SpO<sub>2</sub> = Oxygen saturation as measured by pulse oximetry.
To estimate potency of remifentanil alone, these thermal
recovery effects were relatively small when blood
concentrations of GR90291 were < 170 ng/mL (Figure
2). Hence, values for %TE for this data subset were as-
sumed to principally result from the effects of remif-
entanil rather than from the effects of its metabolite. Thus,
to estimate potency of remifentanil alone, these thermal

Table 2: Mean ± SEM results for physiologic variables and blood concentrations of remifentanil and GR90291 on the basis of the remifentanil infusion rate in 6 awake cats.

<table>
<thead>
<tr>
<th>Infusion rate (µg/kg/min)</th>
<th>Remifentanil concentration (ng/mL)</th>
<th>GR90291 concentration (ng/mL)</th>
<th>Heart rate (beats/min)</th>
<th>Respiration rate (breaths/min)</th>
<th>Body temperature (°C)</th>
<th>Venous pH</th>
<th>Pvco₂ (mm Hg)</th>
<th>Pvco₂ (mm Hg)</th>
<th>Hemoglobin (g/dL)</th>
<th>Dysphoria (No. of cats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Before)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>108 ± 14</td>
<td>71 ± 16</td>
<td>38.7 ± 0.3</td>
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<td>38 ± 1</td>
<td>36 ± 2</td>
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<tr>
<td>0.025</td>
<td>0.13 ± 0.04</td>
<td>146 ± 83</td>
<td>232 ± 19</td>
<td>99 ± 15</td>
<td>39.2 ± 0.4</td>
<td>7.38 ± 0.01</td>
<td>39 ± 1</td>
<td>36 ± 1</td>
<td>12.0 ± 0.5</td>
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<tr>
<td>0.125</td>
<td>0.21 ± 0.06</td>
<td>339 ± 127</td>
<td>205 ± 17</td>
<td>85 ± 19</td>
<td>37.8 ± 0.3</td>
<td>7.37 ± 0.01</td>
<td>42 ± 1</td>
<td>36 ± 1</td>
<td>12.0 ± 0.3</td>
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<tr>
<td>0.25</td>
<td>0.39 ± 0.10</td>
<td>130 ± 50</td>
<td>196 ± 15</td>
<td>70 ± 11</td>
<td>38.6 ± 0.4</td>
<td>7.36 ± 0.01</td>
<td>39 ± 3</td>
<td>36 ± 4</td>
<td>12.0 ± 0.7</td>
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<td>0.5</td>
<td>0.77 ± 0.15</td>
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<td>233 ± 18</td>
<td>55 ± 7</td>
<td>38.7 ± 0.3</td>
<td>7.36 ± 0.01</td>
<td>35 ± 2</td>
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<td>10.9 ± 0.4</td>
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<tr>
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<td>1.83 ± 0.38</td>
<td>176 ± 77</td>
<td>234 ± 15*</td>
<td>44 ± 4</td>
<td>39.0 ± 0.6</td>
<td>7.32 ± 0.01</td>
<td>41 ± 1</td>
<td>41 ± 1</td>
<td>11.1 ± 0.5</td>
<td>2</td>
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<tr>
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<td>4.09 ± 1.40</td>
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<td>240 ± 13</td>
<td>51 ± 4</td>
<td>39.4 ± 0.4</td>
<td>7.28 ± 0.02</td>
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<td>47 ± 1*</td>
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<tr>
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<td>236 ± 22</td>
<td>60 ± 6</td>
<td>40.4 ± 0.5</td>
<td>7.24 ± 0.04</td>
<td>31 ± 4</td>
<td>53 ± 5</td>
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<tr>
<td>8</td>
<td>9.54 ± 2.15</td>
<td>429 ± 81</td>
<td>271 ± 25*</td>
<td>139 ± 58</td>
<td>40.8 ± 0.3</td>
<td>7.23 ± 0.02</td>
<td>33 ± 3</td>
<td>44 ± 3</td>
<td>12.6 ± 0.5</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>23.74 ± 5.19</td>
<td>792 ± 108</td>
<td>243 ± 19</td>
<td>69 ± 12</td>
<td>39.5 ± 0.5</td>
<td>7.21 ± 0.03</td>
<td>29 ± 3</td>
<td>55 ± 2*</td>
<td>12.4 ± 0.2</td>
<td>6</td>
</tr>
<tr>
<td>0 (After)</td>
<td>0 ± 0</td>
<td>303 ± 88</td>
<td>204 ± 12</td>
<td>118 ± 31</td>
<td>37.5 ± 0.3</td>
<td>7.39 ± 0.01</td>
<td>40 ± 2</td>
<td>35 ± 1</td>
<td>11.6 ± 0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Dysphoria represents the number of cats with behavior (defined as frenetic jumping, twisting, or rolling) characteristic of dysphoria. The 0 (Before) and 0 (After) infusion rates represent the periods used to determine baseline values before and after completion of all remifentanil infusions, respectively.

See Table 1 for remainder of key.

Figure 2—The %TE as a function of the blood GR90291 concentration in samples obtained from 6 cats at least 45 minutes after cessation of remifentanil infusions. Each symbol represents results for 1 cat. Remifentanil was undetectable in blood of all 6 cats. Line for the least-squares regression (R² = 0.8) reveals that the mean ± SEM EC₅₀ for GR90291 is 307 ± 28 ng/mL.

The remifentanil and %TE dose response could not be clearly described with a pharmacodynamic model without taking into account the effects of the active metabolite, GR90291. At the end of each experiment, postinfusion thermal threshold responses were not influenced by remifentanil; remifentanil concentrations were not detectable in blood samples obtained at that time (Table 2). Hence, final %TE versus GR90291 concentration was plotted (Figure 2). Results for that plot were described by use of the following equation: %TE = (0.24 × GR90291 concentration) – 23, which had an R² of 0.8. Mean ± SEM predicted GR90291 EC₅₀ was 307 ± 28 ng/mL.

Effects of GR90291 were relatively small when blood concentrations of GR90291 were < 170 ng/mL (Figure 2). Hence, values for %TE for this data subset were assumed to principally result from the effects of remifentanil rather than from the effects of its metabolite. Thus, to estimate potency of remifentanil alone, these thermal

Figure 3—The %TE in 6 cats as a function of the ERC, as calculated by use of the equation ERC = Rₕₑₜ + (GR90291 concentration/307), where Rₕₑₜ is the blood remifentanil concentration. Each symbol represents results for 1 cat. Remifentanil is 307 times as potent as its metabolite, GR90291. Data are described by the equation %TE = (1100 X ERC⁻¹/[ERC⁻¹ + 1.271]), where mean ± SEM ERC is 1.17 ± 0.14 (horizontal bar on the solid sigmoidal line).

Threshold responses were modeled by use of the equation %TE = ([1100 X Rₕₑₜ⁻⁰.⁴⁹]/[(Rₕₑₜ⁻⁰.⁴⁹ + 1.00)], where Rₕₑₜ is the blood remifentanil concentration. Mean ± SEM predicted ERC for remifentanil alone was 1.00 ± 0.35 ng/mL, and the mean of the Hill coefficient was 0.91 ± 0.30. Least-squares regression between infusion rate and blood remifentanil concentration in conscious cats yielded the relationship Rₕₑₜ = 1.44 X infusion rate. Thus, the remifentanil EC₅₀ should have been achieved by infusions at the rate of 0.7 µg/kg/min.

Because the EC₅₀ values for GR90291 and remifentanil were 307 and 1.00 ng/mL, respectively, their ratio yielded a remifentanil-versus-metabolite potency of 307. Hence, remifentanil analgesic potency, as assessed on the basis of thermal threshold testing, was 307 times that of its metabolite. The ERC was calculated to correct thermal threshold responses to account for analgesic effects of GR90291 (which was especially important during low infusion rates of remifentanil) by use of the equation ERC = Rₕₑₜ + (GR90291 concentration/307). All %TE responses could be described as a function of the ERC by use of the equation %TE = (1100 X ERC⁻¹/ERC⁻¹ + 1.271).
(ERC$_{50}^{1.49}$ + 1.27)). Mean ± SEM ERC EC$_{50}$ was 1.17 ± 0.14 ng/mL, and the Hill coefficient was 1.49 ± 0.28 (Figure 3).

Discussion

We did not detect a relationship between remifentanil immobilizing potency (as assessed on the basis of reduction in isoflurane MAC) and remifentanil analgesic potency (as assessed on the basis of results of thermal threshold testing). Isoflurane MAC in this study was similar to values reported elsewhere for cats$^{8,10}$; however, there was no discernable opioid effect, despite a 236-fold range in remifentanil dose. In contrast, both remifentanil and its metabolite induce analgesic effects, and their relative potencies are within the range published elsewhere.$^{20}$

Three explanations exist for these divergent MAC and analgesia findings. First, immobilizing mechanisms of action for inhalation anesthetics do not involve $\mu$-opioid receptors.$^{11}$ Therefore, an analgesic effect need not imply an MAC-sparing effect (and vice versa). Second, opioids suppress high-threshold nociceptive neurons to a greater extent than they suppress WDR neurons.$^{21}$ If most of the noxious heat stimulus is transmitted by WDR neurons, then opioids should greatly increase thermal threshold. Conversely, intense stimulation of both WDR and high-threshold nociceptive neurons by use of a tail clamp may minimize opioid efficacy. Third, neuronal sensitivity to opioids may vary as a function of anatomic location within the spinal cord. In WDR neurons located in lamina I within the spinothalamic tract of cats, morphine effectively attenuates responses to both heat and pinch stimuli. However, in WDR neurons located in lamina I outside of the spinothalamic tract, morphine inhibits responses to heat but not to pinch stimuli.$^{22}$ Thus, activation of large numbers of WDR neurons outside the spinothalamic tract during application of a tail clamp in cats may explain the lack of remifentanil effects on isoflurane MAC.

High doses of remifentanil induced dysphoria in all cats and evoked physiologic changes consistent with an increase in metabolic rate (Table 2). Activation of $\delta$-opioid receptors by endogenous enkephalins is associated with increased cumulative locomotor activity in wild-type and $\mu$-opioid receptor-knockout mice$^{23}$ and may help explain the behavioral excitement in the cats of our study. However, opioid excitement in awake cats did not translate to an increase in isoflurane MAC in anesthetized cats. Hence, those molecular targets that mediate dysphoria may be excluded as modulators of immobility induced by inhalation anesthetics.

Although remifentanil does not change the isoflurane MAC in cats, remifentanil decreases inhalation anesthetic requirements by up to 63% in dogs$^{24}$ and up to 91% in humans.$^{25}$ However, even with other $\mu$- or $\kappa$-agonists (or a combination of $\mu$- and $\kappa$-agonists), reduction of isoflurane MAC in cats is a maximum of 11% to 35%.$^{26,27}$ These modest effects are typically achieved only with high doses of opioids and may reflect activity at nonopioid receptors. It is possible that differences in remifentanil activity at nonopioid targets in cats account for the lack of effects on isoflurane MAC in the study reported here.

Acute opioid tolerance may contribute to hyperalgesia on termination of a remifentanil infusion$^{28}$ because the drug is rapidly metabolized.$^{13}$ However, in the study reported here, postinfusion baseline measurements of thermal threshold were consistently higher than preinfusion baseline measurements. Prolonged analgesic effects may arise from accumulation of GR90291; thus, low-level effects of $\mu$-agonists can persist despite a lack of detectable remifentanil concentration in the blood. Remifentanil, particularly at high concentrations, also causes direct activation of N-methyl-d-aspartic acid receptors in humans,$^{29}$ which could have played a role in preventing acute opioid tolerance in the cats of our study.

It has been proposed$^3$ that assessment of MAC-sparing effects may allow comparisons among analgesic substances. However, if inhalation anesthetic requirements are to provide meaningful inferences with respect to analgesia, then analgesic drugs must, at some reasonable multiple of analgesic potency, have an MAC-sparing effect. However, at the highest infusion rate, there was no change in isoflurane requirement, even though the mean blood concentration of remifentanil was approximately 75 times the thermal analgesic EC$_{50}$.

Perhaps cats represent an extreme example of complete dissociation between the immobilizing and analgesic effects of a narcotic. Effective analgesic doses of remifentanil may induce varying degrees of reduction in isoflurane MAC in other species. However, as determined in cats, the extent or existence of an analgesic-MAC relationship cannot simply be assumed for all analgesics in all species. Therefore, we concluded that measures of anesthetic immobility need not bear any relation to measures of analgesic efficacy. Consequently, MAC-sparing effects should not be used to infer analgesic effects without prior validation of the nature of such a relationship for a specific agent and species.

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j. Ultiva, Abbott Laboratories, North Chicago, Ill.
l. 85 components, Corby, Northamptonshire, England.
m. ABL3, Radiometer America, Westlake, Ohio.
n. OSM3, Radiometer America, Westlake, Ohio.
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r. Cerilliant, Round Rock, Tex.
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t. 1100 series, Agilent Technologies, Palo Alto, Calif.
u. Discovery HS C18, Supelco Inc, Bellefonte, Pa.
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