Opioids are commonly administered because of their analgesic properties. Fentanyl is a potent short-acting member of the class of µ-opioid agonists that is used as an analgesic and, in some species, to reduce inhalation anesthetic requirements (defined by a reduction in MAC). In addition to providing analgesia, opioid–inhalation anesthetic combinations have been associated with beneficial cardiovascular effects, which are thought to be related to opioid-induced MAC reduction. Fentanyl is considered to have minimal effects on the cardiovascular system, except that it reduces heart rate in some species. Fentanyl is, however, a potent respiratory depressant. The mortality rate related to anesthesia is higher in rabbits than in dogs and cats. This may, in part, result from the extreme cardiovascular depression associated with inhalation anesthesia in this species.

The magnitude of opioid-induced sparing of the MAC differs among species. Potent opioids can result in a MAC reduction of 60% or greater in dogs, rats, and goats but moderate to no reduction in cats and horses. There has been minimal investigation of the MAC-sparing effects of opioids in rabbits, and the authors are aware of no such investigations conducted with µ-opioid receptor agonists. Therefore, the purpose of the study reported here was to determine the effects of fentanyl at 6 targeted plasma concentrations on the isoflurane MAC in rabbits.

Materials and Methods

ANIMALS

Six adult female New Zealand White rabbits (Oryctolagus cuniculus) with a mean ± SD body weight of 4.1 ± 0.4 kg were used in the study. Rabbits were deemed healthy on the basis of no abnormal results detected during physical examination. Rabbits were housed individually in wire cages (45 X 55 X 50 cm)
in a room that contained only rabbits. The room was on a light cycle of 12 hours of light to 12 hours of darkness and maintained at a mean ± SD temperature of 21 ± 1°C. Rabbits were fed 142 g of pelleted chow/d and had unlimited access to water. The study was conducted with institutional animal care and use committee approval.

ANESTHESIA AND INSTRUMENTATION
Rabbits were anesthetized with isoflurane in oxygen; each anesthetic episode began at approximately the same time of day. Rabbits initially were placed in a transparent acrylic chamber, and anesthetic was delivered into that chamber by use of a Bain circuit with oxygen flow of 7 L/min and vaporizer setting of 5% isoflurane. Once rabbits lost the righting reflex, they were removed from the chamber, and the anesthetic was then delivered by face mask attached to the Bain circuit with O2 flow reduced to 3 L/min. Once rabbits were at an appropriate anesthetic depth, judged by lack of withdrawal to a toe pinch, an orotracheal tube was placed (inside diameter, 3.5 to 4 mm) and connected to the Bain circuit with an O2 flow of 1 L/min and vaporizer setting of 2.5%.

A 22-gauge, 0.75-inch catheter was placed in a lateral saphenous vein for fluid and drug administration, and a 20-gauge, 1.88-inch catheter was placed percutaneously in a jugular vein for collection of blood samples. Throughout anesthesia, lactated Ringer’s solution was delivered IV at a rate of 3 mL/kg/h with a fluid pump. Three sets of paired 25-gauge hypodermic needles were placed subcutaneously on the shaved ventral aspect of the tail. Rabbits were mechanically ventilated with a pneumatically driven, pressure-limited ventilator to a peak inspiratory pressure of 12 cm H2O, and respiratory rate was adjusted to maintain end-expired PCO2 between 30 and 35 mm Hg.

A Doppler piezoelectric crystal and occluding cuff were placed over a median artery for SBP measurement at 5-minute intervals. A calibrated temperature probe was placed into the esophagus, advanced to the level of the heart, and connected to a physiograph with acquisition software for continuous measurement of body temperature. External heat was applied in the form of a circulating water blanket and forced air warming unit, as needed, to maintain body temperature between 38.5° and 39.5°C. At the end of the experiment, instruments were removed, buprenorphine (0.05 mg/kg, IV) and meloxicam (0.5 mg/kg, SC) were administered, and the rabbits were allowed to recover.

FENTANYL ADMINISTRATION AND MAC DETERMINATION
The pharmacokinetics of fentanyl in isoflurane-anesthetized rabbits has been determined in a preliminary unpublished study conducted by our laboratory group. Isoflurane MAC was determined in duplicate before the administration of fentanyl (baseline) and at each of 6 target plasma fentanyl concentrations (2, 4, 8, 16, 32, and 64 ng/mL). Each rabbit was anesthetized 2 times with an interval of at least 7 days between anesthetic episodes. Target plasma concentrations for each of these 2 days were selected at random out of a hat and then arranged in ascending order for that day (ie, MAC was determined at baseline and for 3 fentanyl concentrations during the first day of anesthesia and for the remaining 3 fentanyl concentrations during the second day of anesthesia).

Anesthetized rabbits were positioned in left lateral recumbency, and fentanyl was administered via the catheter in the lateral saphenous vein by use of a target-controlled infusion system and syringe pump. This system rapidly loaded the central compartment to the desired concentration, and the infusion rate was updated every 10 seconds to maintain a pseudo-steady-state plasma concentration in accordance with the following equation:

\[ r = C_{\text{target}} \times 0.581 \times (0.138 + [0.530 \times e^{-0.176t}] + [0.148 \times e^{-0.016t}]) \]

where \( r \) is the infusion rate, \( C_{\text{target}} \) is the target plasma concentration, \( e \) is the base of the natural logarithm, and \( t \) is the infusion time in minutes.

End-tidal gas samples were collected manually in a glass syringe during a period of 6 to 10 breaths, and end-tidal isoflurane concentration was determined with an infrared analyzer. Each sample was collected in triplicate, and the mean was used as the end-tidal isoflurane concentration. The analyzer was calibrated daily with 3 isoflurane standards that spanned the range of concentrations measured in the study.

A supramaximal electrical stimulus (50 Hz; 6.5 milliseconds; 15 V) was delivered via 1 of the 3 pairs of needles in rotating order. After rabbits had a period of at least 15 minutes of equilibration at a stable end-tidal isoflurane concentration, the supramaximal stimulus was applied for 1 minute or until gross movement was detected, whichever occurred first. A consensus decision regarding whether a rabbit moved in response to the stimulus was made by 2 of the authors (LSB and MGH) who were aware of the fentanyl concentration. If the rabbit had purposeful movement, the isoflurane concentration was increased by 10% and the procedure repeated. Alternatively, if the rabbit did not have purposeful movement, the end-tidal concentration was decreased by 10% and the procedure repeated. The arithmetic mean of the 2 concentrations that permitted and prevented movement was deemed the MAC measurement. The MAC was determined in duplicate and the mean calculated at baseline and each of the 6 target plasma fentanyl concentrations.

MEASUREMENT OF PLASMA FENTANYL CONCENTRATIONS
Immediately after determination of each MAC, 0.5 mL of blood was collected via the catheter in the jugular vein and transferred to tubes containing lithium heparin. Samples were centrifuged for 10 minutes, and the plasma then was removed and stored at −20°C until analysis. Plasma fentanyl concentrations were
determined by use of a liquid chromatography–mass spectrometry method. The limit of quantification of the assay was 0.025 ng/mL with an overall accuracy of 103% and coefficient of variation of 2%. At each target plasma concentration, the mean of the 2 measured plasma fentanyl concentrations was used for data analysis.

**STATISTICAL ANALYSIS**

Data were reported as mean ± SD. The effect of treatment (ie, plasma fentanyl concentration) was assessed by use of a repeated-measures 1-way ANOVA followed, when indicated, by Holm-Sidak multiple comparison tests. Significance was set at values of $P < 0.05$.

**Results**

Baseline isoflurane MAC was 1.92 ± 0.16%. Mean plasma fentanyl concentrations ≥ 2.2 ± 0.3 ng/mL induced a significant reduction in MAC, compared with the baseline value (Figure 1). Fentanyl administration reduced isoflurane MAC by up to 63% at the highest plasma concentration (36.8 ± 2.4 ng/mL). At the highest target plasma fentanyl concentration, MAC could not be determined in 3 rabbits because of excessive spontaneous movement characterized by muscle rigidity, extension of extremities, and paddling. Data collected at this plasma concentration were not included in statistical analyses because of the missing data points.

Mean of the Doppler SBP and heart rate recorded at the time of each MAC determination were calculated for each plasma fentanyl concentration (Table 1). There was no significant effect of treatment on heart rate, but there was for SBP. Post hoc analysis of SBP data did not yield significant differences for pairwise comparisons despite a pattern of increases in SBP at increasing plasma fentanyl concentrations.

**Discussion**

The purpose of the study reported here was to evaluate the effects of a wide range of plasma fentanyl concentrations on isoflurane MAC. Fentanyl was successful at reducing isoflurane MAC in New Zealand White rabbits by up to 63% at the plasma concentrations evaluated. This degree of MAC reduction is similar to that reported in dogs, rats, and goats.

It is unclear whether a ceiling effect on MAC was reached with fentanyl at the plasma concentrations used in the present study because MAC could not be determined in some rabbits at the highest target plasma fentanyl concentration. Excessive spontaneous movement made it difficult to determine whether a rabbit was responding to the noxious stimulus. This has also been evident in cats during experiments conducted to evaluate the effects of potent µ-opioid receptor agonists on isoflurane MAC. Additionally, administration of large doses of potent µ-opioid receptor agonists has been associated with muscle rigidity and seizure-like activity in rabbits. Irrespective of the effect on isoflurane MAC, the excessive movement and associated muscle rigidity would negate the clinical use of infusions at, or greater than, the highest plasma fentanyl concentration (38 ng/mL) evaluated in the present study. The mechanism underlying this increased locomotor activity in species in which µ-opioid receptor agonists induce excitation remains to be elucidated.

Anesthetic protocols based on drugs that reduce the MAC of inhalation agents are clinically useful only if they provide a benefit above that for the inhalation anesthetic alone and are not associated with unmanageable adverse effects. Fentanyl plasma concentrations in the midrange of those evaluated in the present study may be the basis of such a protocol. There was a nonsignificant pattern for increases in SBP at increasing plasma fentanyl concentrations in this study. Admittedly, SBP measurement via a Doppler ultrasonography.

### Figure 1

 Isoflurane MAC measured in duplicate in 6 New Zealand White rabbits (*Oryctolagus cuniculus*) before (baseline) and after administration of fentanyl at 6 targeted plasma concentrations. Values reported are mean ± SD. *Values with different letters differ significantly ($P < 0.05$). †Data obtained for only 3 rabbits and not included in the statistical analysis.

### Table 1

Plasma fentanyl concentration, heart rate, and SBP determined by use of Doppler ultrasonography in 6 isoflurane-anesthetized New Zealand White rabbits (*Oryctolagus cuniculus*) at the time of isoflurane MAC measurement.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 ± 0</th>
<th>1.2 ± 0.1</th>
<th>2.2 ± 0.3</th>
<th>4.4 ± 0.4</th>
<th>9.2 ± 0.4</th>
<th>17.5 ± 2.6</th>
<th>36.8 ± 2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)†</td>
<td>212 ± 35</td>
<td>226 ± 15</td>
<td>211 ± 35</td>
<td>211 ± 35</td>
<td>198 ± 32</td>
<td>207 ± 16</td>
<td>220 ± 26</td>
</tr>
<tr>
<td>SBP (mm/Hg)†</td>
<td>64 ± 4</td>
<td>60 ± 9</td>
<td>65 ± 12</td>
<td>72 ± 14</td>
<td>77 ± 13</td>
<td>82 ± 19</td>
<td>103 ± 12</td>
</tr>
</tbody>
</table>

Values reported are mean ± SD. *Represents results for only 3 rabbits; results were not included in the statistical analysis. †Within a variable, values did not differ significantly ($P > 0.05$) among fentanyl concentrations.
graphic technique is not the most accurate method for blood pressure assessment in rabbits\(^a\); however, it is noninvasive, and evaluation of the effects of fentanyl on blood pressure was not the aim of the study. Further studies conducted to evaluate the cardiovascular effects of equipotent doses of isoflurane-fentanyl combinations will be necessary to determine whether this combination has cardiovascular benefits over those for isoflurane alone.

Measured plasma fentanyl concentrations were within narrow ranges but were only approximately half of the target concentration. The source of this discrepancy could not be identified and may have included incorrect pharmacokinetic estimates, errors in programming of the infusion system, or errors in the delivery system. Further experiments will be necessary to confirm the accuracy of the pharmacokinetic data for fentanyl in rabbits.

An electric stimulus was chosen for this experiment because mechanical stimulation is problematic in rabbits owing to tissue trauma and skin damage. Multiple pairs of electrodes were selected because it has been reported that repeated stimulation of the same set of electrodes can result in desensitization.\(^b\)\(^c\) Additionally, to reduce tissue damage and minimize the risk of desensitization, we used a voltage < 50 V, which has been used in many studies of MAC determination. In a study\(^d\)\(^e\)\(^f\) of rats in which electric stimulation was compared with a tail clamp for MAC determination, there was no difference in MAC of halothane, isoflurane, or desflurane between stimulation with 15 V and a tail clamp and no difference in MAC of isoflurane between stimulation regimens with 10, 20, and 40 V. Lack of differences in MAC values between stimulation with 15 and 50 V was confirmed in preliminary unpublished experiments in rabbits conducted by our laboratory group, and the baseline isoflurane MAC for the present study was consistent with that of other reports in rabbits.\(^g\)\(^h\)

Because the rabbits of the present study had been anesthetized and receiving high doses of fentanyl for long periods, buprenorphine was administered IV at the completion of the study to provide analgesia and at least partially reverse the respiratory depressant and behavioral effects caused by fentanyl.\(^i\)\(^j\)\(^k\) This may not be applicable to clinical scenarios involving moderate to severely painful procedures, and the effects of fentanyl on animals during the recovery period should be closely monitored.

In the present study, fentanyl reduced isoflurane MAC in New Zealand White rabbits and may prove to be a useful adjunct during anesthesia in this species. Further studies are needed to evaluate the potential cardiovascular benefits of a fentanyl-isoflurane combination over those for isoflurane alone.

**Acknowledgments**

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**Footnotes**

b. High fiber rabbit diet No. 5326, PMI Nutrition International, Brentwood, Miss.
c. Insys catheter, Becton-Dickinson, Sandy, Utah.
d. Baxter Healthcare, Deerfield, Ill.
f. Bird Mark 4, Bird Products Corp, Palm Springs, Calif.
g. Parks Medical Electronics Inc, Aloha, Ore.
h. Yellow Springs Instruments, Yellow Springs, Ohio.
k. Bair Hugger, Arizant Healthcare Inc, Eden Prairie, Minn.
m. Metacam, Boehringer Ingelheim, Ingelheim am Rhein, Germany.
n. Fentanyl citrate, Hospira Inc, Lake Forest, Ill.
o. RUGLOOP I, Demed, Temse, Belgium.
q. Beckman Medical gas analyzer LBI, Beckman Instruments, Schiller Park, Ill.
r. Grass Instruments, Quincy, Mass.
s. Prism version 6, GraphPad Software Inc, La Jolla, Calif.

**References**