Effects of diazepam and flumazenil on minimum alveolar concentrations for dogs anesthetized with isoflurane or a combination of isoflurane and fentanyl

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Objective—To determine the effect of a constant-rate infusion of fentanyl on minimum alveolar concentration (MAC) of isoflurane and to determine the interaction between fentanyl and a benzodiazepine agonist (diazepam) and antagonist (flumazenil) in isoflurane-anesthetized dogs.

Animals—8 mixed-breed adult dogs.

Procedure—Dogs were anesthetized with isoflurane 3 times during a 6-week period. After a 30-minute equilibration period, each MAC determination was performed in triplicate, using standard techniques. Fentanyl was administered as a bolus (10 µg/kg of body weight, IV) that was followed by a constant infusion (0.3 µg/kg per min, IV) throughout the remainder of the experiment. After determining isoflurane-fentanyl MAC in triplicate, each dog received saline (0.9% NaCl) solution, diazepam, or flumazenil. After 30 minutes, MAC was determined again.

Results—Fentanyl significantly decreased isoflurane MAC (corrected to a barometric pressure of 760 mm Hg) from 1.80 ± 0.21 to 0.85 ± 0.14%, a reduction of 53%. Isoflurane-fentanyl-diazepam MAC (0.48 ± 0.29%) was significantly less than isoflurane-fentanyl-saline MAC (0.79 ± 0.21%). Percentage reduction in isoflurane MAC was significantly greater for fentanyl-diazepam (74%), compared with fentanyl-saline (54%) or fentanyl-flumazenil (61%). Mean fentanyl concentrations for the entire experiment were increased over time and were higher in the diazepam group than the saline or flumazenil groups.

Conclusions and Clinical Relevance—Fentanyl markedly decreased isoflurane MAC in dogs. Diazepam, but not flumazenil, further decreased isoflurane-fentanyl MAC. Our results indicate that diazepam enhances, whereas flumazenil does not affect, opioid-induced CNS depression and, possibly, analgesia in dogs. (Am J Vet Res 2001;62:555–560)

Fentanyl is a synthetic µ-opioid agonist with an analgesic potency that is 75 to 125 times the potency of morphine. Fentanyl is much more lipid soluble than morphine, resulting in rapid onset and short duration of action.1 Fentanyl may be used as a preanesthetic medication, part of an induction sequence, or as a supplement to inhalant anesthesia.2,4 Because of its short duration of action (approx 30 minutes), fentanyl is administered as a constant-rate infusion or as multiple boluses to provide analgesia in the intraoperative and postoperative periods.3,5

Benzodiazepines (eg, diazepam, midazolam) facilitate the effects of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and increase the availability of the inhibitory neurotransmitter glycine, resulting in sedation and skeletal muscle relaxation.1 Diazepam often is used as part of a balanced regimen for the induction of general anesthesia in dogs. Diazepam significantly reduces the dose of thiobarbiturate required to induce general anesthesia, thereby minimizing the adverse cardiovascular depressant effects associated with anesthetic induction.6 Although diazepam does not exert analgesic effects, it often is used in combination with opioid analgesics to decrease anxiety and enhance the sedative effects of opioid analgesics in the pre- and postoperative periods. Paradoxically, stimulation of GABA receptors purportedly inhibits descending pain modulation (opioid) pathways that serve to suppress the transmission of pain impulses to the brain.7–12 Opioid-benzodiazepine interactions have been investigated in people administered diazepam and morphine for postoperative dental pain.13 Intriguingly, patients administered the benzodiazepine antagonist flumazenil used significantly less ibuprofen for analgesia, compared with patients in which the benzodiazepine antagonist was not administered. Thus, results from laboratory and clinical investigations into the interactions of benzodiazepines and opioids are conflicting.

In the study reported here, we tested the hypothesis that fentanyl would reduce the minimum alveolar concentration (MAC) of isoflurane and that diazepam (benzodiazepine agonist) would attenuate fentanyl-induced reduction of the isoflurane MAC. Our specific objectives were to determine effects of a constant-rate infusion of fentanyl on isoflurane MAC, effects of a constant-rate infusion of diazepam on isoflurane-fen-
tanyl MAC, and effects of a constant-rate infusion of flumazenil (benzodiazepine antagonist) on isoflurane-fentanyl MAC in dogs.

Materials and Methods

Animals—Eight mixed-breed sexually intact female dogs that weighed (mean ± SD) 19.8 ± 2.0 kg and were 1.8 ± 0.4 years old were used in the study. All dogs were determined to be in good health on the basis of results of physical examination and a CBC. Dogs exercised and were allowed to socialize with people on a daily basis. All dogs were treated humanely in accordance with guidelines established by a university animal care and use committee for the treatment of experimental animals. Food, but not water, was withheld for 12 hours prior to the start of each experiment.

Instrumentation—Dogs were anesthetized with isoflurane in O2 administered by a face mask connected to a standard small-animal semiclosed circle-anesthetic breathing system, using an out-of-circle agent-specific vaporizer. Anesthesia was induced by use of an oxygen flow rate of 5 L/min with a vaporizer setting of 5%. After orotracheal intubation, dogs were mechanically ventilated to achieve normocapnia (Paco2, 38 to 42 mm Hg). Inspired and expired isoflurane and CO2 concentrations were continuously monitored by use of samples collected through a nylon catheter, the tip of which was positioned in the distal end of the endotracheal tube. Inspired and expired isoflurane concentrations and respiratory CO2 concentrations were measured, using a quartz-crystal anesthetic analyzer and infrared capnometer. The anesthetic analyzer was calibrated before, during, and after each experiment, using 3 standard concentrations of isoflurane. During instrumentation, the oxygen flow rate was decreased to 2 L/min, and end-tidal isoflurane concentration was maintained between 2.5 and 3%. Dogs were positioned in left-lateral recumbency, and core body temperature was maintained at approximately 38 C with the use of circulating warm water blankets and a heat lamp.

Using aseptic technique, a 20-gauge over-the-needle catheter was percutaneously placed in a cephalic vein for the administration of fluids and drugs. An infusion pump was used to administer lactated Ringer’s solution at a rate of 10 ml/kg of body weight per hour for the first 3 hours, followed by 5 ml/kg per h for the remainder of the experiment. Using aseptic technique, a 20-gauge 1.5-inch catheter was placed in a dorsal pedal artery. Arterial blood pressure was measured, using a balanced transducer, with the middle of the sternum considered to be zero pressure. An arterial blood sample was collected after placement of the arterial catheter and at hourly intervals; samples were used for determination of arterial pH and blood gas analyses. Ventilator settings were adjusted to maintain normocapnia, as determined on the basis of results of blood gas analysis for Paco2.

Calibrated temperature probes were passed per os into the thoracic portion of the esophagus and rectally into the distal aspect of the colon. The higher of the 2 temperatures was considered to be the core body temperature. Arterial blood pressure, ECG, oxygen saturation determined by use of a pulse oximeter (Spo2), and esophageal temperature were continuously displayed on a multiple-channel patient monitor. Temperature was recorded, using a rectal thermometer probe. Two 22-gauge 50-mm insulated needle electrodes were inserted into the buccal mucosa at locations dorsal and caudal to the incisors. These electrodes were used to produce supramaximal stimulation for determination of MAC.

Procedure—Each dog was anesthetized 3 times during a 6-week period, with a minimum of 1 week between anesthetic episodes. The order of MAC determinations was the same for each dog during each anesthetic episode: isoflurane MAC, isoflurane-fentanyl MAC, and isoflurane-fentanyl-treatment MAC. Treatments consisted of administration of saline (0.9% NaCl) solution, diazepam, or flumazenil. Only 1 treatment was given during each anesthetic episode, and the order for administration of treatments was randomized.

After isoflurane MAC was determined (in triplicate), glycopyrrolate (0.015 mg/kg, IM) was administered to prevent the profound bradycardias that can accompany high doses of fentanyl. Glycopyrrolate was used instead of atropine, because glycopyrrolate does not cross the blood-brain barrier and should not affect the determination of MAC. Five minutes after administration of glycopyrrolate, a loading dose of fentanyl (10 µg/kg, IV) was administered during a 60-second period. The loading dose was followed by a constant-rate infusion of fentanyl (0.3 µg/kg per min); the fentanyl infusion was continued until the end of the experiment. Thirty minutes after onset of the constant-rate infusion of fentanyl, isoflurane-fentanyl MAC was determined (in triplicate). After determination of isoflurane-fentanyl MAC, dogs were randomly administered diazepam, saline solution, or flumazenil. Diazepam was administered as a loading dose (0.5 mg/kg, IV) during a 60-second period followed by a constant-rate infusion (0.5 mg/kg per h). Saline solution was administered as a loading dose followed by a constant-rate infusion, using volumes equal to those of diazepam. Flumazenil was administered as a loading dose (0.1 mg/kg, IV) administered during a 60-second period followed by a constant-rate infusion (0.1 mg/kg per h). Because the volume of flumazenil was greater than that of diazepam or saline solution, the rate for IV administration of maintenance fluids was adjusted to ensure that all dogs received the same volume of fluids. Isoflurane-fentanyl-treatment MAC (in triplicate) was determined 30 minutes after onset of constant-rate infusion of the treatment. Arterial blood samples were collected at 30-minute intervals immediately prior to MAC determinations; serum was used for assay of fentanyl concentrations. Nalbuphine (0.1 mg/kg, IM) was administered after extubation at the end of each experiment to hasten recovery. Dogs were constantly observed during recovery, and time to extubation, time to ambulation, and total duration of anesthesia were recorded.

Determination of MAC—After instrumentation, a constant end-tidal isoflurane concentration (2.52 ± 0.32%) that was predicted to be greater than isoflurane MAC was maintained for at least 20 minutes to allow for equilibration between arterial (brain)-alveolar anesthetic partial pressures. The initial end-tidal isoflurane concentration was based on the blood gas analysis for Paco2. The second and third time each dog was anesthetized, the initial end-tidal isoflurane concentration was based on the isoflurane MAC determined during the first anesthetic episode for that dog. Monitored variables (heart rate, mean arterial blood pressure, inspired and expired isoflurane concentration, end-tidal CO2, Spo2, body temperature) were obtained prior to stimulation. Stimulation consisted of an electrical stimulus (30 volts, 5 Hz, 10 milliseconds) applied to the buccal mucosa for a period up to 1 minute; the response to stimulation was recorded. The stimulus was discontinued when gross purposeful movement was detected prior to completion of 1 minute of stimulation. A positive response was considered to be gross purposeful movement of the head or extremities. Slight flexion of the neck, withdrawal of a limb, coughing, or swallowing was not considered to be a positive response. Isoflurane concentration was reduced (after a negative response) or increased (after a positive response) by 10 to 20%. Multiple determinations were averaged for each dog. End-tidal isoflurane concentrations were corrected on the basis of calibration curves for the anesthetic analyzer and
adjusted to sea-level barometric pressure (760 mm Hg), using the following formula:

\[ \text{altitude-adjusted MAC} = \text{measured MAC} \times (\text{measured ambient barometric pressure/sea-level barometric pressure}) \]

Average ambient barometric pressure in our facility at Fort Collins, Colo, is approximately 640 mm Hg. Barometric pressure was recorded daily and used to calculate altitude-adjusted MAC.

**Analysis of compounds**—Beginning after isoflurane MAC was determined and prior to administration of fentanyl, arterial blood samples were collected at 30-minute intervals. For each sample, 10 ml of arterial blood was collected into a red-top (clot) tube, and serum was separated by centrifugation. Serum samples were stored at –40 °C until subsequent analysis. Serum concentrations of fentanyl, flumazenil, diazepam, and oxazepam and desmethyldiazepam were determined by high-performance liquid chromatography-mass spectrometry, using a mass spectrometer with electrospray interface. Extraction of serum drug concentrations was performed, using a modification to a published method. Serum calibrators were prepared by adding appropriate volumes of standard solutions to serum from control dogs. Concentrations used for serum calibrators ranged from 10 to 500 ng/ml for diazepam and oxazepam, 25 to 1,000 ng/ml for desmethyldiazepam, 1 to 50 ng/ml for fentanyl, and 10 to 160 ng/ml for flumazenil.

**Statistical analysis**—Data were reported as mean ± SD. Data were compared, using a 2-way ANOVA for repeated measures. Post-hoc tests, using the Tukey honestly significant difference test, were performed to compare means when significant differences existed. Computations were performed, using a computer statistical program. Because the duration of each experiment varied, analysis of serum drug concentrations was performed for the same times within each treatment. Thus, fentanyl serum concentrations for all treatments were compared for 180 minutes of fentanyl infusion, flumazenil concentrations were compared for 120 minutes of flumazenil administration, and diazepam, oxazepam, and desmethyldiazepam concentrations were compared for 150 minutes of diazepam administration. Significance was defined as values of \( P < 0.05 \).

**Results**

Mean (± SD) time from beginning of mask induction to intubation was 3.7 ± 0.9 minutes. Mean time from discontinuing isoflurane to extubation was 16.1 ± 8.2 minutes, and mean time from extubation until a dog was able to stand was 9.6 ± 7.1 minutes. Total duration of anesthesia from induction to standing was 42.4 ± 7.6 minutes. Total fentanyl dose administered did not differ significantly among treatments (saline solution, 1.6 ± 0.23 mg; diazepam, 1.87 ± 0.27 mg; and flumazenil, 1.79 ± 0.0 mg).

Mean body temperature during the experiments was 38.2 ± 0.4 °C and did not differ significantly among treatments. Mean arterial pH (7.35 ± 0.03), \( P_{aCO_2} \) (39.8 ± 3.6 mm Hg), and \( P_{aO_2} \) (42.8 ± 30 mm Hg) did not differ significantly among treatments. Heart rate measured immediately prior to discontinuation of isoflurane MAC did not differ among dogs or from heart rate measured immediately prior to determination of isoflurane-fentanyl MAC (Table 1). Heart rate measured immediately prior to termination of isoflurane-fentanyl treatment MAC was significantly lower than heart rate measured immediately prior to determination of isoflurane-fentanyl MAC; however, heart rate did not differ significantly among treatments.

Mean arterial pressure immediately prior to determination of isoflurane MAC did not differ significantly among dogs or from mean arterial pressure measured immediately prior to determination of isoflurane-fentanyl MAC (Table 1). Mean arterial pressure immediately prior to determination of isoflurane-fentanyl MAC was significantly lower than mean arterial pressure measured immediately prior to determination of isoflurane-fentanyl MAC. Mean arterial pressure recorded during administration of diazepam was significantly lower than during administration of saline solution or flumazenil.

Isoflurane MAC was determined in each dog during 3 separate anesthetic episodes. Overall, isoflurane MAC corrected to sea-level barometric pressure was 1.80 ± 0.21%. Isoflurane MAC did not differ significantly among the 3 treatment episodes. Isoflurane-fentanyl MAC also did not differ significantly among the 3 treatments (mean, 0.85 ± 0.14%) but represented a reduction of 52.8% from isoflurane MAC. Isoflurane-fentanyl MAC was significantly less than isoflurane MAC. Values for isoflurane-fentanyl-treatment MAC were 0.79 ± 0.20, 0.48 ± 0.29, and 0.71 ± 0.09% for saline solution, diazepam, and flumazenil, respectively. Isoflurane-fentanyl-diazepam MAC was significantly less than isoflurane-fentanyl MAC, isoflurane-fentanyl-saline solution MAC, and isoflurane-fentanyl-flumazenil MAC. Isoflurane-fentanyl-saline solution MAC and isoflurane-fentanyl-flumazenil MAC were not significantly different from isoflurane-fentanyl MAC.

Evaluation of serum fentanyl concentrations was complicated by the variable duration of each experiment. Comparison of the first 180 minutes of fentanyl administration indicated significant differences among treatments; however, we did not detect confounding or
The altitude-adjusted isoflurane MAC determined here (1.80 ± 0.21%) was higher than that reported elsewhere in dogs (1.28%). The supramaximal stimulus used in that study was not reported, and a difference in supramaximal stimulus could have accounted for some of the differences in MAC. We used a standard supramaximal stimulation (electrical stimulus applied to the buccal mucosa) to minimize individual responses. Factors known to influence MAC such as body temperature, arterial pH, P\textsubscript{CO\textsubscript{2}}, and P\textsubscript{O\textsubscript{2}} were all maintained within acceptable limits in our study. In another study, young dogs (2 years old) had a higher altitude-adjusted isoflurane MAC (1.45%) than old dogs (11 years old, 1.22%), indicating the importance of age on MAC. All of our dogs were young healthy dogs; thus, they may have had a higher MAC because of their age.

Following the determination of isoflurane MAC, fentanyl was administered, and isoflurane-fentanyl MAC was determined after a 30-minute equilibration period. Fentanyl has a rapid onset of action, as documented by kinetic studies in rats. A bolus of fentanyl (50 µg/kg, IV) administered to rats had visible effects within 10 seconds, and concentrations within the brain equilibrated with plasma within 1.5 minutes. Therefore, the fentanyl bolus we administered and the 30-minute equilibration period between the start of fentanyl and determination of isoflurane-fentanyl MAC in the study reported here likely was sufficient to allow for complete equilibration of plasma and brain concentrations. Pharmacokinetics of fentanyl in dogs are dose-independent for doses in the range of 6.4 to 640 µg/kg, IV. In that study, investigators did not detect evidence of saturation of biotransformation or tissue-uptake mechanisms. In contrast, enflurane-anesthetized dogs administered high doses of fentanyl (100 µg/kg, IV) in another study had persistent plasma concentrations of fentanyl and a prolonged duration of action, compared with enflurane-anesthetized dogs administered a lower dose of fentanyl (10 µg/kg, IV). In our study, the increase in serum concentrations of fentanyl over time (isoflurane-fentanyl vs isoflurane-fentanyl-treatment) suggests that some degree of saturation was evident in the dogs for our model. It is likely that the increase in fentanyl concentrations over time was attributable in part to the high doses of fentanyl used and the concurrent use of isoflurane.

Effects of fentanyl on cardiovascular, respiratory, and analgesic variables for the range of plasma concentrations between 5 and 30 ng of fentanyl/ml were studied in unanesthetized spontaneously breathing dogs. Maximal effects (response to tail clamping, decreases in heart rate and blood pressure, decreases in respiratory rate and arterial oxygen tension) of fentanyl were observed at plasma concentrations of 30 ng/ml. Similarly, enflurane MAC was reduced a maximum of 65% in dogs following fentanyl infusions that resulted in plasma concentrations of approximately 30 ng of fentanyl/ml. Concentrations of fentanyl that were 3-fold higher did not cause a further reduction of enflurane MAC. In another study, enflurane MAC was reduced 65% in dogs by use of a fentanyl loading dose (13.9 µg/kg, IV, during a 20-minute period) followed by an infusion of fentanyl (1.063 µg/kg per min). That rate

### Table 2—Mean serum concentrations (ng/ml) of flumazenil, diazepam, desmethyldiazepam, and oxazepam obtained for samples obtained from isoflurane-anesthetized dogs given a bolus of fentanyl (10 µg/kg, IV) during a 1-minute period followed by a constant-rate infusion (0.3 µg/kg per min)

<table>
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<th>Treatment* (n)</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>SD</th>
<th>Overall SD</th>
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<tr>
<td>Flumazenil (6)</td>
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<td>78.6</td>
<td>79.8</td>
<td>85.7</td>
<td>ND</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Diazepam (7)</td>
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<td>416</td>
<td>379</td>
<td>453</td>
<td>392</td>
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<tr>
<td>Desmethyldiazepam (7)</td>
<td>474</td>
<td>514</td>
<td>559</td>
<td>609</td>
<td>662</td>
<td>51.1</td>
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<tr>
<td>Oxazepam (7)</td>
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<td>58.7</td>
<td>70.8</td>
<td>77.9</td>
<td>86.8</td>
<td>6.6</td>
<td></td>
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</table>

*Time 0 = Start of fentanyl infusion. †Values differed significantly (P < 0.05) over time. See Table 1 for remainder of key. ND = Not determined.

**Discussion**

Perception of pain is the result of an exceedingly complex interaction involving neurotransmitters that serve to facilitate or inhibit transmission of nociceptive impulses from the periphery to the CNS. Opioids are a diverse group of natural and synthetic drugs used primarily for their analgesic activity, including morphine, fentanyl, and butorphanol. Opioids stimulate specific opioid receptors in the brain and spinal cord, thereby inhibiting transmission of nociceptive impulses to the brain. Thus, opioids constitute the mainstay of analgesic protocols for the treatment of acute postoperative pain in people and small animals.

In the study reported here, we investigated the effect of fentanyl on isoflurane MAC and the interaction of fentanyl with a benzodiazepine agonist (diazepam) and antagonist (flumazenil) in isoflurane-anesthetized dogs. Fentanyl significantly decreased isoflurane MAC from 1.80 ± 0.21% to 0.85 ± 0.14%, a reduction of 52.8%. Analysis of our results suggests that diazepam enhanced, whereas flumazenil did not affect, opioid (fentanyl)-induced antinociceptive effects. The mechanism whereby diazepam enhanced reduction of the fentanyl-induced isoflurane MAC was not determined in this study. Fentanyl serum concentrations were higher when dogs were given diazepam, compared with saline and flumazenil treatments; however, the concentrations of fentanyl were higher prior to the start of treatment with diazepam, saline solution, and flumazenil.
of drug administration resulted in plasma fentanyl concentrations of 71.7 ± 14.4 ng/ml, well above the plasma concentration of 30 ng/ml documented to cause maximal reduction of enflurane MAC. In the study reported here, we calculated that fentanyl administered as a bolus (10 μg/kg, IV) followed by a constant-rate infusion (0.3 μg/kg per min) would result in a reduction of approximately 50% in isoflurane MAC. We hypothesized that reducing MAC by 50% with fentanyl would allow us to detect further MAC reductions or increases during treatment with saline solution, diazepam, or flumazenil. Although serum concentrations of fentanyl obtained during isoflurane-fentanyl MAC in our study were significantly less than values reported in other studies, isoflurane MAC was reduced by 52.8%. Although we did not intend to examine the anesthetic effects of fentanyl infusion per se, it is worth mentioning that the dose of fentanyl used in our study is clinically relevant and is consistent with doses used in isoflurane-anesthetized dogs at our institution.

Although diazepam is highly (96%) protein bound in plasma, it rapidly crosses the blood-brain barrier.8 The dose of diazepam (0.5 mg/kg, IV followed by 0.5 mg/kg/h) used in the study reported here is consistent with doses used clinically in dogs, and it was calculated to maintain steady-state serum concentrations based on pharmacokinetic studies in dogs.10,31 Diazepam is metabolized to N-desmethyl-diazepam, 3-hydroxy-diazepam, and oxazepam, which are pharmacologically active.8 Serum concentrations of diazepam and desmethyl-diazepam did not change significantly over time, whereas oxazepam concentrations increased significantly in our study. The significant reduction of isoflurane-fentanyl MAC after administration of diazepam may be the result of a decreased clearance of fentanyl, increased availability of fentanyl attributable to competition for protein binding, or an enhancement of the CNS-depressant effects of isoflurane-fentanyl. Regardless of the mechanism responsible for the reduction in isoflurane-fentanyl MAC, it is clear from our results that diazepam enhanced the anesthetic effects of isoflurane-fentanyl, rather than antagonizing the effects of fentanyl. Conducting our study by using isoflurane-anesthetized dogs may have drastically affected the interaction of fentanyl and diazepam; therefore, these results should not be extrapolated to conscious dogs.

Diazepam exerts part of its CNS effects through enhancement of GABA activity. It is postulated that there may be an interaction between GABA and opioid receptors at the level of the midbrain periaqueductal gray matter, rostral ventromedial medulla, and dorsal horn of the spinal cord.22-24 Opioids are proposed to activate and enhance the descending pain modulation system. There have been conflicting reports to suggest that GABA agonists may act to inhibit or potentiate the antinociceptive effects of opioid agonists. Intrathecal muscimol, a GABA_A agonist, and baclofen, a GABA_B agonist, potentiated somatic and visceral antinociceptive effects of intrathecally administered morphine as assessed by the tail flick test and colorectal distention test in rats.26 In contrast, administration of benzodiazepine midazolam (0.5 mg/kg, IP) reduced the morphine-induced analgesia in rats by half, whereas flumazenil, a specific benzodiazepine antagonist, prevented the inhibition of morphine antinociception by midazolam.8 Similarly, injection of a GABA agonist into the periaqueductal gray matter reversed morphine-induced analgesia, whereas injection of picrotxin (a GABA antagonist) potentiated the same dose of morphine in rats.29 These results suggest that the interaction between midazolam and morphine is mediated by specific benzodiazepine receptors. In a similar manner, midazolam reduced enflurane-fentanyl MAC in dogs less than predicted, assuming the drugs had an additive effect.28 The antagonistic effects of diazepam on morphine-induced analgesia and the reversal of antagonism with specific benzodiazepine receptor antagonists have been documented in mice.8,21 It has been hypothesized that the antagonistic effect of benzodiazepines on opioid-induced analgesia may be mediated by the release of dynorphin in the spinal cord.35 Clinical implications of these interactions observed in rats and mice are that diazepam may reduce the analgesic effects of opioids administered for the treatment of postoperative pain. In contrast, our results revealed that diazepam enhanced the MAC-reducing effects of fentanyl and further support the clinical impression that balanced anesthetic regimens that incorporate diazepam and opioids decrease the requirement for inhalant anesthetics. The noxious stimulus used in our study was applied to the head; therefore, drug interactions at the level of the spinal cord probably would not have affected the response to our stimulation.

Flumazenil was used in this study to determine whether antagonism of endogenous benzodiazepines would affect isoflurane-fentanyl MAC. If benzodiazepines exert an antianalgesic effect, it is possible that endogenous benzodiazepine ligands would serve a similar function. Flumazenil did not change isoflurane-fentanyl MAC, suggesting that endogenous benzodiazepines did not play a substantial role in the pain modulatory system in this model. The dose of flumazenil (0.1 mg/kg, IV, followed by constant-rate infusion of 0.1 mg/kg/h) was chosen empirically after a review of the literature. Reported doses of flumazenil in dogs range from 0.01 to 1.5 mg/kg.36-40 The CNS effects of an intentional overdose of diazepam (2 mg/kg) were effectively antagonized by flumazenil administered at 0.075 and 0.1 mg/kg, IV.47 Thus, we chose to use a midrange dose that has been effective in reversing diazepam in dogs. In the study reported here, we did not administer flumazenil to isoflurane-fentanyl-diazepam treated dogs to determine whether there would be a return to isoflurane-fentanyl MAC values. Our reasoning was that our experiments were fairly long (424.1 ± 77.6 minutes), and we did not think it was appropriate to keep the dogs anesthetized for an additional determination of MAC. Heart rate and blood pressure were significantly lower during the determination of isoflurane-fentanyl-treatment MAC, compared with values during determination of isoflurane-fentanyl MAC. It is likely that at least some of the decrease in heart rate and blood pressure observed was attributable to a waning of the effects of the glycopyrrolate administered 5 minutes before the administration of fentanyl and 35 minutes.
before beginning to determine isoflurane-fentanyl MAC. Our objective was to avoid extremes in heart rate (bradycardia or tachycardia) during MAC determination. Our experience with the administration of anticholinergics is that it is often not possible to administer them in the amount necessary to obtain the desired effect on heart rate in anesthetized animals. Thus, glycopyrrolate was administered only once during the experiment to avoid wide fluctuations in heart rate.

References


5. MDE Escort II, Medical Data Electronics Inc, Arleta, Calif.

6. Grass S 44, Astromed-Grass Inc, West Warwick, RI.


8. Disposable needle electrodes, Dantac Medical, Allendale, NJ.

9. Romazicon, Roche Pharmaceuticals, Roche Laboratories, Nutley, NJ.

10. Disposable needle electrodes, Dantac Medical, Allendale, NJ.


12. Finnigan LCQ quadrupole ion-trap, company, city, state.


14. MDE Escort II, Medical Data Electronics Inc, Arleta, Calif.

15. Disposable needle electrodes, Dantac Medical, Allendale, NJ.


