Less Than Additive Antinociceptive Interaction Between Midazolam and Fentanyl in Enflurane-anesthetized Dogs

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The anesthetic interactions of midazolam and fentanyl were determined in terms of enflurane MAC reduction in dogs. In part 1, 8 animals received an intravenous (iv) loading dose of fentanyl followed by a constant infusion at 0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ to produce a stable enflurane MAC reduction of approximately 20%. Midazolam was then administered in a series of three incremental loading doses and infusions (2.4, 9.6, and 28.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) previously determined to produce enflurane MAC reductions of approximately 30, 45, and 60%, respectively. Enflurane MAC was determined for each infusion. Then fentanyl was discontinued; naloxone 1 mg/kg was administered; and enflurane MAC was determined. In part 2, six dogs received a loading dose and a continuous infusion of fentanyl (0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) designed to produce a stable enflurane MAC reduction of approximately 40%. A series of two incremental loading doses and infusions of midazolam (2.4 and 28.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were added, and MAC determinations were repeated at each infusion rate. Then midazolam was discontinued; flumazenil (RO 15-1788) 1.5 mg/kg was administered; and enflurane MAC was determined. The fentanyl concentrations in plasma remained stable at 1.0 ± 0.3 ng/ml (mean $\pm$ standard deviation [SD], part 1) and 3.1 ± 0.5 ng/ml (part 2) throughout the study and, in the absence of midazolam, reduced enflurane MAC by 28 ± 11 and 44 ± 5%, respectively. The addition of midazolam produced significant further reductions in enflurane MAC, but the reductions were less than those predicted on the basis of an additive interaction. Naloxone returned enflurane MAC reduction to that expected for midazolam alone (part 1). The degree of enflurane MAC reduction after administration of flumazenil and in the presence of the continuing infusion of fentanyl was significantly less than predicted (part 2). We conclude that there is a less than additive interaction between fentanyl and midazolam with respect to enflurane MAC reduction. (Key words: Anesthetic techniques: infusions. Anesthetics, intravenous: fentanyl; midazolam. Anesthetics, volatile: enflurane. Antagonists: flumazenil; Ro 15-1788; naloxone. Hypnotics: benzodiazepines, midazolam. Opioids: fentanyl. Potency: MAC; enflurane. ED_{50}; midazolam, fentanyl.)

INTRAVENOUS FENTANYL AND MIDAZOLAM are routinely used as adjuncts to anesthesia. These agents act as agonists at stereospecific receptors of which there are finite numbers in the central nervous system (CNS). Accordingly, a ceiling to their effects is expected when all receptors are occupied. This hypothesis has been confirmed in humans and dogs for fentanyl[1-3] and midazolam.[4] In addition, the plasma concentrations required to reduce enflurane MAC in the dog have been found to be close to those required to produce anesthesia in humans.[1,4-6] Despite their frequent combination, the nature of the interactions between fentanyl and midazolam remains uncertain. In a previous study,[7] the interaction of a constant infusion of midazolam with additional incremental infusions of fentanyl was studied and found to be additive at lower concentrations of midazolam and fentanyl. At higher concentrations, the observed enflurane MAC reduction was statistically less than that extrapolated from concentration *versus* enflurane MAC reduction curves for midazolam and fentanyl. The primary purpose of the current study was to investigate further the interaction between fentanyl and midazolam (additive, synergistic, and antagonistic), this time from the perspective of adding midazolam to fentanyl rather than *vice versa*.

Materials and Methods

The protocol was approved by the Emory University Animal Use and Care Committee and followed guidelines established by the National Institutes of Health for the ethical use of animals in research studies. All data are expressed as mean $\pm$ standard deviation (SD).

Male mongrel dogs (n = 14) weighing 15 ± 1 kg were allowed free access to water while fasting overnight. Each was given intravenous (iv) succinylcholine (0.1 mg/kg) and atropine (0.12 mg/kg), and anesthesia was immediately induced with 5% enflurane in oxygen delivered by a Bain anesthetic circuit and a specialized mask.[8] This induction sequence was used to avoid any confounding influence of other CNS depressants (*e.g.*, barbiturates) on the interpretation of the results. Also, the use of iv succinylcholine permits the immediate administration of high concentrations of enflurane and thereby reduces the duration of induction of anesthesia and the potential discomfort the animal may experience while struggling during a slower induction of anesthesia without the use of a muscle relaxant. Since the animals are naive with respect to the use of muscle relaxants, anticipatory anxiety about paralysis is not a factor.

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A cuffed endotracheal tube was introduced, and ventilation with oxygen by a Harvard respirator maintained arterial blood gases in the normal range. An iv catheter was inserted into a foreleg vein, and lactated Ringer's solution was infused at a rate of 10 ml·kg^{-1}·h^{-1}. A urinary catheter was inserted and the tail shaved. Temperature was monitored with an esophageal temperature probe and, with the use of a warming blanket, was maintained over the course of the anesthetic within 1° C of that measured in the animal just after the induction of anesthesia. The electrocardiogram was monitored throughout the experimental period. A femoral arterial catheter was used for continuous blood pressure recording and for periodic sampling of blood for gas analysis and determination of plasma concentrations of fentanyl and midazolam. End-tidal enflurane concentration was measured by a Beckman LB-2 infrared analyzer. MAC determinations using the tail-clamp method were made as described previously.[1] MAC was defined as the end-tidal concentration (to the nearest 0.1%) midway between the end-tidal concentrations of enflurane at which the animals did or did not move in response to the applied stimulus. Measurements of hemodynamic responses (i.e., changes in heart rate and mean systemic blood pressure) were made during each application of the tail-clamp.

PART 1

At least 1 h after the induction of anesthesia, control enflurane MAC was determined in eight dogs (fig. 1). Then, each was given a loading dose of fentanyl (15 μg/

kg iv over 20 min) followed by a constant infusion at 0.05 μg·kg^{-1}·min^{-1} to reduce enflurane MAC by approximately 20%.[1] This infusion rate was maintained until the administration of naloxone (see below). After a 60-min observation period, enflurane MAC was again determined, and then midazolam was administered to each animal in a series of three individual loading doses (118, 352, and 3,970 μg/kg, each administered over 20 min) and continuous infusions (2.4, 9.6, and 28.8 μg·kg^{-1}·min^{-1}) that were expected to produce additional reductions of enflurane MAC (30, 45, and 60%, respectively, based on a previously obtained enflurane MAC reduction vs. midazolam plasma concentration curve[4]). Enflurane MAC was determined after a 60-min observation period for each new loading dose and infusion rate.

After enflurane MAC determination at the highest midazolam infusion rate (28.8 μg·kg^{-1}·min^{-1}) in combination with the fentanyl infusion, the midazolam infusion was maintained while the fentanyl infusion was stopped, and naloxone 1 mg/kg was administered iv every 10 min (two or three doses were used in each of the 8 dogs) until the last determination of enflurane MAC was completed.

PART 2

At least 1 h after the induction of anesthesia, control enflurane MAC was determined in six dogs (fig. 2). Then each was given a loading dose of fentanyl (45 μg/kg iv over 20 min) followed by a constant infusion of 0.2 μg·kg^{-1}·min^{-1} to reduce enflurane MAC by approxi-

FIG. 1. The experimental protocol for part 1. Time in hours is shown on the horizontal axis, and MAC on the vertical axis. The sum of the anesthetic effects produced by each combination always equals 1 MAC, and the observed contribution of each drug (enflurane, fentanyl, and midazolam) is represented at each step. The maintenance infusion rates are shown within the vertical bars. The loading dose of fentanyl was 15 μg/kg administered over 20 min. The sequential loading doses of midazolam were 118, 352, and 3,970 μg/kg, respectively, each administered over the first 20 min after the determination of enflurane MAC (EMAC) for the previous drug administration. In the last phase of the experiment, the fentanyl infusion was stopped and naloxone 1 mg/kg was injected iv every 10 min; that is, two or three doses were injected over the 20–30 min required to complete the final determination of enflurane MAC. See Materials and Methods for details.
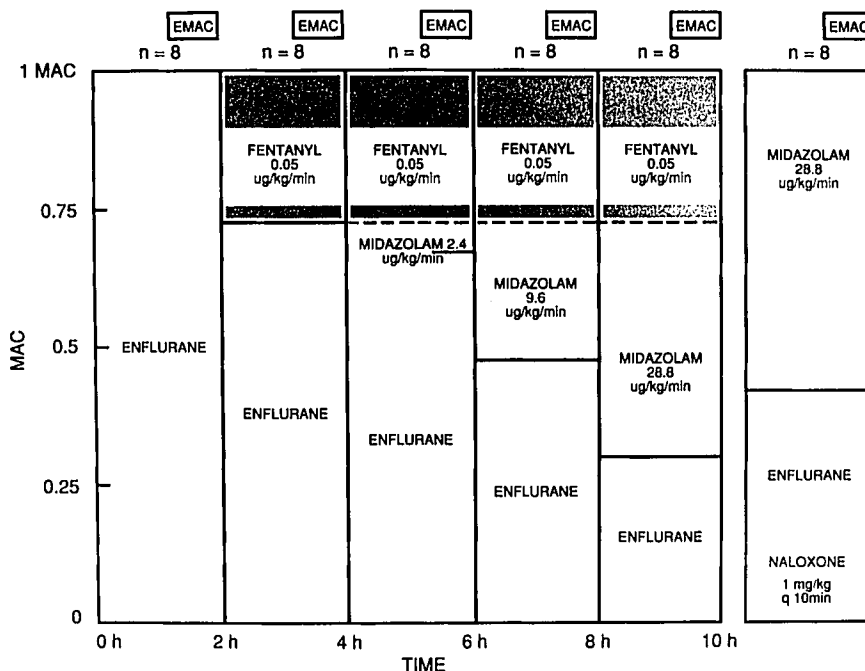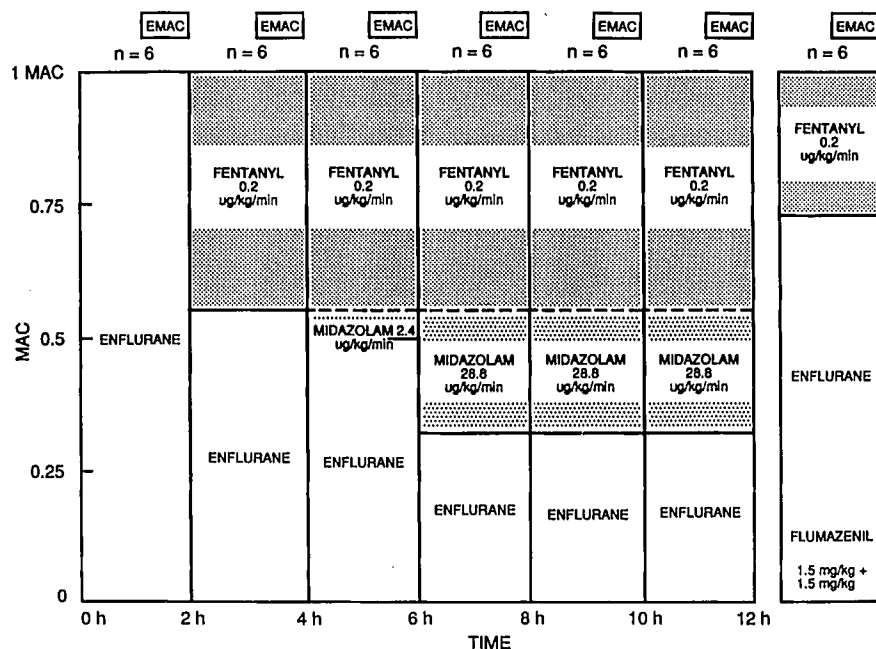
FIG. 2. The experimental protocol for part 2. Time in hours is shown on the horizontal axis and MAC on the vertical axis. The sum of the anesthetic effects produced by each combination always equals 1 MAC. The maintenance infusion rates are shown within the vertical bars. The loading dose of fentanyl was 45 μg/kg administered over 20 min. The sequential loading doses of midazolam were 118 and 1,298 μg/kg, respectively, each administered over the first 20 min after the determination of enflurane MAC (EMAC) for the previous drug administration. In the last phase of the experiment, the midazolam infusion was stopped and flumazenil 1.5 mg/kg was injected iv; enflurane MAC was determined; another 1.5 mg/kg dose of flumazenil was injected iv; and the final determination of enflurane MAC was completed. The last two determinations of enflurane MAC were averaged because they did not differ after the first and second doses of flumazenil. See Materials and Methods for details.

mately 40%.[1] This infusion was maintained for the duration of the experiment. After a 60-min observation period, enflurane MAC was again determined, and then midazolam was administered to each animal in a series of two incremental loading doses (118 and 1,298 μg/kg each administered over 20 min) and infusions (2.4 and 28.8 $μg \cdot kg^{-1} \cdot min^{-1}$) that were expected to produce additional reductions of enflurane MAC (30 and 60%, respectively, again based on the previously obtained enflurane MAC reduction *vs.* midazolam plasma concentration curve[4]). Enflurane MAC was determined after a 60-min observation period for each new infusion rate. The higher midazolam infusion rate (28.8 $μg \cdot kg^{-1} \cdot min^{-1}$) was maintained for approximately 6 h, long enough for three determinations of enflurane MAC with a 60-min observation period between each.

After the enflurane MAC determination at the highest midazolam infusion (28.8 $μg \cdot kg^{-1} \cdot min^{-1}$) in combination with the fentanyl infusion, the fentanyl infusion was maintained; the midazolam infusion was stopped; flumazenil 1.5 mg/kg was administered iv; and enflurane MAC was determined over the next 15–20 min. Then a second 1.5 mg/kg dose of flumazenil was administered, and enflurane MAC was determined over the next 15–30 min. The determinations of enflurane MAC after each dose of flumazenil did not differ; the data are presented as the average of both determinations.

Plasma concentrations of midazolam were determined by gas–liquid chromatography[9] (intraassay variation 7.4% at 25 ng/ml and 3.5% at 100 ng/ml, and interassay variation 7.5% at 25 ng/ml and 4.5% at 100 ng/ml; sensitivity 2–3 ng/ml). Plasma concentrations of fentanyl were de-

termined by radioimmunoassay (absolute sensitivity 100 pg; coefficient of variation 5–7%).§

The enflurane MAC reductions produced by the infusions of fentanyl alone in this study were added to all of the data on enflurane MAC reduction *versus* fentanyl plasma concentration obtained in previous experiments from this laboratory[1,7] and were analyzed by nonlinear regression techniques[10] to obtain a nonlinear correlation of fentanyl plasma concentration *versus* enflurane MAC reduction (fig. 3).

Student's t test was used to identify significant differences ($P < 0.05$) between the degree of enflurane MAC reduction observed and that extrapolated from the nonlinear regression analysis of enflurane MAC reduction *versus* drug concentration in plasma. One-way analysis of variance was used to identify concentration-related differences in the degree of MAC reduction produced by subsequent infusions. Corrections for multiple comparisons were made (by Scheffe's *post hoc* test).[11] Values represent the mean ± SD.

Results

FENTANYL CONCENTRATION VERSUS EFFECT

All measurements of enflurane MAC reduction *versus* fentanyl concentration in plasma made in our laboratory

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§ Fentanyl Radioimmunoassay Kit: Instruction manual for the measurement of fentanyl. Catalog No S1, June 1983. Janssen Life Sciences Products, Beerse, Belgium. Modified according to Schuttler J, White PF: Optimization of the radioimmunoassays for measuring fentanyl and alfentanil in human serum. ANESTHESIOLOGY 61:315–320, 1984.
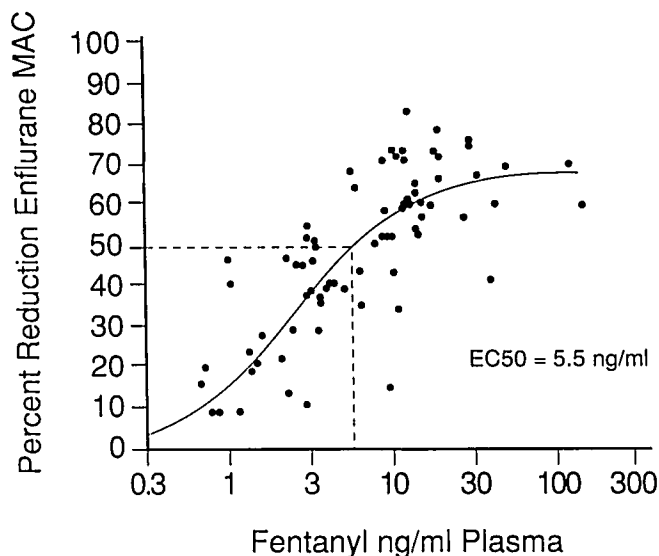
FIG. 3. Reduction of enflurane MAC (vertical axis) *versus* plasma concentration of fentanyl (nanograms per milliliter) on a log scale (horizontal axis). Each dot represents one set of measurements in each of 74 dogs, and the line was determined by nonlinear regression analysis. The equation for the line is

$$y = \frac{67.7}{1 - 2.91e^{-2.74 \log x}}$$

where y = percent reduction of enflurane MAC and x = fentanyl concentration in nanograms per milliliter of plasma.[10] A ceiling effect (maximum enflurane MAC reduction) is evident.

during the past 10 yr are plotted in figure 3. The equation[10] linking percent reduction (y) of enflurane MAC and fentanyl concentration in plasma (x) is:

$$y = \frac{67.7}{1 - 2.91e^{-2.74 \log x}} \tag{1}$$

The ceiling effect or maximum enflurane MAC reduction of 67.7% corresponds to that originally observed by Murphy and Hug.[1]

PART 1

Fentanyl plasma concentrations remained stable in each animal throughout the experimental period (fig. 4), and, in the absence of midazolam, reduced enflurane MAC by 28 ± 11% (predicted enflurane MAC reduction 19 ± 5%, $P > 0.05$; table 1). The incremental midazolam infusions produced significant increases in plasma midazolam concentrations and significant further reductions in enflurane MAC. However, the incremental increases in the observed enflurane MAC reduction attributed to midazolam (obtained by subtracting the initial enflurane MAC reduction in the presence of fentanyl alone from the enflurane MAC

determined for each fentanyl–midazolam combination) were significantly less than those predicted from reference 4 at all three midazolam infusion rates (table 1).

After discontinuation of fentanyl and administration of the specific antagonist naloxone, the degree of enflurane MAC reduction returned to that predicted for midazolam alone (see reference 4 and table 1).

PART 2

Plasma concentrations of fentanyl remained stable in each animal throughout the experimental period (fig. 4) and, in the absence of midazolam, reduced enflurane MAC by 44 ± 5% (predicted enflurane MAC reduction 40 ± 3%, $P > 0.05$; table 2). The incremental midazolam infusions produced significant increases in plasma concentrations of midazolam and significant further reductions in enflurane MAC. The higher midazolam infusion rate was maintained for 234 ± 20 min. The plasma concentrations of midazolam remained stable throughout this period, as did the reductions of enflurane MAC (table 2).
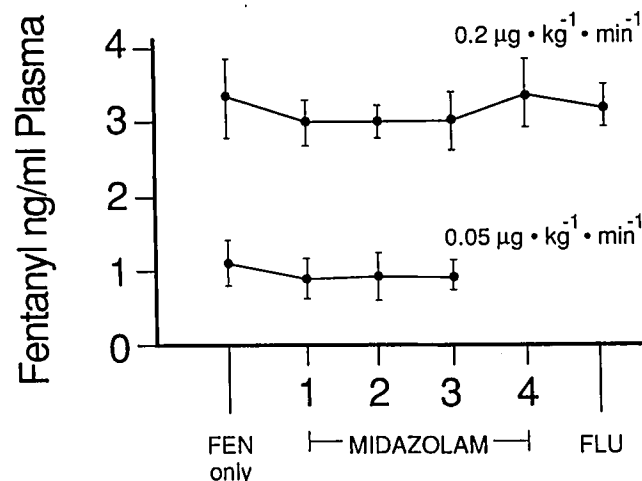

FIG. 4. Average (±SD) fentanyl plasma concentrations during determinations of enflurane MAC. In part 1, 8 dogs received fentanyl 15 μg/kg over 20 min followed by a continuous infusion of 0.05 μg·kg⁻¹·min⁻¹ for the duration of the experiment. In part 2, another 6 dogs received fentanyl 45 μg/kg over 20 min followed by a continuous infusion of 0.2μg·kg⁻¹·min⁻¹. FEN only = the fentanyl concentration during the enflurane MAC measurement (control) made approximately 2 h after beginning fentanyl administration in parts 1 and 2; FLU = the average fentanyl concentration during two enflurane MAC measurements made after injection of flumazenil at the end of the experiments, in part 2, approximately 12 h after starting fentanyl administration. The numbers of MAC determinations at 1.5–2-h intervals during midazolam administration are shown. In part 1, determinations 1–3 occurred during midazolam infusions of 2.4, 9.6, and 28.8 μg·kg⁻¹·min⁻¹. In part 2, the first MAC determination occurred during a midazolam infusion of 2.4 μg· kg⁻¹· min⁻¹; then there were three MAC determinations over approximately 6 h during which time midazolam was infused at a rate of 28.8 μg·kg⁻¹· min⁻¹.

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TABLE 1. Enflurane MAC Reduction Attributable to the Addition of Midazolam to a Continuous Infusion of Fentanyl in Eight Dogs

Infusion (µg·kg⁻¹·min⁻¹)	Midazolam (ng/ml of plasma)	% Reduction of Enflurane MAC			Attributed Minus Predicted
		Observed	Attributed to Midazolam	Predicted for Midazolam*	
Fentanyl 0.05	0	28 ± 11	—	—	—
Fentanyl + midazolam 2.4	129 ± 18	35 ± 11†	7 ± 11	32 ± 1	−25 ± 11
Fentanyl + midazolam 9.6	530 ± 75	53 ± 11‡·§·¶	25 ± 12	46 ± 1	−21 ± 13
Fentanyl + midazolam 28.8	2,319 ± 358	70 ± 9‡·§·¶·**	42 ± 12	61 ± 2	−19 ± 12
Midazolam 28.8 + naloxone	1,795 ± 358	59 ± 10‡	59 ± 10	59 ± 2	0

Values are mean ± SD. The fentanyl infusion was constant at 0.05 µg·kg⁻¹·min⁻¹ throughout the experiment and maintained fentanyl plasma concentrations averaging 1.0 ± 0.3 ng/ml (see fig. 3). Control enflurane MAC = 2.24 ± 0.25%.

* Predicted reduction of enflurane MAC by midazolam (MID) as estimated from concurrent studies of the relationship of midazolam plasma concentration *versus* enflurane MAC reduction (see reference 4).

† $P < 0.01$ *versus* predicted for this combination of fentanyl and midazolam.

‡ $P < 0.01$ *versus* enflurane MAC fentanyl alone.

§ $P < 0.01$ *versus* fentanyl + midazolam 2.4.

¶ $P < 0.05$ *versus* predicted for this combination of fentanyl and midazolam.

** $P < 0.01$ *versus* fentanyl + midazolam 9.6.

†† This determination of enflurane MAC began after discontinuation of the fentanyl infusion and 5 min after the first dose of naloxone (1 mg/kg). In order to ensure antagonism of residual fentanyl, the supramaximal dose of naloxone (1 mg/kg) was repeated every 10 min during this final determination of enflurane MAC, which took 20–30 min.

This combination of midazolam and fentanyl was sufficient to replace enflurane in only one of six dogs.

The incremental increases in the enflurane MAC reduction attributed to midazolam (obtained by subtracting the initial enflurane MAC reduction in the presence of fentanyl alone from the enflurane MAC determined for each fentanyl–midazolam combination) were significantly less than those predicted (table 2), and this difference remained approximately the same throughout the experiment.

After discontinuation of midazolam and administration of flumazenil, the degree of enflurane MAC reduction produced by the continuing infusion of fentanyl was significantly less than that observed for fentanyl alone at the beginning of the experiment (26 ± 6% *vs.* 44 ± 5%; $P < 0.01$) in this study and also was significantly less ($P < 0.01$) than the enflurane MAC reduction predicted for fentanyl alone from our previous studies (39 ± 2%). That is, the average fentanyl concentration in the current study

during EMAC determinations after flumazenil was 3.2 ± 0.3 ng/ml, which would be predicted to reduce enflurane MAC by 39 ± 2% (fig. 3). Flumazenil has been shown to have no effect on either enflurane MAC or the reduction of enflurane MAC by fentanyl.[12]

There were no consistent or statistically significant changes in heart rate or blood pressure associated either with the lower infusion rate of fentanyl alone (atropine had been given at the induction of anesthesia) or with the commencement of the incremental loading doses and maintenance infusions of midazolam. In part 2, the higher infusion rate of fentanyl (0.2 µg·kg⁻¹·min⁻¹) reduced heart rate from the control value (108 ± 10 beats per min, enflurane alone) to 76 ± 13 beats per min ($P < 0.01$). The addition of midazolam produced no further change in heart rate and no change in blood pressure. There was a statistically insignificant tendency for heart rate and blood pressure to increase when the dogs responded to the tail-clamp stimulus. No consistent or significant he-

TABLE 2. Enflurane MAC Reduction Attributable to the Addition of Midazolam to a Continuous Infusion of Fentanyl in Six Dogs

Infusion (µg·kg⁻¹·min⁻¹)	Midazolam (ng/ml of plasma)	% Reduction of Enflurane MAC			Attributed Minus Predicted
		Observed	Attributed to Midazolam	Predicted for Midazolam*	
Fentanyl 0.2	0	44 ± 5	—	—	—
Fentanyl + midazolam 2.4	84 ± 16	47 ± 14†	3 ± 12	27 ± 2	−24 ± 13
Fentanyl + midazolam 28.8	1154 ± 376	66 ± 11†·‡	22 ± 11	54 ± 3	−32 ± 14
Fentanyl + midazolam 28.8	1196 ± 590	67 ± 14†·‡	23 ± 12	54 ± 5	−31 ± 13
Fentanyl + midazolam 28.8	1179 ± 615	65 ± 14†·‡	21 ± 13	54 ± 5	−33 ± 15

Values are mean ± SD. The fentanyl infusion was constant at 0.2 µg·kg⁻¹·min⁻¹ throughout the experiment and maintained fentanyl plasma concentrations averaging 3.1 ± 0.5 ng/ml (see fig. 4). Control enflurane MAC = 2.12 ± 0.28%.

* Predicted reduction of EMAC by midazolam as estimated from

concurrent studies of the relationship of midazolam plasma concentration *versus* enflurane MAC reduction (see reference 4).

† $P < 0.05$ *versus* predicted for this combination of fentanyl + midazolam.

‡ $P < 0.01$ *versus* fentanyl alone and *versus* fentanyl + midazolam 2.4 µg·kg⁻¹·min⁻¹.

modynamic changes were recorded after the administration of flumazenil. Naloxone administration was associated with an increase in heart rate (78 ± 27 to 138 ± 28 beats per min, $P < 0.01$) but with no change in blood pressure.

Discussion

By pooling all measurements of enflurane MAC reduction *versus* fentanyl concentration in plasma that have been made in our laboratory over the past 10 yr, we have confirmed and expanded the observations initially reported by Murphy and Hug.[1] There is a ceiling effect, or maximum enflurane MAC reduction, by fentanyl of approximately 60–80% at plasma concentrations at or above 30 ng/ml. This ceiling is comparable to that observed for morphine, sufentanil, and alfentanil in the dog.[13–15] The explanation for the ceiling effect is not clear, but the effect is compatible with the notion of saturation of a finite number of opioid (mu) receptors in the CNS. The ceiling effect to volatile anesthetic MAC reduction by opioids in the dog is also reminiscent of the inability of extremely high doses of opioids alone to suppress all somatic and sympathetic–hemodynamic responses to noxious stimulation in all human patients.[2,3,6,16]

In a previous study of enflurane MAC reduction accomplished by adding progressively larger amounts of fentanyl to a stable concentration of midazolam, we concluded the drugs were interacting additively.[7] We noted that the highest fentanyl concentration (45 ng/ml) in combination with midazolam (440 ng/ml, which would be expected to produce a 40–50% MAC reduction) was able to replace enflurane virtually completely in only 7 of the 18 dogs. (We said "virtually" because we could not completely eliminate enflurane from the dog after 6 or more h of inspiring the volatile anesthetic.) We attributed the failure to replace enflurane in 11 of the 18 dogs to the development of acute tolerance to fentanyl. It is now clear, from the current study, that this earlier speculation was incomplete.

First, tolerance development is expected to be progressive over time (as appeared to be the case in our previous study of midazolam and fentanyl[7] and also in a previous study of sufentanil[14]). In the current study we observed a less than additive interaction with the first addition of midazolam to fentanyl (*i.e.*, after an only 2–4-h exposure to fentanyl), and the differences between observed and predicted contributions of midazolam to MAC reduction remained the same or decreased slightly with time (contrary to expectations if tolerance were developing progressively).

Second, the magnitude of differences between observed and expected MAC reductions for the combinations of fentanyl and midazolam in part 2 of this study (table 2)

exceed those that can be attributed to tolerance development in either the previous (9–11%)[7] or the current study (13–18%, after flumazenil).¶ It appears that factors in addition to tolerance are responsible for the less than additive interaction of midazolam and fentanyl. There is no evidence of tolerance development to midazolam.[4] In the current study, naloxone consistently returned the degree of MAC reduction to that predicted for midazolam alone.

Also, there was no evidence of a change in enflurane MAC over periods of 5–8 h in our laboratory.[1,12,17]

We believe that in the current experiments, both fentanyl tolerance and a less than additive interaction between midazolam and fentanyl occurred. The latter conclusion is compatible with the concept that hypnotics interact less than additively in terms of the analgesic effects of opioids under certain conditions. Using a similar end point in another species (the tail-clamp method in rats), Kissin *et al.* showed that both fentanyl and, to a lesser degree, morphine had a less than additive interaction with thiopental.[18] More recently, using similar methodology, Daghero *et al.* demonstrated that midazolam decreased the morphine-induced increase in the rat's reaction threshold to pressure applied to the tail by one half and that flumazenil prevented this inhibition of opioid antinociception.[19]

The type of interaction between an opioid and a hypnotic may vary according to the endpoint that is determined; combinations that are synergistic with regard to their hypnotic effect[20,21] may be partially antagonistic with

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¶ In part 1, fentanyl alone reduced enflurane MAC at the beginning of the experiment by $28 \pm 11\%$ (table 1). The same infusion of fentanyl combined with midazolam 28.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$ produced a total MAC reduction of $70 \pm 9\%$ before and $59 \pm 10\%$ after stopping the fentanyl infusion and administering naloxone; it appears that the fentanyl infusion just before naloxone administration was contributing only 11% of the enflurane MAC reduction and that an enflurane MAC reduction of 17% had been lost over the course of the experiment (approximately 8 h).

In part 2, fentanyl alone reduced enflurane MAC at the beginning of the experiment by $44 \pm 5\%$ (table 2). After stopping the midazolam infusion and administering flumazenil, the same continuous infusion rate of fentanyl was associated with an enflurane MAC reduction of $26 \pm 6\%$ (see Results, part 2). Thus, an enflurane MAC reduction of 18% had been lost over the course of the experiment (approximately 10 h).

In part 2, there is another way of presenting the data. It is possible to predict the enflurane MAC reduction by fentanyl on the basis of its measured concentrations in plasma at the beginning and end of the 10 hr experiment. At the beginning, fentanyl 3.3 ± 0.5 ng/ml would be predicted to reduce enflurane MAC by $40 \pm 3\%$, and the observed reduction was $44 \pm 5\%$. At the end, fentanyl 3.2 ± 0.3 ng/ml would be predicted to reduce enflurane MAC by $39 \pm 2\%$, whereas the observed reduction was only $26 \pm 6\%$. Apparently, an enflurane MAC reduction of 13% had been lost during the 10-h experiment.

regard to antinociception (motor response to tail clamping).[18] Opioids act as analgesics in part by activating descending inhibitory control systems within the CNS.[22] Midazolam may inhibit these control systems and thereby decrease or limit the antinociceptive effect of fentanyl.[23] Our results are also compatible with the concept that anesthesia represents a number of different components[6] and that the overall effect of the combination of midazolam and fentanyl depends on which component of "anesthesia" is being studied.

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