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Anesthetic Interactions of Midazolam and Fentanyl: Is There Acute Tolerance to the Opioid?

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The anesthetic effects and interactions of midazolam and fentanyl were determined in terms of their reduction of enflurane MAC in dogs, and the effects of their specific antagonists were also investigated. Control enflurane MAC was determined by the tail clamp method in 18 mongrel dogs. Each animal then received an iv loading dose of midazolam followed by a constant infusion at 9.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ designed to produce a stable enflurane MAC reduction of approximately 40%, and enflurane MAC was determined following a 60-min observation period during which time the midazolam concentration in plasma stabilized. Fentanyl was then administered in a series of three incremental loading doses (15, 30, and 225 $\mu g/kg$) and infusions (0.05, 0.2, and 3.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) designed to produce enflurane MAC reductions of 30%, 50%, and 65%, respectively. Enflurane MAC was again determined following a 60-min observation period for each new infusion. In nine dogs after the fourth determination of enflurane MAC, fentanyl was discontinued and 1 mg/kg naloxone was administered iv every 10 min until enflurane MAC was determined for the last time. In the other nine dogs, midazolam was discontinued and 1.5 mg/kg flumazenil (RO 15-1788) was administered and enflurane MAC determined for the last time. The midazolam concentration in plasma remained stable at 414 ± 134 ng/ml throughout the study, and in the absence of fentanyl reduced enflurane MAC by 40 ± 10% (mean ± SD). The addition of fentanyl produced significant further reductions in enflurane MAC. The additional enflurane MAC reduction attributable to fentanyl was slightly less but not significantly different from that predicted from the fentanyl concentration in plasma except for the highest infusion rate. After administration of naloxone, the degree of enflurane MAC reduction returned to that produced initially by midazolam alone. In contrast, the degree of enflurane MAC reduction after administration of flumazenil and in the presence of a continuous infusion of fentanyl was significantly less than that expected. Lower plasma concentrations of fentanyl interacted additively with midazolam. The development of acute tolerance to fentanyl may be an explanation for the less than additive interaction between midazolam and the highest fentanyl concentrations. This explanation is supported by the lower than expected MAC reduction attributable to the highest infusion of fentanyl after antagonism of midazolam by flumazenil. (Key words: Anesthetics, intravenous: fentanyl. Anesthetics, hypnotics: midazolam. Anesthetics, volatile: enflurane. Antagonists: flumazenil, naloxone. Potency: MAC, ED_{50}.)

INTRAVENOUS DRUGS, such as fentanyl and midazolam, are used as adjuncts to anesthesia. They act as agonists at stereospecific receptors, which are finite in number within the CNS. Accordingly, a ceiling (or maximum) effect would be expected. A ceiling effect has been demonstrated both in humans and dogs, for fentanyl[1,2] and midazolam[3] alone, and the plasma concentrations required to produce the maximum reduction of enflurane MAC in the dog are close to those required to produce anesthesia in humans.[2,4] Despite their frequent use together, the nature of the interaction between fentanyl and midazolam as anesthetics remains unknown. The purposes of this study were as follows: 1) to study the nature of the interaction between fentanyl and midazolam (additive, synergistic, and antagonistic); 2) to determine if the combination of fentanyl and midazolam is a complete anesthetic (*i.e.*, able to replace enflurane); and 3) to examine the effects of a specific opioid antagonist (naloxone) and a specific benzodiazepine antagonist (flumazenil) on the enflurane MAC reduction produced by fentanyl and midazolam.

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.

Male mongrel dogs (n = 18) allowed free access to water while fasting overnight and weighing 19 ± 2 kg (all results expressed as mean ± SD) were each given 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 mask and a specialized mask.[5] This induction sequence was employed to avoid any confounding influence of other CNS depressants (*e.g.*, barbiturates) upon 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. Because the animals are naive with respect to the use of muscle relaxants, anticipatory anxiety about paralysis is not a factor.

A cuffed endotracheal tube was inserted, and ventilation with a Harvard respirator was adjusted to maintain arterial blood gases in the normal range. A catheter was inserted into a foreleg vein, and lactated Ringer's solution was infused at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$. A urinary catheter was inserted and the tail shaved. Temperature was

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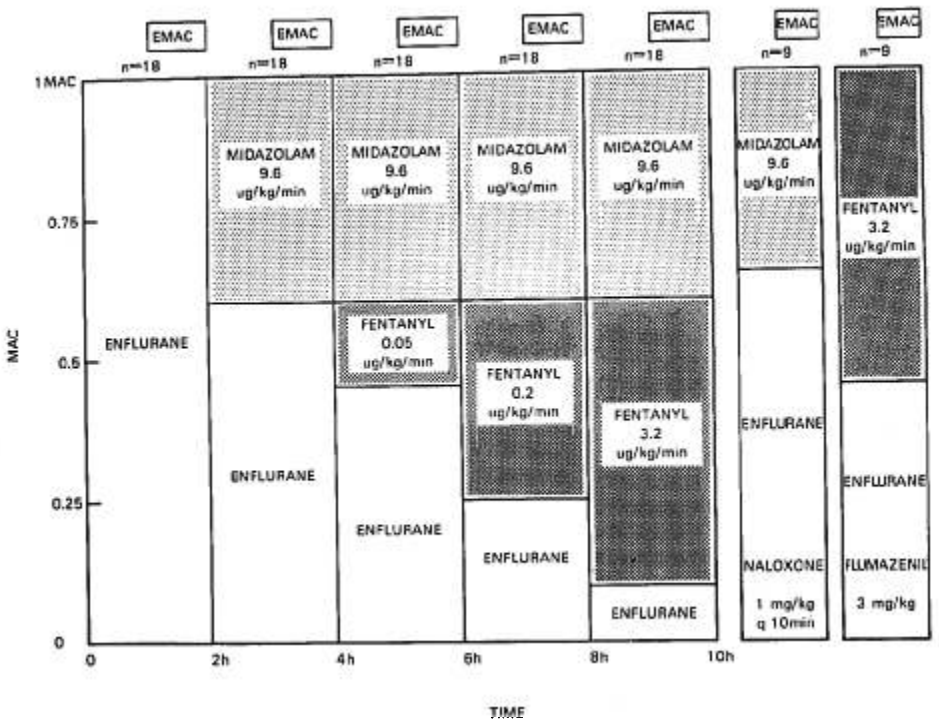

FIG. 1. Schematic representation of the experimental protocol. Time in hours is shown on the horizontal axis and MAC on the vertical axis. The sum of the anesthetic effects produced by each combination always equals 1 MAC, and the contribution of each drug (enflurane, midazolam, and fentanyl) is represented at each step. The maintenance infusion rates are shown within the vertical bars. The loading dose of midazolam was 352 μg/kg administered over 20 min. The sequential loading doses of fentanyl were 15, 30, and 225 μg/kg, respectively, each administered over the first 20 min after the determination of enflurane MAC (EMAC) for the previous drug combination. In the last phase of the experiment, the 18 dogs were divided into two groups of nine dogs each. In one group, the fentanyl infusion was stopped and 1 mg/kg naloxone was injected iv every 10 min until the final determination of EMAC was completed. The midazolam infusion was discontinued in the other group, which received 3 mg/kg iv flumazenil.

monitored using an esophageal temperature probe and maintained over the course of the anesthetic within 1° C of that measured in the animal just after the induction of anesthesia by the use of a warming blanket. The electrocardiogram was monitored throughout the experimental period. A femoral arterial catheter was utilized 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 previously described.[1] MAC was defined as that 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.

Figure 1 presents a schematic diagram of the protocol. After determination of the control enflurane MAC, 18 dogs were each given a loading dose of midazolam (352 μg/kg iv over 20 min) followed by a constant infusion of 9.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ to reduce enflurane MAC by approximately 40% (based on a previously obtained concentration *vs.* enflurane MAC reduction curve).[3] This infusion was maintained for the duration of the experiment. Following a 60-min observation period, enflurane MAC was again determined to verify the MAC reduction due to midazolam, and then fentanyl was administered to each animal in a series of three incremental loading doses (15, 30, and 225 μg/kg) each administered over 20 min and continuous infusions (0.05, 0.2, and 3.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) that were expected to produce 30%, 50%, and 65% reductions of enflurane MAC, respectively (again based on a previously obtained concentration *vs.* enflurane MAC curve).[1] Enflurane MAC was determined following a 60-min observation period for each new infusion rate.

After the enflurane MAC determination at the highest fentanyl infusion rate (3.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in combination with the midazolam infusion), the 18 dogs were divided into two groups of nine dogs each:

In group 1 (n = 9) the highest infusion rate of fentanyl was discontinued, the midazolam infusion was maintained, and 1 mg/kg naloxone was administered every 10 min until the last determination of enflurane MAC was completed.

In group 2 (n = 9) the midazolam infusion was discontinued, the highest fentanyl infusion (3.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was maintained, 1.5 mg/kg flumazenil was administered, and enflurane MAC was determined. Then a second 1.5-mg/kg dose of flumazenil was administered and enflurane MAC was determined for the last time. (We previously demonstrated that 1.5 mg/kg iv flumazenil completely

TABLE 1. Enflurane MAC Reduction *Versus* Plasma Concentrations of Midazolam (MID) and Fentanyl (FEN) Produced by Incremental Fentanyl Infusion Rates in Dogs Receiving Enflurane and a Continuous Infusion of Midazolam (control EMAC = 2.31 ± 0.27%)

Infusion ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	% EMAC Reduction Observed	Plasma (ng/ml)		Predicted* % ↓ EMAC			Observed Minus Predicted
		FEN	MID	FEN	MID	FEN + MID	
MID 9.6	40 ± 10	—	431 ± 134	—	44 ± 3	—	−4
+FEN 0.05	55 ± 15†	1.4 ± 0.8	415 ± 120	13 ± 4	43 ± 3	57 ± 7	−2
+FEN 0.2	75 ± 13†‡	3.8 ± 1.0	418 ± 136	39 ± 9	43 ± 4	82 ± 10	−7
+FEN 3.2	89 ± 7†‡§	45 ± 13	442 ± 156	64 ± 1	44 ± 4	100¶	−11
MID 9.6 + NOX 1 mg/kg (n = 9)	36 ± 17	—	344 ± 141	—	41 ± 4	—	−5
FEN 3.2 + FLU 3 mg/kg (n = 9)	55 ± 11	45.0 ± 2.1	—	64 ± 1¶	—	—	−9

Values are mean ± SD for 18 dogs before and nine dogs after administration of each of the antagonists: flumazemil (FLU) and naloxone (NOX).

* Comparison with observed enflurane MAC reduction based on data obtained from previous publications.[1,3]

† $P < 0.01$ *versus* MID alone.
‡ $P < 0.01$ *versus* MID + FEN 0.05.
§ $P < 0.01$ *versus* MID + FEN 0.2.
¶ $P < 0.01$ *versus* observed.

antagonized the enflurane MAC reduction by these plasma concentrations of midazolam.[8])

Plasma concentrations of midazolam were determined by gas liquid chromatography[6] (within assay variation 7.4% at 25 ng/ml, 3.5% at 100 ng/ml; between assay variation 7.5% at 25 ng/ml, 4.5% at 100 ng/ml; sensitivity 2–3 ng/ml). Plasma concentrations of fentanyl were determined by radioimmunoassay¶ (absolute sensitivity, 100 pg; coefficient of variation, 5–7%).

The 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 concentration *versus* enflurane MAC reduction curve obtained in previous experiments[1,3] and significant changes in mean arterial pressure (MAP) and heart rate (HR) after the administration of naloxone in nine dogs or flumazenil in the other nine dogs. One-way analysis of variance was used to identify concentration related differences in the degree of MAC reduction produced by subsequent infusions and to compare mean changes in HR and MAP produced by any two infusion rates. Corrections for multiple comparisons were made (Scheffe's post-hoc test). Values represent the mean ± SD.

Results

The plasma concentration of midazolam remained relatively stable at 414 ± 134 ng/ml throughout the experiment (table 1) and, in the absence of fentanyl, reduced enflurane MAC by 40 ± 10% (predicted enflurane MAC reduction 44 ± 3%).[3] The addition of fentanyl produced significant further reductions in enflurane MAC (table 1). The enflurane MAC reductions produced by the addition of fentanyl infusions of 0.05 and 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ were not significantly different from those predicted from previously determined concentration *versus* enflurane MAC reduction curves, indicating additive interactions of midazolam and fentanyl at these concentrations. A significant difference from the predicted value was found at the highest fentanyl infusion rate in combination with midazolam. In seven animals a combination of fentanyl and midazolam completely replaced enflurane as demonstrated by no movement in response to tail clamping repeatedly over a 60-min period. The fentanyl concentrations (45.3 ± 19.1 ng/ml) in these dogs did not differ from those (45.6 ± 8.2 ng/ml) achieved in the 11 dogs that did move in response to tail clamping while receiving the highest fentanyl infusion rate and had an average MAC reduction of 86 ± 8%.

After discontinuing fentanyl and administering naloxone, the enflurane MAC reduction returned to that produced initially by midazolam alone (table 1). In contrast, the degree of enflurane MAC reduction evident after administering flumazenil and discontinuing midazolam and while continuing the highest fentanyl infusion rate was significantly less than predicted.

In this study a significantly greater MAP was measured at the highest infusion rate of fentanyl (3.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in presence of the constant midazolam infusion (9.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$) compared with the control MAP (enflurane only) ($P < 0.05$). The addition of fentanyl infusions of 0.2 and 3.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in the presence of constant plasma concentration of midazolam significantly decreased mean HR values ($P < 0.05$ and $P < 0.01$, respectively; table 2).

Table 3 presents the hemodynamic effects of the addition of naloxone in nine dogs and of flumazenil in the other nine dogs. The addition of 1 mg/kg naloxone after

¶ Fentanyl radioimmunoassay kit: Instruction manual for the measurement of fentanyl. Catalog No S1, June 1983. Janssen Life Sciences Products; Beerse, Belgium. Modified according to Schüttler J, White PF: Optimization of the radioimmunoassays for measuring fentanyl and alfentanil in human serum. ANESTHESIOLOGY 61:315–320, 1984

TABLE 2. Hemodynamic Variables during Each Infusion Rate

Maintenance Infusion Rate ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	Heart Rate (beats/min)	Mean Systemic Blood Pressure (mmHg)
Control	119 ± 19	82 ± 17
MID 9.6	124 ± 18	95 ± 22
+FEN 0.05	90 ± 29*	92 ± 18
+FEN 0.2	87 ± 29†	92 ± 21
+FEN 3.2	65 ± 20‡	101 ± 14§

Values are mean ± SD for 18 dogs.
* $P < 0.05$ *versus* MID alone.
† $P < 0.05$ *versus* control and MID alone.
‡ $P < 0.01$ *versus* control and MID alone.
§ $P < 0.05$ *versus* control.

discontinuing fentanyl produced a small but insignificant increase in MAP and a significant increase in HR. The administration of flumazenil after discontinuing midazolam produced no significant changes in MAP or HR.

Discussion

Lower concentrations of fentanyl in plasma interacted additively with midazolam. The highest fentanyl concentration was less than additive, and this combination of fentanyl and midazolam did not completely replace enflurane in 11 of 18 dogs. One possible explanation is the development of acute tolerance to fentanyl in some dogs as described for sufentanil.[7] It is interesting to note that tolerance developed in only 50% of the dogs receiving moderate doses (concentrations) of sufentanil. Acute opioid tolerance is also compatible with the lower than expected enflurane MAC reduction attributable to fentanyl after administration of flumazenil (specific benzodiazepine antagonist).[8] Naloxone returned the enflurane MAC reduction to that produced by midazolam alone, making tolerance to midazolam an unlikely possibility. The rate and degree of opioid tolerance development is thought to be time- and dose-dependent,[9] which may explain its being apparent only at the highest fentanyl concentrations that were always produced at the later part of these experiments. Tolerance was not evident during continuous infusion of fentanyl at a lower rate 0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$ producing plasma concentrations of 12–14 ng/ml.[8] Acute tolerance to the cardiovascular effects of fentanyl in dogs has been shown to occur within 3 h[9] and has developed within hours in humans whether administered as a bolus[10] or during prolonged infusion.[11]

We have previously shown using a similar study design[3] that incremental infusions producing midazolam concentrations of 21–9,763 ng/ml of plasma caused no significant change in HR from that measured in the presence of enflurane alone, but there was a progressive increase in MAP with each increment of midazolam infusion rate. In addition, there were no significant differences between the slight hemodynamic responses observed upon application of the tail clamp stimulus when there was movement *versus* when movement did not occur. In this study we confirmed the absence of significant differences between the hemodynamic responses observed upon application of the tail clamp stimulus when there was movement *versus* when no movement occurred. The addition of midazolam slightly increased (not statistically significant) HR and MAP compared with control (enflurane alone). Incremental infusions of fentanyl (0.05 and 0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) did not change MAP but significantly decreased HR at all three infusion rates when compared with that obtained by midazolam alone, and at the higher two rates when compared with control.

TABLE 3. Effects of Naloxone or Flumazenil on Mean Arterial Pressure and Heart Rate in 2 Groups of 9 Dogs Each

	Flumazenil*		Naloxone†	
	Before	After	Before	After
Mean arterial pressure (mmHg)	79 ± 12	80 ± 12	105 ± 13	116 ± 12
Heart rate (beats/min)	56 ± 9	59 ± 9	79 ± 20	136 ± 18‡

Values are mean ± SD.
* After determining enflurane MAC during infusions of midazolam and fentanyl (highest infusion rate), the midazolam infusion was stopped and 3 mg/kg flumazenil was injected iv and the hemodynamic variables were recorded 1 min later.
† After determining enflurane MAC during infusions of midazolam and fentanyl (highest infusion rate), the fentanyl infusion was discontinued and 1 mg/kg naloxone was injected iv and the hemodynamic variables were recorded 1 min later.
‡ $P < 0.01$ when compared with values obtained before the administration of 1 mg/kg naloxone.

The administration of naloxone in the presence of fentanyl and enflurane increased HR but not MAP. It appears that the opioid antagonist simply reversed the vagotonic effect of fentanyl. Others have observed increases in both HR and MAP indicating a sympathetic response to naloxone antagonism and have suggested that it represented precipitation of an acute abstinence syndrome under general anesthesia.[12–14]

The addition of flumazenil caused no change in HR or MAP when compared with measurements made during the infusions of midazolam and the highest fentanyl infusion rate before the addition of flumazenil, confirming previous results from our laboratory[8] as well as those of O'Boyle *et al.*[15] and Dodgson *et al.*[16]

In conclusion, fentanyl in the lower two infusion rates interacted additively with midazolam in reducing the anesthetic requirement for enflurane. The highest infusion rate of fentanyl resulted in a somewhat less than additive interaction. The combination of midazolam with the

highest concentration of fentanyl would be predicted to replace enflurane, but this actually occurred in only seven of the 18 dogs. Tolerance development may explain the less than additive interaction of high doses (concentrations) of fentanyl with midazolam and the failure of the combination to replace enflurane in 61% of the dogs in this study.

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