Title

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RESEARCH PAPER

Effects of trazodone and dexmedetomidine on fentanyl-mediated reduction of isoflurane minimum alveolar concentration in cats

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Abstract

Objective To screen modulators of biogenic amine (BA) neurotransmission for the ability to cause fentanyl to decrease isoflurane minimum alveolar concentration (MAC) in cats, and to test whether fentanyl plus a combination of modulators decreases isoflurane MAC more than fentanyl alone.

Study design Prospective, experimental study.

Animals A total of six adult male Domestic Short Hair cats.

Methods Each cat was anesthetized in three phases with a 1 week washout between studies. In phase 1, anesthesia was induced and maintained with isoflurane, and MAC was measured in duplicate using a tail clamp stimulus and standard bracketing technique. A 21 ng mL^{-1} fentanyl target-controlled infusion was then administered and MAC measured again. In phase 2, a single cat was administered a single BA modulator (buspirone, haloperidol, dexmedetomidine, pregabalin, ramelteon or trazodone) in a pilot drug screen, and isoflurane MAC was measured before and after fentanyl administration. In phase 3, isoflurane MAC was measured before and after fentanyl administration in cats co-administered trazodone and dexmedetomidine, the two BA modulator drugs associated with fentanyl MAC-sparing in the screen. Isoflurane MAC-sparing by fentanyl alone, trazodone–dexmedetomidine and trazodone–dexmedetomidine–fentanyl was evaluated using paired t tests with $p < 0.05$ denoting significant effects.

Results The MAC of isoflurane was 1.87% ± 0.09 and was not significantly affected by fentanyl administration ($p = 0.09$). In the BA screen, cats administered trazodone or dexmedetomidine exhibited 26% and 22% fentanyl MAC-sparing, respectively. Trazodone–dexmedetomidine co-administration decreased isoflurane MAC to 1.50% ± 0.14 ($p < 0.001$), and the addition of fentanyl further decreased MAC to 0.95% ± 0.16 ($p < 0.001$).

Conclusions and clinical relevance Fentanyl alone does not affect isoflurane MAC in cats, but co-administration of trazodone and dexmedetomidine causes fentanyl to significantly decrease isoflurane requirement.

Keywords anesthesia, dexmedetomidine, fentanyl, feline, isoflurane, trazodone.

Introduction

Although phenylpiperidine opioids, such as fentanyl, decrease inhaled anesthetic requirements in most mammals, they generally have little or no effect on minimum alveolar concentration (MAC) in cats (Brosnan et al. 2009, 2020). However, when administered with the phenothiazine acepromazine, fentanyl exhibits isoflurane MAC-sparing effects in cats (Brosnan & Pypendop 2021). The mechanism posited is that μ-opioid receptor agonists stimulate release of catecholamines within the cat brain (Reis et al. 1969; Chesselet et al. 1981; Gaumann et al. 1988) that increase anesthetic requirement and thus oppose typical MAC-sparing effects of opioids. Acepromazine antagonizes α_1-adrenoreceptors and D_2 dopamine receptors (Brosnan & Pypendop 2021), thereby blocking central catecholamine actions and allowing fentanyl to contribute immobilizing effects in isoflurane-anesthetized cats. However, it is unclear which effect of acepromazine mediates the fentanyl MAC-sparing in cats. In addition to actions on catecholamine receptors, acepromazine (and phenothiazines as a drug class) exert antagonistic effects at multiple dopamine receptor types as well as at receptors for other biogenic amine (BA) neurotransmitters, such as serotonin and histamine (Mlambo et al. 2023). At increased doses, some

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phenothiazines may even inhibit muscarinic acetylcholine receptors.

Acepromazine causes fentanyl to decrease isoflurane MAC in cats, but acepromazine α_1-adrenoreceptor antagonism contributes to hypotension during anesthesia (Sinclair & Dyson 2012; Grasso et al. 2015). Fentanyl is often used as part of a balanced technique to simultaneously decrease inhaled anesthetic requirement and reduce the cardiovascular depression caused by the inhaled anesthetic. However, hemodynamic benefits from fentanyl in cats may be lost if high doses of a hypotension-inducing drug such as acepromazine are necessary to achieve inhalant MAC reduction. Pharmaceuticals with safety data available in cats might be used as probes to affect either the release or reuptake of BAs or to directly modulate specific neurotransmitter receptor targets and therefore help clarify the mechanism underlying effects of acepromazine on fentanyl MAC reduction. As a second aim, clinical drug candidates might be identified that likewise facilitate fentanyl MAC-sparing in cats without the adverse hypotension associated with acepromazine.

Six different BA neurotransmitter modulating agents with different molecular mechanisms of action and prior clinical or research use in cats were used as pharmaceutical probes. Buspirone is an azaspirondecandione anxiolytic that is a partial serotonin 5-HT_{1A} receptor agonist, thereby inhibiting serotonin release in the brain, and a dopamine D_2 receptor antagonist (Gobert et al. 1999). Haloperidol is a butyrophenone agonist of serotonin 5-HT_{1A} receptors and either antagonist or inverse agonist at 5-HT_{2A} and $5HT_7$ receptors and D_2, D_3 and D_4 dopamine receptors (Burstein et al. 2005). It also exhibits some nonselective α-adrenoreceptor antagonism. Trazodone is a phenylpiperazine antidepressant that functions as a weak serotonin reuptake inhibitor with additional partial agonist effects at 5-HT_1 receptors and an antagonism at 5-HT_{2A} and 5-HT_{2B} receptors that is shared with its active metabolite, metachlorophenylpiperazine (Tucker et al. 2023). Trazodone is also an α-adrenoreceptor antagonist, but with much greater affinity for the α_1-adenoreceptor than for the α_2-adrenoreceptor (Owens et al. 1997). Dexmedetomidine is an imidazole α_2-adrenoreceptor agonist that downregulates neuronal release of the catecholamine norepinephrine (Jorm & Stamford 1993). Pregabalin is a gabapentinoid that antagonizes presynaptic neuronal-type voltage-gated calcium channels necessary for neurotransmitter release, including norepinephrine, dopamine and serotonin (Li et al. 2011). Ramelteon is a high-affinity agonist for the both the melatonin MT_1 and MT_2 receptors that, in turn, inhibit dopamine release in the medulla, pons and hypothalamus (Zisapel 2001) and thus may prevent opioid-induced stimulation of dopaminergic arousal centers in the ascending reticular activating system and ventral tegmentum of the midbrain responsible for unconsciousness during anesthesia.

Each of these drug probes exerts direct and/or indirect effects on at least some of the same mechanistic targets as acepromazine. Although fentanyl alone does not appear to affect anesthetic requirement in cats, it is hypothesized that administration of one or more of these BA modulator drugs will cause fentanyl to exhibit MAC-sparing effects. As drugs producing similar pharmacologic end points via different receptor mechanisms often interact synergistically, it is also hypothesized that fentanyl-induced MAC-sparing might be enhanced by coadministration of two mechanistically distinct modulator drugs without causing increased adverse effects.

Materials and methods

Assuming MAC measurements with standard deviations (SD) equal to 10% of the mean, at least five animals would be required to detect a 15% difference in anesthetic EC_{50} with $p <$ 5% and with 80% power using a one-sided paired Student *t* test. Accordingly, six university-bred, male, Domestic Short Hair cats, aged 1−2 years and weighing 4.8−6.2 kg were studied. Animals were housed indoors at approximately 23 °C with a 12 hour light cycle in accordance with current US Department of Agriculture guidelines at an Association for Assessment and Accreditation of Laboratory Animal Care accredited institution. Health was assessed using a history and physical examination, and cats were fasted overnight before anesthesia. This research protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Davis (Protocol #22844).

Anesthetic induction and instrumentation

The study was performed approximately 15 m above sea level. Each cat was anesthetized three separate times at 1 week intervals (Fig. 1). Anesthesia was induced using an acrylic chamber in unmedicated cats with isoflurane (Dechra, Overland Park, KS) in O_2. Following orotracheal intubation with a cuffed 4.5 mm tube, anesthesia was maintained using isoflurane in O_2, with an 80 mL tidal volume achieved by a pressure-controlled, volume-guaranteed, intermittent positive pressure ventilation mode (Carestation 620; GE Healthcare). Respiratory rate was adjusted to maintain end-tidal partial pressure of carbon dioxide ($P_E'CO_2$) at 35−45 mmHg, but hyperventilation was permitted for cats breathing spontaneously during the ventilator pause (intermittent mandatory ventilation). Lactated Ringer's solution (Baxter Healthcare) was administered during anesthesia via an infusion pump at 5 mL kg^{-1} $hour^{-1}$ through a cephalic venous catheter. Noninvasive arterial blood pressure was monitored using an oscillometric device (Cardell 9401; Midmark) attached to a cuff (2.5 cm wide) placed above the tarsal joint. An intravenous (IV) infusion of norepinephrine bitartrate (Baxter Healthcare) was administered as needed to maintain mean arterial blood

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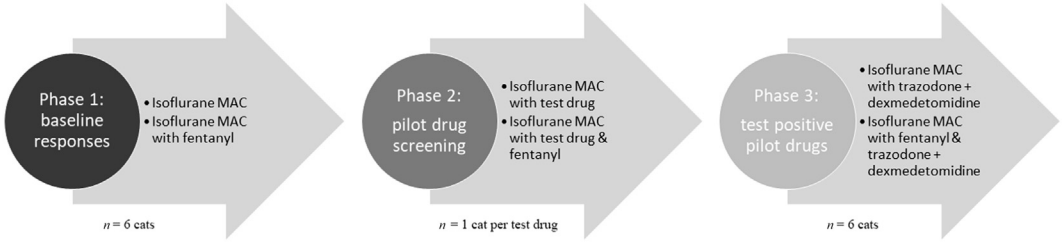

Figure 1 Overview of the three experimental phases of this study. Each phase was separated by a 1 week drug washout period. MAC, minimum alveolar concentration.

pressure ≥ 60 mmHg. Body temperature was monitored using an esophageal probe (ADInstruments) and external forced-air heating or cooling was used to as needed to maintain body temperature at 38−39 °C. Heart rate and blood oxygen saturation were monitored using a pulse oximeter, and $P_E'CO_2$ measured by side-stream capnography (Carescape Monitor B650; GE Healthcare). End-tidal isoflurane concentrations (F_E'Iso) were measured from hand-sampled gases collected in a glass syringe from a port connected to coaxial tubing extending the length of the endotracheal tube. Anesthetic concentrations were measured to the nearest 100 ppm using an infrared analyzer (Carescape Monitor B650; GE Healthcare) that was calibrated daily against multiple gas standards that spanned the range of isoflurane concentrations measured in this study.

Phase 1: isoflurane MAC and fentanyl effect

After instrumentation, the inspired concentration of isoflurane was adjusted to maintain F_E'Iso constant at 1.8% for 20 minutes. Next, after baseline physiologic responses were measured, a Bozeman forceps was clamped onto the distal tail over the caudal vertebrae to the first ratchet for 1 minute or until the cat exhibited movement of the limbs or head. If no movement, F_E'Iso was decreased by 0.15−0.20%; otherwise F_E'Iso was increased by 0.15−0.20%. After 20 minutes equilibration at the new concentration, physiologic parameters and response to tail clamp placed immediately cranial to the prior test site were measured as before. This process was repeated until two crossover events (a change from move to no-move, or vice versa) were recorded. A single isoflurane MAC was defined as the mean of the highest concentration that permitted movement and the lowest concentration that prevented movement. Duplicate MAC measurements were averaged to obtain the individual baseline isoflurane MAC value for each cat.

Next, fentanyl citrate (Hospira) was administered IV using a target-controlled infusion (TCI) syringe pump (PHD 2000; Harvard Apparatus, MA, USA) controlled by software (Rugloop I, Demed, Temse, Belgium) programmed with previously measured cat-specific pharmacokinetic constants (Pypendop et al. 2014) to achieve a plasma fentanyl concentration of 21 ng mL^{-1}, equivalent to the effective concentration that causes 95% the maximal reduction in isoflurane requirement (EC_{95}) in dogs (Machado et al. 2022). After a 30 minute equilibration period, MAC of isoflurane was remeasured in duplicate using the same methods as described for baseline determinations. When complete, the effects of fentanyl were antagonized with 0.6 mg kg^{-1} naltrexone intramuscularly (IM), and cats were administered 2 mg kg^{-1} robenacoxib (Onsior; Elanco) subcutaneously (SC) and recovered from anesthesia (Pypendop et al. 2011b).

Phase 2: screening of biogenic amine modulator effects

After a 1 week washout period, the anesthetic effects of six different potential modulators of BA function were examined in pilot studies consisting of a single cat. Each experiment used a different cat, and no cat was used to screen more than one BA. A summary of drugs used for these screenings is shown in Table 1.

For experiments with buspirone, haloperidol, ramelteon and trazodone, each drug was administered orally 1 hour before anesthetic induction with isoflurane. Pregabalin was administered 12 hours and again 1 hour before anesthesia induction. Preanesthetic sedation (none, mild, moderate, severe) from each oral medication was subjectively assessed by at least two investigators not blinded to treatment. Dexmedetomidine was administered IV as a TCI to achieve a plasma concentration of 1.05 ng mL^{-1} once anesthesia induction was completed. The dexmedetomidine TCI was administered using a syringe pump (PHD 2000; Harvard Apparatus) and software (Rugloop I) programmed with pharmacokinetic constants previously measured in cats (Escobar et al. 2012b).

Isoflurane MAC with the BA modulator alone was determined in duplicate using identical methods to those described for baseline isoflurane MAC measurements in unpremedicated cats. Next, the fentanyl TCI was administered to achieve a plasma concentration of 21 ng mL^{-1} as previously described, and isoflurane MAC was redetermined in duplicate. If the resulting decrease in isoflurane MAC was < 15% compared with phase 1 baseline measurements, the test modulator drug was considered unlikely to exhibit a consistently large effect on

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Table 1 List of drugs, doses and routes used for pilot studies to test for modulation of fentanyl-induced isoflurane MAC-sparing effects. Each drug was screened using a single cat, with a different cat for each drug tested.

Drug (trade name)	Manufacturer	How supplied	Dosage mg (mg kg^{-1})	Route	Time of administration
Buspirone	Teva Pharmaceuticals (NJ, USA)	5 mg tablet	5 mg (0.9 mg kg^{-1})	Orally	Once, 1 hour before anesthesia induction
Dexmedetomidine (Dexdomitor)	Zoetis (NJ, USA)	0.5 mg mL^{-1} injectable solution	1.05 ng mL^{-1} plasma TCI	Intravenously	Continuously during dexmedetomidine TCI study conditions
Haloperidol (Haldol)	Mylan (PA, USA)	2 mg tablet	2 mg (0.4 mg kg^{-1})	Orally	Once, 1 hour before anesthesia induction
Pregabalin	Ascend Laboratories (NJ, USA)	20 mg mL^{-1} oral solution	22 mg (4 mg kg^{-1})	Orally	12 hours and 1 hour before anesthesia induction
Ramelteon (Rozerem)	Takeda Pharmaceuticals (MA, USA)	8 mg tablet	4 mg (0.8 mg kg^{-1})	Orally	Once, 1 hour before anesthesia induction
Trazodone	Teva Pharmaceuticals (NJ, USA)	50 mg tablet	50 mg (8 mg kg^{-1})	Orally	Once, 1 hour before anesthesia induction

fentanyl MAC-sparing in a fully powered study and would not be evaluated further. Cats were recovered at the end of the experiment following administration of 0.6 mg kg^{-1} naltrexone IM and 2 mg kg^{-1} robenacoxib SC.

Phase 3: trazodone—dexmedetomidine isoflurane MAC and fentanyl effect

One week following the drug screening studies, cats were administered 50 mg trazodone orally. One hour later, anesthesia was induced and maintained with isoflurane, and cats were noninvasively instrumented and physiologically supported as previously described. A dexmedetomidine TCI was administered IV to achieve a plasma concentration of 1.05 ng mL^{-1}, as previously described. Isoflurane MAC in the presence of trazodone—dexmedetomidine treatment was measured in duplicate using methods identical to those previously described. Finally, a fentanyl TCI was administered to achieve a target plasma concentration of 21 ng mL^{-1}, and isoflurane MAC was remeasured. At the end of the study, cats were administered 0.6 mg kg^{-1} naltrexone IM and 2 mg kg^{-1} robenacoxib SC and were recovered from anesthesia.

Statistical analysis

Project data has been deposited in the Dryad database (https://doi.org/10.25338/B8Q937). Data were summarized using mean ± SD. Values of $p < 0.05$ indicated statistical significance for inferential tests. Using commercial statistical software (IBM SPSS Statistics, version 28; IBM), the mean norepinephrine infusion rate and the percentage of time that animals were spontaneously breathing were compared between different study conditions using Wilcoxon sign-rank tests. The normality of MAC measurements and other physiologic responses for isoflurane alone (Iso), isoflurane + fentanyl (IsoF), isoflurane + trazodone/dexmedetomidine (IsoTD), isoflurane + trazodone/dexmedetomidine + fentanyl (IsoTDF), the differences between IsoF and Iso (IsoFvIso) and the differences between IsoTDF and IsoTD (IsoTDFvIsoTD) were confirmed using Shapiro—Wilk tests. Assumptions of homoscedasticity for MAC measurements and their differences were assessed using Bartlett's test. Paired two-tailed *t* tests were used to compare physiologic responses between Iso *versus* IsoF, Iso *versus* IsoTD, and IsoTD *versus* IsoTDF. Paired one-tailed *t* tests were used to evaluate the following hypotheses: 1) fentanyl decreases isoflurane MAC (IsoF − Iso < 0); 2) trazodone + dexmedetomidine decreases isoflurane MAC (IsoTD − Iso < 0); 3) fentanyl decreases isoflurane MAC in cats treated with trazodone + dexmedetomidine (IsoTDF − IsoTD < 0); and 4) fentanyl causes a greater MAC-sparing effect in cats treated with trazodone + dexmedetomidine than in cats anesthetized with isoflurane alone (IsoTDFvIsoTD − IsoFvIso < 0).

Results

The baseline isoflurane MAC in unpremedicated cats was 1.87 ± 0.09% atm (Table 2). Isoflurane MAC was not significantly affected by fentanyl administration ($p = 0.09$).

In the pilot study, treatment of cats before or during anesthesia with drugs intended to modulate BA activity had variable effects on isoflurane MAC and on fentanyl MAC-sparing. Dexmedetomidine, haloperidol and pregabalin decreased

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Table 2 Mean (± standard deviation) physiologic responses and median (25% quartile, 75% quartile) norepinephrine infusion rates during isoflurane minimum alveolar concentration (MAC) measurements from study phase 1 in unpremedicated cats without (Isoflurane) and with fentanyl (Iso + Fentanyl). These same measurements are shown in cats premedicated in study phase 3 with 50 mg trazodone orally and a 1.05 ng mL^{-1} plasma target-controlled infusion of dexmedetomidine delivered intravenously, without (Iso + Traz + Dexmed) and with fentanyl (Iso + Traz + Dexmed + Fentanyl). IsoFvIso is the individual animal response difference in unpremedicated animals administered isoflurane + fentanyl *versus* isoflurane alone. IsoTDFvIsoTD is the individual response difference in trazodone–dexmedetomidine medicated animals administered isoflurane + fentanyl *versus* isoflurane alone. HR, heart rate; MAP, mean arterial blood pressure; Norepi, mean infusion dose of norepinephrine administered during the testing period; $P_E'CO_2$, end-tidal partial pressure of carbon dioxide; SB, percentage of the study period during which the animal was spontaneously breathing during the ventilator expiratory pause; SpO_2, pulse oximetry (oxygen saturation of arterial hemoglobin); Temp, esophageal temperature. Statistically significant differences ($p < 0.05$) are denoted by superscripts for the following comparisons: *Isoflurane *versus* Iso + Fentanyl, †Isoflurane *versus* Iso + Traz + Dexmed, ‡Iso + Traz + Dexmed *versus* Iso + Traz + Dexmed + Fentanyl, §IsoFvIso *versus* IsoTDFvIsoTD.

Measurement	Isoflurane	Iso + Fentanyl	Iso + Traz + Dexmed	Iso + Traz + Dexmed + Fentanyl	IsoFvIso	IsoTDFvIsoTD
Isoflurane MAC (%)	1.87 ± 0.09	1.82 ± 0.12	1.50 ± 0.14†	0.95 ± 0.16‡	−0.05 ± 0.08	−0.55 ± 0.10§
$P_E'CO_2$ (mmHg)	36 ± 2	40 ± 2	38 ± 1	39 ± 1	4 ± 2	2 ± 2
SB (% time)	38 ± 34	25 ± 42	0 ± 0†	4 ± 10	−13 ± 26	4 ± 10
SpO_2	98 ± 1	98 ± 1	99 ± 1	99 ± 1	−1 ± 1	0 ± 1
HR (min^{-1})	161 ± 18	205 ± 36*	105 ± 14†	106 ± 9	44 ± 27	0 ± 6§
MAP (mmHg)	87 ± 13	93 ± 15	79 ± 8	93 ± 17‡	6 ± 16	14 ± 12
Norepi (μg $kg^{-1}min^{-1}$)	0.05 (0.00, 0.12)	0.05 (0.00, 0.10)	0.01 (0.00, 0.03)	0.00 (0.00, 0.00)	0.00 (−0.02, 0.00)	−0.01 (−0.03, 0.00)
Temp (°C)	38.5 ± 0.4	38.4 ± 0.2	38.2 ± 0.4	38.3 ± 0.3	- 0.1 ± 0.5	0.1 ± 0.3

isoflurane MAC by 9–10% at the doses tested (Table 3). Haloperidol and pregabalin premedication also caused sedation in cats, but sedation did not necessarily translate to MAC reduction, as was the case with trazodone premedication. Conversely, direct MAC-sparing effects caused by the test modulator drug was not always commensurate with enhancement of fentanyl anesthetic efficacy. Fentanyl decreased isoflurane MAC by 22% in the cat administered the dexmedetomidine TCI, and by 26% in the cat premedicated with trazodone. In cats screened with other test agents, fentanyl either decreased isoflurane MAC by < 12% or did not decrease MAC at all.

Adverse effects encountered during the BA modulator pilot study were relatively mild. The buspirone-treated cat exhibited mild hyperthermia that required active cooling during anesthesia. All cats recovered well from anesthesia and no cat exhibited a noticeably prolonged recovery time.

The combination of trazodone–dexmedetomidine in all six unpremedicated cats decreased mean isoflurane MAC by 20 ± 7% ($p < 0.001$) compared to anesthesia with isoflurane alone (Table 2). Co-administration of fentanyl decreased isoflurane MAC an additional 37 ± 7% compared with isoflurane with trazodone–dexmedetomidine ($p < 0.001$). Trazodone–dexmedetomidine plus fentanyl decreased isoflurane requirement by 49 ± 7% ($p \leq 0.001$) when compared with isoflurane MAC in otherwise unmedicated cats. The MAC-sparing effect of fentanyl was also significantly greater in trazodone–dexmedetomidine medicated cats compared with cats anesthetized with isoflurane alone ($p < 0.001$).

Intermittent mandatory ventilation allowed for spontaneous breathing during the ventilator expiratory pause. Without premedication, cats breathed spontaneously on average about one quarter to one-third of the time while anesthetized with isoflurane (Table 2), despite ventilator settings being sufficient to maintain normocapnia. Addition of trazodone and dexmedetomidine eliminated spontaneous breathing in all cats, and this was significantly different compared to time spent spontaneously breathing during isoflurane anesthesia in otherwise unmedicated cats ($p = 0.04$). Although one trazodone–dexmedetomidine medicated cat did breathe spontaneously about 25% of the time after fentanyl was added and isoflurane concentrations were reduced, the overall change among all cats was not significant ($p = 0.3$).

In cats administered only isoflurane, fentanyl administration caused a mean 27 ± 17% increase in heart rate ($p = 0.01$, Table 1). By contrast, trazodone–dexmedetomidine reduced heart rate by 35 ± 5% compared to anesthesia with isoflurane alone ($p < 0.001$) and the addition of fentanyl had no effect on heart rate ($p = 0.9$). The MAC reduction afforded by fentanyl in cats treated with trazodone–dexmedetomidine also resulted in a 17 ± 14% increase in mean arterial blood pressure ($p = 0.03$). Although the same three cats did require norepinephrine infusions to maintain normotension during both the unpremedicated and the trazodone–dexmedetomidine treatment arms, there was no significant difference in vasopressor

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Table 3 Pharmacologic and physiologic responses for the biogenic amine modulator screening pilot experiments (one cat per agent) from study phase 2. Preanesthetic sedation is a subjective assessment made by an investigator not blinded to treatment; no assessment was performed for dexmedetomidine as this agent was administered after anesthetic induction. Isoflurane minimum alveolar concentration (MAC) measured after test drug modulator administration is followed by the percentage change from baseline MAC of isoflurane in the same unpremedicated cat from study phase 1 in parentheses. Iso + fentanyl is the isoflurane MAC measured during co-administration of a 21 ng mL^{-1} plasma fentanyl target-controlled infusion. HR, heart rate; MAP, mean arterial blood pressure; Norepi, mean infusion dose of norepinephrine administered during the testing period; $P_{E'}CO_2$, end-tidal partial pressure of carbon dioxide; SpO_2, pulse oximetry (oxygen saturation of arterial hemoglobin); Temp, esophageal temperature.

Drug treatment	Preanesthetic sedation	Isoflurane MAC (%)	$P_{E'}CO_2$ (mmHg)	S_pO_2 (%)	HR (min^{-1})	MAP (mmHg)	Norepi ($\mu g\ kg^{-1}min^{-1}$)	Temp (°C)
Buspirone	None	1.86 (+2%)	32	99	178	128	0.10	39.4
Isoflurane		1.88	36	97	238	116	0	39.5
Iso + Fentanyl								
Dexmedetomidine	–	1.66 (−10%)	41	98	141	93	0.08	39.0
Isoflurane		1.29	40	98	118	109	0.08	38.9
Iso + Fentanyl								
Haloperidol	Mild	1.67 (− 10%)	32	96	156	90	0	38.1
Isoflurane		1.67	36	97	236	114	0	38.4
Iso + Fentanyl								
Pregabalin	Moderate	1.60 (− 9%)	37	98	127	82	0.1	37.9
Isoflurane		1.50	39	96	155	78	0.1	37.9
Iso + Fentanyl								
Ramelteon	None	2.09 (+8%)	38	97	150	81	0.08	38.4
Isoflurane		1.85	41	98	256	96	0.02	38.5
Iso + Fentanyl								
Trazodone	Mild	1.99 (− 1%)	35	97	104	70	0.02	38.0
Isoflurane		1.48	40	97	123	84	0.22	38.1
Iso + Fentanyl								

infusion between any parts of these studies ($p = 0.1–0.6$) to account for the changes in heart rate and blood pressure.

In the trazodone–dexmedetomidine study arm, five cats exhibited mild sedation 1 hour after trazodone premedication. The remaining cat (which was not administered trazodone during the BA modulator pilot study) showed no sedation but did develop facial and head twitching that ceased following isoflurane induction. Postanesthetic recovery was calm and unremarkable for all cats.

Discussion

Fentanyl infusions are reported to have minimal effect on isoflurane MAC in cats (Brosnan et al. 2020), a finding confirmed by the present study. As shown in Table 2, co-administration of trazodone and a low dexmedetomidine plasma TCI does modestly reduce isoflurane MAC. However, the addition of fentanyl in cats treated with trazodone–dexmedetomidine effectively reduces isoflurane requirements by half when compared with MAC in cats anesthetized with isoflurane alone. This is similar to fentanyl MAC reduction that has been observed in cats administered 0.1 mg kg^{-1} acepromazine IV (Brosnan & Pypendop 2021).

The pilot drug screening study was not statistically powered to perform hypothesis testing, but several patterns emerge that may provide insights to molecular mechanisms for why fentanyl alone does not contribute to immobilization during isoflurane anesthesia in cats. For example, pregabalin inhibits voltage-gated calcium channels in the nerve terminal bouton, thereby nonselectively reducing release of excitatory central nervous system (CNS) neurotransmitters (Li et al. 2011). However, if antagonism of fentanyl MAC-sparing is primarily mediated by large effects at one or a small number of receptor targets, then modest global depression of neurotransmission at clinical concentrations might only yield a minimal fentanyl MAC-sparing response. Buspirone and haloperidol both directly inhibit dopamine receptors (Gobert et al. 1999; Burstein et al. 2005), and the melatonin agonist ramelteon indirectly reduces dopamine release in the brain (Zisapel 2001). However, all these drugs failed to alter fentanyl anesthetic efficacy, suggesting that the dopamine receptor may be a less likely target to account for antagonism of fentanyl MAC-sparing. As buspirone and haloperidol both also alter serotonin neurotransmission (Gobert et al. 1999), the 5-HT receptor might also play a limited role.

Although trazodone alters serotonergic neurotransmission, it also antagonizes α_1-adrenoreceptors with very little effect on α_2-adrenoreceptors (Valeri et al. 1988). Inhibition of α_1-adrenoreceptors may block the effects of increased brain norepinephrine release that is stimulated by μ-opioid receptor

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agonist administration in cats (Reis et al. 1969; Gaumann et al. 1988). Haloperidol also antagonizes α-adrenoreceptors but in a more nonselective manner, with an α_1:α_2 binding ratio of only 8:35 and an affinity for dopamine receptors that is an order of magnitude higher (Shahid et al. 2009). Therefore, the behavioral effects observed from haloperidol may be mediated primarily by dopaminergic actions, and brain haloperidol concentrations at clinical doses might only be able to antagonize a small fraction of adrenergic receptors. Furthermore, antagonism of α_2-adrenoreceptors by haloperidol could interfere with negative feedback mechanisms following noradrenergic neuron stimulation, resulting in even greater norepinephrine release onto α_1-adrenoreceptors in the brain. This might then explain why fentanyl could reduce isoflurane MAC in the screening study after premedication with trazodone, but not with haloperidol.

Dexmedetomidine also interferes with noradrenergic neurotransmission through selective presynaptic binding of α_2-adrenoreceptors that, via an inhibitory G-protein second-messenger system, decreases intracellular calcium in the nerve terminal required for synaptic norepinephrine vesicle release (Jorm & Stamford 1993). Therefore, dexmedetomidine probably prevents fentanyl-induced α_1-adrenergic receptor stimulation not by blocking the receptor directly, but by blocking release of norepinephrine in the first place.

Aside from the findings of the present study, there are at least two additional pieces of evidence that point to α_1-adrenergic receptor stimulation, or lack thereof, as determining whether μ-opioid receptor agonists are MAC-sparing in cats. The first is that acepromazine, a drug with central α_1-adrenorecepor antagonist effects, causes fentanyl to reduce isoflurane requirements in cats (Brosnan & Pypendop 2021). The second is that sympathomimetic drugs that cross the blood—brain barrier, such as ephedrine, can increase inhaled anesthetic MAC by around 50% (Steffey & Eger 1975). Moreover, this MAC increase is approximately equal and opposite to MAC-sparing in the present study resulting from fentanyl administration when noradrenergic neurotransmission is inhibited. In other words, the magnitude of MAC increase possible with central noradrenergic stimulation is sufficient to mask the magnitude of MAC-sparing that otherwise would occur from fentanyl.

Several factors limit further mechanistic inferences regarding the pharmacology of fentanyl interactions with BA modulators in anesthetized cats. For one, using a single cat for the drug screening study could easily have resulted in a β error in which there was failure to recognize that another pharmaceutical, besides trazodone and dexmedetomidine, could cause fentanyl to decrease isoflurane MAC. Furthermore, the BA modulators were selected in part because they all shared either direct or indirect receptor actions with acepromazine which, as previously noted, is known to cause fentanyl MAC-sparing in cats. However, phenothiazines such as acepromazine and chlorpromazine antagonize other BA neurotransmitter receptors that were not examined in this study, such as histamine receptors and muscarinic cholinergic receptors (Bolden et al. 1992; Appl et al. 2012). Indeed, fentanyl can alter cholinergic activity in some species (Reitan et al. 1978; Mortazavi et al. 1999), but there is no evidence that either increased or decreased cholinergic neurotransmission affects anesthetic requirement (Eger et al. 2002; Paraskeva et al. 2002). Inhibition of neuronal histamine receptors or brain histamine release does decrease MAC (Mammoto et al. 1997), but there is no evidence that fentanyl stimulates histamine release. As a result, it seems doubtful that either histamine or acetylcholine significantly contributes to reversal of fentanyl MAC-sparing in cats.

Plasma drug concentrations were not measured in the present study, and it is quite possible that null effects observed for some BA modulator drugs could have been as a result of the dose administered or inter-cat variability in bioavailability, time to maximum plasma concentration or volumes of distribution. In particular, oral haloperidol pharmacokinetics can vary considerably in humans (Kudo & Ishizaki 1999), although cat-specific data is not currently available. The doses of BA modulators used in the present study are known to produce behavioral effects in cats, but they may have been insufficient to affect fentanyl pharmacodynamics. However, higher doses might also have increased adverse effects, such as excessive sedation and prolonged recovery time, none of which were observed at the doses administered here. Additionally, no effects of sex on drug interactions could be assessed. Only male cats were studied to avoid measurements during estrous as progesterone can decrease MAC and potentially add intra-individual variability (Datta et al. 1989).

Dexmedetomidine infusions targeted a drug concentration of 1.05 ng mL^{-1}, equal to the median effective concentration (EC_{50}) needed for MAC reduction, and this should have caused a 40% decrease in isoflurane requirement in cats (Escobar et al. 2012a). Yet, the cat administered dexmedetomidine as part of the BA modulator screen showed only a 10% decrease in isoflurane requirement, an effect size that is associated with one-tenth of the plasma dexmedetomidine concentration that was targeted (Escobar et al. 2012a). Furthermore, administration of dexmedetomidine and trazodone only resulted in a 20% mean reduction in MAC; a much smaller effect than expected for dexmedetomidine alone. The reason for this discrepancy probably arises from the dexmedetomidine infusion dose used to generate the pharmacokinetic model which was more than 16 fold greater than the concentrations targeted here (Escobar et al. 2012b). Dexmedetomidine causes a dose-dependent increase in vascular resistance and decreases cardiac index in cats during isoflurane anesthesia (Pypendop et al. 2011a). Consequently, the volume of distribution in the central compartment and the hepatic clearance of dexmedetomidine in the

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pharmacokinetic study were both likely reduced as a function of the higher dexmedetomidine plasma concentration. Because of context-sensitive pharmacokinetics, as well as increased cardiac output from norepinephrine infusions used to correct hypotension, the constants used to program the infusion pump might have been too low to allow targeted dexmedetomidine concentrations to be reached.

Trazodone and dexmedetomidine were studied in combination to maximize modulating effects on fentanyl MAC-sparing. Drugs that cause a pharmacologic action via different molecular mechanisms often interact synergistically. Although synergy between trazodone and dexmedetomidine is suspected, separate measurements of trazodone dose-dependent effects and dexmedetomidine dose-dependent effects on fentanyl MAC-sparing would be needed for verification by isobolographic analysis (Shafer et al. 2008). Nonetheless, because fentanyl decreases isoflurane MAC when co-administered with trazodone—dexmedetomidine but not when administered alone, the interaction between fentanyl and trazodone—dexmedetomidine is synergistic by definition (Hendrickx et al. 2008).

Preanesthetic sedation with trazodone and intraoperative infusions of dexmedetomidine might offer translation to feline anesthetic practice in which μ-opioid receptor agonist administration is anticipated and inhaled anesthetic MAC reduction is desired. Some caution accompanies these clinical considerations. As a serotonin reuptake inhibitor, trazodone can excessively increase in intra-synaptic serotonin concentrations in the brain, known as serotonin syndrome (Baldo & Rose 2020). This can be exacerbated by co-administration of certain opioids, such as fentanyl, which stimulate serotonin release and bind some serotonin receptors. One cat in this study did exhibit preanesthetic facial myoclonus after trazodone administration, consistent with serotonin syndrome, although fentanyl administration during isoflurane—dexmedetomidine anesthesia did not produce physiologic signs of worsening toxicity, such as tachycardia, hypertension or hyperthermia. Also unknown is whether trazodone—dexmedetomidine—fentanyl MAC reduction improves cardiac output and blood pressure compared with anesthesia with isoflurane alone. Although dexmedetomidine potently reduces cardiac output in anesthetized cats at a median inhibitory plasma concentration of only 0.45 ng mL^{-1} (Pypendop et al. 2011a), phenylpiperidine opioids increase cardiac output in cats (Pascoe et al. 1997), and trazodone appears to produce little effect on cardiovascular function (Fries et al. 2019). Therefore, this drug combination could plausibly reduce both isoflurane requirement and anesthetic risk, necessary components of a balanced anesthetic technique (Lundy 1926).

Finally, there is potential to refine drug strategies around MAC-sparing with μ-opioid agonists. This study targeted very high plasma fentanyl concentrations, possibly much higher than necessary to achieve a maximum effect. If so, then similar isoflurane MAC-sparing might be possible using much lower fentanyl doses. By contrast, higher dexmedetomidine doses might give rise to greater central sympatholysis and lesser CNS norepinephrine release during fentanyl administration. This could further enhance fentanyl MAC-sparing by eliminating any residual α_1-adrenoreceptor agonism responsible for CNS excitation. Lastly, if trazodone is indeed modulating fentanyl anesthetic effects through the α_1-adrenoreceptor, then perhaps drug combinations incorporating phenothiazines, which more potently block α_1-adrenoreceptors, could yield better outcomes than trazodone. Consequently, fentanyl MAC-sparing and cardiovascular effects from combinations of low-dose acepromazine and dexmedetomidine in cats might warrant exploration.

Conclusions

Fentanyl alone does not significantly affect isoflurane MAC in healthy cats. However, administration of trazodone and dexmedetomidine does cause fentanyl to decrease isoflurane anesthetic requirements. These findings support the hypothesis that reducing noradrenergic neurotransmission in the CNS can reveal significant fentanyl contributions to anesthetic immobility.

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Authors' contributions

RJB: conceived project, designed study, participated in all experiments, analyzed data, wrote the manuscript. BHP: assisted with study design, participated in all experiments, revised the manuscript. AC: participated in experiments, revised the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

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