The effects of i.v. fentanyl administration on the minimum alveolar concentration of isoflurane in horses

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Background. Fentanyl decreases the minimum alveolar concentration (MAC) of inhaled anaesthetics and has been used clinically to reduce the requirements of other anaesthetic drugs in humans and small animals. We hypothesized that i.v. fentanyl would decrease the MAC of isoflurane in horses in a dose-dependent manner.

Methods. Following determination of baseline MAC of isoflurane, fentanyl was administered i.v. to target plasma concentrations of 1, 8, and 16 ng ml⁻¹. Each horse was randomly assigned two of three target concentrations administered in ascending order. Loading and infusion doses for each horse were determined from previously derived individual pharmacokinetic values. Isoflurane MAC determination began 45 min after fentanyl administration at each target fentanyl concentration. Venous blood was collected at fixed intervals during the infusion for measurement of plasma fentanyl concentrations.

Results. Mean actual fentanyl plasma concentrations were 0 (baseline), and 0.72 (SD 0.26), 8.43 (3.22), and 13.31 (6.66) ng ml⁻¹ for the target concentrations of 1, 8, and 16 ng ml⁻¹, respectively. The corresponding isoflurane MAC values were a baseline of 1.57 (0.23), and 1.51 (0.24), 1.41 (0.23) and 1.37 (0.09)%, respectively. The fentanyl concentrations of 0.72 and 8.43 ng ml⁻¹ did not significantly alter the MAC of isoflurane, but an 18 (7)% ISO-MAC reduction was observed at the 13.31 ng ml⁻¹ concentration.

Conclusions. These results cautiously encourage further study of fentanyl as an opioid anaesthetic adjunct to inhalant anaesthesia in horses.

Keywords: anaesthetics volatile, isoflurane; analgesics opioid, fentanyl; MAC, horse; potency, anaesthetic

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Balanced anaesthesia is the concomitant administration of multiple anaesthetic drugs so that no single drug is given at a dosage sufficient to produce toxicity during or after surgery. In humans and small animals, balanced anaesthesia with opioids provides perioperative analgesia and improves haemodynamics by minimizing the necessary inhalation concentration for general anaesthesia.¹⁻³ Fentanyl, a synthetic (µ)-receptor agonist, is the most commonly used intraoperative opioid analgesic in human and small animal medicine.¹,² I.V. administration of fentanyl has been reported to decrease the minimum alveolar concentration (MAC) of inhalation anaesthetics in humans, dogs and pigs, but its effect in horses has not been documented.⁴⁻⁶ However, previous studies demonstrated that two µ-opioid agonists, morphine and alfentanil, did not consistently alter the MAC of volatile anaesthetics in horses.⁷⁻⁸ Preliminary clinical studies evaluating the analgesic efficacy of transdermal fentanyl in conscious horses suggest that fentanyl patches provide significant analgesia without undesirable side-effects.⁹⁻¹⁰ Therefore, the purpose of this study was to determine the effect of three pseudo steady-state plasma fentanyl concentrations on the MAC of isoflurane in horses. We hypothesized that fentanyl dose-dependently decreases the MAC of isoflurane.

Materials and methods
The study was approved by the Institutional Animal Care and Use Committee of the University of California at Davis.
Effects of fentanyl on isoflurane in horses

Eight healthy, adult horses [4 females, 4 castrated males; mean (sd) weight 539 (32) kg and mean (range) age 9 (4–13) yr] were studied on two occasions. Food, but not water, was withheld from the horses for 12 h before beginning each study.

Anaesthesia and instrumentation
Catheters were placed in both external jugular veins before induction of anaesthesia for i.v. drug administration and blood collection, respectively. Anaesthesia was induced in unpremedicated horses by administration of isoflurane in oxygen as described elsewhere.1 Briefly, isoflurane was delivered to each horse via a mask connected to a large animal anaesthetic circle system. The horses were in the left lateral recumbency for induction and maintenance of anaesthesia. Orotbral intubation was performed when anaesthetic depth was suitable. Anaesthesia was maintained using intermittent positive pressure ventilation (Modified Mark 14, Bird Corp., Palm Springs, CA, USA), with peak airway pressure of 23 (1) cm H₂O and a variable rate of 7 (2) bpm to maintain arterial carbon dioxide tension (PᵃCO₂) between 45 and 55 mm Hg. A base-apex lead ECG (Grass Instruments, Quincy, MA, USA) was used to monitor heart rate (HR) and rhythm. A calibrated thermistor probe (Yellow Springs Instrument Co., Yellow Springs, OH, USA) was positioned in the nasopharynx to measure body temperature which was maintained constant at 37.5 (0.4)°C using heat lamps if it decreased to less than 37°C or by pouring ethanol on the skin if it increased to greater than 38°C. A 20-gauge catheter was inserted percutaneously in the right facial artery for direct measurement of systemic arterial pressure. The catheter was connected to a strain gauge (Model P23D, Division of Mark IV Industries, Oxnard, CA, USA) positioned level with the sternum. The strain gauge was calibrated at the beginning of each experimental day using a mercury column. The arterial catheter was also used for collection of blood samples for measurement of PᵃCO₂, arterial oxygen tension (PᵃO₂), pH (pHₐ), packed cell volume (PCV) and total protein concentration (TP). Lactated Ringer’s solution was infused at a rate of 2–4 ml kg⁻¹ h⁻¹ via a 16 gauge catheter placed in the left jugular vein. The urinary bladder was aseptically catheterized, and the catheter was connected to a receptacle to permit continuous drainage throughout anaesthesia and minimize urinary bladder distention during recumbency and before anaesthetic recovery.

Fentanyl delivery and anaesthetic monitoring
Individual pharmacokinetic data obtained from a previous study were used to determine the loading and infusion doses for each horse.12 The loading dose was determined by the following equation: C_T × V_{d(area)} , where C_T is the target plasma concentration and V_{d(area)} is the apparent volume of distribution at steady state. The loading dose was administered over 12 min to minimize possible central nervous system (CNS) excitation and subsequent locomotor activity induced by fentanyl.13 The fentanyl loading doses administered were 0.28 (0.10), 3.03 (0.85) and 4.69 (2.14) µg kg⁻¹ for the target plasma concentrations of 1, 8 and 16 ng ml⁻¹, respectively. The infusion rate per minute was determined by the equation: C_T × Cl, where Cl is total body clearance, and was begun immediately after loading dose administration was completed. The fentanyl infusions administered were 0.006 (0.002), 0.059 (0.018) and 0.113 (0.037) µg kg⁻¹ min⁻¹ for the target plasma concentrations of 1, 8 and 16 ng ml⁻¹, respectively. Each horse was randomly assigned two of three target concentrations of 1 (n=6), 8 (n=5), and 16 (n=5) ng ml⁻¹ and the target concentrations were arranged in ascending order. Isoflurane MAC was determined in duplicate at each fentanyl concentration for every animal.11

Values for pharyngeal temperature, HR, systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) and long and short axes of the pupil were recorded immediately before and at the conclusion of the fentanyl loading dose, then every 15 min. Blood samples (12 ml) were collected from the right jugular catheter immediately before and at the conclusion of the loading dose, then every 15 min. All blood samples were transferred to a tube containing sodium heparin, centrifuged for 10 min, and the plasma collected and frozen at −20°C for future determination of fentanyl concentration by a previously described method using liquid chromatography–mass spectrometry.12 Arterial blood was collected in heparinized syringes immediately before and at the conclusion of the fentanyl loading dose, then every 30 min. Samples were analysed immediately for measurement of PᵃO₂, PᵃCO₂, and pHₐ (ABL 330, Radiometer America, Cleveland, OH, USA), as well as PCV and TP. Results for PᵃO₂, PᵃCO₂, and pHₐ were corrected to the horse’s pharyngeal temperature.

During recovery from anaesthesia, several variables were monitored including time to tracheal tube removal, time to standing, number of attempts to standing and total recovery time. Once the horse attained standing position, a venous blood sample was collected for future plasma fentanyl determination.

MAC determination
Approximately 1 h after anaesthetic induction, determination of predrug (baseline) MAC was begun using previously described techniques.11 Briefly, end-tidal isoflurane concentration was maintained constant for at least 20 min and anaesthetic step changes were usually near 10%, but no more than 15% of the previous concentration. End-expired gas samples were obtained by intermittent manual collection from a nylon catheter positioned near the caudal tip of the tracheal tube. Isoflurane was measured by an infrared gas analyser (LB-2 anaesthetic analyser, Sensormedics Corp., Anaheim, CA, USA) that was calibrated before the start of each experiment against multi-point calibration standards.
(Primary gas standards, Matheson Gas Products, Newark, CA, USA). Calibration checks were also performed throughout the day of each experiment. The concentration of O₂ and CO₂ in the tracheal tube were also intermittently monitored by the use of calibrated polarographic (OM-11, Sensormedics Corp., Anaheim, CA, USA) and infrared (LB-2 CO₂ analyser, Sensormedics Corp., Anaheim, CA, USA) analysers, respectively. A response was judged as positive if some purposeful movement such as lifting or rotating of the head or neck occurred in response to up to 60 s of electrical stimulation (50 V, 5 Hz, 10 ms) of oral mucous membranes. Isoflurane MAC was defined as the average of the isoflurane concentrations, allowing and preventing gross purposeful movement in response to electrical stimulation. Baseline MAC was determined in triplicate and the mean was reported. To determine the effect of noxious stimulation on cardiopulmonary measurements, six values for HR, SAP, MAP and DAP over three breaths were obtained immediately before and after noxious stimulation.

**Statistical analysis**

Statistical analyses were conducted to assess the effects of fentanyl concentration on isoflurane MAC, temperature, pupil area, PCV, TP, $P_{\text{aO}_2}$, $P_{\text{aCO}_2}$ and pHa. A mixed-effect statistical model using SAS (version 9.1, Cary, NC) that included the categorical, fixed effect of treatment (target fentanyl concentration adjusted by actual fentanyl concentration), and a random horse effect was used to assess whether fentanyl had a significant effect on the above variables. For cardiovascular variables (HR, SAP, MAP, DAP), an additional categorical variable (stimulus) was added to the mixed-effect model. Significance was set at $P<0.05$. Data are reported as mean (SD).

**Results**

Fentanyl plasma concentrations were stable over time as shown in Figure 1. Actual fentanyl plasma concentrations were 0.72 (0.26), 8.43 (3.22) and 13.31 (6.66) ng ml⁻¹ for the target concentrations of 1, 8 and 16 ng ml⁻¹, respectively. The MAC of isoflurane in this study was 1.57 (0.23), 1.51 (0.24), 1.41 (0.23) and 1.37 (0.09)% at fentanyl plasma concentrations of 0, 0.72, 8.43 and 13.31 ng ml⁻¹, respectively, as shown in Figure 2. The fentanyl concentrations of 0.72 and 8.43 ng ml⁻¹ did not significantly alter the MAC of isoflurane, but a statistically significant 18 (7)% reduction was observed at the 13.3 ng ml⁻¹ fentanyl concentration as shown in Figure 3. PCV, TP, $P_{\text{aO}_2}$ and pupil size were not significantly altered by fentanyl administration, but $P_{\text{aCO}_2}$ and pHa were significantly different (Table 1). Fentanyl administration had a significant effect on HR and MAP, while noxious stimulation had a significant effect on MAP but not HR as shown in Figure 4.

Total anaesthesia time, determined as the time from a horse’s first breath of isoflurane to the time the breathing circuit was disconnected from the tracheal tube, was 464 (69) min and time to tracheal tube removal was 30 (13) min. All individuals in both experiments recovered from anaesthesia in 38 (31) min without serious complication. Recovery behaviour was variable with median 2 (range 1–5) attempts to attain standing position. One horse exhibited recovery behaviours that have been previously associated with opioid administration to horses. The horse went from

![Fig 1](image_url)
Effects of fentanyl on isoflurane in horses

![Graph showing isoflurane MAC at different plasma fentanyl concentrations in eight horses.]

**Fig 2** Isoflurane MAC at different plasma fentanyl concentrations in eight horses.

![Graph showing percentage reduction in isoflurane MAC at three different plasma fentanyl concentrations in eight horses. *P<0.05 from baseline.]

**Fig 3** Percentage reduction in isoflurane MAC [mean (SD)] at three different plasma fentanyl concentrations in eight horses. *P<0.05 from baseline.

lateral recumbency to standing at 13 min, then frantically attempted to circle in both directions and fell down several times. Plasma fentanyl concentration immediately after standing was 2.78 (1.47) ng ml\(^{-1}\).

**Discussion**

The MAC for isoflurane [1.57 (0.23)%] determined in this study was similar to that reported in previous studies of horses.\(^7\)\(^1\) Measured plasma fentanyl concentrations of 0.72 and 8.43 ng ml\(^{-1}\) did not have a consistent or significant effect on the MAC of isoflurane. However, fentanyl at 13.31 ng ml\(^{-1}\) significantly decreased the MAC of isoflurane in comparison to baseline, 0.72 and 8.43 ng ml\(^{-1}\) of fentanyl. The 18% reduction in isoflurane requirement observed with 13.31 ng ml\(^{-1}\) of fentanyl is similar to that found for swine\(^6\) (14 ng ml\(^{-1}\) fentanyl decreased isoflurane MAC by 25%), but is small in comparison to other species studied. For example, fentanyl at approximately 6–10 ng ml\(^{-1}\) decreased the MAC of isoflurane by 53% in dogs and fentanyl at 10 ng ml\(^{-1}\) decreased the MAC of isoflurane by 82% in humans.\(^4\)\(^5\) Perhaps
expectedly, the effect of morphine on isoflurane anaesthetic requirement in swine, dogs and primates is also qualitatively and quantitatively similar to that for fentanyl (i.e. primates > dogs > swine). However, with the same dose of morphine (2 mg kg\(^{-1}\)) the response of the isoflurane-anaesthetized horse was more variable than that noted in these other species and included increases in MAC.\(^7\) Reasons for the varied response in the horse to morphine remain speculative.\(^7\) Regardless, our present finding of a uniform decrease in MAC provides some encouragement for further study of fentanyl as an anaesthetic adjuvant in horses. This is further supported by a previous report of a small but measurable increase in nociceptive threshold in awake horses administered 10 \(\mu\)g kg\(^{-1}\) i.v. fentanyl\(^14\) or approximately twice the loading dose given to achieve a plasma fentanyl concentration of 13.3 ng ml\(^{-1}\) in this study.

Baseline cardiovascular parameters were typical of horses anaesthetized with a low dose (1.0 MAC) of isoflurane in \(O_2.\)\(^{15,16}\) Administration of fentanyl was associated with a significant and dose-dependent increase in MAP, SAP and DAP. For example, MAP was 85 (9), 89 (6), 100 (7) and 104 (11) mm Hg at fentanyl concentrations of 0, 0.72, 8.43 and 13.31 ng ml\(^{-1}\), respectively. Temporal effects may contribute to the observed increase in MAP, but MAP has been reported to increase after the first hour of anaesthesia then stabilize after 2–4 h of anaesthesia.\(^17\) As fentanyl administration did not begin until 3.6 (0.8) h after the start of anaesthesia, it is unlikely that temporal effects could completely explain the increase in MAP. A small but statistically significant increase in HR from a baseline of 32 (3) to 34 (5) and 35 (4) beats min\(^{-1}\) was noted for the fentanyl concentrations of 8.43 and 13.3 ng ml\(^{-1}\), respectively. Other studies have noted an increase in MAP and HR after the administration of opioids to anaesthetized horses and postulated that it may be because of CNS stimulation and subsequent increased sympathetic tone.\(^7\)\(^8\)

Fentanyl concentrations were variable among studied horses despite using individual pharmacokinetic data from a previous study to determine loading and infusion doses (Fig. 3).\(^12\) However, actual plasma fentanyl concentrations were unchanged in individuals over time during the determination of MAC as shown in Figure 1. In addition, low intra-individual variability between doses was noted. Factors that may have contributed to the variability between actual and target plasma fentanyl concentrations include high inter-individual and intra-individual variability in the disposition of fentanyl, differences in concentration of isoflurane between the present and previous study where the pharmacokinetics were determined, and poor performance of the pharmacokinetic models.

### Table 1 Minimum alveolar concentration (MAC), temperature, pupil area, packed cell volume (PCV), total protein (TP), arterial pH (pH\(_a\)), and arterial oxygen and carbon dioxide tensions (\(P_{\text{aCO}_2}, P_{\text{aO}_2}\)) in eight isoflurane-anaesthetized horses at fentanyl concentrations (FC) of 0, 0.72, 8.43 and 13.31 ng ml\(^{-1}\). Data are mean (SD). *\(P<0.05\) from 0 FC. †\(P<0.05\) from 0.72 FC. ‡\(P<0.05\) from 8.43 FC.

<table>
<thead>
<tr>
<th>Fentanyl concentration (ng ml(^{-1}))</th>
<th>0 (n=8)</th>
<th>0.72 (n=6)</th>
<th>8.43 (n=5)</th>
<th>13.31 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC (%)</td>
<td>1.57 (0.23)</td>
<td>1.51 (0.24)</td>
<td>1.41 (0.23)</td>
<td>1.37 (0.09)*†,‡</td>
</tr>
<tr>
<td>Temperature ((^\circ)C)</td>
<td>37.4 (0.4)</td>
<td>37.6 (0.3)</td>
<td>37.7 (0.6)*</td>
<td>37.5 (0.4)*</td>
</tr>
<tr>
<td>Pupil area (mm(^2))</td>
<td>1100 (250)</td>
<td>870 (90)</td>
<td>1000 (300)</td>
<td>1100 (350)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>31 (2)</td>
<td>32 (2)</td>
<td>33 (6)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>TP (mg dl(^{-1}))</td>
<td>5.6 (0.2)</td>
<td>5.7 (0.3)</td>
<td>5.7 (0.4)</td>
<td>5.3 (0.4)</td>
</tr>
<tr>
<td>(P_{\text{aCO}_2}) (mm Hg)</td>
<td>417 (167)</td>
<td>439 (137)</td>
<td>438 (155)</td>
<td>359 (142)</td>
</tr>
<tr>
<td>(P_{\text{aO}_2}) (mm Hg)</td>
<td>45.6 (4.1)</td>
<td>47.7 (3.2)*</td>
<td>47.4 (3.2)</td>
<td>48.4 (2.6)*</td>
</tr>
<tr>
<td>pH(_a) (units)</td>
<td>7.42 (0.02)</td>
<td>7.40 (0.03)</td>
<td>7.40 (0.04)</td>
<td>7.40 (0.02)*</td>
</tr>
</tbody>
</table>

**Fig 4** Mean (+SD) HR (A) and MAP (B) in eight isoflurane-anaesthetized horses immediately before and after noxious stimulation at fentanyl concentrations (FC) of 0, 0.72, 8.43 and 13.31 ng ml\(^{-1}\). *\(P<0.05\) from 0 FC. †\(P<0.05\) from 0.72 FC. ‡\(P<0.05\) from 8.43 FC. *\(P<0.05\) from pre-noxious stimulation.
A small but statistically significant increase in temperature was noted between baseline and the fentanyl concentrations of 8.43 and 13.31 ng ml\(^{-1}\). In this study, an attempt was made to tightly regulate pharyngeal temperature between 37 and 38°C because halothane MAC has been shown to increase linearly by 8% per °C from 37.3 to 40.7°C in dogs.\(^{18}\) Two horses required active cooling by applying ethanol to the skin when their temperatures reached 38.2°C but no horse attained a temperature higher than 38.6°C.

In spontaneously ventilated isoflurane-anæsthetized horses, morphine increased \(P_{aCO_2}\) to 102 mm Hg.\(^{7}\) As hypercapnia has been shown to decrease halothane MAC in dogs,\(^{19}\) horses were mechanically ventilated in this study to maintain normocapnia and prevent hypventilation from confounding the effects of fentanyl on isoflurane MAC. As described, \(P_{aCO_2}\) was maintained between 45 and 55 mm Hg. However, \(P_{aCO_2}\) was slightly but significantly increased from a baseline of 45.6 to 47.7 mm Hg and 48.4 mm Hg for the 0.72 and 13.31 ng ml\(^{-1}\) fentanyl concentrations, respectively. We consider these changes in \(P_{aCO_2}\) to be of no consequence to the magnitude of isoflurane MAC determined in this study. However, it is of interest that during these studies, small upward adjustments in mechanical ventilation were required to maintain \(P_{aCO_2}\) within our target range as the dose of fentanyl increased. Such observations may be attributable to normal variability or may represent small changes in metabolic output associated with increased opioid-induced sympathetic tone, and are consistent with previously noted changes in HR and MAP.\(^{7,8}\)

All horses recovered from anaesthesia without serious complication. Seven of eight recoveries were similar to those previously described for horses recovering from inhalation anaesthesia.\(^{20}\) One horse showed some signs consistent with opioid stimulation during recovery.\(^{7,8}\) There was no correlation between overall recovery score and plasma fentanyl concentration at the time of recovery. Fentanyl concentrations declined rapidly from 11.0 (6.52) to 2.78 (1.47) ng ml\(^{-1}\) 64 (20) min after discontinuation of the fentanyl continuous-rate infusion (CRI).

In conclusion, this study is both similar to and in conflict with current knowledge of opioid action in horses, but suggests that there may be a therapeutic range of fentanyl that consistently decreases anaesthetic requirement. Accordingly, this study encourages further investigation of the use of fentanyl as part of a balanced anaesthetic technique in horses.

The results of this study further suggest that the anaesthetic potency of fentanyl in horses is similar to swine, but less than that of humans or dogs.

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References