RESEARCH PAPER

Effects of a single intravenous bolus of fentanyl on the minimum anesthetic concentration of isoflurane in chickens (*Gallus gallus domesticus*)

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Abstract

Objective To assess the temporal effects of a single fentanyl intravenous (IV) bolus on the minimum anesthetic concentration (MAC) of isoflurane in chickens and to evaluate the effects of this combination on heart rate (HR) and rhythm, systemic arterial pressures (sAP) and ventilation.

Study design Prospective experimental trial.

Animals Seventeen adult chickens weighing 1.8 ± 0.2 kg.

Methods Individual isoflurane MAC for 17 chickens was previously determined using the bracketing method. Chickens were anesthetized with isoflurane to evaluate the effects of a single IV fentanyl bolus (10 or 30 μg kg^{-1}) on isoflurane MAC over time using the up-and-down method. Ventilation was controlled. The isoflurane MAC reduction was estimated by logistic regression at 5 and 15 minutes after fentanyl administration. In the second phase, seven chickens were anesthetized with isoflurane, and fentanyl was administered (30 μg kg^{-1}) IV over 1 minute during spontaneous ventilation and HR and rhythm, sAP and ventilation variables were measured.

Results At 5 minutes after IV administration of fentanyl (10 or 30 μg kg^{-1}), isoflurane MAC was significantly reduced by 17.6% (6.1–29.1%) [logistic regression estimate (95% Wald confidence interval)] and 42.6% (13.3–71.9%), respectively. Isoflurane MAC reduction at 15 minutes after IV administration of fentanyl (10 or 30 μg kg^{-1}) was 6.2% (−0.6 to 12.9%) and 13.2% (−0.9 to 27.3%), respectively; however, this reduction was not significant. No clinically significant cardiopulmonary changes or arrhythmias were detected after the administration of fentanyl (30 μg kg^{-1}).

Conclusions and clinical relevance Administration of a single fentanyl bolus induced a dose-dependent and short-lasting reduction in isoflurane MAC. The higher dose induced no significant cardiopulmonary depression in isoflurane-anesthetized chickens during spontaneous ventilation. In chickens anesthetized with isoflurane, the clinical usefulness of a single fentanyl bolus is limited by its short duration of effect.

Keywords balanced anesthesia, birds, fentanyl, inhalation anesthesia, opioids, ventilation.

Introduction

Isoflurane is the most commonly used inhalation anesthetic agent in birds owing to its low blood—gas partition coefficient, which allows both rapid induction of, and recovery from, anesthesia and also because its elimination is not dependent on metabolic or excretory pathways (Ludders 2015). Concomitant administration of injectable and inhalation anesthetics may reduce the dose of inhalation agent required, resulting in not only less cardiopulmonary depression but also possible blunting of the sympathetic response to stimulation during surgery (Gunkel & Lafortune 2005).

Morphine decreased the minimum anesthetic concentration (MAC) of isoflurane by 52% in chickens (Concannon et al. 1995), and butorphanol reduced the isoflurane MAC in cockatoos by 25% (Curro et al. 1994). However, neither study evaluated the temporal effect of the opioid administration on isoflurane MAC. The duration of the anesthetic-sparing effect of opioids during inhalation anesthesia in birds is reported to be short. In the guinea-fowl, intravenous (IV) administration of butorphanol (4 mg kg^{-1}) reduced the sevoflurane MAC at 15 and 30 minutes by 21% and 11%, respectively (Escobar et al. 2012); however, this dosage was considered unsafe (Escobar et al. 2014). Intramuscular (IM) administration of morphine (3 or 6 mg kg^{-1}) in chickens reduced the isoflurane MAC at 15 minutes by 15% and 22%, respectively (Vela et al. 2014). Methadone (6 mg kg^{-1}) IM decreased isoflurane MAC by 30% at 15 minutes after administration in chickens; however, the effect was gone by 30 minutes (Escobar et al. 2016).

Fentanyl is a μ-opioid agonist with a short onset and duration of effect when administered as a single IV bolus (Kukanich & Wiese 2015). The results of a study of target-controlled infusions of fentanyl on isoflurane MAC in red-tailed hawks identified a plasma concentration-dependent reduction in MAC of up to 55% (Pavez et al. 2011). That study maintained constant plasma fentanyl concentrations; thus, the temporal effects of a bolus of fentanyl on isoflurane MAC were not examined.

The purpose of this study was to evaluate the temporal effects of an IV fentanyl bolus on isoflurane MAC in chickens and to measure selected cardiopulmonary variables. We hypothesized that a single bolus of fentanyl would induce a dose-dependent reduction in isoflurane MAC for a minimum of 15 minutes in chickens. We also hypothesized that the combination of fentanyl and isoflurane would not result in a greater cardiopulmonary depression when compared with an equipotent isoflurane concentration.

Material and methods

This study was approved by the Institutional Animal Care and Use Committee of the School of Agricultural and Veterinarian Sciences, São Paulo State University (no. 008078/13).

First phase: effects of fentanyl administration on the isoflurane MAC

Animals

Seventeen ISA Brown laying chickens, aged 8—13 months and weighing 1.8 ± 0.2 kg [mean ± standard deviation (SD)], were used in the study. Birds were maintained in a stall (3 × 3 × 4 m) and poultry feed and water were available *ad libitum*. All animals were dewormed with piperazine citrate tetrahydrate (Proverme; Tortuga Cia Zootécnica Agrária, Brazil), and only birds considered healthy after physical examination, complete blood count, uric acid, and aspartate aminotransferase tests were included in the study. Animals were not fasted before anesthesia. Time allowed between successive experiments was ≥ 1 week.

Anesthesia and instrumentation

Chickens were physically restrained and anesthesia was induced with isoflurane in oxygen (Isoforine; Cristália Produtos Químicos e Farmacêuticos Ltda, Brazil) delivered from a precision vaporizer (Isoflurane Sigma Delta Vaporizer; Penlon Inc., MN, USA) through a face mask connected to a Bain circuit. The vaporizer setting was 5% and oxygen flow was 3 L $minute^{-1}$ for the induction of anesthesia. When muscle tone and palpebral reflex were lost, a 3.0 mm endotracheal tube was inserted into the trachea, the cuff was left deflated and isoflurane administration decreased to a vaporizer setting of 2% and oxygen 1 L $minute^{-1}$. Birds were positioned in dorsal recumbency and intermittent positive pressure ventilation (HB Conquest 2003; Hospitalar Indústria e Comércio Ltda, Brazil) was initiated, with a respiratory rate (f_R) of 8—12 cycles $minute^{-1}$, a maximum peak inspiratory pressure (PIP) of 15 cm H_2O, and an inspiration:expiration ratio of 1:3. f_R and PIP were adjusted to maintain an end-tidal partial pressure of carbon dioxide ($P_E'CO_2$) of 30—40 mmHg

(4.0−5.3 kPa). End-tidal gas samples were collected during 7−10 breaths using a glass syringe and a 3.5 Fr catheter (Tom Cat 3.5 Fr; Ortovet Produtos Veterinarios, Brazil) introduced within the lumen of the endotracheal tube, with the tip of the catheter close to the distal end of the endotracheal tube. $P_E'CO_2$ and end-tidal isoflurane concentration (F_E'Iso) were measured using an infrared spectrophotometer (Infra-red gas analyzer DX-AJAGA-1 (AGA); Dixtal, Brazil). The spectrophotometer was calibrated daily using room air and three standards of known isoflurane concentrations (0.5%, 1.5% and 3.0%) (Isoflurane, N_2, O_2; White Martins Gases Industriais SA, Brazil). Values measured by the spectrophotometer were corrected by means of linear regression based on the calibration values (Rudolff et al. 2014).

The ulnar vein was catheterized with a 24 gauge, 19 mm catheter (BD Angiocath; BD, Brazil) for the infusion of fentanyl (Fentanest; Cristália Produtos Químicos e Farmacêuticos Ltda) and NaCl 0.9% (5 mL kg^{-1} $hour^{-1}$) using a syringe infusion pump (Medfusion 2010i; Medex Inc., GA, USA). A pulse oximeter sensor (Dixtal 2010; Dixtal Biomédica Indústria e Comércio Ltda, Brazil) was positioned over one of the digits of the bird to determine the pulse rate (PR). Noninvasive systolic arterial pressure (SAP) was measured by a Doppler ultrasound probe (model 812; Parks Medical Electronics Inc., Brazil) placed over the median metatarsal artery and a cuff around the thigh with a width of 40−50% of the circumference. A mercury thermometer was used to measure the cloacal temperature (T) (Veterinary Thermometer; Incoterm, Brazil). Body temperature was maintained at 40−41 °C with a heat lamp, hot water blanket (T/Pump; Gaymar Industries Inc., NY, USA) and procedure gloves filled with hot water. These variables were measured immediately before each electrical stimulus.

Determination of isoflurane MAC

The individual isoflurane MAC of 13 chickens had been previously determined in triplicate using the bracketing method and electrical stimulation (Escobar et al. 2016). The MAC of isoflurane was determined in four additional chickens.

In these four animals, F_E'Iso was adjusted to 1.2% and kept constant for 15 minutes before stimulation. Two needles were inserted into the subcutaneous tissue of the medial aspect of the thigh and connected to an electrical stimulator (SD9 square pulse stimulator; Astro-Med Inc., RI, USA). An electrical stimulus (15 V, 50 Hz, 6.5 ms) was applied for 1 minute or until a positive response was observed, whichever occurred first (Laster et al. 1993). A positive response was defined as movement, such as beating of wings, or movement of the contralateral leg, head or tail. Subsequently, F_E'Iso increased or decreased by 10%, after a positive or negative response was observed, respectively. The stimulation was repeated 15 minutes after F_E'Iso was changed. The MAC was defined as the average of two consecutive values of F_E'Iso that allowed and prevented movement and was determined in triplicate. Because the study was conducted at an altitude of 605 m, each bird's isoflurane MAC was corrected to atmospheric pressure at sea level using the following equation: MAC (%) at sea level = Individual MAC (%) × (716/760).

For these four birds, the effect of fentanyl on isoflurane MAC was assessed immediately after isoflurane MAC determination.

Fentanyl effect on the isoflurane MAC

The effect of a single fentanyl bolus on isoflurane MAC was assessed using the up-and-down method (Dixon 1965). This method provides an estimate of isoflurane MAC reduction at predetermined time points in a population of animals. The response (movement or no movement) of each animal at a single isoflurane concentration was assessed at different times after fentanyl administration and analyzed by logistic regression to estimate the isoflurane MAC (50% probability of movement).

F_E'Iso was set at 0.8 individual MAC (20% decrease in MAC) for the first bird, and 15 minutes were allowed for equilibration. Expired gas samples were collected in triplicate for F_E'Iso determination, and the mean value was recorded. After recording PR, SAP and T, fentanyl (10 μg kg^{-1}) was administered over 1 minute into the ulnar vein by syringe infusion pump (Medfusion 2010i; Medex Inc.). After 4 minutes, PR, $P_E'CO_2$, SAP and T were recorded. At 5 minutes after fentanyl administration, an electrical stimulus was applied as previously described. If the animal did not move after the first stimulus, it was assumed that the anesthetic effects of isoflurane-fentanyl were >1.0 MAC. The electrical stimulus was repeated at 15 minutes after administration of fentanyl and then every 15 minutes until a positive response was observed, at which time it was assumed that the effects of isoflurane-fentanyl were <1.0 MAC. Anesthesia was discontinued when movement was observed, and the bird was allowed to recover from

anesthesia. For statistical analysis, it was assumed that the bird would move at subsequent time points. Subsequently, F_E'Iso for each bird was administered according to the response of the previous bird. Thus, if the previous bird moved in response to the electrical stimulus at 5 minutes after fentanyl administration, F_E'Iso in the next bird was increased by 0.1 × individual MAC (i.e. to 0.9 × MAC). Conversely, if the previous bird did not move in response to the electrical stimulus, F_E'Iso was decreased by 0.1 × individual MAC (i.e. to 0.7 × MAC). The study was continued until at least four independent crossovers were observed at 5 minutes after fentanyl administration and at least three crossovers were observed at 15 minutes after fentanyl administration. Birds in which F_E'Iso was 1.0 × isoflurane MAC were only tested 5 minutes after fentanyl administration. Because the probability of movement at 1.0 × MAC is 50%, i.e., if the stimulation is repeated, the response is likely to change; for statistical analysis, it was assumed that the same response would be observed at the subsequent times.

After a washout period of 1 week, chickens were anesthetized a second time using the same methods to assess the effects of fentanyl (30 μg kg^{-1}) administered IV on isoflurane MAC.

Data collected

Additional data were collected during the study: anesthesia induction time (time from start of administration of isoflurane to tracheal intubation), instrumentation time (time from tracheal intubation to complete placement of the monitoring equipment), study time (time from the injection of fentanyl to the end of anesthetic administration), time to extubation (time from the end of anesthetic administration to endotracheal tube removal) and time to stand (time from the end of anesthetic administration to a standing position).

Statistical analyses

The physiological variables were tested for normality using the Shapiro–Wilk test. Normally distributed data were evaluated using a one-way analysis of variance (ANOVA), followed by Tukey's test. Time to extubation and time to stand for animals administered fentanyl (10 or 30 μg kg^{-1}) were compared using the unpaired *t* test or the Mann–Whitney test if the data were normally distributed or not, respectively. Tests were considered significant when $p < 0.05$.

To calculate the isoflurane MAC reduction after administration of fentanyl, isoflurane MAC reduction-response data at each time point were analyzed by logistic regression. The isoflurane MAC reduction at the time point considered was defined as the MAC reduction resulting in a 50% probability of movement. The 95% Wald confidence interval was calculated and the reduction of MAC was considered statistically significant if the lower limit of the 95% confidence interval was positive. MAC reduction data are reported as estimate (95% Wald confidence interval). Statistical analysis was performed using the SigmaPlot, Version 11.0 (Systat Software, CA, USA) and JMP Pro Version 12.2 (SAS Institute, NC, USA).

Second phase: cardiopulmonary response after fentanyl administration

Animals

Seven chickens used in the first phase were randomly selected. Birds were aged 11 months and weighed 1.8 ± 0.2 kg.

Anesthesia and instrumentation

Anesthesia was induced, as described for the first phase. Birds remained in dorsal recumbency, breathing spontaneously, and F_E'Iso was adjusted to 1.0 × MAC. f_R, $P_E'CO_2$ and F_E'Iso were measured (Infra-red gas analyzer DX-AJAGA-1 (AGA); Dixtal). Gas samples for $P_E'CO_2$ and F_E'Iso measurement were collected and the spectrophotometer was calibrated, as described in the first phase.

The ulnar vein was catheterized for fluid administration. A 24 gauge, 19 mm catheter (BD Angiocath; BD) was inserted in the ulnar artery for blood pressure measurement using a pressure transducer, which was positioned and zeroed at the level of the heart, and connected to a multiparameter monitor (Dixtal 2010; Dixtal Biomédica Indústria e Comércio Ltda). Arterial blood samples (0.5 mL) were collected using syringes containing sodium heparin (Hemofol; Cristália Produtos Químicos e Farmacêuticos Ltda) for blood gas and electrolyte measurement (Omni C; Roche Diagnostics, Brazil), and values were corrected for body temperature. Electrodes were placed on both wings and legs, and the lead II electrocardiogram (ECG) was recorded (ECGPC; Tecnologia Electrônica Brasileira Ltda, Brazil) during the study. Temperature was measured and controlled as described in the first phase.

Evaluation of physiologic variables

At the end of instrumentation, heart rate (HR), f_R, $P_E'CO_2$, invasive SAP, diastolic arterial pressure (DAP) and mean (MAP) arterial pressure were measured when birds were anesthetized with 1.0 × individual isoflurane MAC (time point T1.0MAC). Arterial pH, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), bicarbonate concentration (HCO_3^-), base excess (BE) and concentrations of sodium, chloride and ionized calcium were measured. The F_E'Iso was decreased to 0.8 × individual MAC and measurements were made 15 minutes later (time point T0.8MAC). Subsequently, fentanyl (30 μg kg^{-1}) was administered IV over 1 minute using a syringe infusion pump, and cardiopulmonary variables were measured at 1, 5, 10, 15 and 30 minutes. pH, PaO_2, $PaCO_2$ and electrolyte concentrations were measured at T1.0MAC, T0.8MAC, and at T5, T15, and T30 minutes after administration of fentanyl.

Statistical analyses

The physiological variables were tested for normality using the Shapiro–Wilk test. Data at different times after fentanyl administration were compared with T1.0MAC. Normally distributed data were analyzed by the one-way repeated measures ANOVA followed by Dunnett's test and were reported as mean ± SD. Tests were considered significant when $p < 0.05$. Statistical analyses were performed with the assistance of SigmaPlot Version 11.0 (Systat Software).

Results

First phase

Individual isoflurane MAC was determined in three of the four birds in which it had not previously been determined. Mean ± SD isoflurane MAC for the three birds was 1.1 ± 0.1%. The fourth bird simultaneously extubated itself and regurgitated and aspirated gastric content during MAC determination, resulting in death. No adverse effects were noted during and after fentanyl administration. At 5 and 15 minutes after fentanyl (10 μg kg^{-1}) administration, isoflurane MAC was reduced by 17.6% (6.1–29.1%) and 6.2% (−0.6 to 12.9%), respectively (Figs 1a & b). Twelve birds were used to study this dose, and six moved at 5 minutes after fentanyl administration. Two birds were anesthetized at 1.0 MAC, and the study

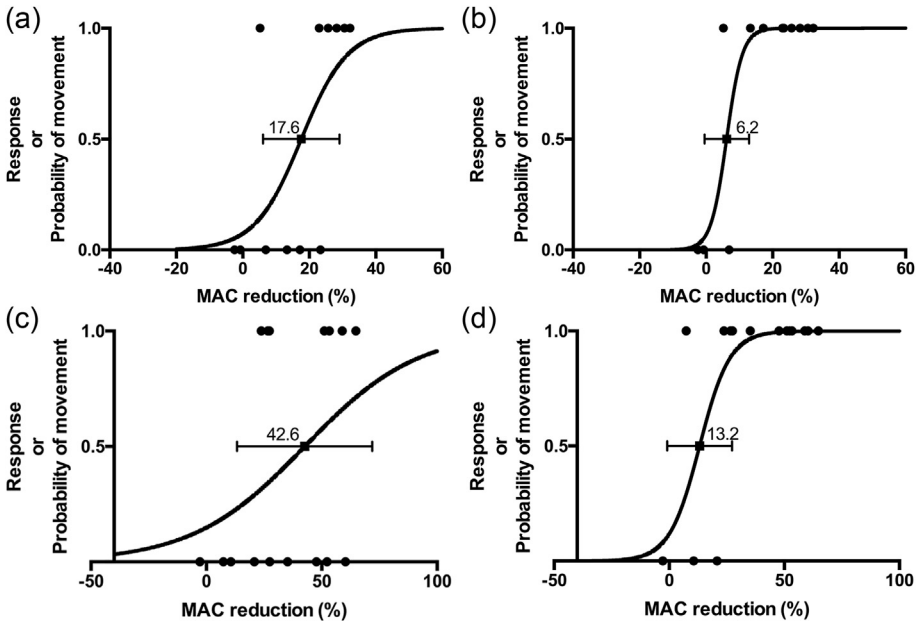

Figure 1 MAC reduction obtained by logistic regression (line) of observed response (closed circles) after intravenous (IV) administration of 10 μg kg^{-1} or 30 μg kg^{-1} of fentanyl in isoflurane-anesthetized chickens with different end-tidal isoflurane concentrations, computed as MAC reduction. Data at (a) 5 and (b) 15 minutes after IV fentanyl administration of 10 μg kg^{-1}. Data at (c) 5 and (d) 15 minutes after IV fentanyl administration of 30 μg kg^{-1}. The error bar represents the 95% Wald confidence interval, and the MAC reduction at the respective time points is considered to be the MAC reduction resulting in a 50% probability of movement. Note that some data points are superimposed.

terminated for them at 5 minutes after the injection of fentanyl. At 15 minutes after fentanyl administration, three of the four remaining birds moved, and the remaining bird moved only at 30 minutes after fentanyl administration.

Fentanyl (30 μg kg^{-1}) reduced isoflurane MAC by 42.6% (13.3−71.9%) and 13.2% (−0.9 to 27.3%) at 5 and 15 minutes after administration, respectively (Figs 1c & d). Of the 16 birds, 7 moved 5 minutes after fentanyl injection. One bird was anesthetized with 1.0 isoflurane MAC, and the study terminated at 5 minutes after injection. Six birds moved at 15 minutes and two birds at 30 minutes after fentanyl administration.

Most physiological data after the administration of the two fentanyl doses were normally distributed (Table 1). The recorded variables in chickens subjected to the two fentanyl dose rate experiments were not significantly different between doses.

Times are expressed as median (range). Induction time for all anesthetic episodes was 2 (1−13) minutes and instrumentation time was 10 (7−14) minutes. The study times for fentanyl doses 10 or 30 μg kg^{-1} were 16 (6−36) and 15 (6−32) minutes, respectively ($p = 0.468$). Times to extubation were 4 (1−14) and 5 (1−18) minutes for birds administered fentanyl at 10 or 30 μg kg^{-1}, respectively ($p = 0.291$). Times to standing were 15 (7−47) and 11 (3−24) minutes after administration of fentanyl at 10 or 30 μg kg^{-1}, respectively, significantly longer for birds administered the lower dose ($p = 0.030$).

Second phase

Instrumentation time was 43 ± 19 minutes. All cardiopulmonary values, blood gases and electrolyte concentrations were considered normally distributed. $P_{E'}CO_2$ was increased at T0.8MAC, T15 and T30 compared with that at T1.0MAC ($p = 0.008$) (Table 2). $PaCO_2$ values were significantly higher at T15 and T30 ($p = 0.029$) than at T1.0MAC. HR, f_R, SAP, DAP, MAP, T, arterial pH, PaO_2, HCO_3^-, BE and electrolytes after fentanyl administration did not differ from T1.0MAC (Table 2). No arrhythmias were observed before or after fentanyl administration.

Discussion

The present study reports that the administration of a fentanyl bolus dose-dependently reduces the isoflurane MAC in chickens for <15 minutes. The higher dose tested reduced isoflurane MAC by 43%, which was similar to another study involving red-tailed hawks, in which isoflurane MAC reduction ranged from 31% to 55% after fentanyl target-controlled infusions (Pavez et al. 2011). However, neither dose of fentanyl studied caused significant reduction in isoflurane MAC at 15 minutes after administration.

The bracketing method was used to determine the MAC of isoflurane in each bird, while the up-and-down method was used to evaluate the temporal effects of fentanyl on isoflurane MAC. Quantal analysis was used to evaluate the probability of movement of the population of chickens at specific time points, presuming that fentanyl concentration was similar among the birds. The bracketing method does not allow the determination of MAC at specific time points because the time to perform the study is generally long and unpredictable.

One study in red-tailed hawks evaluated the effect of three plasma fentanyl concentrations on isoflurane MAC using target-controlled infusions to maintain plasma fentanyl concentration constant during the study. The highest reduction (43%) reported in the present study was obtained 5 minutes after the administration of 30 μg kg^{-1} of fentanyl as a slow IV

Table 1 Mean ± standard deviation for pulse rate (PR), noninvasive systolic arterial pressure (SAP), end-tidal partial pressure of carbon dioxide ($P_{E'}CO_2$) and cloacal temperature (T) at 5 and 15 minutes after intravenous administration of fentanyl (10 or 30 μg kg^{-1}) in chickens anesthetized with different end-tidal isoflurane concentrations

Variable	Fentanyl 10 μg kg^{-1}		Fentanyl 30 μg kg^{-1}	
	5 minutes ($n = 12$)	15 minutes ($n = 4$)	5 minutes ($n = 16$)	15 minutes ($n = 8$)
PR (beats $minute^{-1}$)	217 ± 75	218 ± 74	212 ± 50	233 ± 42
SAP (mmHg)	90 ± 16	80 ± 14	105 ± 25	101 ± 22
$P_{E'}CO_2$ (mmHg)	33 ± 6	30 ± 9	35 ± 5	38 ± 7
$P_{E'}CO_2$ (kPa)	4.4 ± 0.8	4.0 ± 1.2	4.7 ± 0.7	5.1 ± 0.9
T (°C)	40.6 ± 0.4	40.6 ± 0.1	40.5 ± 0.3	40.5 ± 0.2

n, number of chickens.

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Table 2 Mean values ± standard deviation for heart rate (HR), respiratory rate (f_R), end-tidal partial pressure of carbon dioxide ($P_{E'}CO_2$), systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressures, cloacal temperature (T), blood gas variables and arterial electrolyte concentrations in seven spontaneously breathing chickens anesthetized with isoflurane at 1.0 MAC (T1.0MAC) and 0.8 MAC before (T0.8MAC) and for 30 minutes after intravenous administration of fentanyl (30 μg kg^{-1})

Variables	Time points						
	T1.0MAC	T0.8MAC	T1	T5	T10	T15	T30
HR (beats $minute^{-1}$)	200 ± 32	210 ± 28	219 ± 25	211 ± 28	212 ± 29	210 ± 29	205 ± 26
f_R (breaths $minute^{-1}$)	16 ± 11	14 ± 3	14 ± 4	14 ± 3	14 ± 3	14 ± 3	14 ± 3
$P_{E'}CO_2$ (mmHg)	44 ± 4	50 ± 6*	46 ± 5	46 ± 6	45 ± 6	50 ± 7*	50 ± 6*
$P_{E'}CO_2$ (kPa)	5.9 ± 0.5	6.7 ± 0.8*	6.1 ± 0.7	6.1 ± 0.8	6.0 ± 0.8	6.7 ± 0.9*	6.7 ± 0.8*
SAP (mmHg)	99 ± 9	107 ± 6	106 ± 5	105 ± 6	105 ± 9	104 ± 6	105 ± 5
DAP (mmHg)	90 ± 12	96 ± 7	97 ± 4	96 ± 6	96 ± 8	95 ± 6	94 ± 4
MAP (mmHg)	96 ± 10	102 ± 6	103 ± 5	101 ± 6	102 ± 9	101 ± 6	101 ± 4
T (°C)	40.5 ± 0.2	40.6 ± 0.3	40.5 ± 0.3	40.5 ± 0.3	40.5 ± 0.3	40.4 ± 0.3	40.4 ± 0.3
PaO_2 (mmHg)	365 ± 50	350 ± 76		340 ± 52		332 ± 38	344 ± 42
PaO_2 (kPa)	48.7 ± 6.7	46.7 ± 10.1		45.3 ± 6.9		44.3 ± 5.1	45.9 ± 5.6
$PaCO_2$ (mmHg)	36 ± 6	38 ± 4		38 ± 6		39 ± 5*	39 ± 4*
$PaCO_2$ (kPa)	4.8 ± 0.8	5.1 ± 0.5		5.1 ± 0.8		5.2 ± 0.7*	5.2 ± 0.5*
pH	7.46 ± 0.04	7.43 ± 0.07		7.45 ± 0.06		7.44 ± 0.06	7.44 ± 0.05
BE (mmol L^{-1})	0.8 ± 3.9	1.9 ± 4.1		1.6 ± 2.6		2.2 ± 2.8	2.6 ± 3.6
HCO_3^- (mmol L^{-1})	24 ± 3	25 ± 4		25 ± 2		26 ± 2	26 ± 3
Na (mmol L^-)	152 ± 4	153 ± 2		154 ± 3		154 ± 3	153 ± 3
Cl (mmol L^{-1})	119 ± 7	118 ± 9		122 ± 14		117 ± 8	115 ± 2
iCa (mmol L^{-1})	1.12 ± 0.20	1.20 ± 0.20		1.12 ± 0.20		1.17 ± 0.20	1.13 ± 0.20

BE, base excess; HCO_3^-, bicarbonate; Na, sodium; Cl, chloride; iCa, ionized calcium.
*Significantly different from values at T1.0MAC ($p < 0.05$).

bolus and was similar to the highest reduction on isoflurane MAC in red-tailed hawks (55%) when the mean plasma fentanyl concentration was 29 ng mL^{-1} (Pavez et al. 2011). The experimental design of the present study allowed the measurement of the fentanyl effect offset after a bolus administration and is more representative of clinical practice than the use of target-controlled infusions. However, the rapid offset of fentanyl immobilizing effect in chickens suggests that its usefulness when administered as an IV bolus is limited and that an IV infusion would be necessary to utilize its potential benefits.

Studies addressing the effect of a single bolus of fentanyl have only been conducted in humans. An IV fentanyl bolus of 1.5 or 3 μg kg^{-1} administered 5 minutes before surgical incision significantly decreased the isoflurane MAC for the blockade of autonomic responses (MAC-BAR) by 60% and 70%, respectively, in adult human patients (Daniel et al. 1998). A similar study in children reported that a fentanyl IV bolus at 2 or 4 μg kg^{-1} administered 5 minutes before surgical incision decreased the sevoflurane MAC-BAR by 56% and 74%, respectively (Katoh et al. 2000). In neonates, administration of a single dose of fentanyl at 1 or 2 μg kg^{-1} over 1 minute reduced the sevoflurane MAC by 13% and 20%, respectively (She et al. 2014). However, the duration of effect was not determined in these studies. In any case, fentanyl appears more potent for its effect on MAC in adult and young humans than in chickens.

There is one study addressing the pharmacokinetics of fentanyl in birds. The maximum plasma drug concentration and terminal half-life were 2.23 and 3.33 ng mL^{-1} and 1.2 and 1.4 hours after IM fentanyl administration of 10 or 20 μg kg^{-1}, respectively, in white cockatoos (Hoppes et al. 2003). The pharmacokinetic profile of a drug can vary considerably between species and even between individuals within the species; therefore, extrapolation of data from cockatoos to chickens should be conducted with caution. In addition, concentrations reported after IM administration are not representative of concentrations reached after IV administration because IM administration results in prolonged absorption and likely incomplete bioavailability.

The offset of the fentanyl MAC-reducing effect was observed to be short in this study and may be caused

by rapid distribution and/or metabolism. The volume of distribution at steady state (V_{ss}) has been reported to be large in dogs (6.16 L kg^{-1}) and cats (2.18 L kg^{-1}) (Kukanich & Allen 2014; Pypendop et al. 2014), as would be expected from fentanyl's high lipid solubility, and suggests extensive distribution out of the central compartment. Although V_{ss} of fentanyl has not been reported in birds, it is expected to be large.

There were no differences in PR, $P_E'CO_2$, noninvasive SAP and cloacal temperature in the first phase of the study. However, this assessment was limited because data were not compared to records when birds were anesthetized with isoflurane only, and ECG and invasive blood pressure were not monitored. In addition, controlled ventilation was used, which did not allow the evaluation of the effects of fentanyl on ventilation.

Time to extubation was approximately 4 minutes from the end of anesthetic administration. However, one bird (anesthetized with 0.8 × individual MAC) was extubated 18 minutes after the discontinuation of isoflurane. This large variability may be the result of anesthetizing birds with different fractions of their individual MAC. Time to standing was longer after the administration of 10 μg kg^{-1}, presumably because birds tested at this dose were anesthetized with a higher $F_E'Iso$ than those tested at the dose of 30 μg kg^{-1}. Thus, fentanyl did not appear to influence extubation and standing times, contrary to methadone in chickens anesthetized with isoflurane, which induced prolonged recovery times (Escobar et al. 2016).

In the second phase of the study, there were no significant changes in cardiopulmonary variables before and after administration of fentanyl (30 μg kg^{-1}) IV over 1 minute. Other studies in birds have reported the cardiopulmonary effects of equipotent concentrations of inhalation anesthetics combined or not with opioids (Escobar et al. 2014, 2016). A combination of sevoflurane with butorphanol resulted in cardiopulmonary arrest in two guineafowl (Escobar et al. 2014). Administration of isoflurane combined with methadone to chickens decreased the HR and increased sAP when compared with anesthesia using an equipotent concentration of isoflurane (Escobar et al. 2016). However, equipotent concentrations of isoflurane with and without fentanyl were not maintained in this study as the MAC reduction at 5 minutes after fentanyl (>40%) was much larger than the MAC reduction used in the second phase (20%). As a consequence of the rapidly waning effect of fentanyl, chickens were anesthetized at <1.0 MAC toward the end of the monitoring period.

$P_E'CO_2$ in chickens anesthetized with isoflurane 0.8MAC (50 ± 6 mmHg; 6.7 ± 0.8 kPa) was greater than that in chickens anesthetized with 1.0MAC (44 ± 4 mmHg; 5.9 ± 0.5 kPa). It is unclear why the $P_E'CO_2$ values increased at T0.8MAC when animals were more lightly anesthetized. One possible explanation is the contamination of end-tidal gases with inspired gases; however, an increase in respiratory amplitude at T0.8MAC could have induced a greater elimination of CO_2 during expiration. The crosscurrent gas exchange system observed in birds describes the relationship between the gas and blood flows into the lungs, and is the reason why $P_E'CO_2$ may exceed $PaCO_2$ as observed in this study (Davies & Dutton 1975). Fentanyl administration (30 μg kg^{-1}) during 0.8MAC resulted in $PaCO_2$ at 15 and 30 minutes higher than that during anesthesia with 1.0MAC. Although the $PaCO_2$ values after fentanyl administration were within the normal range for birds (24–40 mmHg; 3.2–5.3 kPa) (Powell 2000), inclusion of birds anesthetized only with isoflurane as a control group may have provided information about a change in the ventilatory pattern. In addition, administration of fentanyl over 1 minute in this study may have minimized subsequent cardiopulmonary changes in comparison with a rapid IV bolus administration in another study that induced cardiac arrest (Escobar et al. 2014).

In conclusion, fentanyl IV administration resulted in a dose-dependent reduction in isoflurane MAC in chickens, and this effect was detected only at 5 minutes after administration. The clinical usefulness of a single fentanyl bolus in chickens is limited since the duration of effect appears short. No significant cardiopulmonary depression was identified after fentanyl (30 μg kg^{-1}) administration. Further studies should be conducted to evaluate the effects of IV fentanyl infusions on isoflurane MAC in chickens.

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Authors' contributions

RWdaR: acquisition of birds, protocol approval, data acquisition, statistical analysis, preparation of

manuscript. AE: study design, data acquisition, statistical analysis, preparation of manuscript. BHP: statistical analysis. DZF, RT: data acquisition. FNG: electrocardiographic evaluations. All authors contributed to critical revisions of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

References

Concannon KT, Dodam JR, Hellyer PW (1995) Influence of a mu- and kappa-opioid agonist on isoflurane minimal anesthetic concentration in chickens. Am J Vet Res 56, 806–811.

Curro TG, Brunson DB, Paul-Murphy JR (1994) Determination of the ED50 of isoflurane and evaluation of the isoflurane-sparing effect of butorphanol in cockatoos (*Cacatua spp.*). Vet Surg 23, 429–433.

Daniel M, Weiskopf RB, Noorani M, Eger EI 2nd (1998) Fentanyl augments the blockade of the sympathetic response to incision (MAC-BAR) produced by desflurane and isoflurane: desflurane and isoflurane MAC-BAR without and with fentanyl. Anesthesiology 88, 43–49.

Davies DG, Dutton RE (1975) Gas-blood PCO_2 gradients during avian gas exchange. J Appl Physiol 39, 405–410.

Dixon WJ (1965) The up-and-down method for small samples. J Am Stat Assoc 60, 967–978.

Escobar A, Rocha RW, Pypendop BH et al. (2016) Effects of methadone on the minimum anesthetic concentration of isoflurane, and its effects on heart rate, blood pressure and ventilation during isoflurane anesthesia in chickens (*Gallus gallus domesticus*). PLoS One 11, e0152546.

Escobar A, Valadão CAA, Brosnan RJ et al. (2012) Effects of butorphanol on the minimum anesthetic concentration for sevoflurane in guineafowl (*Numida meleagris*). Am J Vet Res 73, 183–188.

Escobar A, Valadão CAA, Brosnan RJ et al. (2014) Cardiopulmonary effects of butorphanol in sevoflurane-anesthetized guineafowl (*Numida meleagris*). Vet Anaesth Analg 41, 284–289.

Gunkel C, Lafortune M (2005) Current techniques in avian anesthesia. Seminars Avian Exot Pet Med 14, 263–276.

Hoppes S, Flammer K, Hoersch K et al. (2003) Disposition and analgesic effects of fentanyl in white cockatoos (*Cacatua alba*). J Avian Med Surg 17, 124–130.

Katoh T, Kobayashi S, Suzuki A et al. (2000) Fentanyl augments block of sympathetic responses to skin incision during sevoflurane anaesthesia in children. Br J Anaesth 84, 63–66.

Kukanich B, Allen P (2014) Comparative pharmacokinetics of intravenous fentanyl and buprenorphine in healthy greyhound dogs. J Vet Pharmacol Ther 37, 595–597.

Kukanich B, Wiese AJ (2015) Opioids. In: Lumb and Jones' Veterinary Anesthesia and Analgesia (5th edn). Grimm KA, Lamont LA, Tranquilli WJ et al. (eds). Wiley Blackwell, USA. pp. 207–226.

Laster MJ, Liu J, Eger EI 2nd, Taheri S (1993) Electrical stimulation as a substitute for the tail clamp in the determination of minimum alveolar concentration. Anesth Analg 76, 1310–1312.

Ludders JW (2015) Comparative anesthesia and analgesia of birds. In: Lumb and Jones' Veterinary Anesthesia and Analgesia (5th edn). Grimm KA, Lamont LA, Tranquilli WJ et al. (eds). Wiley Blackwell, USA. pp. 800–816.

Pavez JC, Hawkins MG, Pascoe PJ et al. (2011) Effect of fentanyl target-controlled infusions on isoflurane minimum anaesthetic concentration and cardiovascular function in red-tailed hawks (*Buteo jamaicensis*). Vet Anaesth Analg 38, 344–351.

Powell FL (2000) Respiration. In: Sturkie's Avian Physiology (5th edn). Whittow CG (ed.). Academic Press Inc, USA. pp. 233–264.

Pypendop BH, Brosnan RJ, Majewski-Tiedeken CR et al. (2014) Pharmacokinetics of fentanyl, alfentanil, and sufentanil in isoflurane-anesthetized cats. J Vet Pharmacol Ther 37, 13–17.

Rudolff AS, Moens YP, Driessen B et al. (2014) Comparison of an infrared anaesthetic agent analyser (Datex-Ohmeda) with refractometry for measurement of isoflurane, sevoflurane and desflurane concentrations. Vet Anaesth Analg 41, 386–392.

She YJ, Wang HZ, Huang JX et al. (2014) Effect of a bolus dose of fentanyl on the ED_{50} and ED_{95} of sevoflurane in neonates. Med Sci Monit 14, 2658–2665.

Vela DB, Rocha RW, Sousa SS et al. (2014) Effects of morphine on the minimum anesthetic concentration of isoflurane in chickens (*Gallus gallus domesticus*). In: XI Congresso Brasileiro de Anestesiologia Veterinária, 30. Ars Veterinária. p. 89. http://dx.doi.org/10.15361/2175-0106.2014v30n3p1-108.

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