Cardiovascular effects of dobutamine, norepinephrine and þÿ p h e n y l e p h r i n e i n i s o f l u r a n e - a n a e s t h e t i z e d d o g s

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RESEARCH PAPER

Cardiovascular effects of dobutamine, norepinephrine and phenylephrine in isoflurane-anaesthetized dogs administered dexmedetomidine–vatinoxan

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Abstract

Objective To determine whether dobutamine, norepinephrine or phenylephrine infusions alleviate hypotension in isoflurane-anaesthetized dogs administered dexmedetomidine with vatinoxan.

Study design Balanced, randomized crossover trial.

Animals A total of eight healthy Beagle dogs.

Methods Each dog was anaesthetized with isoflurane (end-tidal isoflurane 1.3%) and five treatments: dexmedetomidine hydrochloride (2.5 μg kg^{-1}) bolus followed by 0.9% saline infusion (DEX-S); dexmedetomidine and vatinoxan hydrochloride (100 μg kg^{-1}) bolus followed by an infusion of 0.9% saline (DEX-VAT-S), dobutamine (DEX-VAT-D), norepinephrine (DEX-VAT-N) or phenylephrine (DEX-VAT-P). The dexmedetomidine and vatinoxan boluses were administered at baseline (T0) and the treatment infusion was started after 15 minutes (T15) if mean arterial pressure (MAP) was < 90 mmHg. The treatment infusion rate was adjusted every 5 minutes as required. Systemic haemodynamics were recorded at T0 and 10 (T10) and 45 (T45) minutes. A repeated measures analysis of covariance model was used.

Results Most dogs had a MAP < 70 mmHg at T0 before treatment. Treatments DEX-S and DEX-VAT all significantly increased MAP at T10, but systemic vascular resistance index (SVRI) was significantly higher and cardiac index (CI) lower after DEX-S than after DEX-VAT. CI did not significantly differ between DEX-S and DEX-VAT-S at T45, while SVRI remained higher with DEX-S. Normotension was achieved by all vasoactive infusions in every dog, whereas MAP was below baseline with DEX-VAT-S, and higher than baseline with DEX-S at T45. Median infusion rates were 3.75, 0.25 and 0.5 μg kg^{-1} $minute^{-1}$ for dobutamine, norepinephrine and phenylephrine, respectively. Dobutamine and norepinephrine increased CI (mean ± standard deviation, 3.35 ± 0.70 and 3.97 ± 1.24 L $minute^{-1}$ m^{-2}, respectively) and decreased SVRI, whereas phenylephrine had the opposite effect (CI 2.13 ± 0.45 L $minute^{-1}$ m^{-2}).

Conclusions and clinical relevance Hypotension in isoflurane-anaesthetized dogs administered dexmedetomidine and vatinoxan can be treated with either dobutamine or norepinephrine.

Keywords dexmedetomidine, dobutamine, hypotension, norepinephrine, phenylephrine, vatinoxan.

Introduction

Medetomidine, or more appropriately the active ingredient dexmedetomidine, produces central nervous system depression by stimulating central α_2-adrenoceptors and reducing norepinephrine release (Vainio & Palmu 1989). However, it also has significant cardiovascular effects. Dexmedetomidine initially induces a considerable increase in systemic vascular resistance (SVR), mediated by postjunctional peripheral α_2-adrenoceptors located on vascular smooth muscle (Link et al. 1996). This increases arterial blood pressure which in turn leads to bradyarrhythmias commonly associated with α_2-agonist administration (Savola 1989). The increase in SVR and decrease in heart rate (HR) result in decreased cardiac output (CO) (Pypendop & Verstegen 1998), which may compromise old or sick animals. As these adverse cardiovascular effects occur at a dose < 1 μg kg^{-1} and magnitude and duration of sedation are dose-dependent, minimizing the dexmedetomidine dose to avoid associated negative cardiovascular

effects is seldom practical in clinical veterinary patients (Pypendop & Verstegen 1998).

Vatinoxan is an α_2-adrenoceptor antagonist that selectively blocks the canine α_2-adrenoceptors in peripheral tissues with minimal central nervous system penetration (Clineschmidt et al. 1988; Honkavaara et al. 2020). In dogs, it can be added to medetomidine or dexmedetomidine to prevent the α_2-agonist—induced, peripherally mediated increase in SVR and decrease in CO without direct effects on sedation. This widens the therapeutic margin of α_2-agonists and allows a clinically relevant dose of an agonist to be administered with reduced cardiovascular adverse effects (Pagel et al. 1998; Honkavaara et al. 2008, 2011). However, vatinoxan decreases blood pressure (Honkavaara et al. 2011), which can progress to hypotension during general anaesthesia when combined with the negative inotropic and vasodilatory effects of inhalant anaesthetic agents, such as isoflurane (Kaartinen et al. 2014; Salla et al. 2014, 2017). Whereas hypotension associated with inhalant anaesthetics can be treated with pharmacological interventions, such as vasopressors and/or positive inotropes, no data exists about the effect of vatinoxan on their efficacy in supporting cardiovascular function and blood pressure in dogs.

The aim of this study was to determine if and how dobutamine, norepinephrine and phenylephrine infusions would increase blood pressure in isoflurane-anaesthetized healthy dogs administered dexmedetomidine—vatinoxan, and the infusion rates required. Anaesthesia without vatinoxan or inotrope/vasopressor infusion was included in the study for comparison. The hypotheses of the study were that: 1) the vasoactive infusions would restore normotension in anaesthetized dogs administered dexmedetomidine—vatinoxan, and 2) the addition of vatinoxan and an inotrope/vasopressor infusion would result in less negative effects on both CO and SVR than use of dexmedetomidine alone.

Materials and methods

Animals

The study protocol was approved by the Finnish National Animal Ethics Committee, license number ESAVI/7187/04.10.03/2012. A group of eight healthy, neutered, purpose-bred laboratory Beagle dogs, two female and six male, were studied. The dogs were housed together with access to outdoors and were fed commercial dog food twice daily with fresh water freely available. They weighed (mean ± standard deviation; SD) 14.7 ± 1.3 kg and were aged approximately 2.5 years at the time of the study. The dogs were considered healthy based on clinical examination, complete blood count and routine serum biochemistry results. Food, but not water, was withheld for a minimum of 8 hours prior to drug administration. The dogs were familiar with the study environment and the personnel performing the experiments. All dogs have since been adopted.

Study design

This was an exploratory study with a balanced, randomized, assessor-blinded, five-way crossover trial design. The randomization was performed on a 5 × 8 matrix which was filled so that it satisfied the following constraints: each column must contain all five treatments, each row must contain each treatment at least once, and no treatment could be duplicated more than once per row. After the matrix was constructed, the rows were allocated to a treatment period and the columns were randomly allocated to a dog by writing the dog identification on separate pieces of paper, placing these pieces of paper into an envelope, and withdrawing them blindly from the envelope.

Each dog was assigned five treatments during isoflurane anaesthesia, with all drugs administered intravenously (IV) and at least 2 weeks between treatments: dexmedetomidine hydrochloride (Dexdomitor, 0.5 mg mL^{-1}; Orion Pharma Ltd, Finland), 2.5 μg kg^{-1} bolus followed 15 minutes later by 0.9% saline infusion (treatment DEX-S); dexmedetomidine (2.5 μg kg^{-1}) and vatinoxan hydrochloride (Vatinoxan; Vetcare Ltd, Finland), 100 μg kg^{-1} bolus followed 15 minutes later by 0.9% saline infusion (treatment DEX-VAT-S); or dexmedetomidine (2.5 μg kg^{-1}) and vatinoxan (100 μg kg^{-1}) followed 15 minutes later by infusions of dobutamine (treatment DEX-VAT-D), norepinephrine (treatment DEX-VAT-N) or phenylephrine (treatment DEX-VAT-P). Dexmedetomidine and vatinoxan were mixed in the same syringe, and dexmedetomidine and dexmedetomidine—vatinoxan were diluted with 0.9% saline to a total volume of 10 mL and injected IV over 30 seconds at T0. On the morning of the experiment, dobutamine, norepinephrine and phenylephrine were diluted in 0.9% saline as follows: dobutamine (Dobuject, 12.5 mg mL^{-1}; Primex Pharmaceuticals Ltd, Finland) to a concentration of 750 μg mL^{-1}; norepinephrine (Noradrenalin Abcur, 1 mg mL^{-1}; Laboratoires Renaudin, France) to 75 μg mL^{-1}; and phenylephrine (Fenylefrin Unimedic, 0.1 mg mL^{-1}; Unimedic Pharma AB, Sweden) to 75 μg mL^{-1}. Once diluted, the infusions were kept in a dark cupboard until used.

Anaesthesia and instrumentation

At the start of each experimental session, a 20 gauge catheter (Terumo Europe NV, Belgium) was aseptically inserted into a cephalic vein, after which the dogs were preoxygenated with 100% oxygen and anaesthesia induced with IV propofol (6.1 ± 0.7 mg kg^{-1}; Vetofol, 1%; Norbrook Laboratories Ltd, UK). Anaesthesia was maintained with isoflurane (Isoflo; Orion Pharma Ltd) in oxygen administered with a circle rebreathing system (Fig. 1). Positive pressure ventilation (Hallowell EMC

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Model 2000 ventilator; Hallowell Engineering & Manufacturing Corp., MA, USA) was initiated to maintain end-tidal carbon dioxide partial pressure ($P_{E'}CO_2$) within 35–45 mmHg (4.6–6.0 kPa), and acetated Ringer's solution was administered at 5 mL kg^{-1} $hour^{-1}$. Body temperature was maintained at 36–38 °C using an electric heat pad and a blanket.

During instrumentation, the depth of anaesthesia, $P_{E'}CO_2$, end-tidal isoflurane concentration ($F_{E'}$Iso), HR and rhythm (electrocardiogram), respiratory rate (f_R), noninvasive oscillometric arterial blood pressure, haemoglobin oxygen saturation and oesophageal temperature were monitored. The infrared gas analyser of the multiparameter monitor (Datex S/5; GE Healthcare, Finland) was calibrated prior to each experiment with a standardized, single-point calibration gas (Quick-Cal 755583; GE Healthcare). A 22 gauge catheter (Terumo Europe NV) was placed in the coccygeal or metatarsal artery, a second cephalic catheter inserted for the administration of treatment infusions and a 7 Fr, 16 cm two-lumen central venous catheter (CV-12702; Arrow International Inc., PA, USA) inserted in either the left or right jugular vein through an introducer needle. The jugular catheter was advanced until a typical central venous pressure (CVP) waveform was observed on the monitor screen and used for blood sampling, lithium chloride (LiCl) injection and CVP measurements. The arterial catheter was used for measurements of invasive systolic (SAP), mean (MAP) and diastolic (DAP) blood pressures, CO and for collection of blood for blood gas analysis. The arterial catheter was connected by low compliance tubing to a pressure transducer (Gabarith PMSET; Becton Dickinson, UT, USA), and the transducer was connected to a multiparameter monitor, zeroed to atmospheric pressure, and placed at the level of the manubrium. For determination of CO, the lithium sensor of the CO measuring device (LiDCO Plus Haemodynamic Monitor; LiDCO Ltd, UK) was attached to the arterial catheter using a three-way valve, and 1 mL of LiCl (Lithium Chloride, 0.15 mmol mL^{-1}; LiDCO Ltd) was injected into an extension line connected to the central venous catheter and flushed with 10 mL 0.9% saline (Mason et al. 2001). The arterial haemoglobin (Hb) and sodium concentrations required for the determination of CO by lithium dilution were measured with a blood gas analyser (ABL800 Flex; Radiometer Medical, Denmark) before each CO measurement.

The experiment

After instrumentation, $F_{E'}$Iso was stabilized at 1.3% prior to the start of the experiment. HR, SAP, MAP, DAP and CVP were recorded at 5 minute intervals. CO was measured and arterial blood collected for analysis at baseline and 10 (T10) and 45 (T45) minutes after dexmedetomidine ± vatinoxan administration (Fig. 1). Cardiac index (CI), SVR index (SVRI), stroke volume index (SVI) and left cardiac work index were calculated as described by Haskins et al. (2005). After 15 minutes from dexmedetomidine ± vatinoxan administration (T15), the vasoactive drug or saline infusion was started and adjusted every 5 minutes depending on MAP (Table 1). After T45 measurements, all instrumentation was removed, isoflurane and mechanical ventilation were discontinued, meloxicam (0.2 mg kg^{-1}, Metacam 5 mg mL^{-1}; Boehringer Ingelheim, Germany) was administered IV and the dog was allowed to recover.

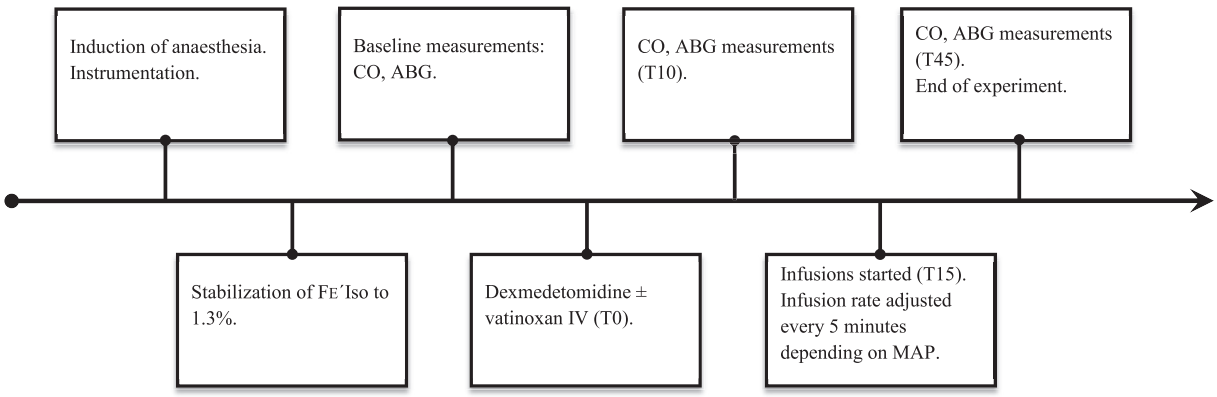

Figure 1 Timeline of events. The following five treatments were administered to eight dogs under isoflurane anaesthesia (end-tidal isoflurane 1.3%): dexmedetomidine (2.5 μg kg^{-1}) bolus followed by saline infusion (DEX-S); or dexmedetomidine and vatinoxan (100 μg kg^{-1}) bolus followed by an infusion of saline (DEX-VAT-S), dobutamine (DEX-VAT-D), norepinephrine (DEX-VAT-N) or phenylephrine (DEX-VAT-P). The bolus was administered at T0, and the infusion was started 15 minutes thereafter (T15) if mean arterial pressure (MAP) was below 90 mmHg. The infusion rate was adjusted every 5 minutes as required. Systemic haemodynamics were recorded at baseline and 10 (T10) and 45 (T45) minutes. ABG, arterial blood gas; CO, cardiac output; $F_{E'}$Iso, end-tidal isoflurane concentration; IV, intravenous.

Table 1 The following five treatments were administered to eight isoflurane-anesthetized dogs (end-tidal isoflurane 1.3%): dexmedetomidine (2.5 μg kg^{-1}) bolus followed by 0.9% saline infusion (treatment DEX-S); or dexmedetomidine and vatinoxan (100 μg kg^{-1}) bolus followed by an infusion of 0.9% saline (treatment DEX-VAT-S), dobutamine (treatment DEX-VAT-D), norepinephrine (treatment DEX-VAT-N) or phenylephrine (treatment DEX-VAT-P). The DEX or DEX-VAT bolus was administered at T0 and the saline or vasoactive infusion was started 15 minutes later (T15) if mean arterial pressure (MAP) was < 90 mmHg, at an infusion rate determined by the measured MAP. MAP was recorded every 5 minutes and infusion rate adjusted: continued at the same rate if MAP 70–90 mmHg, infusion rate increased to the value indicated on the table when MAP < 70 mmHg, infusion discontinued when MAP > 90 mmHg.

MAP at T15 (mmHg)	Infusion rate (mL kg^{-1} $hour^{-1}$)	DEX-VAT-D Dobutamine dosage (μg kg^{-1} $minute^{-1}$)	DEX-VAT-N Norepinephrine dosage (μg kg^{-1} $minute^{-1}$)	DEX-VAT-P Phenylephrine dosage (μg kg^{-1} $minute^{-1}$)
>90	No infusion	–	–	–
70–90	0.1	1.25	0.125	0.125
60–69	0.2	2.5	0.25	0.25
50–59	0.4	5.0	0.5	0.5
40–49	0.8	10.0	1.0	1.0
<40	1.6	20.0	2.0	2.0

Plasma drug concentration analysis

Blood samples for plasma drug concentration analysis were collected into ethylenediamine tetra-acetic acid (EDTA) tubes at T10 and T45. The concentrations of dexmedetomidine (reference standard: dexmedetomidine; Toronto Research Chemicals, ON, Canada) in dog plasma were determined with high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) after solid phase extraction (Sep-Pak tC18 96-well extraction plates; Waters Co., MA, USA), with 4,5-diphenylimidazole (Sigma-Aldrich, Germany) as internal standard. After separation with a Gemini C18 column (2 × 150 mm, 5 μm; Phenomenex, CA, USA) with a gradient flow system (0.1% formic acid in water and methanol), quantitative detection was performed in multi-reaction monitoring mode (MRM) with a triple quadrupole mass spectrometer (API 4000; MDS Sciex, ON, Canada). For dexmedetomidine and diphenylimidazole, the respective precursor ions (m z^{-1}) were 201.2 and 221.1. The fragment ions (m z^{-1}) monitored and used for quantitation were 95.1 for dexmedetomidine and 194.0 for diphenylimidazole. The chromatograms were processed using Applied Biosystems/MDS Sciex software (Analyst Version 1.4.1). The linear concentration range was 0.03 to 5 ng mL^{-1}. The inter-assay accuracy of the quality control samples (at three concentration levels, 0.08, 1.5 and 4.33 ng mL^{-1}) ranged from 104.5% to 105.6%.

The concentrations of vatinoxan (reference standard: MK-467; PCAS Finland Oy, Finland) in dog plasma were determined with HPLC–MS/MS after solid phase extraction using Sep-Pak tC18 96-well extraction plates, with RS-79948 (Tocris Bioscience, UK) as internal standard. Reversed-phase separation with a SunFire C18 column (2.1 × 150 mm, 3.5 μm, Waters) and a gradient solvent system (0.1% formic acid in water and acetonitrile) was used. Quantitative detection was performed in MRM with a triple quadrupole mass spectrometer (4000 Qtrap; MDS Sciex). For vatinoxan and RS-79948, the respective precursor ions (m z^{-1}) scanned were 419.3 and 365.3. The fragment ions monitored and used for quantitation were 200.1 for vatinoxan and 190.2 for RS-79948. The chromatograms were analysed and processed using Applied Biosystems/MDS Sciex software (Analyst Version 1.6.1). The linear range of the assay was 25 to 460 ng mL^{-1}. The inter-assay accuracy of the quality control samples (at three concentration levels, 70, 250 and 380 ng mL^{-1}) ranged from 94.4% to 111.6%.

Statistical analysis

The sample size was dictated by the number of dogs available and since the primary outcome of MAP > 70 mmHg was predefined, no formal power analysis was performed. All statistical analyses were performed at 4Pharma Ltd, Finland, using SAS System for Windows Version 9.3 (SAS Institute Inc., NC, USA). The normality of model residuals was confirmed visually and with Shapiro–Wilk test, to detect deviations from model assumptions. Changes in cardiovascular variables at T10 and T45 were compared with the baseline and with DEX-S and DEX-VAT-S (controls) at each time point. Plasma concentrations of dexmedetomidine and vatinoxan at T10 and T45 were compared with the controls. Differences were evaluated with repeated measures analysis of covariance model with treatment, time point, two-way interaction of treatment*time point, and the baseline covariate as fixed effects. If needed, standard transformations (logarithm, square root) of the data were performed. After transformation, the residual distribution of HR and SAP deviated from normal and their comparisons were performed using Wilcoxon signed-rank test.

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Table 2 Cardiovascular variables in eight isoflurane-anaesthetized dogs assigned to five treatments: dexmedetomidine (2.5 μg kg^{-1}) bolus followed by 0.9% saline infusion (treatment DEX-S); or dexmedetomidine and vatinoxan (100 μg kg^{-1}) bolus followed by an infusion of 0.9% saline (treatment DEX-VAT-S), dobutamine (treatment DEX-VAT-D), norepinephrine (treatment DEX-VAT-N) or phenylephrine (treatment DEX-VAT-P). Variables were recorded at T0 (baseline) immediately before injection of dexmedetomidine—vatinoxan, at 10 minutes (T10) before start of infusions, and at 45 minutes (T45). Data are presented as mean ± standard deviation.

Variable	DEX-S			DEX-VAT-S			DEX-VAT-D			DEX-VAT-N			DEX-VAT-P		
	T0	T10	T45	T0	T10	T45	T0	T10	T45	T0	T10	T45	T0	T10	T45
HR (beats $minute^{-1}$)	78 ± 11	56 ± 15*,‡	69 ± 10	74 ± 11	74 ± 13†	75 ± 13	77 ± 12	71 ± 7	58 ± 8*,‡	77 ± 8	77 ± 11†	60 ± 7*,†,‡	75 ± 14	71 ± 15	52 ± 16*,†,‡
SAP (mmHg)	109 ± 24	139 ± 11*	119 ± 15‡	113 ± 19	128 ± 12*	100 ± 11†	111 ± 25	123 ± 14*	177 ± 37*,†,‡	113 ± 21	121 ± 10	171 ± 38*,†,‡	114 ± 23	127 ± 21*	148 ± 31*,‡
MAP (mmHg)	62 ± 10	99 ± 4*,‡	72 ± 7*,‡	58 ± 7	73 ± 2*,†	54 ± 4*,†	61 ± 6	74 ± 10*,†	73 ± 9*,‡	63 ± 8	75 ± 7*,†	75 ± 4*,‡	62 ± 9	76 ± 13*,†	78 ± 11*,‡
DAP (mmHg)	48 ± 8	85 ± 4*,‡	57 ± 6*,‡	44 ± 6	57 ± 2*,†	41 ± 4†	46 ± 5	58 ± 11*,†	50 ± 8‡	48 ± 6	60 ± 8*,†	52 ± 3‡	47 ± 8	61 ± 13*,†	58 ± 10*,‡
CVP (mmHg)	2 ± 3	6 ± 2.5*,‡	2.5 ± 3*,‡	1 ± 2	2 ± 2.5*,†	1 ± 2†	1 ± 2	2 ± 2*,†	3.5 ± 2.5*,‡	2 ± 2.5	3 ± 3†	4 ± 2*,‡	2 ± 3	4 ± 3.5*,†	7 ± 3*,†,‡
CI (L $minute^{-1}$ m^{-2})	2.86 ± 0.66	1.73 ± 0.19*,‡	2.70 ± 0.45	2.31 ± 0.36	2.68 ± 0.47†	2.76 ± 0.50	2.46 ± 0.24	2.53 ± 0.49†	3.35 ± 0.70*,†	2.68 ± 0.55	2.90 ± 0.51†	3.97 ± 1.24*,†,‡	2.78 ± 0.75	2.92 ± 0.98†	2.13 ± 0.45*,†,‡
SVRI (dynes second cm^{-5} m^{-2})	1735 ± 367	4360 ± 501*,‡	2088 ± 333*,‡	2000 ± 348	2149 ± 325†	1558 ± 254*,†	1984 ± 379	2337 ± 449*,†	1657 ± 237*,†	1862 ± 340	2027 ± 280†	1553 ± 437*,†	1815 ± 454	2160 ± 814†	2771 ± 598*,†,‡
SVI (mL $beat^{-1}$ kg^{-1})	1.56 ± 0.39	1.36 ± 0.37	1.66 ± 0.40	1.32 ± 0.21	1.53 ± 0.28	1.55 ± 0.32	1.36 ± 0.22	1.48 ± 0.23	2.46 ± 0.45*,†,‡	1.50 ± 0.52	1.58 ± 0.26	2.76 ± 0.74*,†,‡	1.55 ± 0.28	1.71 ± 0.32	1.79 ± 0.42*
LCWI (kg minute m^{-2})	2.54 ± 0.75	2.46 ± 0.30	2.80 ± 0.61	1.93 ± 0.39	2.82 ± 0.54*	2.13 ± 0.48	2.15 ± 0.10	2.71 ± 0.74	3.49 ± 1.08*,‡	2.45 ± 0.64	3.17 ± 0.78*	4.34 ± 1.52*,†,‡	2.50 ± 0.86	3.21 ± 1.30*,†	2.42 ± 0.70
Lactate (mmol L^{-1})	1.65 ± 0.42	2.05 ± 0.62*	1.48 ± 0.45	1.48 ± 0.60	1.47 ± 0.56	1.35 ± 0.62	1.65 ± 0.28	1.88 ± 0.25	1.45 ± 0.26	1.61 ± 0.89	1.67 ± 0.78	0.79 ± 0.59*,†,‡	1.65 ± 0.42	1.70 ± 0.35	2.15 ± 0.87*,†,‡

CI, cardiac index; CVP, central venous pressure; DAP, diastolic arterial pressure; HR, heart rate; LCWI, left cardiac work index; MAP, mean arterial pressure; SAP, systolic arterial pressure; SVI, stroke volume index; SVRI, systemic vascular resistance index.

*Significantly different from baseline within the same treatment ($p < 0.05$).

†Significantly different from treatment DEX-S at the same time point ($p < 0.05$).

‡Significantly different from treatment DEX-VAT-S at the same time point ($p < 0.05$).

Observation correlations were modelled using compound symmetry covariance structure. Treatment differences on change from baseline were estimated with 95% confidence intervals and two-sided p values for all analysed time points and $p < 0.05$ was considered statistically significant. The p values were not adjusted for multiple testing.

Results

A majority of the dogs (32/40) under isoflurane anaesthesia had a MAP < 70 mmHg at baseline prior to the administration of dexmedetomidine ± vatinoxan, and 21/40 dogs were hypotensive (MAP < 60 mmHg). In treatments DEX-S and DEX-VAT-S, MAP significantly increased at T10, and at T45 in treatment DEX-S (Table 2).

CI was significantly decreased and SVRI increased at T10 in treatment DEX-S compared with all other treatments. At T45, CI was not different between treatments DEX-S and DEX-VAT-S. Simultaneously, CI was significantly increased in treatments DEX-VAT-D and DEX-VAT-N, but decreased in treatment DEX-VAT-P, in comparison with treatment DEX-S. At the same time point (T45), SVRI was significantly higher in treatments DEX-S and DEX-VAT-P than all other treatments.

In treatment DEX-S, plasma lactate concentration was significantly higher at T10 than at baseline (Table 2). Lactate concentration in treatment DEX-VAT-P was significantly higher at T45 than treatment DEX-VAT-S at the same time point. Arterial partial pressures of carbon dioxide and oxygen ($PaCO_2$ and PaO_2) were not different among treatments or time points. Plasma dexmedetomidine concentrations in treatments DEX-VAT-S, DEX-VAT-D, DEX-VAT-N and DEX-VAT-P were significantly lower at T10 than in treatment DEX-S, and lower at T45 in treatments DEX-VAT-S and DEX-VAT-N than in treatment DEX-S (Table 3).

MAP < 90 mmHg was recorded at T15 in all dogs administered vatinoxan, except for one dog in treatment DEX-VAT-P, and an infusion was started in all but that one animal. The MAP of that dog in treatment DEX-VAT-P decreased at 30 minutes to < 90 mmHg and phenylephrine infusion was started at the lowest dose rate. Treatment infusions were administered up to T45 in all dogs, except one dog in treatment DEX-VAT-P in which MAP was > 90 mmHg at 40 minutes and the infusion was stopped. At T45, the median (range) infusion rates of dobutamine, norepinephrine and phenylephrine were 3.75 (1.25–10.0), 0.25 (0.125–1.0) and 0.5 (0–2.0) μg kg^{-1} $minute^{-1}$, respectively, and the average dosages administered over the 30 minute infusion period were 3.53, 0.35 and 0.7 μg kg^{-1} $minute^{-1}$, respectively. Normotension was achieved by all drug infusions in every dog. All dogs in treatment DEX-VAT-S were hypotensive at T45.

Table 3 Plasma dexmedetomidine and vatinoxan concentrations in eight isoflurane-anaesthetized dogs assigned to five treatments: dexmedetomidine (2.5 μg kg^{-1}) bolus followed by 0.9% saline infusion (treatment DEX-S); or dexmedetomidine and vatinoxan (100 μg kg^{-1}) bolus followed by an infusion of 0.9% saline (treatment DEX-VAT-S), dobutamine (treatment DEX-VAT-D), norepinephrine (treatment DEX-VAT-N) or phenylephrine (treatment DEX-VAT-P). Measurements were performed 10 minutes (T10) after injection of boluses of dexmedetomidine and vatinoxan and before start of infusions, and at 45 minutes (T45). Data are presented as mean ± standard deviation.

Drug	DEX-S		DEX-VAT-S		DEX-VAT-D		DEX-VAT-N		DEX-VAT-P	
	T10	T45	T10	T45	T10	T45	T10	T45	T10	T45
Dexmedetomidine (ng mL^{-1})	1.77 ± 0.39†	0.32 ± 0.10†	1.39 ± 0.52*	0.24 ± 0.08*	1.24 ± 0.23*	0.29 ± 0.08	1.17 ± 0.20*	0.19 ± 0.04*	1.25 ± 0.20*	0.36 ± 0.12†
Vatinoxan (ng mL^{-1})	ND	ND	419 ± 93	253 ± 111	447 ± 85	268 ± 81	465 ± 89	259 ± 106	431 ± 91	282 ± 98

ND, not determined.
*Significantly different from treatment DEX-S at the same time point ($p < 0.05$).
†Significantly different from treatment DEX-VAT-S at the same time point ($p < 0.05$).

Discussion

Vatinoxan did not prevent the action of dobutamine, norepinephrine or phenylephrine, and all drug infusions maintained the MAP at or above 60 mmHg regardless of the different mechanism(s) of action. Dobutamine and norepinephrine increased CI and decreased SVRI, whereas phenylephrine had the opposite effect.

Dobutamine is a synthetic inotrope that mainly expresses β_1-and β_2-adrenergic receptor agonist effects (Ruffolo 1987). Consequently, stroke volume (SV) is increased and afterload reduced through decreased SVR, significantly augmenting CO without markedly affecting MAP (Ruffolo 1987). The plasma half-life of dobutamine in dogs is short, 1−2 minutes (Murphy et al. 1976). In the present study, dobutamine infusion significantly increased MAP in dogs administered dexmedetomidine−vatinoxan via increased SVI and CI, but also decreased HR and SVRI, indicating peripheral vasodilation. When compared with treatment DEX-VAT-S at T45, the CI and SVRI in treatment DEX-VAT-D did not differ significantly, although blood pressures and SVI were significantly higher. Compared with the administration of dexmedetomidine alone (DEX-S), treatment DEX-VAT-D resulted in significantly better SVI and CI and lower SVRI, although there were no differences in MAP at T45. The haemodynamic effects of dobutamine observed in the present study were similar to those in previous studies that used comparable dosages in isoflurane-anaesthetized cats, dogs, foals and alpacas (Pascoe et al. 2006; Craig et al. 2007; Rosati et al. 2007; Vincent et al. 2009).

Norepinephrine is a naturally occurring catecholamine that has agonistic effects on both α_1-and α_2-adrenoceptors and β_1-and β_2-adrenoceptors (Stokland et al. 1983; Stowe & Ebert 2011). The main clinical indication for norepinephrine is treatment of hypotension associated with vasodilation during sepsis, for which it is administered 'to effect' over a wide dosage range of 0.01−3.3 $\mu g^{-1}\,kg^{-1}\,minute^{-1}$ (Dellinger et al. 2013). The half-life of norepinephrine is short, approximately 2 minutes (Beloeil et al. 2005). The norepinephrine infusion dosages required in the present study to achieve and maintain normotension were comparable with the dosages published in foals, alpacas and rabbits to treat isoflurane-induced hypotension (Valverde et al. 2006; Vincent et al. 2009; Uccello et al. 2020). In the present study, the administration of norepinephrine infusion significantly improved blood pressure (SAP and MAP) through enhanced SVI and CI. Similar to dobutamine, norepinephrine also decreased HR and SVRI. The reduction in SVRI was unexpected, because norepinephrine is generally considered a vasopressor. Interestingly, Kojima et al. (2021) recently reported a haemodynamic outcome comparable with the present study in healthy, isoflurane-anaesthetized dogs, suggesting that the effects of norepinephrine may be context-sensitive. Furthermore, Brick et al. (1967) suggested that in the presence of the nonselective α-adrenoceptor antagonist phentolamine, the vasoconstrictive α-adrenoceptor effect of norepinephrine is either reduced, abolished or reversed in a dose-dependent manner, allowing the vasodilatory β-adrenoceptor effect to become dominant. However, vatinoxan is a selective α_2-adrenoceptor antagonist, with an α_2:α_1 selectivity of 105 (Clineschmidt et al. 1988). Bryant et al. (1998) administered vatinoxan to horses and sheep that had been pretreated with methoxamine, an α_1-adrenoceptor agonist, and concluded that vatinoxan had no effect on α_1-adrenoceptors, at least with the dose that was investigated. The activation of central α_2-adrenoceptors decreases sympathetic outflow and reduces blood pressure and HR (Guyenet 1997), whereas the activation of peripheral α_2-adrenergic receptors leads to vasoconstriction and hypertension (Hein 2001). It is possible that the prior administration of vatinoxan prevented peripheral α_2-adrenoceptor activation by norepinephrine; this, together with the central actions of both dexmedetomidine and isoflurane, lead to vasodilation overruling the peripheral α_1-adrenoceptor effect of norepinephrine.

In the present study, the administration of norepinephrine infusion to dexmedetomidine−vatinoxan treated dogs improved arterial blood pressures, SVI and CI. Compared with the administration of dexmedetomidine alone, treatment DEX-VAT-N resulted in significantly better SVI and CI, and lower SVRI and plasma lactate concentration.

Phenylephrine is a synthetic non-catecholamine that has mostly α_1-adrenoceptor agonist properties; it has no activity at α_2-adrenoceptors and exerts β-adrenoceptor effects only at very high doses (Stowe & Ebert 2011; Thiele et al. 2011). In humans, phenylephrine has a relatively long elimination half-life of 2−3 hours (Hengstmann & Goronzy 1982). It is usually administered as an infusion, although it can also be administered as a bolus (Thiele et al. 2011). The main indication for phenylephrine is to increase blood pressure through vasoconstriction without increasing myocardial contractility (Stowe & Ebert 2011). In the present study, the administration of phenylephrine to dexmedetomidine−vatinoxan treated dogs improved blood pressure by significantly increasing SVRI, at the expense of decreased HR and CI and increased plasma lactate concentration. In clinical veterinary practice, CO is not routinely measured and the intraoperative haemodynamic assessment is based on arterial blood pressure measurements. Consequently, treating hypotension exclusively by increasing SVRI may further compromise tissue perfusion, especially if the hypotension was due to decreased CI. The phenylephrine infusion dosages used in this experiment, and the haemodynamic effects observed, are comparable to previous studies in anaesthetized cats and horses (Pascoe et al. 2006; Ohta et al. 2013).

In treatment DEX-VAT-S, the isoflurane-anaesthetized dogs became hypotensive by T45, whereas in treatment DEX-S

normotension was maintained throughout the study period. However, CI is a major component of oxygen delivery to tissues and thus a better determinant of tissue perfusion than blood pressure (Peterson & Moses 2011), and at T45 the CI of dogs in treatment DEX-VAT-S did not significantly differ from those in treatment DEX-S despite the hypotension. CI is the product of HR and SV corrected for body surface area (or body weight), and at T45 the HR and SV were not significantly different between treatments DEX-VAT-S and DEX-S. The CI of dogs administered dexmedetomidine alone was significantly lower at T45 than in treatments DEX-VAT-D and DEX-VAT-N.

The plasma dexmedetomidine concentrations at T10 were significantly lower in all treatments that included vatinoxan compared with dexmedetomidine alone owing to the preservation of CI by vatinoxan, leading to higher organ perfusion and increased dexmedetomidine clearance (Honkavaara et al. 2012; Bennett et al. 2016). Also, norepinephrine enhanced the CI and organ perfusion, as demonstrated by the lower plasma dexmedetomidine concentrations in treatment DEX-VAT-N compared with treatment DEX-S at T45. Again, phenylephrine had the opposite effect: as a consequence of the decreased CI and increased SVRI, DEX-VAT-P resulted in significantly higher plasma dexmedetomidine concentrations than DEX-VAT-S at T45 indicating decreased organ perfusion.

It is important to mention that α_2-adrenoceptor agonists, including dexmedetomidine, may influence the accuracy of LiDCO sensors by increasing sensor voltage resulting in overestimation of CO compared with the thermodilution method (Ambrisko et al. 2013). However, in an *in vitro* study by Ambrisko et al. (2013) the concentration of dexmedetomidine required for the measurement bias to occur, far exceeded the plasma concentrations measured in the present study. Catecholamines are another source of positive bias causing a dose-dependent overestimation of LiDCO measurements (Hopster et al. 2017). The median dobutamine infusion rate used in the present study corresponds with the high dobutamine infusion rate administered by Hopster et al. (2017) to isoflurane-anaesthetized horses. In that study, a gross overestimation of the LiDCO values was recorded when high doses of catecholamines were infused. Based on the dosages used in the present study, it is possible that the dobutamine infusion administered in treatment DEX-VAT-D influenced the CO results. However, that cannot explain the differences at T45 compared with other treatments in HR, blood pressure and CVP, nor does it explain the measurements obtained in other treatments.

A potential limitation of this study is our choice of infusion doses and rates, particularly those of phenylephrine and norepinephrine. Because the assessor monitoring the study was blinded to treatment assignments, the infusions had to be increased in a similar step-wise manner where each step doubled the dose. This limited the choice of dosages. Based on *in vivo* and *in vitro* vasoconstrictor studies in humans, the potency ratio of phenylephrine to norepinephrine ranges from 1.1:1 in septic patients to approximately 10:1 in anaesthetized patients (Goradia et al. 2021). Therefore, it is possible that our study did not use equipotent doses of phenylephrine and norepinephrine. However, all infusions were administered until the target MAP was achieved, and Pascoe et al. (2006) used similar phenylephrine dose rates in cats, derived from those used in clinical veterinary patients. Furthermore, the half-life of phenylephrine is longer than that of dobutamine and norepinephrine, and during administration the plasma concentration increases over time until steady state is reached (Hengstmann & Goronzy 1982; Ohta et al. 2013). For this reason, Ohta et al. (2013) recommended that in clinical use, at least in horses, phenylephrine infusion should be stopped when MAP reaches the target value. In the present study, phenylephrine infusion was continued until MAP was 90 mmHg, and the total infusion period was short at $\leq$ 30 minutes, meaning that steady state could not have been reached. Another limitation is that the co-oximeter of the blood gas analyser used at the time of the experiment did not work, prohibiting arterial haemoglobin saturation (SaO_2) measurement. The SaO_2 is required for the calculation of arterial oxygen content, which in turn is required for the calculation of oxygen delivery, oxygen extraction and oxygen consumption. Also of note is that the dogs in the present study were mechanically ventilated with a time-cycled and pressure-limited 'bag in a bottle'-type ventilator throughout the instrumentation and study periods. Increased intrathoracic pressure during positive pressure ventilation may negatively affect CO by decreasing venous return and SV; however, positive pressure ventilation was used equally in all dogs and all treatments. Further limitations of this study include not adjusting F_E'Iso to the anaesthetic requirements of the dogs, the short observational period and the fact that the CI was determined only once after starting the infusions.

Conclusions

Dobutamine and norepinephrine infusions not only restored normotension in isoflurane-anaesthetized dogs administered dexmedetomidine—vatinoxan but also significantly improved the CI when compared with dogs administered dexmedetomidine alone. With the doses used in this study, the addition of vatinoxan to dexmedetomidine has the potential to improve the haemodynamic stability of healthy dogs anaesthetized with isoflurane, and hypotension can be effectively treated by the administration of either dobutamine or norepinephrine.

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Author's contributions

VH: study design, collection, analysis and interpretation of data, writing of the manuscript. FR: study design, collection, analysis and interpretation of data. MR: study design, interpretation of data, supervised the study. JH: study design, collection and interpretation of data. TP: statistical analysis of data. OV: study design and supervised the study. All authors contributed to the critical revision of the paper and approved the final version of the manuscript for publication.

Conflict of interest statement

FR received financial support from Vetcare Ltd during this study. However, Vetcare Ltd had no involvement in the study design, data collection, data analysis or interpretation, nor in the writing and publication of the manuscript. The authors declare no other conflicts of interest.

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