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Cardiovascular effects of two adenosine constant rate infusions in anaesthetized dogs

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Abstract

The aim of the study was to investigate cardiovascular effects of two adenosine constant rate infusion (CRI) doses in dogs.
Ten healthy beagle dogs were included into the experimental study. After sedation with butorphanol, anaesthesia was induced with propofol and maintained with sevoflurane. Each dog received 2 consecutive adenosine CRI's: 140 μg $kg^{-1}minute^{-1}$ (A140) followed by 280 μg $kg^{-1}minute^{-1}$ (A280). Pulse rate, invasive arterial blood pressures and stroke volume [measured by magnetic resonance phase contrast angiography] were measured at baseline, 3 minutes after starting and 3 and 10 minutes after discontinuing adenosine. Cardiac output, cardiac index and approximated systemic vascular resistance (approxSVR) were calculated. Arterial blood gases, co-oximetry, electrolytes, glucose and lactate were measured and oxygen content and delivery calculated. Data were analysed using one-way repeated measures ANOVA ($p < 0.05$).
A140 and A280 resulted in a significant decrease in arterial blood pressures and approxSVR. No significant changes were detected for the other variables. All values returned to baseline within 3 minutes after adenosine discontinuation.
Adenosine decreases arterial blood pressures by vasodilatation in healthy dogs. The observed effects were not dose-dependent.

Adenosine, anaesthesia, cardiovascular, cMRI, dog

Zusammenfassung

Das Ziel der Studie war es, die kardiovaskulären Effekte von zwei Adenosin Dauertropfinfusionen (DTI) zu untersuchen.
Zehn gesunde Beagle Hunde wurden in die experimentelle Studie einbezogen. Nach einer Sedation mit Butorphanol wurde die Anästhesie mit Propofol eingeleitet und mit Sevofluran aufrechterhalten. Jeder Hund bekam eine tiefere Dosis von 140 µg $kg^{-1}minute^{-1}$, gefolgt von der höheren mit 280 µg $kg^{-1}minute^{-1}$ Adenosin. Kardiovaskuläre Parameter wie Pulsfrequenz, invasive arterielle Blutdrucke und Schlagvolumen (Messung mittels MRT Phasenkontrast Angiographie) wurden vor Start der DTI, 3 Minuten nach Start und 3 und 10 Minuten nach Stopp der jeweiligen DTI gemessen. Herzminutenvolumen, Herzminutenvolumenindex und angenäherter peripherer Gefässwiderstand (approxSVR) wurden berechnet. Arterielle Blutgase, Cooximetrie, Elektrolyte, Glucose und Lactat wurden gemessen und der Sauerstoffgehalt des Blutes sowie der Sauerstoffverbrauch berechnet. Die Daten wurden mittels einfaktorieller-ANOVA mit Messwiederholung ausgewertet ($p < 0.05$).
Beide Dosen führten zu einem Abfall der arteriellen Blutdrucke sowie des approxSVR. Für die restlichen Variablen wurden keine signifikanten Änderungen festgestellt. Alle Messungen kehrten 3 Minuten nach Stopp von Adenosin auf die Ausgangswerte zurück.
Adenosin reduziert den arteriellen Blutdruck bei gesunden Hunden mittels Vasodilatation. Der beobachtete Effekt war nicht Dosis abhängig.

Adenosin, Anästhesie, kardiovaskulär, cMRI, Hund

RESEARCH PAPER

Cardiovascular effects of two adenosine constant rate infusions in anaesthetized dogs

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Abstract

Objective Adenosine induces vasodilatation. The aim of this study was to investigate cardiovascular effects of two adenosine constant rate infusion (CRI) doses in dogs.

Study design Experimental, longitudinal repeated measure design.

Animals Ten healthy purpose-bred Beagle dogs.

Methods Each dog was sedated with butorphanol. Anaesthesia was induced with propofol intravenously and maintained with sevoflurane (inspired oxygen fraction = 47–55%). Controlled mechanical ventilation was used to maintain normocapnia. Two doses of adenosine were administered as CRIs to each dog: 140 μg kg^{-1} $minute^{-1}$ (A140) followed by 280 μg kg^{-1} $minute^{-1}$ (A280). Pulse rate, invasive arterial pressure and stroke volume (by magnetic resonance phase contrast angiography) were measured at baseline, 3 minutes after starting adenosine and 3 and 10 minutes after discontinuing adenosine. Cardiac output, cardiac index and approximated systemic vascular resistances (approximate SVR) were calculated. Additionally, arterial blood gases, co-oximetry, electrolytes, glucose and lactate were measured and oxygen content and delivery calculated. One-way repeated measures analysis of variance ($p < 0.05$) was used for data analysis.

Results A140 and A280 resulted in a significant decrease in arterial blood pressure [systolic ($p = 0.008$), mean ($p = 0.003$), and diastolic arterial pressure ($p = 0.004$)] and approximate SVR ($p = 0.008$) compared with baseline. No significant changes were detected for the other variables. All values returned to baseline within 3 minutes after adenosine discontinuation.

Conclusions and clinical relevance Adenosine CRI decreases arterial pressure by vasodilatation in healthy dogs. No additional effects were observed with the higher dose. The effects in compromised dogs remain to be investigated.

Keywords adenosine, anaesthesia, cardiovascular, cMRI, dog.

Introduction

Adenosine is an extracellular purine nucleoside signalling molecule that is present in every cell of the body. Its half-life is seconds to minutes (Moser et al. 1989) and its biological effects are mediated via four receptor subtypes (A_1, A_{2A}, A_{2B}, A_3). Adenosine receptors seem to play a dynamic role not only in normal cell physiology but also as modulators in disease processes (Sheth et al. 2014).

In humans, adenosine depresses atrioventricular conduction and induces coronary vasodilatation (Faulds et al. 1991). Therefore, and based on its short duration of action, adenosine is commonly used for diagnosis of coronary heart disease, and for diagnosis

and emergency treatment of complex tachycardias (DiMarco et al. 1983; Rankin et al. 1989; Verani et al. 1990; Jenni et al. 2009; Greenwood et al. 2012; Bailey et al. 2016). Furthermore, adenosine has been extensively investigated for its anti-nociceptive and anti-inflammatory properties and its importance during ischemia and shock (Sjolund et al. 2001; Skrabanja et al. 2005; Hayashida et al. 2006; Granfeldt et al. 2014). Due to its wide field of applicability, adenosine is expected to gain importance in veterinary medicine. Therefore, more controlled trials, especially investigating cardiovascular effects, in companion animals are necessary.

The cardiovascular effects of adenosine, in particular hypotension, have been studied in humans and dogs (Lagerkranser et al. 1984; Sollevi et al. 1984; Asakawa et al. 2007). However, to the authors' knowledge, cardiovascular effects have not been investigated in anaesthetized dogs using modern anaesthetic agents.

The aims of this study were: 1) To investigate the effects of adenosine on the cardiovascular system of healthy anaesthetized dogs; and 2) to compare two different adenosine doses administered as continuous rate infusions (CRI).

We hypothesized that adenosine CRI will result in a dose-dependent hypotension induced primarily by vasodilatation rather than by decreasing cardiac output.

Methods

All examinations were performed with written permission of the Committee for Animal Experimentation of the Canton Zürich, Switzerland (License number ZH001-15) and were performed in consensus with the ARRIVE guidelines.

Animals

Ten healthy, adult purpose-bred Beagle dogs were recruited for this study. They were included if deemed healthy based on clinical and routine blood examination (haematocrit and standard biochemical blood analysis).

Exclusion criteria included suspicion of cardiovascular disease and cardiovascular instability [mean arterial blood pressure (MAP) < 60 mmHg and pulse rate (PR) > 130 beats minutes^{-1}] during anaesthesia prior to starting baseline measurements. The dogs were housed in groups of two and fasted for 11 hours before the experiment but had free access to water.

Preanaesthetic preparation and anaesthesia

Dogs were premedicated with butorphanol (0.2 mg kg^{-1}; Alvegesic 1% forte; Virbac AG, Switzerland) intramuscularly. After 10 minutes, catheters (Vaso-Vet 22 gauge; Eickemeyer, Switzerland) were inserted into both the cephalic veins. One catheter was used exclusively for adenosine infusion. After 3 minutes of preoxygenation, anaesthesia was induced with propofol 4−6 mg kg^{-1} intravenously (IV) (Propofol 1% MCT Fresenius; Fresenius Kabi AG, Switzerland). Dogs were orotracheally intubated with a cuffed endotracheal tube, and the tube was connected to an anaesthetic machine via a circle system (Aestivia S5, 7900; SmartVent, Switzerland). Anaesthesia was maintained with sevoflurane (Sevorane; abbVie AG, Switzerland) in an oxygen-air mixture (O_2-air: 1:1; inspired oxygen fraction = 47−55%]. Volume-controlled mechanical ventilation with a tidal volume (V_T) of 10−15 mL kg^{-1} and respiratory rate (f_R) of 12−25 breaths minute^{-1} ensured normocapnia [end-tidal carbon dioxide ($P_E'CO_2$) between 36 and 38 mmHg (4.8−5.1 kPa)]. A 22 gauge arterial catheter (BD Insyte-A; Becton, Dickinson and Company, China) was advanced into a metatarsal artery for continuous blood pressure measurements and arterial blood sampling. A new pressure transducer for each dog (DPT-6000 system; Codan Medical AG, Switzerland) was zeroed to atmospheric pressure and levelled to the height of the shoulder joint. Monitoring was performed with a multiparameter monitor (Datex-Ohmeda N-MRI2-01; GE Healthcare, Finland) and consisted of pulse oximetry, invasive arterial pressure, inspired and expired gases including end-tidal sevoflurane concentration in % (F_E'Sevo), spirometry and capnography.

All dogs received Ringer's lactate solution at 5 mL kg^{-1} hour^{-1} (Ringer-Lactate; Fresenius Kabi AG) IV. If mean arterial blood pressure dropped below 60 mmHg and/or PR raised above 130 beats minute^{-1}, boluses of Ringer's lactate (3 mL kg^{-1} over 10 minutes up to a maximum of 20 mL kg^{-1} total dose), if necessary, followed by balanced hydroxyethyl starch 130/0.4 (Voluven 6% balanced; Fresenius Kabi AG) (3 mL kg^{-1} over 10 minutes up to 10 mL kg^{-1} total dose) were administered IV until reaching MAP > 60 mmHg and PR < 130 beats minute^{-1}.

Study design

Baseline measurements were taken once basic magnetic resonance imaging (MRI) studies were

completed and anaesthesia was considered stable. Measurements always followed the same order: 1) recording of variables from the multiparameter monitor [PR, MAP, systolic (SAP) and diastolic arterial pressure (DAP), expired and inspired gases, f_R]; 2) cardiac MRI scans; and 3) arterial blood sampling.

After recording baseline measurements, adenosine was started as a CRI. Adenosine (Krenosin 6 mg/2 mL; Sanofi-aventis, Switzerland) was administered through the right cephalic catheter using a calibrated syringe driver (Syramed μSP6000; Arcomed, Switzerland). All dogs were first administered a lower infusion dose of adenosine (A140: 140 μg kg^{-1} $minute^{-1}$), followed by a higher dose (A280: 280 μg kg^{-1} $minute^{-1}$).

There was a minimum 3 minute stabilization period before taking measurements after starting A140, and only if no further changes in MAP were observed. As soon as all measurements were recorded, the adenosine dose was increased and measurements for A280 were taken after an additional 3 minute stabilization period. After the adenosine CRI was discontinued, measurements were repeated after 3 and 10 minutes (Fig. 1).

Cardiac magnetic resonance imaging

For the cardiac MRI (cMRI) scans, the dogs were placed in dorsal recumbency with the head towards the gantry. A board-certified radiologist (MD) performed cMRI with a 3 Tesla system (Philips Ingenia 3T with a dStream body coil Solution; Philips AG, Switzerland). Vector cardiography with four MR-safe electrodes (750 Clear Tape Electrodes; Kendall Anandic Medical System SA; Switzerland) attached to both sides of the chest wall over the heart and a peripheral pulse unit (Philips AG, Switzerland) placed on the tongue monitored cardiac action.

Initial scout views were performed to determine cardiac location. Cine gradient echo sequences of the cardiac anatomy allowed planning of the flow sensitive sequences perpendicular to the flow direction. Parameters used were time to echo (2 ms), time to repetition (4 ms), turbo field echo factor (8), number of signal averages (1), sensitivity encoding (yes), echo train length (6), slice thickness (4 mm), spacing between slices (5 mm), field of view (200 × 181); flip angle (45°) and matrix (200 × 190). The parameters for quantitative phase contrast angiography were set at time to echo (2.9 ms), time to repetition (4.7 ms); turbo field echo factor (2), number of signal averages (2), sensitivity encoding (yes), echo train length (2), slice thickness (8 mm), spacing between slices (5 mm), field of view (180 × 180), flip angle (10°), matrix (120 × 120). Measurements were acquired in a supravalvular position over the aortic valve perpendicular to flow direction.

Every quantitative phase contrast angiography followed a perfusion scan for which Gadolinium (Omniscan 78.67 mg Gd mL^{-1}; GE Healthcare AG, Switzerland) 0.05 mmoL kg^{-1} IV was administered through the venous catheter.

Quantitative phase contrast angiography and perfusion scans required apnoea. To guarantee apnoea for the scans, ventilation was increased for 2 minutes (either increase in V_T or f_R) to reach $P_E'CO_2$ of 35−38 mmHg (4.7−5.1 kPa) before switching off the ventilator. Apnoea duration was 10−25 seconds.

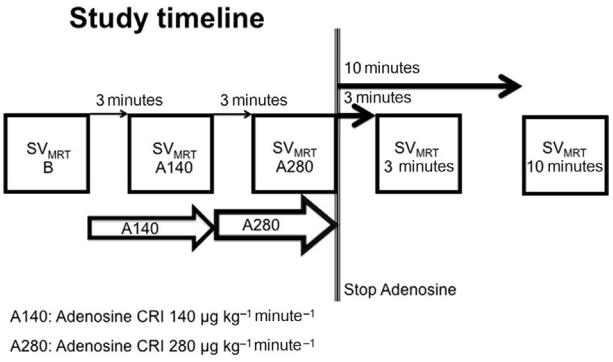

Figure 1 Study timeline showing magnetic resonance imaging Q-Flow stroke volume measurements (SV_{MRT}) at baseline (B) during different adenosine constant rate infusions (CRI) (A140: 140 μg kg^{-1} $minute^{-1}$; A280: 280 μg kg^{-1} $minute^{-1}$) and 3 and 10 minutes after discontinuing adenosine treatment.

Arterial blood gases, electrolytes, haemoglobin, glucose and lactate

First, 3 mL of blood was removed from the arterial catheter and discarded before 1 mL of blood was collected anaerobically into a heparinized syringe (BD Preset; Becton, Dickinson and Company, Belgium) and analysed immediately (RapidPoint 500; Siemens Healthcare Diagnostics Inc., NY, USA). The catheter was flushed with heparinized saline. Arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen (PaO_2), pH, total haemoglobin (tHB), fraction of oxyhaemoglobin (FO_2Hb), sodium (Na^+), potassium (K^+), ionized calcium (Ca^{2+}), chloride (Cl^-), glucose and lactate were measured.

Retrospective data analysis

MRI image processing and analysis

The DICOM-images were exported to a dedicated workstation (Extended MR Workspace; Philips AG). The same radiologist quantified flow information with an imaging software (QFlow Analysis; Philips AG) in a post-processing step by drawing a region of interest around the aorta. The software propagated the region of interest to each phase of the cardiac cycle. The automatically generated region of interests were reviewed and manually corrected. The software calculated flow parameters based on the signal intensity of the voxel representing flow velocity and the square area of the region of interest. The analysis included the following parameters: stroke volume, forward flow, backward flow, regurgitant fraction, absolute stroke volume (SV_{MRT}), mean flux, stroke distance and mean velocity. The results were exported to an excel sheet.

Calculated haemodynamic variables

Cardiac index (CI), cardiac output (CO), oxygen delivery (DO_2) and arterial oxygen content (CaO_2) were calculated using conventional formulas and the measured SV_{MRT}. Systemic vascular resistance (SVR) was calculated based on Stoelting and Hillier (Stoelting & Hillier 2012) and defined as approximated SVR:

$$CO = PR \times SV_{MRT}$$

$$CI = CO/(\text{bodyweight in kg})$$

$$\text{Approximate SVR} = MAP/CO \times 80$$

$$CaO_2 = (1.34 \times Hb \times SaO_2) + (0.003 \times PaO_2)$$

$$DO_2 = CO \times CaO_2$$

where Hb, haemoglobin concentration (g dL^{-1}); SaO_2, oxyhaemoglobin saturation; PaO_2, partial pressure of oxygen in the blood (Grimm et al. 2015).

Recovery

After finalizing data acquisition for the study described here, dogs were transported out of the MRI and underwent a continuous positive airway pressure study (Meira et al. 2018). After data collection for the ventilation study, dogs were allowed to recover. Each dog was administered a single dose of robenacoxib 2 mg kg^{-1} IV (Onsior; Novartis, Switzerland).

Data handling and statistical analysis

The present study was a subproject of an imaging trial of the canine femoral and sciatic nerves (license number: 26164-ZH001/15) (Sievert et al. 2017), which predetermined the total number of dogs. Therefore, no prospective power analysis was performed.

Statistical analyses were performed with GraphPad Prism (GraphPad Software, Inc., CA, USA). Normal distribution of all variables was confirmed using the Shapiro–Wilk normality test. Consequently, one-way repeated measures analysis of variance was performed followed by Sidak's multiple comparisons test for multiple pairwise comparisons. All time points were compared with baseline, and values collected at A140 were compared with those at A280. Results are reported as mean ± standard deviation (SD), and significance was set at $p < 0.05$. Adjusted p values (p_{adj}) are presented for *post hoc* tests.

Values of MAP 1 minute before (A140-1min, A280-1min) and at A140 and A280, respectively, were compared to verify stabilization of the effects of adenosine. A Wilcoxon test was used for that purpose ($p < 0.05$).

A power calculation was retrospectively performed (SigmaPlot Version 14; Systat Software Inc., Germany) for parameters showing a tendency to reach significance (SV_{MRT}).

Results

The dogs weighed 14.11 ± 2.5 kg (mean ± SD) and were aged 4.5 ± 0.5 years old; half of them were male and the other half were female, all of them entire. One dog was excluded from analysis due to a mild mitral insufficiency detected by MRI during the study. Aortic Q-Flow data was excluded in one dog because of technical problems to measure quantitative phase contrast angiography at high velocity flows; therefore, aortic Q-Flow data were analysed in eight dogs only. Due to technical problems related to the analyser, complete blood gas analysis was only obtained for seven dogs.

The initial dose of butorphanol (0.2 mg kg^{-1} IV) was repeated in two dogs. This was decided based on clinical experience of the anaesthetist and butorphanol was administered at least 30 minutes before baseline measurements. Baseline measurements were taken 168.7 ± 22.3 minutes after induction of anaesthesia. All dogs recovered uneventfully.

No changes in MAP were detected from A140-1min and A280-1min to A140 and A280, respectively. This led to the assumption that stable adenosine effects were achieved before starting A140 and A280 measurements.

Cardiovascular data, CaO_2 and DO_2 are presented in Figs 2–4. Changes over time were detected for SAP ($p = 0.008$), MAP ($p = 0.003$) and DAP ($p = 0.004$). MAP and DAP decreased with A140 (MAP $p_{adj} = 0.007$; DAP $p_{adj} = 0.005$) and A280 (MAP $p_{adj} = 0.002$; DAP $p_{adj} = 0.003$) compared with baseline. The decrease in SAP was only significant at A280 (SAP $p_{adj} = 0.03$). No differences in SAP were seen between baseline and 3 minutes (SAP $p_{adj} = 0.16$, MAP $p_{adj} = 0.93$, DAP $p_{adj} = 0.99$) and 10 minutes (SAP $p_{adj} = 0.14$, MAP $p_{adj} = 0.60$, DAP $p_{adj} = 0.81$) after discontinuing adenosine. Also no differences were detected between the two dosages, A140 and A280, (SAP $p_{adj} = 0.39$; MAP $p_{adj} = 0.21$; DAP $p_{adj} = 0.29$; Fig. 2).

No changes in PR were detected over time or between time points ($p = 0.134$). A wide range of PR was observed (baseline: 71–120 beats $minute^{-1}$; A140: 106–138 beats $minute^{-1}$; A280: 109–150 beats $minute^{-1}$).

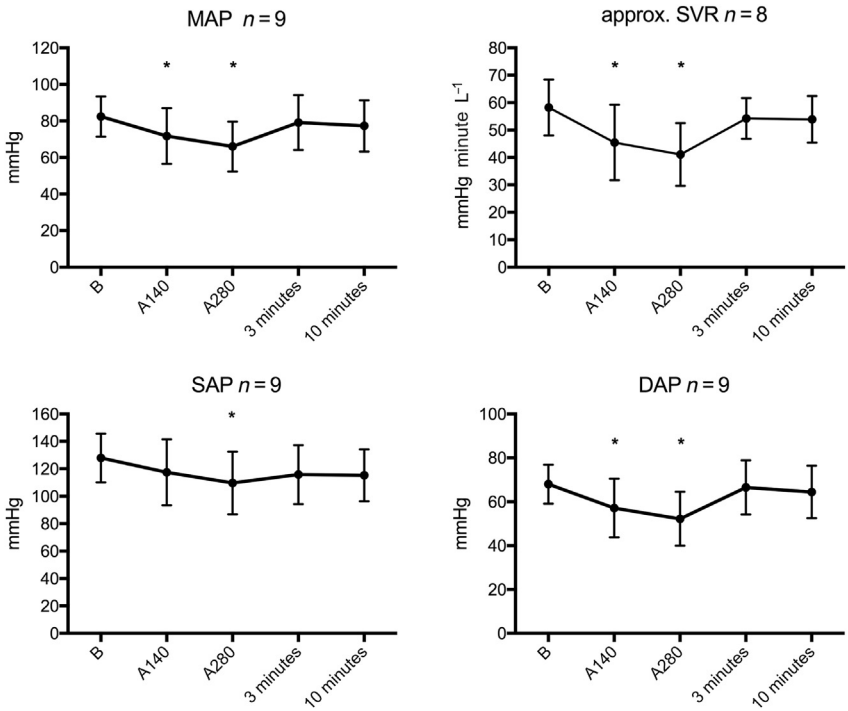

Figure 2 Blood pressures and approximated systemic vascular resistance (SVR) of nine sevoflurane-anaesthetized Beagle dogs at baseline (B), during adenosine infusion (A140: 140 µg kg^{-1} $minute^{-1}$; A280: 280 µg kg^{-1} $minute^{-1}$) and 3 and 10 minutes after discontinuing adenosine (X-axis). Points indicate mean, whiskers indicate standard deviation. *Significantly different to B. DAP, diastolic blood pressure; MAP, mean arterial blood pressure; SAP, systolic blood pressure.

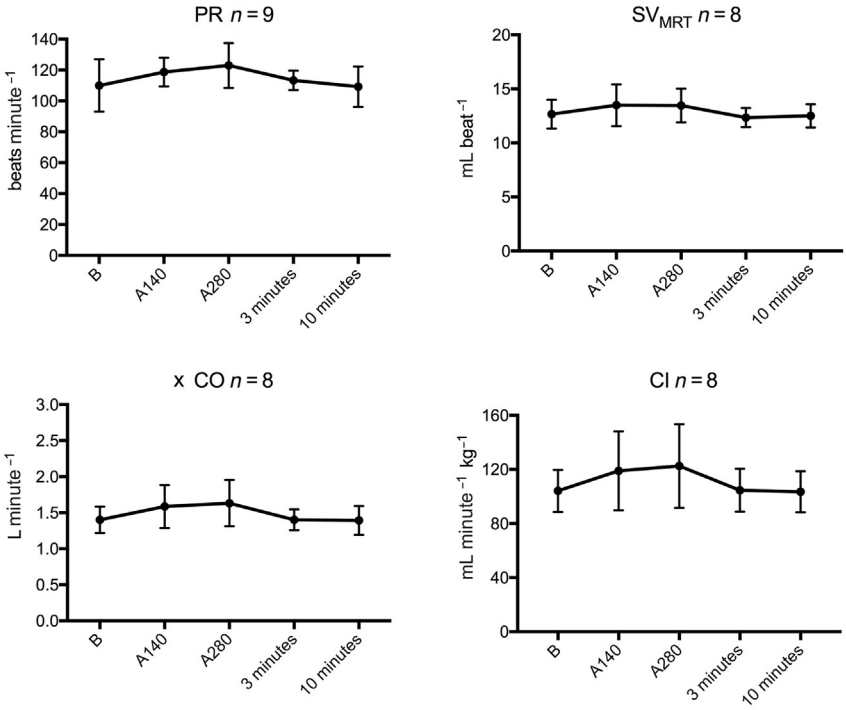

Figure 3 Pulse rate (PR), stroke volume (measured by magnetic resonance imaging; SV_{MRT}), cardiac output (CO) and cardiac index (CI) of nine sevoflurane-anaesthetized Beagle dogs at baseline (B), during adenosine infusion (A 140: 140 μg kg^{-1} $minute^{-1}$; A 280: 280 μg kg^{-1} $minute^{-1}$) and 3 and 10 minutes after discontinuing adenosine (X-axis). Points indicate mean, whiskers indicate standard deviation. *Significant changes over time.

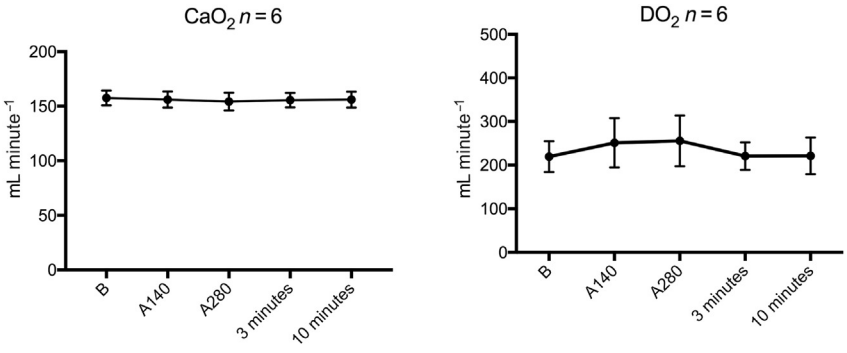

Figure 4 Oxygen content (CaO_2) and oxygen delivery (DO_2) of nine sevoflurane-anaesthetized Beagle dogs at baseline (B) during adenosine infusion (A 140: 140 μg kg^{-1} $minute^{-1}$; A 280: 280 μg kg^{-1} $minute^{-1}$) and 3 and 10 minutes after discontinuing adenosine (X-axis). Points indicate mean, whiskers indicate standard deviation.

The SV_{MRT} ($p = 0.054$) was not different compared with baseline during both A140 and A280 infusions. In addition, no differences were detected between A140 and A280 (SV_{MRT} $p_{adj} > 0.99$). A retrospective power calculation showed a power of 63% to detect a difference in SV_{MRT}. A change in CO was detected over time ($p = 0.043$) but not between individual time points. CI ($p = 0.062$) was not different during both A140 and A280 infusions compared with baseline. Changes over time were detected for the approximate SVR ($p = 0.008$). Approximate SVR was lower with A140 ($p_{adj} = 0.029$) and A280 ($p_{adj} = 0.015$) compared with baseline. However, no differences were detected between the higher and the lower adenosine doses ($p_{adj} = 0.26$). Approximated SVR returned to baseline values 3 minutes after discontinuing adenosine (Fig. 3).

Table 1 Blood gases, electrolytes, glucose, lactate and end-tidal sevoflurane measurements of seven sevoflurane-anaesthetized Beagle dogs at baseline (B) during adenosine infusion (A140: 140 μg kg^{-1} $minute^{-1}$; A280: 280 μg kg^{-1} $minute^{-1}$) and 3 and 10 minutes after discontinuing adenosine

Variable	B	A140	A280	Time points		*p*
				3 minutes	10 minutes	
$PaCO_2$ (mmHg)	38 ± 3	39 ± 3	40 ± 2	40 ± 2	41 ± 3	0.036[a]
$PaCO_2$ (kPa)	5.0 ± 0.3	5.2 ± 0.4	5.3 ± 0.3	5.3 ± 0.2	5.4 ± 0.4	
PaO_2 (mmHg)	276 ± 11	277 ± 13	281 ± 14	282 ± 16	284 ± 21	0.499
PaO_2 (kPa)	36.7 ± 1.5	37.0 ± 1.7	37.5 ± 1.8	37.6 ± 2.2	37.8 ± 3.0	
pH	7.37 ± 0.03	7.37 ± 0.04	7.36 ± 0.04	7.36 ± 0.03[b]	7.35 ± 0.04[b]	0.001
tHB (g dL^{-1})	12.06 ± 0.64	11.95 ± 0.58	11.79 ± 0.61	11.98 ± 0.59	11.91 ± 0.65	0.129
FO_2Hb (%)	96.7 ± 0.3	96.6 ± 0.4	96.7 ± 0.3	96.3 ± 0.7	96.8 ± 0.5	0.135
Na^+ (mmol L^{-1})	141.5 ± 1.2	141.8 ± 1.2	141.6 ± 1.1	141.5 ± 1.0	141.4 ± 1.0	0.428
K^+ (mmol L^{-1})	3.94 ± 0.17	3.91 ± 0.25	3.83 ± 0.19	3.75 ± 0.16	3.77 ± 0.15[b]	0.033
Ca^{2+} (mmol L^{-1})	1.32 ± 0.03	1.30 ± 0.07	1.31 ± 0.04	1.33 ± 0.04	1.33 ± 0.05	0.265
Cl^- (mmol L^{-1})	111.1 ± 1.6	110.9 ± 1.8	111.2 ± 1.8	111.0 ± 1.6	111.2 ± 1.8	0.647
Glucose (mmol L^{-1})	5.61 ± 0.69	5.81 ± 0.38	5.91 ± 0.28	5.76 ± 0.38	5.81 ± 0.31	0.343
Lactate (mmol L^{-1})	1.82 ± 0.63	1.95 ± 0.66	2.14 ± 0.74[c]	1.93 ± 0.58	1.93 ± 0.59	0.034
F_E'Sevo (%)	3.41 ± 0.53	3.46 ± 0.52	3.49 ± 0.51	3.54 ± 0.48[b]	3.54 ± 0.50[b]	0.001

Values are presented as mean ± SD.
Ca^{2+}, ionized calcium; Cl^-, Chloride; F_E'Sevo, end-tidal sevoflurane; FO_2Hb, fraction of oxyhaemoglobin; K^+, potassium; Na^+, sodium; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; tHB, total haemoglobin.
[a]Significant changes over time ($p < 0.05$) with no significant differences detected in *post hoc* tests.
[b]Significantly different to B.
[c]Significant difference between A140 and A280.

CaO_2 ($p = 0.278$) and DO_2 ($p = 0.136$) did not change over time (Fig. 4).

Blood gases, electrolytes, lactate and glucose results are presented in Table 1. Statistically significant but probably clinically irrelevant changes over time were detected for individual parameters (pH, $PaCO_2$, F_E'Sevo, K^+). Lactate was never significantly different from baseline, although it was significantly higher with A280 than with A140 ($p_{adj} = 0.03$).

Discussion

Adenosine at the doses used, produced a decrease in arterial blood pressure due to vasodilatation rather than a decrease in CO. No differences between the two doses could be detected. All values returned to baseline within 3 minutes after discontinuing adenosine infusion.

A dose of 140 μg kg^{-1} $minute^{-1}$ adenosine is routinely used in human medicine to produce maximal vasodilatation for cMRT diagnostics (Wilson et al. 1990; Cerqueira et al. 1994), while lower doses have been shown to reduce requirements of isoflurane and improve postoperative analgesia (Segerdahl et al. 1995). In cats, a maximal dose of 280 μg kg^{-1} $minute^{-1}$ was suggested to achieve maximal hyperaemic myocardial blood flow response by Jenni et al. (2009). Maximal vasodilatation is important to quantify the coronary hyperaemic response to ischaemia and exercise (Layland et al. 2014). Until now, no recommendation exists regarding the dose rate of adenosine for cMRT diagnostics in dogs. Therefore, the dose rates selected for the present study were based on the aforementioned reports.

While no known research studies exist investigating time to steady state, clinical effects can be seen 10–20 seconds after starting administration and last for up to 20 seconds after a bolus of 100 μg kg^{-1} in humans (Wilbur & Marchlinski 1997). An onset time of less than 30 seconds after a single bolus (257 ± 36 μg kg^{-1}) and less than 3 minutes using a CRI (984 ± 225 μg kg^{-1} $minute^{-1}$) has been reported to reduce blood pressure by 45% in rabbits (Fukunaga et al. 1982). Based on the rapid onset time described in other species, an infusion period of 3 minutes was considered sufficient to achieve stable cardiovascular effects in the present study. Stabilization of MAP was observed clinically and could be confirmed retrospectively using statistics.

The observed decrease in blood pressure combined with a decrease in approximate SVR and no changes in SV_{MRT} and PR indicates vasodilatation. The

vasodilatatory effects of adenosine have been previously described in humans (Skrabanja et al. 2005) and in dogs (Lagerkranser et al. 1984). The degree of vasodilatation has been shown to be directly dose-dependent (Lagerkranser et al. 1984; Owall et al. 1987). However, in the present study no differences were detected between the two adenosine CRI doses. In contrast, Lagerkranser et al. (1984) reported lower MAP values than our results using higher doses of adenosine. However, comparison between different studies is problematic as the effect of adenosine on blood pressure could be influenced by external stimulation (e.g., surgery, noise, etc.), co-administered drugs, and might be different in conscious *versus* anaesthetized patients (Cobb et al. 1974; Edlund et al. 1990). Another reason for not detecting a difference between the two doses might have been insufficient statistical power or a relatively small difference between doses.

An adenosine-induced increase in SV and CO was detected in dogs (Lagerkranser et al. 1984) and humans (Skrabanja et al. 2005). In the present study, we only detected a tendency for SV_{MRT} to increase during adenosine CRI (Fig. 2) without reaching statistical significance. However, retrospective power calculation revealed only 63% of power to detect a difference. CO changed significantly over time, but no differences between individual time points were detected. These changes over time for CO could be the result of a parallel, although not significant, increase in SV_{MRT} and PR during adenosine CRI. The tendency to an increased SV_{MRT} is probably rather related to a decrease in afterload than to an increase in contractility. Adenosine has been shown to be negatively dromotropic (Wilbur & Marchlinski 1997), but the effects on the cardiac contractility are poorly investigated. However, some *in vitro* studies report a negative inotropic effect in dog and guinea pig atriae (Chiba & Himori 1975; Gesztelyi et al. 2013; Kiss et al. 2013). Based on our results, the two doses of adenosine investigated did not cause predictable changes in chronotropy. Some dogs showed an increase, while others showed no changes or even a decrease in PR during adenosine CRI. This corresponds with reports in humans and dogs which showed variable responses in heart rate independent of the dose, injection site anaesthesia administration (Cobb et al. 1974; Rembert et al. 1980; Lagerkranser et al. 1984; Owall et al. 1987; Edlund et al. 1990; Sidi & Rush 1992; Layland et al. 2014).

The influence of adenosine infusion on DO_2 has not been investigated earlier. In our dogs, CO and CaO_2 did not change between time points and infusion doses; therefore, DO_2 remained stable throughout the study period. Although changes in $PaCO_2$, pH, K^+, lactate and FE′Sevo were statistically significant, they were considered as clinically irrelevant.

All changes induced by adenosine returned to baseline within 3 minutes after discontinuing administration. A fast return to baseline was expected as adenosine is known to have a very short half-life (Klabunde 1983; Moser et al. 1989; Skrabanja et al. 2005). In humans, an adenosine half-life of 0.6−10 seconds has been described (Wilbur & Marchlinski 1997). The result of our study points towards a short half-life in dogs too; however, future pharmacokinetic studies are needed to confirm this assumption.

The main limitation of the study was the low number of animals and lack of a prospective sample size calculation. Following the three R principles, this study was a subproject of a planned imaging study, which limited the number of dogs to 10. Therefore, this study was underpowered to detect changes in some cardiovascular variables (SV_{MRT}) and maybe also differences between dosages.

cMRI was used to estimate SV_{MRT} (Dennler et al. 2017), which is not a commonly used method to measure CO but is reportedly comparable to thermodilution (Hockings et al. 2003; Senay et al. 2009). Still the estimation of SV_{MRT} with this method may have led to high SDs. The learning effect of the investigator as well as finding the perpendicular alignment of scan plane and flow direction are described to impact the variability of individual calculations (Muthurangu et al. 2012; Sargent et al. 2015). Nonetheless, in the present study, all data were calculated by a single, very experienced radiologist; we assume this impact to be negligible. The MRI-based technique was chosen for the present study because it is noninvasive and suitable during MRI. Furthermore, as we were interested in changes over time (between the two dose rates and the baseline) rather than in absolute values, we consider the method as appropriate for our study aim. Another limitation is that only healthy Beagle dogs were used, which will limit extrapolation to clinical cases.

In conclusion, adenosine, at the doses studied, produced a very short-lasting vasodilatation without changing CO and PR in healthy sevoflurane-anaesthetized dogs. Dose-dependent

effects could not be shown in the present study. The use of higher dosages to achieve larger decreases in blood pressure and also the use of adenosine in animals with heart disease requires further investigation.

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Authors' contributions

FBJ: study design, data acquisition, management and data interpretation and preparation of the manuscript. MD: data acquisition, management and data interpretation and review of the manuscript. CM: data acquisition and review of the manuscript. MM: data acquisition and preparation and review of the manuscript. HR: study design and review of the manuscript. SKR: study design, acquisition, management and data interpretation and preparation and review of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

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