



Original article

Effects of dexmedetomidine and MK-467 on plasma glucose, insulin and glucagon in a glibenclamide-induced canine hypoglycaemia model

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ABSTRACT

The commonly used sedative α_2 -adrenoceptor agonist dexmedetomidine has adverse cardiovascular effects in dogs that can be prevented by concomitant administration of the peripherally acting α_2 -adrenoceptor antagonist MK-467. An ancillary effect of dexmedetomidine is to decrease insulin release from the pancreas, whereas MK-467 stimulates insulin release. This study assessed the effects of co-administered dexmedetomidine and MK-467 in a canine glibenclamide-induced hypoglycaemia model. In a randomised, cross-over experiment, eight beagle dogs received five intravenous treatments, comprising two administrations of saline, with dexmedetomidine or dexmedetomidine and MK-467, and three administrations of glibenclamide, with saline, dexmedetomidine or dexmedetomidine and MK-467. Plasma concentrations of glucose, lactate, insulin, glucagon and the test drugs were monitored. Administration of glibenclamide significantly increased insulin secretion and decreased blood glucose concentrations. Dexmedetomidine counteracted glibenclamide-evoked hypoglycaemia. This was opposed by the α_2 -adrenoceptor antagonist MK-467, but the glibenclamide-evoked hypoglycaemia was not potentiated by co-administration of dexmedetomidine and MK-467. None of the dogs developed uncontrolled hypoglycaemia. Thus, the combination of dexmedetomidine and MK-467 appeared to be safe in this canine hypoglycaemia model. Nevertheless, when MK-467 is used to alleviate the undesired cardiovascular effects of α_2 -adrenoceptor agonists in dogs, it should be used with caution in animals at risk for hypoglycaemia because of its insulin-releasing and hypoglycaemic effects.

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Introduction

Dexmedetomidine is a selective agonist of α_2 -adrenoceptors, an important class of receptors for noradrenaline and adrenaline. In addition to their sedative effects, all drugs in this class depress the cardiovascular system by evoking vasoconstriction, followed by marked baroreflex-mediated bradycardia (Bloor et al., 1992; Flacke et al., 1993; Pypendop and Versteegen, 1998). Dexmedetomidine decreases insulin release from the pancreas (Burton et al., 1997) and increases plasma glucose concentrations in dogs (Benson et al., 2000; Ambrisko and Hikasa, 2002; Restitutti et al., 2012). As demonstrated in mice, α_2 -adrenoceptor agonists inhibit insulin secretion via activation of the α_{2A} -adrenoceptor subtype expressed by pancreatic β -cells (Peterhoff et al., 2003; Fagerholm et al., 2008). In addition, hypothermia and reduced glucose utilisation due to

sedation are likely to contribute to the hyperglycaemic effects of these drugs (Fagerholm et al., 2004).

MK-467 (vatinoxan; previously also known as L-659'066) mainly antagonises peripherally located α_2 -adrenoceptors due to its minimal ability to cross the blood-brain barrier, as demonstrated in rats and marmosets (Clineschmidt et al., 1988). In dogs, MK-467 alleviates the peripheral cardiovascular effects of dexmedetomidine, such as increased arterial blood pressure and bradycardia (Pagel et al., 1998; Enouri et al., 2008; Honkavaara et al., 2011; Rolfe et al., 2012). Co-administration of MK-467 also prevents dexmedetomidine-induced changes in plasma insulin, glucose and lactate concentrations in healthy dogs (Restitutti et al., 2012).

In mice, genetic absence of α_{2A} -adrenoceptors or pharmacological blockade of all α_2 -adrenoceptor subtypes with subtype non-selective antagonists leads to severe hypoglycaemia when insulin release had been stimulated with the clinically used antidiabetic drug glibenclamide (Fagerholm et al., 2008). Similar potentiation of glibenclamide-evoked insulin release and

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hypoglycaemia was also observed following treatment with MK-467 (Ruohonen et al., 2015). These observations have led to concerns about the safety of MK-467 in animals with hyperinsulinaemia and hypoglycaemia.

The aim of the present study was to determine the effects of the combination of dexmedetomidine and MK-467 in a canine glibenclamide-induced hypoglycaemia model to predict its safety in hypoglycaemic dogs undergoing sedation. Ethically, it was not considered to be acceptable to investigate the effects of co-administered dexmedetomidine and MK-467 in a clinical veterinary setting in dogs with spontaneous hypoglycaemia. Instead, a pharmacological hypoglycaemia model was employed in which glibenclamide was used at a dose known to increase insulin secretion and induce hypoglycaemia in dogs (Lorrain et al., 1992; Okamoto et al., 2002).

Materials and methods

Experimental design

The experiment was conducted as a five period, randomised, cross-over study with a 14 day wash-out between treatments (Table 1). The experimental protocol was approved by the National Animal Experiment Board of Finland (approval number ESAVI/355/2015; date of approval 17th February 2015). Eight purpose bred beagle dogs (six castrated males and two spayed females; mean weight \pm standard deviation 14.4 \pm 1.6 kg) were housed and handled according to European Union (EU) guidelines (Directive 2010/63/EU and Annex III) in groups in indoor pens, with access to outdoor runs. All study sessions commenced before noon. Prior to each session, food was withheld for 12 h, but water was freely available.

Treatments

At the beginning of each session, a 22 G catheter was inserted into a cephalic vein and a 16 G single lumen central venous catheter was inserted into a jugular vein of each dog. Thirty minutes before administration of the test treatment (time point –30 min), i.e. dexmedetomidine (DEX), dexmedetomidine and MK-467 (DEX-MK) or saline, the dogs received either 1 mg/kg of glibenclamide (TRC) or an equivalent volume of saline intravenously (IV) (Table 1). Glibenclamide powder was dissolved in dimethyl sulphoxide (20 mg/mL) and then mixed with saline to a total volume of 5 mL before administration.

At time point 0, the dogs received either DEX, DEX-MK or an equivalent volume of saline. MK-467 (Vetcare) and dexmedetomidine (Dexdomitor 0.5 mg/mL, Orion) were drawn into a single syringe, diluted to a total volume of 1 mL with saline and administered over 10 s (time point 0). Blood samples were collected into lithium heparin syringes (PICO 50, Radiometer) immediately prior to administration of each drug and then at 30, 60, 120, 240 and 360 min after treatment to determine plasma glucose and lactate concentrations. Additional 4 mL blood samples were collected into ethylene diamine tetraacetic acid (EDTA) aprotinin tubes (Vacuette, Greiner Bio-One) for analysis of plasma drug concentrations and glucagon and insulin.

The dogs were monitored for adverse effects during and after the treatments. Sedation was assessed subjectively using a composite sedation score (CSS) (Kuusela et al., 2000; Honkavaara et al., 2008). According to the study protocol, plasma glucose concentrations <3 mmol/L led to monitoring of plasma glucose at 15 min intervals, while concentrations <1.5 mmol/L triggered an IV infusion of 5% glucose solution. Rectal temperature was recorded at 30 min intervals until 360 min. Dogs were passively insulated with mattresses and blankets. After completion of each experimental session and removal of the catheter, the dogs were fed and subsequently given 0.2 mg/kg meloxicam subcutaneously (SC) for pain relief.

Biochemical analysis

Plasma glucose and lactate concentrations were determined within 30 min of collection using a blood gas analyser (ABL800 Flex, Radiometer). EDTA-treated

samples were chilled in ice water and the plasma was separated by refrigerated centrifugation. The plasma was divided into 500 μ L aliquots within 30 min after blood sampling and frozen at -80°C . Concentrations of glucagon and insulin in plasma were determined with validated immunoassays (AlphaLISA, Perkin-Elmer). Lower limits of quantitation were 3 pg/mL for glucagon and 20 pg/mL for insulin.

Concentrations of the test drugs in plasma were analysed using reversed phase (C_{18}) high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The analytical methods were validated for range, precision, accuracy, carry-over, interference of analytes and internal standards, analyte stability and matrix effects. For MK-467, plasma aliquots of 100 μ L were treated with acetonitrile to precipitate proteins and propranolol was used as an internal standard. Reference standards and quality control (QC) samples were prepared in blank dog plasma. The column temperature was 40°C and the injection volume was 4 μ L. Gradient elution was performed with 0.5% formic acid in water and acetonitrile employed at 2–2–90–90% acetonitrile in 0–1–2.5–3 min. The eluent flow rate was 0.5 mL/min. Positive ionisation mode was used with capillary voltage set at 1000 V. The linear assay range was 25–460 ng/mL. The inter-assay accuracy of the QC samples (at 70, 250 and 380 ng/mL) ranged from 98.9 to 103.0%.

Concentrations of dexmedetomidine were determined as previously described (Snapir et al., 2006); diphenylimidazole was used as the internal standard. The linear range was 0.03–5 ng/mL. The inter-assay accuracy of QC samples (at 0.08, 1.5 and 4.33 ng/mL) ranged from 99.8 to 106.9%.

Concentrations of glibenclamide were determined after solid-phase extraction (Sep-Pak tC_{18} , Waters Corporation) and lorazepam was used as internal standard. A gradient solvent system (0.1% formic acid in water and acetonitrile) was used for separation and quantitative detection was performed in multiple reaction monitoring mode. The linear assay range was from 0.05 to 5.0 μ g/mL. The inter-assay accuracy of the QC samples (at 0.15, 1.0 and 4.0 μ g/mL) ranged from 93.8 to 108.5%.

Statistical analysis

Statistical analysis was performed by 4Pharma Ltd., Turku, Finland, employing SAS System for Windows, version 9.3 (SAS Institute). Treatment differences were evaluated using change from baseline as the response variable. Baseline was defined as the –30 min measurement value. Differences between treatments were evaluated with repeated measures analysis of covariance (RMANCOVA) models. The models included treatment, time point, treatment time point and the baseline covariate as fixed effects. Dog within treatment was included as the random subject effect. Correlations were modelled using compound symmetry covariance structures. Treatment differences in changes from baseline were estimated with 95% confidence intervals and two-sided *P* values for predefined comparisons (including comparisons over the treatment periods and comparisons by time point). Changes from baseline were also evaluated. Model residuals were checked visually and with the Shapiro–Wilk test to assess the model assumptions. If model assumptions were in doubt, then standard transformations (logarithm, square root) were employed to improve the fit.

The relationships between changes in glucagon versus insulin and glucose were analysed with linear mixed effects regression models. The change from baseline in glucagon was used as the response variable. The change in insulin or glucose and the time point were used as fixed effects and dog as the random subject effect. Estimates were calculated from the models using contrasts. When reporting analysis results with a transformed response variable (insulin), the point estimates and treatment differences were calculated using the log-transformed values. The treatments were also compared by using independent samples *t* tests, as integrated areas under the concentration curves (AUCs) calculated with the trapezoidal method for drug concentrations in plasma. *P* values < 0.05 were considered to be statistically significant. *P* values were not adjusted for multiple testing.

Results

Plasma glucose concentrations were markedly reduced following administration of glibenclamide to dogs, with average reductions of 1.1 mmol/L by 30 min after treatment (Fig. 1). A mean nadir of 2.5 mmol/L was observed 120 min after

Table 1
Experimental design, treatments and doses; all drugs were administered intravenously.

Treatment	Drugs administered	
	Time point –30 min	30 min later (time point 0)
DEX	Saline 1 mL	Dexmedetomidine HCl (5 μ g/kg)
DEX-MK	Saline 1 mL	Dexmedetomidine HCl (5 μ g/kg) + MK-467 (250 μ g/kg)
GLIB	Glibenclamide (1 mg/kg)	Saline 1 mL
GLIB-MK	Glibenclamide (1 mg/kg)	Dexmedetomidine HCl (5 μ g/kg)
GLIB-DEX-MK	Glibenclamide (1 mg/kg)	Dexmedetomidine HCl (5 μ g/kg) + MK-467 (250 μ g/kg)

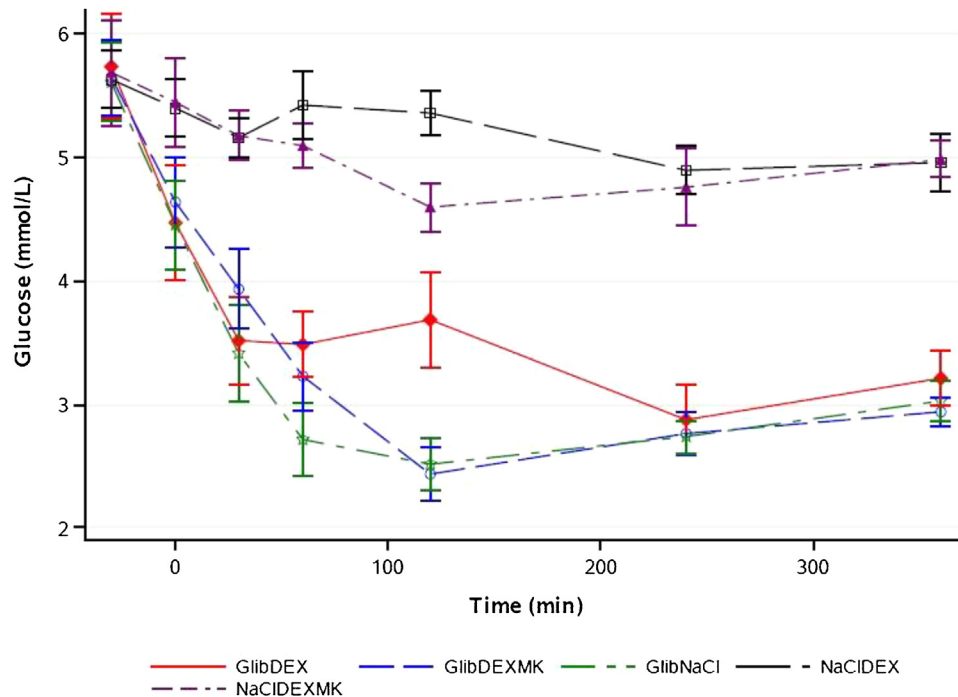


Fig. 1. Mean concentrations of glucose in plasma (mmol/L \pm standard deviation, SD) by time point after administration of: (1) saline; 30 min later dexmedetomidine (5 μ g/kg IV) (DEX); (2) saline; 30 min later dexmedetomidine + MK-467 (250 μ g/kg IV) (DEX-MK); (3) glibenclamide (1 mg/kg); 30 min later saline (GLIB); (4) glibenclamide; 30 min later dexmedetomidine (GLIB-DEX); and (5) glibenclamide; 30 min later dexmedetomidine + MK-467 (GLIB-DEX-MK). Saline or glibenclamide was given at time point -30 min. Saline, DEX or DEX-MK were given at time point 0. All eight dogs received each treatment.

administration of glibenclamide as the only drug (GLIB). When dexmedetomidine was administered to dogs that had received glibenclamide (GLIB-DEX), the hypoglycaemic effect of glibenclamide was attenuated, but not totally reversed. Whilst co-administration of MK-467 blocked this anti-hypoglycaemic effect of dexmedetomidine, glucose responses were not different after glibenclamide as the sole drug (GLIB) and glibenclamide followed by the sedative combination (GLIB-DEX-MK). Plasma glucose levels remained relatively stable after DEX and DEX-MK. Intravenous glucose administration for the treatment of hypoglycaemia was not required in any dog. A change in behaviour was detected when blood glucose concentrations were decreasing; dogs were spontaneously less active, showed some signs of nausea and were less interactive with the investigators. No other significant adverse effects were observed during the experiments. Lactate concentrations in plasma remained in the range 0.3–1.6 mmol/L in all dogs after all treatments (i.e. within the reference range of 0.3–2.5 mmol/L for adult healthy dogs; Hughes et al., 1999).

The changes in plasma glucose concentrations were mirrored by those in plasma insulin concentrations; by 30 min after the administration of glibenclamide, insulin concentrations in plasma were more than doubled. After administration of glibenclamide as the sole drug (GLIB), plasma insulin concentrations remained elevated, but when dexmedetomidine was administered after glibenclamide (GLIB-DEX and GLIB-DEX-MK), concentrations of insulin returned to baseline by 30 min (Fig. 2). When MK-467 was co-administered with dexmedetomidine, concentrations of insulin again were markedly increased at time points 60 and 120 min if the sedative combination was preceded by glibenclamide (GLIB-DEX-MK). In dogs pre-treated with saline, plasma insulin concentrations were significantly higher at 60 and 120 min with DEX-MK compared with DEX. Blood glucagon concentrations after administration of the test drugs did not differ significantly between the treatments or from baseline values (Fig. 3).

Changes in insulin and glucagon concentrations were associated; a 100 U increase in insulin concentration from the baseline value increased the glucagon concentration from baseline by 0.57 U ($P < 0.05$). There was no significant association between changes in glucagon and glucose concentrations.

The AUC of dexmedetomidine (DEX versus DEX-MK and GLIB-DEX versus GLIB-DEX-MK, respectively) was significantly smaller when dexmedetomidine was co-administered with MK-467 (Table 2). Exposure to glibenclamide was reduced by dexmedetomidine, but not by co-administration of dexmedetomidine and MK-467 (Table 2).

Discussion

The main finding of this study is that glibenclamide-induced hypoglycaemia was not exacerbated by MK-467 co-administered with the sedative agent dexmedetomidine. Dexmedetomidine counteracted glibenclamide-induced hypoglycaemia and hyperinsulinaemia, while the α_2 -adrenoceptor antagonist MK-467 prevented this effect of dexmedetomidine.

Whilst another test treatment with glibenclamide followed by saline and MK-467 might have been informative to monitor the expected synergistic actions of MK-467 and glibenclamide (Ruohonen et al., 2015), we did not include such a treatment in this study because it could have resulted in severe and potentially lethal hypoglycaemia, as reported in mice (Khosravi et al., 2006; Fagerholm et al., 2008; Ruohonen et al., 2015). Moreover, in the clinical setting, MK-467 would always be co-administered with an α_2 -adrenoceptor agonist for dogs undergoing sedation; hence, treatment with MK-467 without dexmedetomidine would not have clinical relevance.

Insulin concentrations in plasma peaked at 120 min after the GLIB + DEX-MK treatment, when both MK-467 and glibenclamide probably continued to stimulate insulin release from pancreatic

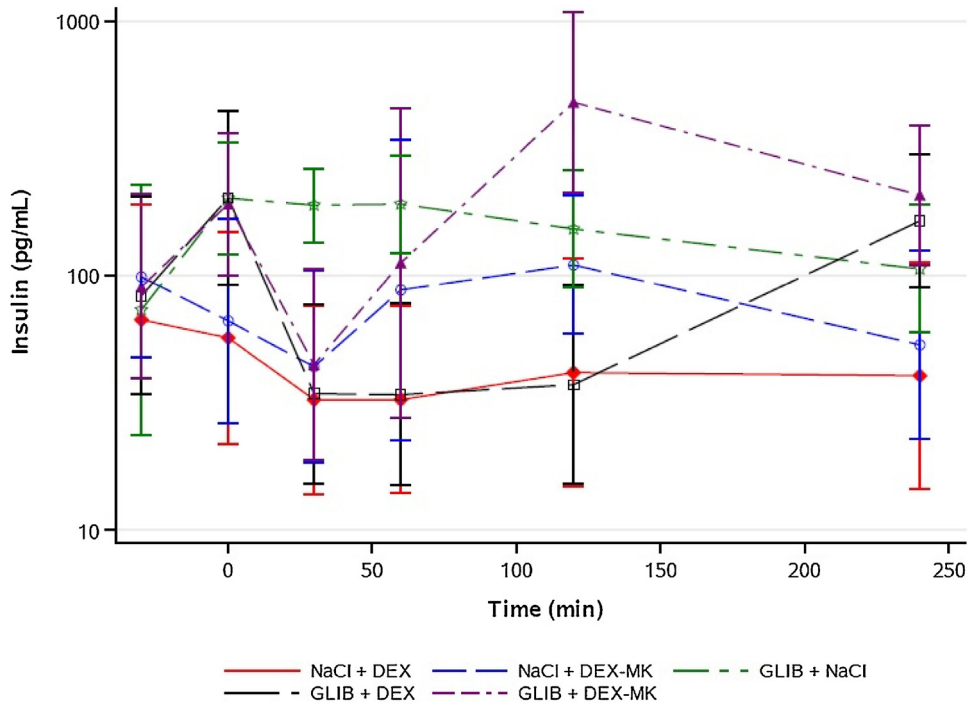


Fig. 2. Geometric mean concentrations of insulin in plasma (pg/mL ± geometric mean/geometric standard deviation, SD; geometric mean * geometric SD) by time point after administration of: (1) saline; 30 min later dexmedetomidine (5 µg/kg IV) (DEX); (2) saline; 30 min later dexmedetomidine + MK-467 (250 µg/kg IV) (DEX-MK); (3) glibenclamide (1 mg/kg); 30 min later saline (GLIB); (4) glibenclamide; 30 min later dexmedetomidine (GLIB-DEX); and (5) glibenclamide; 30 min later dexmedetomidine + MK-467 (GLIB-DEX-MK). Saline or glibenclamide was given at time point -30 min. Saline, DEX or DEX-MK was given at time point 0. All eight dogs received each treatment.

islet cells, while the inhibitory effect of dexmedetomidine was already subsiding. Nevertheless, blood glucose concentrations were maintained at similar levels after GLIB-DEX and GLIB-DEX-MK. This was probably due to dynamic mechanisms maintaining

glucose homeostasis and protecting against hypoglycaemia. It is possible that the concentration of MK-467 in plasma related to dexmedetomidine was not optimal to prevent the effects on insulin and glucose. The dose of MK-467 we selected was clinically

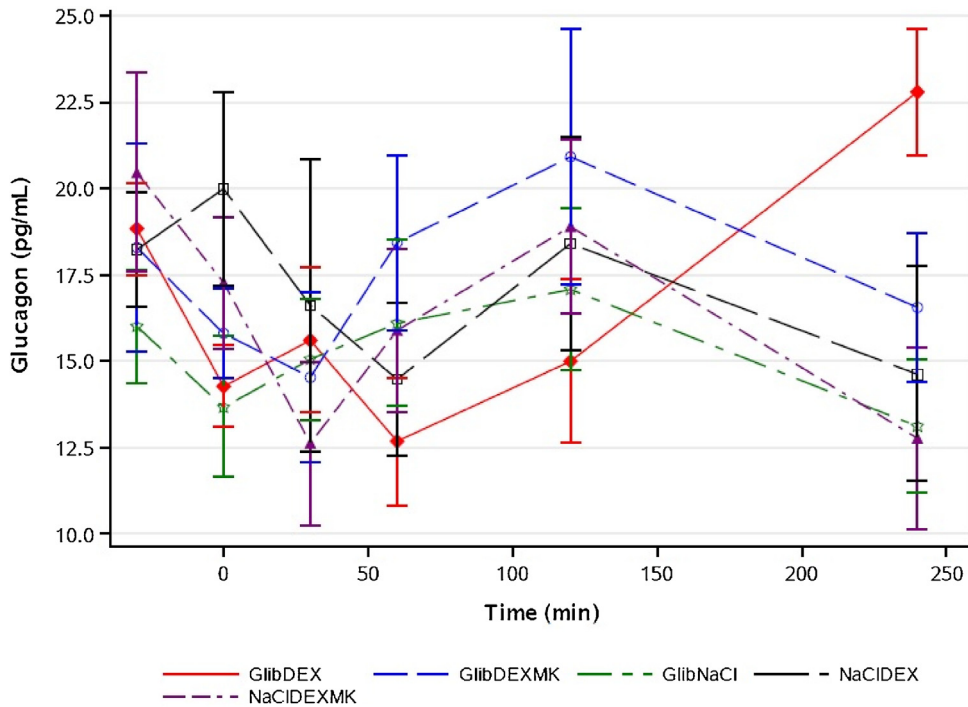


Fig. 3. Mean concentrations of glucagon in plasma (pg/mL ± standard deviation, SD) by time point after administration of: (1) saline; 30 min later dexmedetomidine (5 µg/kg IV) (DEX); (2) saline; 30 min later dexmedetomidine + MK-467 (250 µg/kg IV) (DEX-MK); (3) glibenclamide (1 mg/kg); 30 min later saline (GLIB); (4) glibenclamide; 30 min later dexmedetomidine (GLIB-DEX); and (5) glibenclamide; 30 min later dexmedetomidine + MK-467 (GLIB-DEX-MK). Saline or glibenclamide was given at time point -30 min. Saline, DEX or DEX-MK was given at time point 0. All eight dogs received each treatment.

Table 2

Areas under the concentration-time curves (AUCs) of dexmedetomidine, glibenclamide and MK-467 (expressed as mean \pm standard deviation, ng/mL * min).

Treatment	Dexmedetomidine	Glibenclamide	MK-467
DEX	205 \pm 44 ^a ($P < 0.001$)		
DEX-MK	89 \pm 32		89,500 \pm 42,500
GLIB		867 \pm 173 ^c ($P = 0.002$)	
GLIB-DEX	245 \pm 51 ^b ($P = 0.001$)	580 \pm 171	
GLIB-DEX-MK	137 \pm 40	825 \pm 171 ^b ($P = 0.008$)	84,100 \pm 50,400

^a Significant difference between DEX and DEX-MK ($P < 0.001$).

^b Significant difference between GLIB-DEX and GLIB-DEX-MK.

^c Significant difference between GLIB and GLIB-DEX.

relevant, because the associated MK-467:dexmedetomidine dose ratio provided the best protection of cardiovascular function in healthy dogs (Honkavaara et al. 2011).

Regulation of glucose homeostasis is mediated by hormones, such as glucagon, adrenaline, cortisol and growth hormone, which are released when glucose concentrations in the blood decrease (Schwartz et al., 1987; Cryer, 2001, 2011). We only monitored glucagon levels in plasma; these were not significantly influenced by the treatments. Glucagon is a counter-regulatory hormone secreted by α cells of the islets of Langerhans, increasing plasma glucose concentrations by stimulating glycogenolysis and gluconeogenesis (Cryer, 2001). Increases in blood glucose suppress glucagon release; conversely, hypoglycaemia stimulates glucagon release (Unger et al., 1962). Hypoglycaemia can evoke almost three-fold increases in hepatic glucose production in dogs (Rivera et al., 2010). Our results revealed a statistically significant positive association between changes in plasma insulin and glucagon concentrations. Whilst the exact mechanisms of maintaining blood glucose homeostasis in our dogs after the combined administration of glibenclamide, dexmedetomidine and MK-467 remain uncertain, glucagon still may have an important role.

As expected, administration of MK-467 reduced the plasma exposure of the dogs to dexmedetomidine and glibenclamide, as demonstrated by AUC comparisons. Co-administration of MK-467 approximately halved the AUC of dexmedetomidine, probably by increasing dexmedetomidine hepatic clearance secondary to increased liver blood flow (Honkavaara et al., 2012; Bennett et al., 2016). Preserved cardiac output and liver blood flow may also explain why the plasma concentrations of glibenclamide were higher after GLIB alone and GLIB-DEX-MK compared to GLIB-DEX. These changes in the tissue distribution of dexmedetomidine and glibenclamide may have affected the severity of hypoglycaemia at least to some extent, because the magnitude of their influence on insulin release and blood glucose concentrations may be concentration dependent.

Conclusions

Potential of glibenclamide-evoked hypoglycaemia was not observed when the peripherally acting α_2 -adrenoceptor antagonist MK-467 was co-administered with the veterinary sedative agent dexmedetomidine. Insulin release was to some extent augmented in dogs that received the sedative drug combination after glibenclamide, but this did not result in overt hypoglycaemia. Co-administration of MK-467 appears to be safe in this glibenclamide-induced canine hypoglycaemia model. Nevertheless, MK-467 should be used with caution in dogs at risk of hypoglycaemia because of its insulin-releasing effect.

Conflict of interest statement

Professor Scheinin has been engaged in contract research for Orion Pharma, the manufacturer of dexmedetomidine, and for Vetcare, the developer of MK-467. None of the other authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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