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The effects of an alpha-2-adrenoceptor agonist, antagonist, and their combination on the blood insulin, glucose, and glucagon concentrations in insulin sensitive and dysregulated horses

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ABSTRACT

Alpha-2-adrenoceptor agonists are sedatives that can cause fluctuations in serum insulin and blood glucose (BG) concentrations in horses. The objectives of this study were to investigate the effects of detomidine and vatinoxan on BG, insulin, and glucagon concentrations in horses with and without insulin dysregulation (ID). In a blinded cross-over design, eight horses with ID and eight horses without ID were assigned to each of four treatments: detomidine (0.02 mg/kg; DET), vatinoxan (0.2 mg/kg; VAT), detomidine + vatinoxan (DET + VAT), and saline control (SAL). Blood samples were taken at 0, 1, 2, 4, 6, and 8 h. Change from baseline was used as the response in modelling, and the differences between treatments were evaluated with repeated measures analysis of covariance. P values $\leq$0.05 were considered significant.

Comparing DET vs. SAL and DET vs. DET + VAT, insulin was higher at 2 h in the non-ID group and 2 and 4 h in the ID group. There was no difference in insulin between SAL and DET + VAT or VAT. Comparing DET vs. SAL, BG was higher at 1 and 2 h then was lower at 4 h in both ID and non-ID groups. At 1 h in both groups, BG after DET + VAT was lower than after DET but higher than after SAL. Comparing DET vs. SAL, glucagon was lower at 1 h in the ID group and 1 and 2 h in the non-ID group. Vatinoxan was effective in preventing detomidine-induced hyperglycaemia as well as the subsequent insulin increase in horses with ID.

Introduction

An increase in plasma glucose concentration is induced by all α_2-adrenoceptor agonists commonly used for sedation in horses, i.e. xylazine (Ringer et al., 2013; Kullmann et al., 2014; Kritchevsky et al., 2020), detomidine (Kullmann et al., 2014; Tapio et al., 2018; Kritchevsky et al., 2020), romifidine (Ringer et al., 2013; Kullmann et al., 2014; Pakkanen et al., 2018) and medetomidine (Grimsrud et al., 2012; Tapio et al., 2019). The increase is mediated by activation of α_2-adrenoceptors in pancreatic beta cells of the islets of Langerhans, which inhibits insulin release (Angel et al., 1988). The effects of α_2-adrenoceptor agonists on plasma insulin concentration have also been described in horses (Kritchevsky et al., 2020). Furthermore, in horses with insulin dysregulation (ID), after administration of xylazine, the initial decrease in insulin concentration was followed by a marked increase, and the hyperglycaemia was prolonged compared to control horses without ID (Kritchevsky et al., 2020).

Vatinoxan (formerly MK-467 or L-659,066) is an α_2-adrenoceptor antagonist that minimally crosses the mammalian blood-brain barrier (Clineschmidt et al., 1988; Honkavaara et al., 2020), so its actions are predominantly on peripheral receptors (Clineschmidt et al., 1988). Thus, the centrally mediated sedative effects of α_2-adrenoceptor agonists are not markedly affected (Vainionpää et al., 2013; De Vries et al., 2016; Tapio et al., 2018, 2019), but peripheral vasoconstriction and concomitant cardiovascular changes are alleviated. This has been demonstrated in many species including horses (De Vries et al., 2016; Tapio et al., 2018, 2019). Vatinoxan also alleviates the glucose response to α_2-agonists in horses (Pakkanen et al., 2018; Tapio et al., 2018, 2019). In dogs, vatinoxan prevented both the dexmedetomidine-induced decrease in plasma insulin concentration and increase in glucose (Restitutti et al., 2012). However, one study could not verify the effects of vatinoxan on plasma insulin concentration in horses, likely due to most of the results being below the quantification limit of the assay used (Pakkanen et al., 2018).

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Activation of both α_2- and β-adrenoceptors stimulate the release of glucagon by the alpha cells of the islets of Langerhans (Hirose et al., 1993). Glucagon increases plasma glucose concentration by stimulating glycogenolysis and gluconeogenesis and it is inhibited by elevated blood glucose (BG) concentrations (Iben Rix et al., 2019). In the available literature, no studies on the effects of α_2-adrenoceptor agonists or antagonists on plasma glucagon concentration in horses could be found, but in dogs, neither dexmedetomidine nor vatinoxan affected it significantly (Kallio-Kujala et al., 2018). Since it has been speculated that glucagon production might be downregulated as a compensatory response to hyperinsulinaemia in horses with ID (Newkirk et al., 2018), circulating glucagon concentrations after administration of an α_2-adrenoceptor agonist and/or antagonist were of interest to the investigators.

The objectives of this study were to investigate the effects of an α_2-adrenoceptor agonist (detomidine) and peripherally acting antagonist (vatinoxan) on plasma glucose, insulin, and glucagon concentrations in horses with normal and reduced insulin sensitivity. It was hypothesised that vatinoxan would alleviate the detomidine-induced alterations in blood glucose, insulin, and glucagon concentrations but would not markedly affect them when administered alone.

Materials and methods

Animals and determination of insulin sensitivity

The study protocol was approved by the National Animal Experimentation Board of Finland (Approval number, ESAVI/6728/04; Approval date, 10 July 2017).

Sixteen (5 geldings and 11 non-pregnant mares) adult Finnhorses 11.7 ± 4 years old (mean ± standard deviation) owned by the Natural Resources Institute Finland or Equine College (Ypäjä, Finland) were selected for the study based on three preceding oral sugar tests (OST) conducted by the authors. Briefly, 29 horses were fasted overnight then given 0.45 mL/kg Karo Light corn syrup orally. Serum insulin concentration was measured before syrup and at 60, 90, and 120 min after administration. Horses were tested on three separate occasions (once each in June, August, and October 2018). For this study, horses with insulin concentrations ≥40 μIU/mL (Immulite 1000) at 60, 90, 120, or all time points on at least one occasion were classified as ID (Durham et al., 2019). From the 29 horses, eight horses with low insulin response (0–26.3 μIU/mL) and eight horses with ID were included in the experiment. Furthermore, horses were considered healthy based on physical exam findings, a haematological test, and a serum biochemical profile.

None of the horses had clinical signs consistent with pituitary pars intermedia dysfunction. Plasma ACTH concentration of each horse was measured three times (June, August, October) before the experiments using a chemiluminescent immunoassay (Immulite 1000) and all values were within the seasonally adjusted reference range. The seasonally adjusted ACTH cut-off concentrations used for the study were 44.5 pg/mL in June and 89.4 pg/mL in August and October (Adams et al., 2017).

Test procedure

All horses were fasted overnight and throughout the entire experiment but had access to water. Each horse was assigned to four treatments: saline control (SAL), detomidine hydrochloride (HCl) (Equisedan 10 mg/mL, Vetcare Ltd.) (0.02 mg/kg) (DET), vatinoxan HCl (Vetcare Ltd.) (0.2 mg/kg) (VAT), and a combination of the same doses of detomidine HCl and vatinoxan HCl (DET + VAT) by means of a blinded cross-over design with a minimum 13 day washout period between treatments. Horses' weights were estimated using an equine weight tape (Virbac UK Ltd.). Vatinoxan HCl powder was dissolved in saline to a concentration of 10 mg/mL. Detomidine and vatinoxan, or the same volume of saline, were mixed in the same syringe immediately before administration. Each treatment was given as a single bolus via 18 G 1.3 × 32 mm IV catheter (Terumo Europe N.V.) after which the catheter was removed.

Blood sampling

Blood samples to determine blood glucose (BG), plasma glucagon, and serum insulin were taken before treatment and at 1, 2, 4, 6 and 8 h thereafter. Blood glucose concentration was determined from lithium-heparin blood (Vacuette LH, Greiner Bio-One) immediately after sampling using a handheld glucometer (AlphaTRAK II, Zoetis). Blood for glucagon concentration measurement was collected in 9 mL K_2EDTA tubes (Vacuette K_2EDTA, Greiner Bio-One) and kept cool until centrifugation (within 3 h). Blood for insulin concentration measurement was collected into 6 mL serum tubes (Vacuette, Z serum clot activator, Greiner Bio-One) and was allowed to clot at room temperature for at least 60 min. All plasma and serum samples were centrifuged and separated within 4 h then stored at −80 °C until analysis was performed.

Sedation scoring

All horses were evaluated at 0, 0.5, 1, 2, 4, 6, and 8 h for sedation by one of two investigators (JRB or HET) who were blinded to the treatments. Attitude, standing ability, head position, eye openness, and ear position were scored then a total (0–10) given at each time point (Rohrbach et al., 2009). A score of zero represented a fully alert horse and a ten represented a deeply sedated but still standing horse.

Laboratory analysis

Insulin was analysed with an HI-14 K radioimmunoassay (Merck Millipore, Missouri, USA) that has been validated for use in horses (Tinworth et al., 2011). Glucagon was analysed with a GL-32 K radioimmunoassay (EMD Millipore), which is not validated for use in horses according to the manufacturer. The coefficient of variation of the solid controls varied between 1.1–10.3 % within day and 1.8–6.9 % between days. The sensitivity of assay, as reported by manufacturer, was 18.5 ± 2 pg/mL. All samples in both assays were measured in duplicate.

Statistical analysis

All statistical analyses were done using SAS System for Windows, version 9.4 (SAS Institute). Normality assumptions for all responses were checked with Kolmogorov-Smirnov tests. For insulin and BG, normality could not be verified initially. A logarithmic transformation was used on insulin values, and normality was deemed adequate based on graphical inspection, skewness, and kurtosis values. For BG, no transformation was found to normalise the distribution. The kurtosis value was slightly larger than in normal distribution, but normality was considered adequate for parametric analysis.

Pre-treatment measurement was used as a baseline, and the change was used as the response in modelling. The differences between treatments were evaluated with repeated measures analysis of covariance (RM ANCOVA) models. The model included a baseline covariate, the main effects (treatment, time point, ID status and period), two-way-interactions (treatment*timepoint, treatment*ID-status, ID-status*timepoint, period*timepoint), and three-way-interactions (treatment*ID-status*timepoint) as fixed effects. Additionally, the main effect of horse, and two-way interactions of horse*timepoint and horse*period as random effects were modelled. To verify the RM ANCOVA results of BG, Wilcoxon signed rank sum test for treatment differences in change from baseline in BG by timepoint and ID status was used.

The estimates of selected treatment effects by ID status were calculated over time and by time point from the fitted analysis model with contrasts. Only treatment comparisons to SAL and between DET and DET + VAT were of interest and selected to be calculated. For the estimates, 95 % confidence intervals and P values were calculated. $P < 0.05$ were considered statistically significant.

Results

Insulin

The mean serum insulin concentrations in response to each of the four treatments are presented in Fig. 1a,b. The treatment effect ($P = 0.020$) and the interaction of time point and treatment ($P < 0.0001$) were both significant. No significant difference was detected between the ID and non-ID groups. Fig. 2a illustrates the individual variation in insulin concentrations after DET. Neither DET + VAT nor VAT differed significantly in insulin concentration from SAL at any time point in either group.

Blood glucose

Blood glucose concentrations in response to each of the four treatments are shown in Fig. 1c,d. Again, the treatment effect ($P < 0.0001$) and the interaction of time point and treatment ($P < 0.0001$) were both found to be significant and no significant difference between groups was detected. The increase in BG was less in both groups when comparing DET + VAT vs. DET at 1 and 2 h ($P < 0.0001$). Four horses (2 in each group) at 4 h and one horse with ID had BG values < 3.5 mmol/L at 6 h (confirmed with a duplicate test). Fig. 2b illustrates the individual variation in BG concentrations after DET.

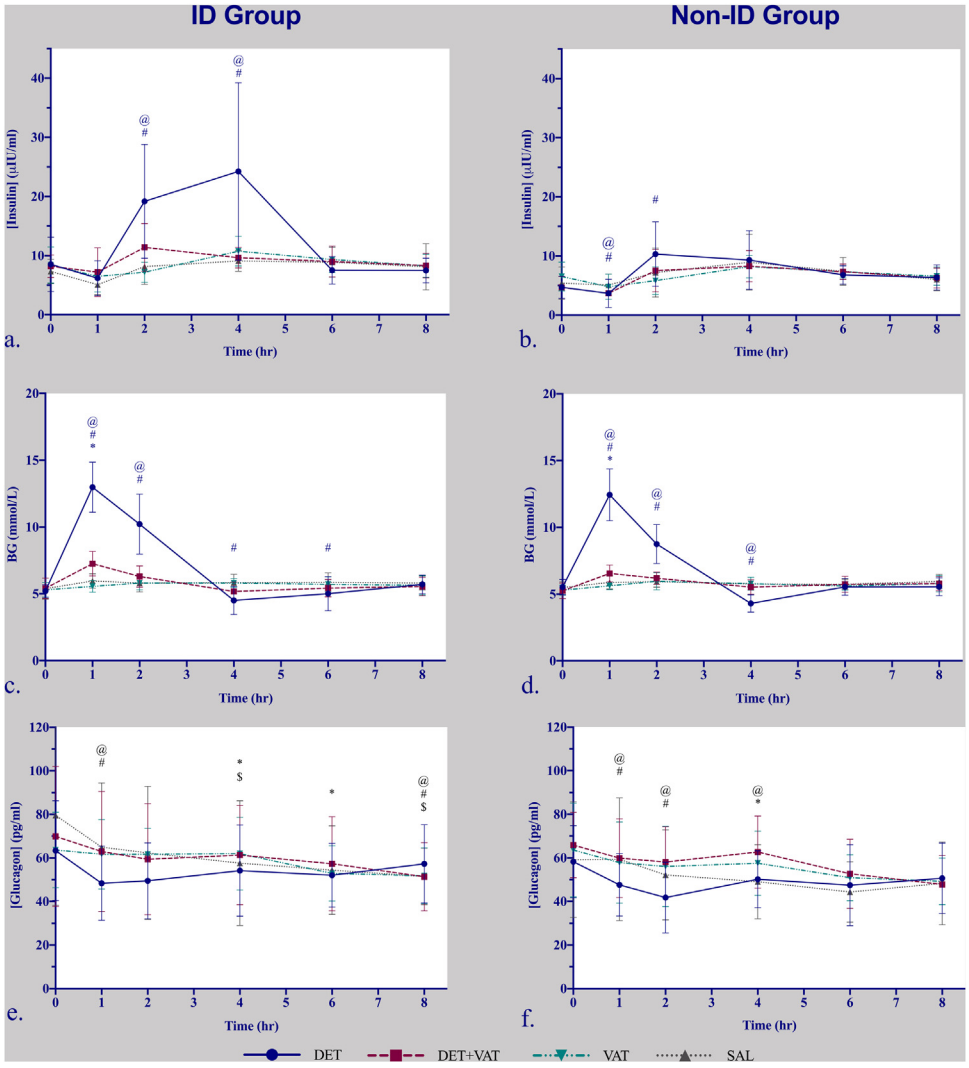

Fig. 1. Mean ± standard deviation insulin (a,b), blood glucose (BG) (c,d), and glucagon (e,f) concentrations in horses with (left column, n = 8) and without (right column, n = 8) insulin dysregulation (ID) after each treatment. Significant differences ($P \leq 0.05$) in the change from baseline within each timepoint and compared to saline control (SAL) are indicated by the symbols # for detomidine (DET), * for detomidine + vatinoxan (DET + VAT), and $ for vatinoxan (VAT). Significant differences between DET and DET + VAT are indicated by @.

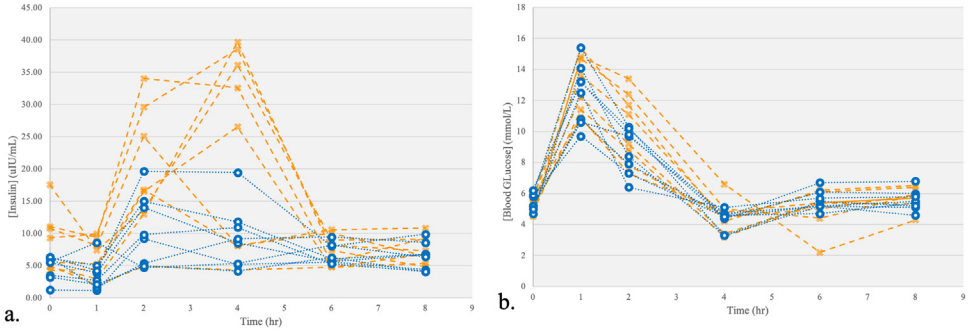

Fig. 2. Individual horse (n = 16) insulin (a) and blood glucose (b) response after detomidine (DET) treatment demonstrating interindividual variability in horses with insulin dysregulation (ID; –x–) and without (non-ID; . . . o . . .).

Glucagon

Plasma glucagon concentrations in response to each of the four treatments are shown in Fig. 1e,f. No significant difference between groups was detected, but treatment effect ($P = 0.018$) and the interaction of time point and treatment ($P < 0.0001$) were both found to be significant. Glucagon was higher after DET + VAT than after DET from 1–4 h in the non-ID group ($P \leq 0.03$) and at 1 h in the ID group ($P = 0.005$).

Sedation scoring

At 0.5 h after DET and DET + VAT, median (range) sedation score was 6 (4–7) and 6 (3–7), respectively. Descriptive data are available (see Appendix A: Supplementary material).

Discussion

This study demonstrated that the α_2-adrenoceptor antagonist vatinoxan can alleviate the detomidine-induced hyperglycaemia in horses with and without ID, and it can prevent the subsequent insulin increase in horses with ID. Furthermore, when vatinoxan was given alone, it did not significantly alter insulin or BG when compared to SAL. Lastly, vatinoxan was able to prevent the reduction in plasma glucagon concentrations detected after DET.

In this study, insulin was initially decreased after DET, and a concurrent hyperglycaemia was detected. Although significance could only be detected in the non-ID group at 1 h, the insulin nadir may have occurred prior to the 1 h sample. It is also possible that the delayed insulin clearance demonstrated in horses with ID (Tóth et al., 2010) reduced the effect of DET on insulin concentration in that group. Yet in a recent study where samples were taken prior to 1 h after detomidine, no significant decrease from baseline was demonstrated in horses with and without ID (Kritchevsky et al., 2020). In another recent study, insulin was suppressed 10 min after detomidine (Kerrigan et al., 2020). In our study, after this initial decrease, insulin concentrations significantly increased after DET in the ID group until the 6 h sample. This prolonged increase could be an exaggerated response to the increased BG. As revealed in this study for the first time, vatinoxan prevented all the detomidine-induced fluctuations in insulin concentrations in plasma in horses with ID.

Detomidine induced a hyperglycaemia of similar magnitude in horses with and without ID, which is supported by another study (Kritchevsky et al., 2020). After DET + VAT, BG was initially significantly increased (compared to SAL) in both groups but the magnitude and duration was markedly less than after DET. Similarly, vatinoxan prevented detomidine-induced hyperglycaemia when administered with romifidine (Pakkanen et al., 2018). Surprisingly, at 4 and 6 h after DET, some horses in both groups became hypoglycaemic (BG < 3.5 mmol/L) but none exhibited clinical sings. Only one of the four had an insulin concentration > 10 μIU/mL at these time points. Hypoglycaemia did not occur after any other treatments at any time point, and it is unclear at this time why it occurred.

Although activation of α_2-adrenoceptors is reported to stimulate glucagon excretion (Hirose et al., 1993) no such effect was detected in our study. On the contrary, in both groups, there was initially a significant decrease in glucagon after DET, which appeared to coincide with the hyperglycaemia. Physiologically, reduced secretion of glucagon in response to hyperglycaemia was to be expected (Iben Rix et al., 2019), so it remains unclear exactly the extent to which α_2- and β-adrenoceptors play a role in the glucagon regulatory process in horses with and without ID.

The results of this study are potentially important because horses are routinely sedated with detomidine and other α_2-adrenoceptor agonists without knowledge of their insulin regulation status. Furthermore, in the present study, the large individual variation in insulin response makes it very difficult to predict which horses will have exaggerated or prolonged insulin increase after the initial insulin inhibition. Due to the fluctuations in insulin concentration, it is also difficult to anticipate the responses in BG. When repeated sedations or constant rate infusions with detomidine are used, the BG and insulin response could be of greater length and magnitude. For example, a 1-h constant rate infusion with medetomidine in healthy horses induced an increase in BG that lasted for all the follow-up period, namely during the infusion and at least 1 h after (Tapio et al., 2019). No reports could be found on the effects of infusions with an α_2-adrenoceptor agonist on insulin concentration. On the other hand, it is known that clinically normal ponies and horses develop clinical laminitis when they have sustained hyperinsulinaemia for approximately 48 h (Asplin et al., 2007; De Laat et al., 2010). Therefore, it could be speculated that lengthy sedation with an α_2-adrenoceptor agonist could predispose some horses to a prolonged hyperinsulinaemia and thus to laminitis. However, more studies are needed to investigate the possible influence of α_2-adrenoceptor agonists on the risk of inducing laminitis. It should be noted that the increased insulin demonstrated in this study is unlikely to induce laminitis, although horses with greater ID may be more significantly affected. Since vatinoxan alleviated the detomidine-induced changes in insulin, BG, and glucagon in both groups with no adverse effects, it could potentially be beneficial to all horses that require sedation with detomidine without the need to test for ID first.

The dose of detomidine used in this study was the labelled dose to induce standing sedation in horses. The dose of vatinoxan used was based on previous studies where it alleviated the cardiovascular effects of detomidine in horses. Vatinoxan did not markedly affect the sedative effects of detomidine which is similar to results from previous studies (Vainionpää et al., 2013; Tapio et al., 2018).

The main limitation of this study was determination and selection of the horses with ID. The ID status of the horses was determined with the OST which may not be the most accurate test for diagnosing ID (Dunbar et al., 2016). This may have allowed for misclassification of the horses which could contribute to the large variation in insulin response to detomidine particularly in the ID group. Ideally the ID status would have been verified with a second dynamic test such as an oral glucose test. Furthermore, the horses without ID in this study were classified as non-ID in each of the three OST performed, but not all horses with ID were classified as ID every time. Annual variation in ID status has been demonstrated in another study conducted by the authors (Box et al., 2019). Therefore, it is possible that this also contributed to the variation in insulin response after detomidine.

Conclusions

Vatinoxan at the dose used in this study prevented detomidine-induced hyperglycaemia in horses with and without ID and the subsequent serum insulin increase observed in horses with ID.

Conflict of interest

The authors declare the following financial interests which may be considered as potential competing interests. The drugs used in this study were manufactured and donated by Vetcare Finland Oy, (Helsinki, Finland). Vetcare also provided funding for the rental of the research horses, the assay kits used to analyse the samples, and statistical analysis. However, Vetcare did not play any role in the study design, sampling process, actual analysis and interpretation of data, or manuscript preparation. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tvjl.2021.105610.

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