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VETERINARY ANAESTHESIA AND ANALGESIA

The impact of Vatinoxan, a peripheral alpha-2 adrenoceptor antagonist, on Medetomidine –Ketamine-Midazolam anaesthesia in Patagonian Maras (Dolichotis patagonum)

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Abstract

Objective The study compared the cardiovascular, respiratory, and anaesthetic effects and the effects on tissue perfusion of a medetomidine-ketamine-midazolam combination with or without vatinoxan (MK-467), a peripherally acting alpha-2 adrenoceptor antagonist.

Study design Randomized, blinded cross-over study.

Animals Nine healthy Patagonian maras (*Dolichotis patagonum)*.

Methods Maras were anaesthetized twice with: 1) medetomidine hydrochloride (0.1 mg kg^{-1}) + ketamine (5 mg kg^{-1}) + midazolam (0.1 mg kg^{-1}) (MKM) + saline, and 2) MKM + vatinoxan hydrochloride (0.8 mg kg^{-1}), intramuscularly, mixed in a syringe. Twenty, 30 and 40 minutes after injection, invasive blood pressure, heart rate, respiration rate, end-tidal CO_2, arterial oxygen saturation (pulse oximetry), arterio-venous oxygen content difference, and muscle oxygenation (near-infrared spectroscopy) were measured. Muscle tone, jaw tone, spontaneous blinking and palpebral reflex were evaluated. Times to initial effect, recumbency, initial arousal and control of the head were recorded. Paired t-test, Wilcoxon matched-pairs signed rank test and ANOVA were used to compare protocols. A seven-day washout period was allowed between protocols.

Results Vatinoxan significantly reduced systolic ($p= 0.0002$), mean ($p < 0.0001$) and diastolic ($p < 0.0001$) arterial blood pressures measured from 20 to 40 minutes. Without vatinoxan, four animals were hypertensive (MAP>120 mmHg) while with vatinoxan, four animals were hypotensive (MAP<60 mmHg). Muscle and jaw tone were significantly more frequently present with MKM (both $p = 0.039$), indicating that anesthesia with vatinoxan was deeper. Other measurements did not significantly differ between protocols.

Conclusion and clinical relevance The study confirmed the hypothesis that the increase in blood pressure induced by medetomidine can be reduced with vatinoxan in Patagonian maras. Notably, it failed to show if the reduction in blood pressure was physiologically beneficial through better muscle perfusion.

Keywords alpha-2 adrenoceptor agonist, anaesthetic depth, blood pressure, MK-467, rodent, muscle oxygenation

Introduction

Medetomidine, an alpha-2 adrenoceptor agonist, is commonly used as an analgesic and sedative drug in veterinary medicine (Sinclair 2003; Rankin 2015; Schmitz et al. 2016). However, medetomidine should not be used in debilitated animals, as it leads to a marked cardiovascular depression in many species, including rodents (Cullen 1996; Paddleford & Harvey 1999, Hawkins & Pascoe 2012). Activation of peripherally located, vascular alpha-2 adrenoceptors is responsible for an initial increase in vascular resistance leading to arterial hypertension, which induces baro-reflex mediated bradycardia and a decrease in cardiac output (Pypendop & Verstegen 1998; Rankin 2015). However, the desired effects of sedation and antinociception originate from alpha-2 adrenoceptors located in the central nervous system, in brain and spinal cord (Doze et al. 1989; Correa-Sales et al. 1992).

Vatinoxan (MK-467), an alpha-2 adrenoceptor antagonist, has attracted increased attention in the last decade. Due to its poor penetration to the central nervous system, as shown in marmosets and rats (Clineschmidt et al. 1988), it primarily acts peripherally. In several species, vatinoxan in combination with alpha-2 adrenoceptor agonists has been shown to reduce cardiopulmonary side-effects while preserving the desired sedative and antinociceptive effects (Salla et al. 2014; Honkavaara et al. 2017; Tapio et al. 2018; Adam et al. 2018a; Sainmaa et al. 2019). When injected intramuscularly, vatinoxan enhanced absorption of medetomidine and dexmedetomidine administered in the same syringe and fastened induction, but it also reduced the duration of sedation (Honkavaara et al. 2017; Restitutti et al. 2017; Adam et al. 2018b; Kallio-Kujala et al. 2018a).

During anesthesia, sufficient tissue perfusion is of paramount importance. Alpha-2 adrenoceptor agonists reduce cardiac output (Pypendop & Verstegen 1998) and induce an initial increase in the systemic vascular resistance (Sinclair 2003). Alpha-2 agonists can decrease tissue perfusion. For

example, dexmedetomidine reduced blood flow to most organs, but mostly in less vital organs such as the spleen and skin in dogs (Lawrence et al. 1996), while medetomidine decreased intestinal and skeletal blood flow in dogs when administered with butorphanol and midazolam (Pypendop & Verstegen 2000). The reduction of tissue oxygen delivery due to dexmedetomidine was attenuated with vatinoxan in conscious dogs (Honkavaara et al. 2011). Actual muscle oxygenation can be measured with near-infrared spectroscopy (NIRS), through non-invasive measurements of the hemoglobin volume and the oxygenation of hemoglobin and myoglobin (Boushel & Piantadosi 2000). Tissue oxygen consumption is the amount of oxygen consumed by tissues per minute. It is a function of tissue metabolism and oxygen availability, and can be calculated via the difference of arterial and venous oxygen content ($_{A\text{-}V}O_2$) multiplied by the cardiac output (Dunn et al. 2016). It has been demonstrated that alpha-adrenoceptor stimulation increases oxygen extraction ratio in peripheral tissues (Ko JC et al. 2007; Ward & Hussain 1996) due to the decrease in oxygen delivery of alpha-2 adrenoceptor agonists given an unchanged rate of consumption. Theoretically, $_{A\text{-}V}O_2$ would increase with alpha-agonists, and through reducing vasoconstriction and increasing oxygen delivery, vatinoxan should reverse this effect.

Alpha-2 adrenoceptor agonist based anesthesia is frequently used in rodents (Longley et al. 2008; Heard 2014) and the addition of vatinoxan could be desired to reduce unwanted cardiopulmonary effects. To investigate this, nine Patagonian maras (*Dolichotis patagonum*) were subjected to a medetomidine-ketamine-midazolam anesthesia with and without vatinoxan in a cross over design. It was hypothesized that with vatinoxan, i) arterial blood pressure would significantly decrease and heart rate significantly increase; ii) there would be a faster onset of anesthesia but no change in anesthetic depth; iii) tissue perfusion would be higher, resulting in higher NIRS readings and in a decreased $_{A\text{-}V}O_2$.

Material and Methods

The study was conducted under a permit from the xx Experimental Animal Inspectorate (permit # xxx). Nine Patagonian maras, four males and five females, with a weight (mean ± SD) of 8.1 ± 1.2 kg, ranging from seven months to six years of age, were housed at xx with access to an indoor and outdoor enclosure. Sample size was based on a rough calculation (Power: 80%, two-sided alpha: 5%, for detecting a mean of the differences of 30mmHg MAP, assuming the standard deviation of the differences to be 20mmHg = 7 animals http://statulator.com), and the availability of animals. Food and water was available at all times. The study was performed in conjunction with an annual health examination, and animals were deemed healthy on the basis of a clinical examination performed during anesthesia as well as a standard hematology and biochemistry panel.

Study design

In a prospective, randomized cross over design, two anesthesia protocols were administrated to each animal with a seven-day washout period. The protocols were randomized via an internet platform (www.random.org). In the first week of the study, the animals were crated and weighed with the crate prior to anesthesia. This weight was used for the calculation of both protocols. The two protocols were 1) medetomidine hydrochloride (0.1 mg kg^{-1}; Domitor Vet. 1 mg ml^{-1}, Orion Pharma, Denmark) + ketamine (5 mg kg^{-1}; Ketaminol Vet, 100 mg mL^{-1}, MSD Animal Health, Denmark) + midazolam (0.1 mg kg^{-1}; Midazolam Hameln 5mg/mL, Hameln plus Gmbh, Germany) (MKM) + vatinoxan hydrochloride (0.8 mg kg^{-1}; Vatinoxan, Vetcare Ltd, Finland), and 2) MKM + saline (Natriumchlorid 0.9%, B. Braun Melsungen AG, Germany) in the same volume as vatinoxan, mixed in a syringe. Immediately prior to use, a pre-weighed amount of vatinoxan hydrochloride was suspended in physiological saline (Natriumchlorid 0.9%, B. Braun) in a 10 mg mL^{-1} solution and vortexed for approx. 10 minutes to secure complete dissolution. The dosage of vatinoxan, which was eight times the dosage of medetomidine, was based on a pilot study, in which it was the dose that had the highest effect on blood pressure without inducing severe hypotension.

After crating, the animal was placed in a quiet room for a minimum of ten minutes. Thereafter, without restraint, the animal was hand-injected in the right or left hind thigh muscle, depending on the position of the animal. After becoming recumbent, the mara was removed from the crate, weighed to verify the initially recorded weight, and instrumented.

Instrumentation and measurements

Oxygen ($\geq$ 2L min^{-1}) was administrated via a loose fitting cone shaped veterinary grade face mask, and body temperature was maintained between 36 and 38°C with an electric heating mat. Following a surgical preparation of the medial lower thigh region, maximally 20 mg of lidocaine hydrochloride (Xylocain 20 mg mL^{-1}, AstraZeneca PLC, United Kingdom) was administrated subcutaneously, and a 45 mm, 20-gauge single lumen arterial catheter (BD Arterial Cannula, Becton Dickinson Infusion Therapy Systems Inc., United States) was placed in the saphenous artery. In most cases, a surgical cut down was necessary to access the vessel. For that purpose, a skin incision of approx. 1 cm was made, and the artery was bluntly isolated from the underlying tissue to facilitate catheter placement. After anesthesia, the skin was closed routinely. Before measurements started, the invasive blood pressure was zeroed to atmospheric pressure at the level of the heart; correct measurement was verified against a 100 cm water column. A 3-way stopcock was attached to the catheter to facilitate arterial blood collection, and connected with a non-distensible arterial blood pressure monitoring line which was attached to a pressure transducer (BD DTXPlus™, Becton Dickinson Critical Care Systems Pte Ltd, Singapore). Invasive blood pressures were monitored with a multiparameter monitor (PM-9000Vet, Mindray Bio-Medical Electronics Co., Ltd, China). In the following week, the contralateral leg was catheterized. The animal was instrumented with a rectal temperature probe, a three-lead electrocardiogram, a pulse oximeter attached to the lip, a unilateral intranasal tube for end-tidal CO_2 ($P_E'CO_2$) measurement (PM-9000Vet, China), and an optode for near-infrared spectroscopy (Adult Soma Sensor, NIRS; Invos™

5100 C Cerebral/Somatic Oximeter, Medtronic Inc, USA). After clipping the hair of the medial thigh of the opposite leg as the arterial catheter, the NIRS optode was attached in the center of the inner thigh in a proximo-distal orientation, and taped in place with lightproof tape.

Using pre-heparinized syringes (Pico Aspirator, Radiometer Medical ApS, Denmark), a venous blood sample from the jugular vein and an arterial blood sample from the arterial catheter were anaerobically collected at 20, 30, and 40 minutes. Blood gases were measured immediately after collection using temperature correction (GEM3500, Instrumentation Laboratory, United States). Hemoglobin concentration was measured once in every animal from venous blood at the laboratory of the veterinary university of Copenhagen (ADVIA2120i, Siemens Healthineers, 2750 Ballerup, Denmark). The oxygen content was calculated (oxygen content = (haemoglobin x 1.36) x (oxygen saturation /100) + (oxygen partial pressure x 0.0031) (Hoiland et al. 2016), for both arterial and venous blood, and thereafter the $_{A\text{-}V}O_2$ for the three time points was derived. Blood gas oxygen saturation values were used to calculate the oxygen content.

At 20, 30, and 40 minutes after injection, the invasive systolic, mean, and diastolic blood pressure, heart rate, respiration rate obtained via the capnogram, $P_E'CO_2$, arterial oxygen saturation (SPO_2) measured by pulse oximetry, the arterial and venous partial pressure of oxygen (PaO_2/PvO_2) and of CO_2 ($PaCO_2/PvCO_2$), the arterial and venous oxygen content (CaO_2, CvO_2), muscle oxygenation measured by NIRS, and the $_{A\text{-}V}O_2$ was compared between the two protocols. The time of initial effects (ataxia, decreased response to the surrounding area, leaning (head or body) against the crate wall, head down), recumbency, initial arousal (first spontaneous movement) and the control (autonomous carrying) of the head was noted by a blinded observer. Between 20 to 45 minutes after injection in a five-minute interval, the muscle tone of one front leg, jaw tone, spontaneous blinking and the palpebral reflex was recorded. The muscle and jaw tone, spontaneous blinking, and palpebral reflex were noted as present or absent.

149 At 45 minutes of initial injection, the medetomidine was reversed with an intramuscular injection in
150 the thigh of atipamezole hydrochloride (0.5 mg kg-1; Antisedan Vet. 5 mg/mL, Orion Pharma A/S,
151 Denmark). Following reversal, invasive blood pressure was recorded every minute for five minutes.

152 **Statistical Methods**

153 Statistical analysis was performed using GraphPad Prism version seven for Windows (GraphPad
154 Software, Inc. La Jolla CA 92037, USA). For the systolic, mean and diastolic invasive blood
155 pressures until reversal at 45 minutes, and after reversal from 46 to 50 min, SPO_2, heart rate,
156 respiration rate, muscle oxygenation, $P_E'CO_2$, PaO_2, PvO_2, $PaCO_2$, $PvCO_2$, CaO_2, CvO_2, and $_{A\text{-}V}O_2$
157 normality was identified with a D´Agostino-Pearson omnibus normality test. Thereafter, the effect
158 of time and protocol was studied using a two-way ANOVA followed by Tukey's multiple
159 comparisons post-test.

160 For muscle tone, jaw tone, spontaneous blinking and palpebral reflex, as score was generated
161 reflecting the sum of positive observations over the 25 minutes (6 observations) and compared
162 between protocols. For these parameters a Wilcoxon matched-pairs signed rank test was performed.
163 For the time points initial effect, recumbency, initial arousal and control of the head normality was
164 tested with the D´Agostino-Pearson omnibus normality test. If normally distributed, a paired t-test
165 was performed, if not, a Wilcoxon matched-pairs signed rank test was used.

166 Differences were considered significant at values of $p < 0.05$.

167 **Results**

168 The systolic ($p = 0.0002$), mean ($p < 0.0001$) and diastolic ($p < 0.0001$) blood pressures were
169 significantly lower with vatinoxan (Table 1), and all three parameters significantly decreased over
170 time ($p < 0.0001$). For at least one-time point between 20 to 40 minutes during anaesthesia, four
171 animals were hypertensive with saline, (MAP>120 mmHg) while four animals were hypotensive

with vatinoxan (MAP<60 mmHg) (Haskins 2015). Invasive blood pressures after medetomidine reversal with atipamezole could be measured in eight animals, one animal awaked too sudden after reversal, so measurements were impeded. Blood pressures were significant lower with vatinoxan (SIPB: $p = 0.0008$; MIBP: $p = 0.0013$, DIBP: $p = 0.0014$) and decreased over time ($p < 0.0001$) (Table 2). For at least one-time point after reversal, MIBP decreased under 60 mmHg in one animal with the MBM protocol, while in six animals in the protocol including vatinoxan.

Muscle tone and jaw tone were more frequently present with MKM (both $p = 0.039$), at every time point the two were equally either present or absent. Spontaneous blinking ($p = 0.31$) and palpebral reflex ($p = 0.06$) were not significantly different between treatments (Figure 1).

The heart rate and other parameters measured did not differ significantly between protocols or over time, and the times to initial effect, recumbency, initial arousal and control of the head did not differ between treatments (Table 3). Blood gas related parameters (PaO_2, PvO_2, $PaCO_2$, $PvCo_2$, CaO_2, CvO_2, and $_{A\text{-}V}O_2$) could only be measured in eight animals as in one animal a blood clot in the venous blood sample inhibited measurements.

Discussion

The study demonstrated that during 20-40 minutes of medetomidine-based anesthesia, vatinoxan significantly reduced the blood pressure in Patagonian maras. Muscle and jaw tone were less frequent, indicating that anesthesia with vatinoxan was deeper. Interestingly, the heart rate and other parameters did not differ between treatments.

In previous studies in dogs and cats, vatinoxan reduced blood pressure but also significantly increased the heart rate when administered with an alpha-2 adrenoceptor agonist. (Honkavaara et al. 2011; Honkavaara et al. 2017; Martin Flores et al. 2018). However, in markhors (*Capra falconeri heptneri*) immobilized with a medetomidine-ketamine combination (Sainmaa et al. 2019), no

195 significant difference could be detected in heart rate although blood pressure decreased significantly
196 due to vatinoxan. Initially, alpha-2 adrenoceptor agonists induce vasoconstriction resulting in high
197 blood pressures, and bradycardia follows from a primarily baroreceptor mediated reflex. In a
198 secondary phase at a later stage, vascular resistance returns to normal, however often bradycardia
199 persists as a result of decreased central sympathetic outflow (Rankin 2015). No physiological data
200 is available of the resting heart rate in Patagonian maras. Although the heart rate did not differ
201 between treatments, it is unknown if the observed heart rates were within normal limits or should be
202 considered bradycardic. Based on a generic formula to calculate physiological hear rates in
203 mammals depending on body weight ($HR = 241 \times BW^{-0,25}$, Kline et al. 2015), the animals would be
204 considered bradycardic during anesthesia with both treatments, however such generalizations
205 should be made cautiously. Regardless, it is conceivable, that the concurrent use of ketamine and
206 midazolam inhibited the vagal component of the baroreceptor reflex dampening the effect on heart
207 rate. In rabbits, ketamine inhibited the vagal component of the baroreceptor reflex and thus
208 bradycardia in the face of hypertension (McGrath et al. 1975; Blake & Korner 1982). Midazolam
209 may also attenuate the heart rate response to the baroreflex (Sakamoto et al. 1994). Still, in sheep
210 sedated with medetomidine and ketamine, vatinoxan increased the heart rate (Adam et al. 2018a).
211 Even though comparisons should made cautiously because of inter-species differences in ketamine
212 pharmacokinetics (Saland et al. 2017), in sheep the dosage of ketamine was low (1 mg kg^{-1})
213 compared to the present study (5 mg kg^{-1}). Respiration rate did not differ, but evidence of
214 hypoventilation (Mean $PaCO_2$ > 50 mm Hg) was present with both protocols, possibly indicating
215 that the respiratory depression either did not arise from medetomidine alone, and/or that the
216 depression is happening centrally, out of reach of the vatinoxan. End-tidal CO_2 was measured via a
217 nasal canula and provided consistently credible values, however as there was a clear gradient
218 between $P_E'CO_2$ and $PaCO_2$; one should be aware that this measurement method can provide

accurate readings, nevertheless they depend more on biological and mechanical factors (Fukuda et al. 1997).

Various studies have shown that vatinoxan increased oxygen delivery towards tissues in alpha-2 adrenoceptor-based sedations. In dogs sedated with dexmedetomidine, significant changes in organ blood flow were prevented with vatinoxan (Restitutti et al. 2013), and with medetomidine oxygen delivery index was significantly higher after vatinoxan treatment (Salla et al. 2014). In horses sedated with detomidine, PvO_2 and CvO_2 decreased significantly from baseline 15 minutes after injection, and these values did not significantly change from baseline when vatinoxan was added (Tapio et al. 2018). In the present study, the arterio-venous oxygen content difference and other blood gas parameters did not differ significantly between treatments. For the $_{A\text{-}V}O_2$ calculation, jugular blood was used reflecting specifically differences in oxygen content from the brain and head area. Ideally, mixed venous blood would be used as it represents the global tissue consumption (Dunn et al. 2016), however this was not technically possible in the current study. Other limitations include that the hemoglobin values used to calculate oxygen content were only measured once from venous blood in every animal during the first week of the trial, regardless of the used protocol. Hemoglobin concentration can be influenced by hypertension (Enawgaw et al. 2017) and sedative drugs like medetomidine (Wolkers et al. 1994), which may have introduced minor inaccuracies.

Various equations to measure oxygen availability for and consumption of tissues have been used in studies with vatinoxan. Commonly tissue oxygen delivery was calculated, for this calculation the cardiac output is needed, which was not measured in this study. However, NIRS measures actual muscle oxygenation and therefore is a valuable indicator of sufficient oxygen delivery towards tissue. That said, no differences between protocols were detected using NIRS, and sometimes it was difficult to obtain consistent readings, as intermittently values were higher and inconsistent with immediate prior readings in the same animal. The maras had a slight skin pigmentation which can

influence NIRS readings (Wassenaar & Van den Brand 2005), also species dependent myoglobin concentrations must be taken into consideration when interpreting NIRS values for muscle oxygenation (Davis & Barstow 2013). Still, these aberrations should be consistent in all animas, so the values should be interpreted with care. The continuous administration of approx. 2 L min^{-1} of oxygen via the face mask, associated with high PaO_2 and PvO_2 values and a 100% hemoglobin saturation, likely masked blood gas differences and changes in muscle oxygenation between treatments.

At each five-minute measurement the presence of muscle and jaw tone was evaluated, and no difference was observed at a single time point. Muscle and jaw tone were more frequently present with MKM, and although not significantly different, spontaneous blinking and palpebral reflex were less frequently recorded with vatinoxan, indicating that anesthesia with vatinoxan was deeper. This finding goes along with several studies. In sheep, the sedation score was higher in the combination with vatinoxan than with medetomidine alone, however sedation score also decreased more rapidly (Adam et al. 2018b). Vatinoxan intensified the early state of sedation in dogs sedated with medetomidine and butorphanol, and shortened the duration of sedation (Kallio-Kujala et al. 2018b).

The onset of sedation also appears to be faster when vatinoxan is mixed in the same syringe with medetomidine for induction and injected intramuscularly (Restiutti et al. 2017; Adam et al. 2018b; Kallio-Kujala et al. 2018a). Medetomidine causes local vasoconstriction, and vatinoxan likely accelerates absorption because it can block the local action of the alpha-2 adrenoceptor agonist on the circulation (Restitutti et al. 2017). This effect was not observed in the present study, and no differences were observed in the time to recumbency, initial arousal and control of the head. More studies are needed to investigate the effects of co-administration of vatinoxan with an alpha-2 adrenoceptor agonist on anaesthetic induction, depth and recovery.

Though in general cut-offs for hypo- and hypertension are guiding and slightly arbitrary (Haskins 2015), hyper- and hypotension was observed in four animals with MKM and with vatinoxan, respectively. Additionally, after reversal of medetomidine with atipamezole, blood pressures quickly dropped and hypotension occurred. It is presently unknown if a transient period of hypertension due to the alpha-2 adrenoceptor agonist does more damage than a hypotensive period due to the vatinoxan. Further studies evaluating the varying ratios of medetomidine to vatinoxan dosages are required to prevent periods of hyper-and hypotension.

In conclusion, vatinoxan significantly reduced the blood pressure in Patagonian maras under medetomidine based anesthesia, when dosed eight times more than medetomidine. Significant benefits in muscle oxygenation were not identified; oxygen administration is always beneficial, but might have precluded the detection of subtle differences between the two protocols. Further studies titrating vatinoxan dosages and investigating the potential benefits of adding vatinoxan to medetomidine-based sedations are highly warranted.

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389 **Figure 1** Median ± interquartile ranges representing the number of times muscle tone, jaw tone,
390 spontaneous blinking, and palpebral reflex was present during a medetomidine-ketamine–
391 midazolam anaesthesia (Saline) with and without vatinoxan (Vatinoxan) in nine Patagonian maras.
392 Parameters were assessed every 5 minutes from 20 to 45 minutes after injection. The (*) indicates
393 significant difference between the two protocols ($p < 0.05$).

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396 **Table 1** Mean ± Standard deviation of the diastolic (DIBP), mean (MIBP), and systolic (SIBP)
397 arterial blood pressure, oxygen saturation (SPO_2), heart rate (HR), respiration rate (RR), end-tidal
398 CO_2 ($P_E'CO_2$), muscle oxygenation (MO), arterial and venous partial pressure of oxygen
399 (PaO_2/PvO_2) and of CO_2 ($PaCo_2/PvCO_2$), arterial and venous oxygen content (CaO_2, CvO_2) and
400 arterio-venous oxygen content difference ($_{A\text{-}V}O$) measured at 20, 30 and 40 minutes during a
401 medetomidine-ketamine–midazolam anaesthesia with and without vatinoxan in Patagonian maras.
402 The (*) indicates significant difference between the two protocols ($p < 0.05$).

Variable	20 min Saline	20 min Vatinoxan	30 min Saline	30 min Vatinoxan	40 min Saline	40 min Vatinoxan
DIBP (mmHg)	91 ± 9 *	66 ± 13 *	85 ± 10 *	57 ± 11*	78 ± 10 *	52 ± 9 *
MIBP (mmHg)	112 ± 12 *	81 ± 16 *	105 ± 12 *	71 ± 14 *	97 ± 12 *	65 ± 12 *
SIPB (mmHg)	153 ± 16*	113 ± 23*	144 ± 19 *	100 ± 21 *	134 ± 17*	92 ± 19*
SPO_2 (%)	97 ± 3	96 ± 5	96 ± 4	97 ± 2	97 ± 3	97 ± 3
HR (bpm)	98 ± 19	100 ± 10	98 ± 18	102 ± 8	101 ± 18	99 ± 10
RR (bpm)	34 ± 7	31 ± 7	35 ± 12	33 ± 7	35 ± 11	32 ± 9
$P_E'CO_2$ (mmHg)	39 ± 8	42 ± 4	41 ± 6	41 ± 7	42 ± 6	38 ± 8
MO (%)	52 ± 7	59 ± 13	54 ± 8	57 ± 10	53 ± 7	54 ± 7
PaO_2 (mmHg)	451 ± 160	432 ± 165	390 ± 165	416 ± 108	445 ± 159	439 ± 115

PvO_2 (mmHg)	131 ± 74	115 ± 54	121 ± 70	114 ± 68	174 ± 117	120 ± 114
$PaCO_2$ (mmHg)	59 ± 7	58 ± 7	56 ± 4	55 ± 4	60 ± 13	53 ± 6
$PvCO_2$ (mmHg)	59 ± 8	59 ± 7	61 ± 8	58 ± 4	62 ± 11	57 ± 4
CaO_2 (mL/dL)	24 ± 2	24 ± 2	24 ± 2	24 ± 2	24 ± 2	24 ± 2
CvO_2 (mL/dL)	22 ± 2	22 ± 2	22 ± 2	22 ± 3	22 ± 3	22 ± 2
$_{A\text{-}V}O$ (mmHg)	1.7 ± 1.2	2.1± 1.7	1.5 ± 0.7	2.1 ± 1.8	1.8 ± 1.6	2.2 ± 1.4

Table 2 Mean (± Standard deviation) values of diastolic (DIBP), mean (MIBP), and systolic (SIBP) arterial blood pressures in the five minutes following reversal with atipamezole at 45 min of a medetomidine-ketamine–midazolam anaesthesia with and without vatinoxan in eight Patagonian maras. Blood pressures were significant lower with vatinoxan ($p < 0.05$), indicated with a (*).

Time (min)	DIBP (mmHg) Saline	DIBP* (mmHg) Vatinoxan	MIBP (mmHg) Saline	MIBP* (mmHg) Vatinoxan	SIBP (mmHg) Saline	SIBP* (mmHg) Vatinoxan
46	71 ± 7	45 ± 13	90 ± 10	59 ± 14	127 ± 13	86 ± 19
48	62 ± 8	46 ± 9	78 ± 9	58 ± 12	112 ± 10	84 ± 18
50	52 ± 7	43 ± 8	68 ± 8	55 ± 11	99 ± 13	75 ± 15

409 **Table 3** Mean (± Standard deviation) time (min) to initial effect, recumbency, initial arousal and
410 control of the head during a medetomidine-ketamine–midazolam anaesthesia with and without
411 vatinoxan in Patagonian maras. There was no significant difference between the two protocols.

	Protocol	
Milestone	Saline	Vatinoxan
Initial effect	2 ± 0.7	1.9 ± 0.3
Recumbency	4.8 ± 3.2	4.6 ± 3.1
Initial arousal	51.2 ± 3.7	55.2 ± 4.3
Control of the head	53.7 ± 5.1	57 ± 5.4

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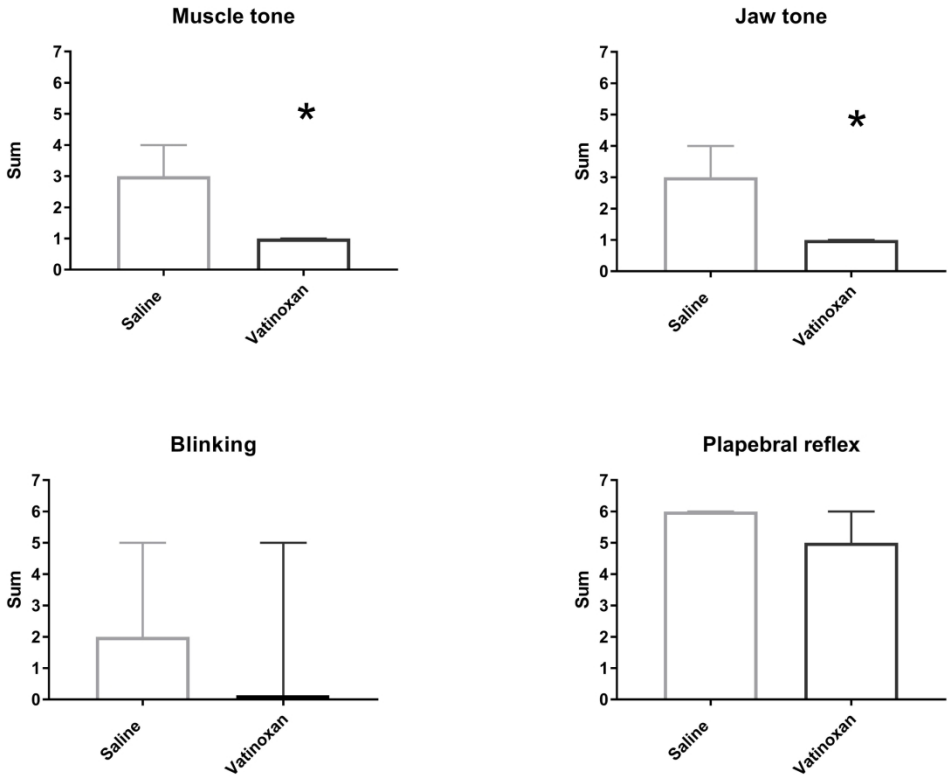

Figure 1 Median ± interquartile ranges representing the number of times muscle tone, jaw tone, spontaneous blinking, and palpebral reflex was present during a medetomidine-ketamine–midazolam anaesthesia (Saline) with and without vatinoxan (Vatinoxan) in nine Patagonian maras. Parameters were assessed every 5 minutes from 20 to 45 minutes after injection. The (*) indicates significant difference between the two protocols ($p < 0.05$).

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