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Original Research

Methadone Increases and Prolongs Detomidine-Induced Arterial Hypertension in Horses, but These Effects Are Not Mediated by Increased Plasma Concentrations of Arginine Vasopressin or Serum Concentrations of Catecholamines

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ABSTRACT

Catecholamines and arginine vasopressin (AVP) release can affect arterial blood pressure (ABP) and hemodynamic stability in standing, sedated horses. Six mature horses were included in this prospective, randomized, crossover, blinded, experimental study. All the horses were sedated with detomidine (DET) alone (0.01 mg/kg, IV) or combined with methadone (MET) (0.01 mg/kg DET and 0.2 mg/kg MET, IV). Cardiopulmonary data and blood samples were collected 30 minutes before (prebaseline and baseline) and for 120 minutes postinjection. The combination DET/MET produced a significant increase (31%) in mean ABP (MAP) 5 minutes after drug administration which lasted for 120 minutes. Detomidine alone induced only a short-term increase in MAP (15%) at 5 minutes compared with baseline. There were significant differences between groups at 5, 15, and from 60 to 120 minutes. Plasma AVP concentrations were higher in horses receiving the treatment DET from 60 to 120 minutes than those in the combination group, for the same period. There were no significant differences in norepinephrine and epinephrine serum concentrations respect to baseline and between treatments. Detomidine induces a short-term MAP increase, and this effect was prolonged and potentiated by MET association. There is no evidence of AVP, norepinephrine, and epinephrine involvement in this effect.

1. Introduction

Detomidine (DET) is a sedative and analgesic drug commonly used in equine practice to produce chemical restrain and sedation for diagnostic or surgical procedures. The cardiovascular effects of this alpha-2 adrenergic agonist include reductions in heart rate (HR) and cardiac output, atrioventricular conduction disturbances, depression of the index of contractility, and increased systemic vascular resistance (SVR) and pulmonary vascular resistance [1,2]. In standing horses, the combination of alpha-2 agonists and

Ethical Considerations: The authors certify that legal and ethical requirements have been met with regards to the humane treatment of animals described in the study and specifying the Universidade Estadual Paulista Animal Care and Use Committee that has overseen this process (under protocol number CEEA 176/2008).

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opioids results in synergistic analgesic effects, reliable sedation, and stable cardiorespiratory function with reduced side effects [3–6].

The use of systemically administered opioids in horses to provide analgesia remains controversial [7,8]. After IV administration, respiratory depression, sympathetic stimulation, and central nervous system (CNS) excitation, increased locomotor activity, and decrease of gastrointestinal tract motility may occur [7,8]. Methadone (MET), a racemic mixture with d- and l-isomers, is a potent synthetic mu (μ) opioid receptor agonist, with similar analgesic properties to morphine, but also with N-methyl-D-aspartate receptor antagonist activity [9]. In horses, IV doses of 0.5 mg/kg increased locomotor activity with poor motor coordination, whereas lower doses (up to 0.2 mg/kg) may reduce the incidence of these adverse behavioral effects [6,10].

Arginine vasopressin (AVP), an essential hormone for both osmotic and cardiovascular homeostasis released by the neurohypophysis, plays an important role in the maintenance of arterial blood pressure (ABP) in the presence of hypotension in compromised patients [11–14]. It acts mainly due to its antidiuretic effects on the kidney and peripheral vasoconstriction via stimulation of V_{1a} receptors leading to increases in ABP [13]. Alpha-2 agonists cause a significant decrease in the plasma AVP concentration in rats [11]. In conscious horses, DET reduced norepinephrine and catecholamine metabolites [15], whereas AVP remained unaffected [16] or with little changes [17] during total intravenous anesthesia (TIVA) with DET, guaiphenesin, and ketamine. Horses anesthetized with inhalant agents showed increases in AVP [17–19], justified as a response to hypotension produced by those agents [17].

In dogs, plasma AVP increased up to 40 times after MET administration [12,20]. It has been suggested that the release of this hormone may lead to a vasoconstrictive response, that is, increased SVR index [21]. Both in conscious and isoflurane anesthetized dogs receiving MET, SVR, ABP, circulating catecholamines, and AVP rose [22]. In horses, cardiovascular stimulation, such as increases in HR, cardiac index, and ABP [23] may follow CNS stimulation after opioid administration, especially at high doses and when not experiencing pain [7,8]. To the authors' knowledge, there is no information about the influence of MET alone or combinations in AVP and catecholamine plasma concentrations and the possible effect of these hormonal changes in cardiovascular stimulation in horses. According to that, the authors hypothesize that increases of plasma AVP and catecholamines produced by MET administration may potentiate DET-induced hypertension by means of peripheral vasoconstriction.

The aim of this study was to evaluate the cardiopulmonary effects and AVP and catecholamine changes of DET alone or in combination with MET in healthy standing, sedated horses.

2. Material and Methods

2.1. Animals

This study was approved by the Institutional Ethics Committee on Animal Use (protocol number 176/2008) and is the Phase 2 of a previously reported article [6]. A washout period of 4 weeks between phases was allowed. Six healthy mature Arabian horses (two males and four females) weighing 415 ± 20 kg were enrolled in the present trial. Health status was assessed on the basis of physical and laboratory investigation (complete blood cell count, serum biochemistry, venous blood gas analysis, and electrocardiogram [ECG] tracing). Before starting the study, the animals were acclimated to the experimentation room, remaining undisturbed and calm while restrained in the stocks.

2.2. Instrumentation and Variables Recorded

Hay and water were provided ad libitum until just before the beginning of each experiment. Concentrate food was not provided during the day of the experiment. Instrumentation was performed with horses restrained in the stocks. Lidocaine [(Xylestesin 2%; Cristália, São Paulo, Brazil), 0.5 mL subcutaneous per site] was topically administered to place both arterial and venous catheters. A 14-gauge Teflon catheter (BD Angiocath; Becton & Dickinson, São Paulo, Brazil) was aseptically inserted into both jugular veins: the left vein for drug administration and the right for blood collection.

A 20-gauge Teflon catheter (BD Insight; Becton & Dickinson, São Paulo, Brazil) was placed into a transverse facial artery and used to collect blood samples in heparinized syringes for blood gas analysis (348 pH Blood Gas Analyzer; Corning Medical and Scientific, MA) and to record ABP values. Proper position of the arterial catheter was confirmed by observation of the characteristic pressure waveform. Arterial blood gas parameters were corrected to body temperature. The arterial catheter was connected to a pressure transducer (TruWave; Edwards Lifesciences Critical Care Division, CA), zeroed at atmospheric pressure and leveled at the level of the right atrium (olecranon in the standing horse). The accuracy of these transducers was confirmed with a mercury column before each experiment. Heart rate and rhythm were assessed by means of a base apex lead ECG (Spacelabs 90,309 Pc Scout; Spacelabs Medical, Ontario, Canada) with adhesive electrodes (Monitoring Electrode 2223; 3M, São Paulo, Brazil). From the ECG printout recorded during 30-second periods, the percentage (%) of second degree atrioventricular block (AVB) was calculated according to the formula: (number of P waves not followed by QRS-T complexes)/(total number of P waves recorded) × 100.

2.3. Study Protocol

With the catheters in place, each horse was allowed to stand undisturbed in the stocks for at least 30 minutes prior the beginning of the experiment. Once prebaseline (T −30 and T −15) and baseline (T 0) values for the different parameters were recorded, the assigned treatment was administered. Prebaseline values were taken to allow acclimatization of the horses. Comparisons with baseline values refer to T 0. Heart rate (from ECG); respiratory rate (direct observation of thorax wall movements); rectal temperature; systolic, mean ABP (MAP), and diastolic ABPs;

blood gas parameters [pH, arterial partial pressures of carbon dioxide ($PaCO_2$) and oxygen (PaO_2), and standard bicarbonate ($HCO_{3\,std}^-$)]; and blood samples for AVP, and catecholamines determination were taken and recorded at 5, 15, 30, 60, 90, and 120 minutes after drug administration. Moreover, at those times and at 1 minute after drug administration, the percentages (%) of second degree AVBs were calculated as described previously.

The main investigator was unaware of the treatment, and the order of treatments was randomly selected by a person not involved in the observations and recordings. Two different treatments were administered to each horse, with at least 1-week washout period: treatment DET (DET 0.01 mg/kg, IV) (detomidine, Dormiun V, Agener União Saúde Animal, São Paulo, Brazil) and treatment DET/MET (detomidine 0.01 mg/kg, IV plus methadone 0.2 mg/kg, IV) (methadone, Mytedom, Cristália, São Paulo, Brazil) in the same syringe. The syringes were prepared by the same person, with the volume of the solution filled up to 20 mL of physiological saline and administered during 1-minute period. All the parameters (cardiorespiratory and those taken from the blood sampling) were recorded by the same researcher, blinded to treatment.

2.4. Serum Catecholamines and Plasma AVP

Blood samples were collected from the right jugular catheter and stored into two collection tubes (each with 10 mL) for determination of catecholamine concentrations in serum and two collection tubes (each with 4 mL) for determination of AVP concentrations in plasma. The containers were immediately centrifuged (1,127g) at −4°C temperature-controlled centrifuge during 15 minutes. The serum and plasma samples were immediately stored in Eppendorf tubes and frozen at −70°C.

Arginine vasopressin concentrations were measured by radioimmunoassay after acetone and petroleum ether extraction from serum as described elsewhere [24]. Vasopressin antiserum (Peninsula Laboratories, San Carlos, CA) and ^{125}I-vasopressin (Dupont NEN Research Products, Boston, MA) were obtained from commercial sources. This technique resulted in extraction rates higher than 89% [25].

Serum catecholamines were measured by high-performance liquid chromatographic technique as previously described [26]. The mobile phase was methanol buffer (90/10, vol/vol), and the buffer was prepared with $Na_2HPO_4.12H_20$ (7.16 g/l), citric acid (4.2 g/L), 0.12 mMEDTA, and 0.0556% (wt/vol) human serum albumine. The pH of the mobile phase was adjusted to 2.64 with 6 M H_3PO_4. This mobile phase was degassed for 15 minutes and cycled through an OS-224, RP-18, 5-llm (4.6 × 220 mm) analytical column at 1.2 mL/min. Electrochemical detection at a potential of 0.5 V was used at 25°C as described previously.

2.5. Data Analysis

A Kolmogorov–Smirnov test was used to determine data normality of the distribution, followed by two-way analyses of variance. Differences attributable to treatment were analyzed by Dunnett's test, whereas differences between groups were compared by Tukey test (SAS 9.2, SAS Institute Inc, NC). Significance was set at the 5% level.

3. Results

3.1. Blood Pressure, HR, and Atrioventricular Block

There was a significant increase in MAP with respect to baseline values after both treatments (T 5) $P = .0049$ and $P < .0001$ with DET and DET/MET, respectively (Fig. 1). Detomidine induced these significant increases in MAP (5 minutes after drug administration) that were higher when MET was coadministered (15 and 31% MAP increases, respectively). These increases in MAP were prolonged up to 2 hours with significant differences between treatments (Fig. 1). When receiving the DET/MET combination, the values were always statistically significant above baseline values, even after 2 hours of drug administration (10.6%).

Heart rate significantly decreased in both groups at 5 minutes, $P < .0001$ and $P = .0118$, respectively, after DET and DET/MET administration (Fig. 1). In treatment DET, this significant difference lasted for 60 minutes when compared with baseline, whereas in treatment DET/MET, this reduction was only significant after 5 minutes. Although not significant between treatments, the bradycardia induced by DET was more pronounced than the combination with MET, 36.1% and 22.2%, respectively, at 5 minutes. A significant difference in HR between groups was only present at 90 minutes ($P = .0101$).

The presence of second degree AVB was significantly increased at 1 minute (35% in both DET and DET/MET groups) and at 5 minutes (11% and 8%, respectively). Normal sinus rhythm was restored after 15 minutes with only 1% of AVB (no significant) in both DET and DET/MET groups. There were no significant differences between treatments at any time.

3.2. Plasma Concentrations for AVP and Serum Concentrations for Catecholamines

In the determination of plasma concentration for AVP, the limit of detection ranged from 0.15 to 0.6 pg/mL. The reported intraassay and interassay coefficients of variation were 7.7% and 11.9%, respectively [25]. Plasma concentrations for AVP were significantly higher in horses receiving DET alone compared with those receiving the combination, from T 60 until the end of the study ($P = .0044$, $P = .0032$, and $P = .0033$ at T 60, T 90, and T 120, respectively) (Fig. 2). Arginine vasopressin plasma concentrations in the treatment DET/MET did not vary over the time. Catecholamine concentrations did not change from baseline values. Differences in norepinephrine and epinephrine concentrations between groups throughout the study were not observed.

3.3. Blood Gas and Electrolyte Analyses

The most relevant changes in blood gases were the lower PaO_2 values at 5 and 15 minutes after DET and DET/MET compared with baseline, respectively, and the lower PaO_2 values at 15 minutes in the combination compared

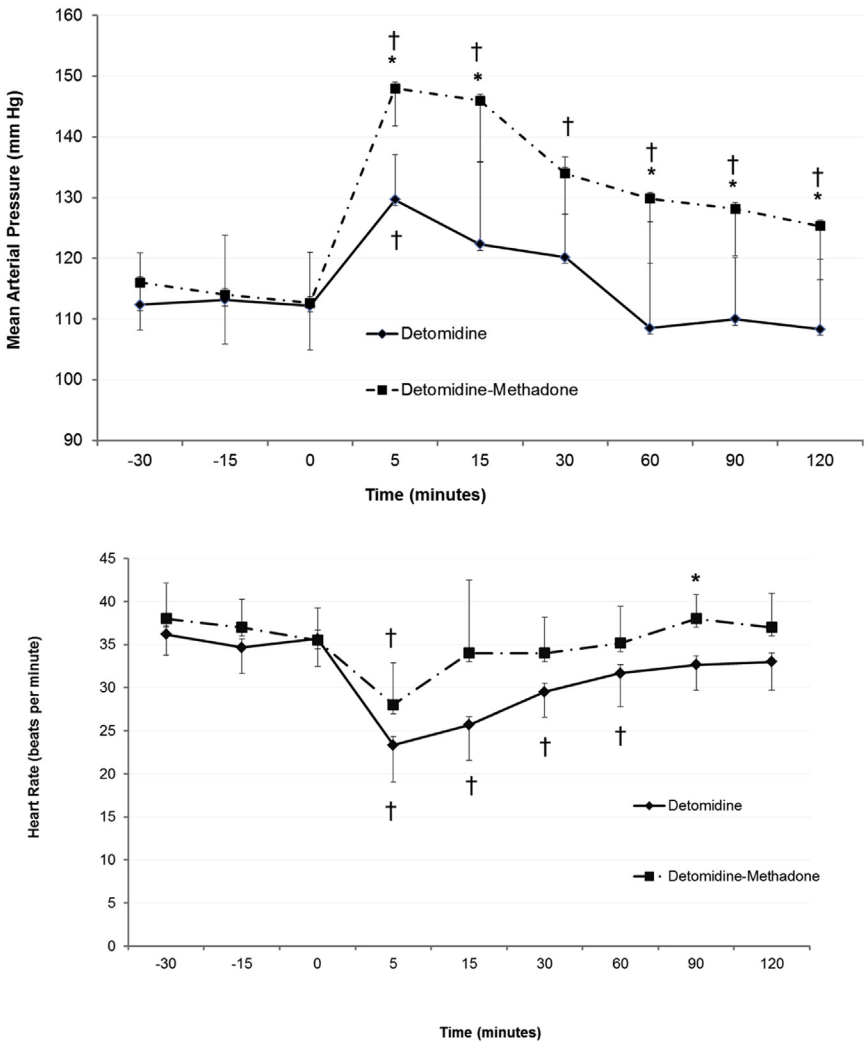

Fig. 1. Mean arterial pressure and heart rate (mean ± standard deviation) recorded in six horses after receiving IV detomidine (0.01 mg/kg) or a detomidine/methadone combination of detomidine (0.01 mg/kg) and methadone (0.2 mg/kg). The drugs were administered after baseline data collection (times −30 and −15) at T 0. Significant differences between treatments are represented as asterisks (*) and with baseline values as daggers (†).

with DET. The other few changes in blood gas were considered of minimal clinical relevance (Table 1).

3.4. Spontaneous Locomotor Activity

All the horses remained calm in the stocks, and none of them showed any sign of excitation.

4. Discussion

Methadone heightens and prolongs the ABP increase in horses sedated with DET and the cardiovascular effects of the combination are not associated with changes in AVP, epinephrine, or norepinephrine.

Before all else, it may be argued that a MET group was not included. This might have allowed us to compare the cardiovascular and hormonal effects of this opioid alone with the other groups. Nevertheless, low doses of MET (0.2 mg/kg, IV) administered alone are apparently not clinically adequate to provide analgesia as they did not increase antinociceptive electrical and thermal thresholds in horses [6]. As a consequence, the authors decided to focus in the study of the cardiovascular changes of more clinically applicable drugs (DET) and combinations (DET and low doses of MET). Higher doses of MET were not considered as they activate the CNS [6,23] and may indirectly stimulate the cardiovascular function. A second limitation could be the absence of a placebo. A saline group was not included as the cardiovascular effects of DET have been widely described [1,2], and our main objective was to compare the cardiovascular effects of DET alone with its combination with MET, not reported yet.

The most unexpected finding was the increase in AVP after 1 hour in the DET group, which was not accompanied by any changes in ABP or HR. Studies in ponies receiving DET-based TIVA suggested that this drug is the most likely component to reduce AVP. During 2 hours of TIVA, AVP levels were reduced and remained low, even 2 hours after

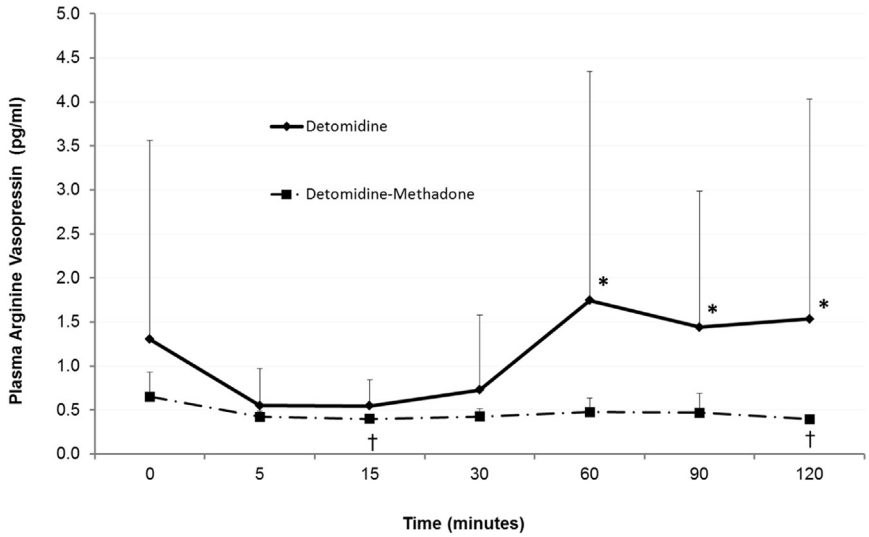

Fig. 2. Plasma arginine vasopressin concentration (mean ± standard deviation) in six horses after receiving IV detomidine (0.01 mg/kg) or a detomidine/methadone combination of detomidine (0.01 mg/kg) and methadone (0.2 mg/kg). The drugs were administered after baseline blood collection (times −30 and −15) at T 0. Significant differences between treatments are represented as asterisks (*) and with baseline values as daggers (†).

the end of the infusion [17]. Although these increases in AVP were of statistical significance in the DET group, they appear not to be of clinical relevance because reference values of AVP in ponies are between 0 and 33 pg/mL [27], the upper limit being far higher than the values observed here.

Detomidine alone increased MAP and decreased HR as expected. In horses, DET causes a short-term hypertension by means of peripheral arterial vasoconstriction induced by α-2b adrenergic receptors stimulation in vascular smooth muscle [1,2,28]. In our horses, the combination of DET and MET produced a higher and more prolonged arterial hypertension than DET alone. However, there is no evidence of the involvement of these hormones in horses as their concentrations remained within normal values [27]. In dogs, plasma AVP concentration was increased after MET administration, but cardiovascular stimulation was not reversed by the use of an AVP antagonist, showing that this effect is not mediated by AVP [22]. Differently, the combination of MET and DET did not increase AVP in horses, which may be explained by DET-induced reduction in stress response [17] or by the fact that MET may decrease AVP concentrations in horses. Furthermore, this increase in AVP may be explained as the physiologic result of renal fluid losses associated with DET administration.

The decrease in HR was slightly more pronounced in horses receiving only DET. Although a significant difference between treatments was only found at 90 minutes after drug administration, the difference with baseline values was significant in the DET group for 1 hour, whereas this was only true in the combination group at T 5. A dose-dependent bradycardia follows the short-term increases in ABP, secondary to a reduction in the sympathetic outflow from the CNS and a vagal reflex from baroreceptor response to hypertension [1,2,28,29]. However, this mechanism seemed to have less importance in the DET/MET combination, maintaining higher HR values. Second degree AVBs were noticed in both groups, with no significant differences. The presence of this arrhythmia is a well-known adverse effect of alpha-2 agonists, especially at high doses [1]. The addition of MET did not decrease the incidence of this type of block.

To the authors' knowledge, there is no written evidence describing the effects of any opioid in AVP plasma concentration in horses. In contrast, the cardiovascular effects of different opioids have been previously studied [7,8]. These effects depend on the drug, doses, route of administration, and coadministration drugs. Stimulation of the CNS may produce cardiovascular hyperdynamism [8], mainly due to the special distribution of μ receptors in the equine brain [30]. Increased ABP and HR linked to dysphoria and euphoria have been reported after MET IV at 0.12 mg/kg [23], high doses of morphine (0.66 mg/kg, IM) [31], and buprenorphine (0.01 mg/kg, IV) [32]. In this last study, hypertension was not related to increases in SVR, but due to increased cardiac output. Otherwise, lower doses of buprenorphine (0.003 mg/kg, IV) produced excitation, with a marked and prolonged hypertension with no increases in HR [33]. Moreover, butorphanol did not cause significant cardiovascular changes, although increasing doses produced excitatory behavioral changes [34].

The cardiovascular changes produced by both treatments were apparently neither related to the increase of AVP nor by excitation. In the combination group, a certain degree of cardiovascular stimulation might have occurred due to the inclusion of MET, leading to higher ABP values without behavioral reactions. Horses receiving MET alone at the same dose (0.2 mg/kg, IV) increased spontaneous locomotor activity when compared with baseline [6], which might lead to a certain degree of cardiovascular stimulation [23,31,32]. In our study, a synergistic cardiovascular stimulation produced by the coadministration of a low dose of MET might have produced higher ABP, not linked to CNS stimulation because DET abates the possible

Table 1
Mean (±standard deviation) rectal temperature (T), respiratory rate (RR), pH, arterial partial pressure of carbon dioxide ($PaCO_2$) and oxygen (PaO_2), and standard bicarbonate ($HCO_{3\,std}$) of six horses after IV detomidine (DET) 0.01 mg/kg or DET/methadone (MET) combination 0.01 mg/kg DET and 0.2 mg/kg MET.

Variable	Group	Time (min)							
		−30	−15	0	5	15	30	60	90	120
T (°C)	DET	37.9 ± 0.3	37.8 ± 0.3	37.8 ± 0.3	37.8 ± 0.3	38.0 ± 0.3	37.9 ± 0.3	37.8 ± 0.2	37.6 ± 0.3	37.7 ± 0.4
	DET/MET	37.9 ± 0.4	37.9 ± 0.3	38.0 ± 0.3	38.0 ± 0.3	38.0 ± 0.3	37.9 ± 0.3	37.6 ± 0.1[a]	37.5 ± 0.3[a]	37.5 ± 0.2[a]
RR (move/min)	DET	22.7 ± 15.7	16.3 ± 6.7	15.5 ± 7.1	14.0 ± 4.2	13.0 ± 3.5	10.7 ± 3.3	11.0 ± 3.0	10.0 ± 2.2	12.0 ± 2.5
	DET/MET	17.0 ± 4.3	16.2 ± 4.4	17.0 ± 6.4	12.0 ± 4.6	12.3 ± 2.7	11.0 ± 1.7[a]	10.5 ± 2.0[a]	11.3 ± 2.7[a]	12.8 ± 2.9
pH	DET	7.45 ± 0.03	7.44 ± 0.01	7.45 ± 0.02	7.44 ± 0.03	7.44 ± 0.03	7.44 ± 0.01	7.47 ± 0.04	7.46 ± 0.01	7.44 ± 0.02
	DET/MET	7.45 ± 0.02	7.45 ± 0.02	7.45 ± 0.03	7.45 ± 0.04	7.46 ± 0.04	7.48 ± 0.03[b]	7.48 ± 0.02	7.47 ± 0.01	7.47 ± 0.02[b]
$PaCO_2$ (mm Hg)	DET	39.0 ± 1.8	38.6 ± 1.7	38.1 ± 2.3	37.9 ± 2.6	39.9 ± 3.3	42.1 ± 2.1	41.6 ± 4.2	39.8 ± 1.4	40.0 ± 3.1
	DET/MET	39.7 ± 1.5	40.1 ± 3.5	39.4 ± 3.6	40.6 ± 4.7	40.9 ± 4.9	39.5 ± 4.2	41.9 ± 3.4	39.9 ± 3.1	39.5 ± 2.0
PaO_2 (mm Hg)	DET	90.7 ± 7.2	94.0 ± 11.8	89.9 ± 4.1	76.0 ± 12.3[b]	89.4 ± 9.1	84.8 ± 7.6	87.5 ± 6.7	85.1 ± 2.1	85.5 ± 5.3
	DET/MET	96.9 ± 10.1	93.0 ± 9.8	93.3 ± 9.7	73.5 ± 15.9[b]	78.4 ± 13.7[b,a]	89.8 ± 13.0	83.5 ± 7.0	85.6 ± 8.0	83.8 ± 6.6
$HCO_{3\,std}$ (mmol/L)	DET	26.6 ± 1.2	25.9 ± 1.0	26.4 ± 2.1	26.0 ± 1.4	26.4 ± 2.0	27.3 ± 1.3	28.9 ± 0.9[a]	27.6 ± 1.0	26.5 ± 1.0
	DET/MET	27.4 ± 1.3	27.4 ± 1.3	27.1 ± 1.5	27.5 ± 1.7	28.3 ± 1.5[a]	29.3 ± 1.1[a]	30.1 ± 1.1[a]	28.6 ± 1.1[a]	28.7 ± 0.8[a]

The drugs were administered at time 0. Baseline values were recorded 30 and 15 minutes before drug administration.
[a] Significant difference between treatments ($P < .05$).
[b] Significant difference between treatments with baseline ($P < .05$).

excitatory effect of MET [6]. According to that we hypothesize that lower doses of opioids may stimulate cardiovascular activity not related to behavioral changes, these and other mechanisms yet to be elucidated as this is beyond the scope of this study.

The blood gas changes were of minimal clinical relevance. The decrease in PaO_2 values is a typical effect of alpha-2 agonists [2] that could have been potentiated by the use of MET. Although the respiratory effects of opioid drugs are not always predictable [8], the combination of some opioids with alpha-2 agonists seems to potentiate their respiratory depressant effects [7,8].

In conclusion, a combination of DET and MET at the doses reported here causes a higher and more prolonged increase in MAP than DET alone in horses. However, this effect is not related to changes in AVP or catecholamine plasma concentrations.

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