Article

The effect of drotaverine on gastrointestinal motility and heart rate variability in normal, resting horses

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Simple Summary: The influence of a spasmolytic drug (drotaverine hydrochloride) on heart rate variability (HRV) and gastrointestinal tract contractions in normal, resting horses was evaluated. Ten adult horses had electrocardiogram recording for three hours. Every horse received the saline and then drotaverine hydrochloride, separated by 1 week at the same time of day. Drotaverine treatment induced gastrointestinal hypomotility for two hours but also it showed drowsiness (relaxed wakefulness). HRV was analyzed in the time domain, frequency domain and nonlinear parameters. Drotaverine caused a significant decrease in heart rate compared to those receiving saline, with values remaining within physiological limits. All frequency domain and non linear parameters showed low values after drotaverine administration pointing a diminished in parasympathetic and sympathetic activity. Decreased HRV was suggestive for a pervasive state of sympathetic hypervigilance of horses a daily state of sympathetic hypervigilance. The significant effect of drotaverine on HRV and gastrointestinal function in normal horses should be considered especially when evaluating a clinical response to this drug in diseases associated with smooth muscle spasm.

Abstract: The impact of a non-anticholinergic spasmolytic drug (drotaverine hydrochloride) on heart rate (HR), HR variability (HRV) and gastrointestinal tract (GIT) motility in the normal, resting horses was determined. Ten adult riding horses had ECG recordings for 180 minutes after treatment with drotaverine (180 mg) or saline. Horses from the drotaverine group presented a decreased of GIT contraction for two hours after treatment, but also showed drowsiness. Drotaverine caused a reduction in cardiac vagal modulation of HR at T30 (P=0.050) and T60 (P=0.008) compared to those receiving saline. RMSSD and high frequency (HF) recorded significantly low values after drotaverine treatment by the end of the study, suggesting a decrease in parasympathetic activity. HRV analysis indicated that drotaverine decreased both the low frequency (LF) and Lf/HF ratio reflecting a decrease in sympathetic system. Non linear parameters registered low values after drotaverine administration pointing a diminished in parasympathetic (for SD1) and sympathetic (for SD2) activity. Decreased HRV was suggestive for a pervasive state of sympathetic hypervigilance of horses. The marked effect of drotaverine on HRV and GIT function in horses should be taken into consideration when evaluating a clinical response to this drug in diseases associated with smooth muscle spasm.

Keywords: antispasmodic; heart rate variability; intestinal function; parasympathetic activity

1. Introduction

Drotaverine is a synthetic derivative of the natural isoquinoline alkaloid of *Papaver somniferum,* papaverine. It is an efficient muscle spasmolytic [1,2]. It acts as a selective inhibitor of phosphodiesterase-IV, stimulates the cyclic adenosine monophosphate (cAMP) synthesis in tissues and has no anticholinergic effects [3–5]. Increased level of

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cAMP affect cellular processes by inhibiting the contractile process in smooth muscle [6,7]. The specific mode of action of drotaverine includes reduction of cytosolic Ca2+ level and/or desensitization of the contractile apparatus [8]. Both processes are fundamental for the development of smooth muscle contraction or relaxation [9] in all types of smooth muscle tissues, involving both spontaneous phasic contractions and maintenance of smooth muscle tone [10].

In humans, drotaverine can suppress spasm-associated pain and is successfully used in the symptomatic treatment of various conditions such as gastrointestinal, urinary, biliary, vasomotor diseases associated with smooth muscle spasms [1,11], it has a cytostatic effect [12] and improves cognitive function [13]. It is available without prescription in many countries of Central and Eastern Europe (Poland [11,14–16], Hungary [17], Estonia, Croatia, Latvia [4], Romania [18,19]), Africa (Nigeria [20]) and Asia (India [21–23], Russia [24], China [25] under several brand names.

In animals, there are few studies on drotaverine despite the fact that there are veterinary products approved without prescription that contain this substance indicated in dogs, cats, horse and cattle: Espa-Vet® (Square Pharmaceuticals Ltd®, Dhaka, Bangladesh); Drotavet® (Romvac®, Ilfov, Romania); Vetalgin® (AVZ Animal Health®, Moscow, Russia). Studies on spasmolytic effect of drotaverine were performed in cows with dystocia [26], vaginal prolaps [27] and in horses with abdominal colic [28]. Other authors suggested stomach emptying inhibition of drotaverine in Beagle dogs [29]. In mice brain, drotaverine at dose of 20, 40 and 80 mg/kg, has increased neurotransmitter levels being associated with improvement of learning and memory [30]. In horses, colic is one of the most common problems reported. Spasmodic colic has been estimated to be as high as 72% of all colic cases examined in general practice [31]. In the management of equine colic characterized by intestinal hyper motility, anticholinergic, spasmolytic agents such as hyoscine-N-butylbromide (hyoscine) and propantheline bromide (propantheline) are usually recommended [32]. These drugs block muscarinic cholinergic receptors of the parasympathetic nervous system, thereby decreasing gastrointestinal motility and pain but elevating heart rate [33].

Heart rate variability (HRV) is an effective, non-invasive and easy-to-apply tool used in humans and animals to investigate the sympathovagal balance of the autonomic nervous system (ANS) which involved in pain and stress reactions [34–38]. The HRV measurement is based on the mathematical analysis of precisely measured variations in successive inter-beat intervals, defined as RR intervals (normal-to-normal intervals). In horses, the HRV has been used in many studies for behavioral research [39–41], to assess different pain conditions [36,42], stress [43], to identify dysautonomia in equine grass sickness [44], and to monitor the influence of special surgical investigations on the autonomous nervous system [45]. There are essentially three approaches for quantifying HRV: time-domain analyses, frequency-domain analysis and nonlinear analysis methods. The time domain analysis is focused on the analyses of the inter-beat interval, which is the time period between consecutive heart beats. The parameters of time domain analyses on short time measurements are: the mean heart rate, the mean RR intervals, standard deviation of all NN intervals (SDNN), root mean square of successive differences between RR intervals (RMSSD) and pNN50 (percentage of differences between NN intervals greater than 50 ms) [37]. The RMSSD typically provides a better assessment of respiratory sinus arrhythmia and most researchers prefer it to the pNN50 [46]. Decreases in time-domain values of the HRV variables SDNN and RMSSD indicate a shift towards more sympathetic dominance, while increases indicates a shift towards parasympathetic dominance [37]. The most widely used tool in the frequency domain is spectral analysis, which means decomposing the heart rate variation in a given period into its fundamental oscillatory components, defining them by their frequency and amplitude. One of the mathematical algorithms most commonly used to determine the number, frequency and amplitude of these components is the fast Fourier transform (FFT) [47]. Frequency domain includes three main spectral components: very low (VLF), low (LF) and high (HF) frequency peaks. The VLF band appears in short time measure-

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ments but should only be interpreted in long-term recordings (24 h). The way in which autonomic nervous system regulation is involved in the VLF component has not yet been determined and some authors even question any direct relationship between the VLF and the ANS [47]. Frequency domain can be used to evaluate the vagal influences and to deduce the sympathetic activity [48].

Nonlinear analysis of HRV uses a variety of approaches. A useful graphical representation of HRV is provided by the Poincaré diagram (a graphical representation of the correlation between RR intervals) with SD1 (standard deviation 1) and SD2 (standard deviation 2). SD1 reflects short-term component of HRV derived from a quantitative analysis of Poincaré plot and it is an index of parasympathetic activity, whereas SD2 reflects long-term component of HRV derived from a quantitative analysis of Poincaré plot and is regarded as an index of sympathetic activity [49].

The effect of a non-anticholinergic spasmolytic drug on HRV has never been previously documented in horses. The aim of the present study was therefore to investigate the influence of drotaverine chlorhidrate on heart rate, HRV (time domain, frequency domain and nonlinear analysis) and gastrointestinal tract contractions in normal, resting horses.

2. Materials and Methods

A total of ten adult riding horses of mixed sex (3 geldings, 3 stallions, and 4 mares) stabled at a private boarding facility were used in this study. The sample size was chosen based on a previous study of spasmolytic agents on HRV and gastrointestinal motility [33] in which a sample of five horses was sufficient to yield statistically significant changes in the HRV. Horses had a mean body weight of 450 ± 40 kg, and were between 5 and 10 years of age. They were kept in individual boxes on sawdust and were fed concentrates 3 times daily (6:00, 12:00, 17:00 hours) and hay twice daily (6:00 and 17:00 hours). Water was provided ad libitum. All horses were habituated to handling and being secured in a stock for the recording period with ECG leads placed.

Horses were ineligible for inclusion in the study if they had any of the following: cardiac disease (congenital or acquired); current or previous 6 months clinical signs attributable to important systemic diseases; received cardiovascular or respiratory medications including corticosteroids or non-steroidal anti-inflammatory within the previous 6 months; or undergone a general anesthetic in the last month. In addition, mere that were pregnant or lactating could not be included. All horses were healthy throughout the study. The horses individually underwent one control (saline) and a treatment (drotaverine) on two separate experimental sessions. The two experiments were as follows: (i) 5 ml saline (solution of sodium chloride 0.9%); (ii) drotaverine hydrochloride 180 mg (9 mL total volume of Drotavet® 20mg/mL, Romvac®, Ilfov, România). All drugs were administered slowly intravenously via a 21-gauge needle. Every horse received the control (saline) and then experimental (drotaverine hydrochloride) treatment, separated by 1 week at the same time of day (2-4 pm), thus allowing for each horse to serve as its own control to account for possible confounding variables such as personality, breed, sex, and age. One recording was made per day. Every horse participated in both the saline and the drotaverine hydrochloride studies.

On the day of experimentation, the individual horse was secured in stocks with free access to food (hay/chaff mix) and water. The horses stood quietly relaxed during electrode attachment, demonstrating minimal, if any, stress related to monitor hook-up stage. They were monitored non-invasively with digital ECG system for small and large animals (Poly-Spectrum-8E/8V, Neurosoft version 4.8.131.0., Ivanovo, Russia) for measurement of heart beat activity, specially interbeat intervals (RR intervals), as described by [50]. ECG parameters have been measured at speed 100 mm/s and amplitude 20 mm/mV in lead II. This program offers the possibility of saving and archiving the electrocardiographic record and subsequent analysis, as well as changing the recording parameters and performing electrocardiographic deflection measurements. ECG elec-

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trodes were connected to the horse using a base-apex lead method with reposition of the ground limb electrode to the xiphoid minimize the variation of ECG morphology, particularly the configuration of the P wave and the QRS complex [51]. Warm water and methylated spirit were used to optimize electrode to skin contact.

Data from ECG recording have been exported of an RR intervals file, which were then processed using a HRV Analysis Software (Kubios HRV 2.2, Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland) as described by Rietmann et al. [35,36], Tarvainen [52] and Gehlen et al. [42]. The RR intervals were measured in milliseconds. To remove trend components, data were detrended with Kubios software and, in addition, an artifact correction was made [53] following established procedures described by Tarvainen [52] and Tarvainen et al [54]. Data for HR and HRV analysis was obtained before (T0) and after drug injection every 30 minutes at T30, T60, T90, T120 and finally at T180. Stable 5 min segments of data for analysis were chosen from a 10 min window around T0, T30, T60, T90, T120, T180 min as previously described by Rietmann et al. [35,36], Thayer et al. [55] and Sundra et al. [33].

HRV parameters were analyzed in the time domain, frequency domain and non-linear components. The parameters of time domain analyzed are: the mean heart rate, the mean RR intervals, Standard Deviation of all NN intervals (SDNN) and Root Mean Square of Successive Differences between RR intervals (RMSSD). Moreover, HRV was analyzed in the frequency domain based on power spectral analysis using a fast Fourier transformation. The HRV parameters in the frequency domain were the high-frequency (HF) ranges, low-frequency (LF) ranges, and the low-/high-frequency ratio (LF/HF). The frequency component thresholds for LF were set at 0.04–0.15 Hz and for HF at 0.15-0.4 Hz [33,56,57]. LF/HF was calculated as an additional variable. Nonlinear variables included standard deviations of the points in the Poincaré plot: SD1 and SD2.

Gastrointestinal motility was assessed by auscultation with a stethoscope for 2 minutes for all horses as described by Sasaki et al. [58]. Gut sounds were assessed in the right and left dorsal and ventral flank regions before (T0) and after treatments, every 30 min at T30, T60, T90, T120 and finally at T180. Gastrointestinal contraction scores were assigned for each quadrant with a score as described by Sundra et al. [33]. Score 0 was recorded when there were no gut sounds, score 1 was when the period of no borborygmi was longer than the period of peristaltic sound and score 2 indicated regular and ongoing peristaltic sound (normal activity). Results from each quadrant were summed to obtain a score: 0 was considered absent, 1-6 was regarded as reduced and 7-8 was deemed as normal sounds.

Statistical analyses were carried out in SPSS (IBM®-SPSS Inc., Chicago, IL, USA, Version 22.0). The normality of data distribution was verified with the Kolmogorov-Smirnov test. Because the data were normally distributed, results were reported as mean and standard deviation (SD). Analyses of variance (ANOVA) tests were done to evaluate the overall effect of saline and drotaverine over time. In the case of significant main effect, the differences between the group means were tested using the Tukey test. Moreover, paired one tailed Student's t-test was calculated to assess the significant differences between the saline and drotaverine group. The level of statistical significance was set at $P \leq 0.05$ as significant, $P \leq 0.01$ as highly significant, and $P \leq 0.001$ as most significant.

3. Results

This A total of ten horses of mixed sex were enrolled in this study. The mean age of these horses was 8 ± 1,6 years with mere predominant (40%). All horses received saline and drotaverine hydrochloride treatment, separated by 1 week.

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Cardiac RR interval recordings and HRV analyses were successfully performed and the horses were healthy throughout the entire study. The results of the HRV parameters are presented in Table 1.

There was no significant difference in the baseline (T0) data for RR intervals, HR, SDNN, RMSSD, LF/HF, SD1 and SD2 prior to the administration of the treatments.

In the saline group there was no effect of time for any of the variables (RR, P=0.255; HR, P=0.393; RMSSD, P=0.381; SDNN, P=0.127; 0.256; LF, P=0.112; HF, P=0.275; LF/HF, P=0.118; SD1, P=0.439; SD2, P=0.185).

RR interval tended to increase an T30 (1771.55±74.76 ms) in response to drotaverine but this increase did not reach statistical significance (P=0.065) instead highly significant differences between groups at T60 (P=0.010) were noted.

Drotaverine caused a significant decrease in HR at T30 (P=0.050) and highly significant at T60 (P=0.008) compared to those receiving saline, with values remaining within physiological limits. Throughout the study HR values in horses of both groups were within normal limits.

RMSSD had significantly decreased values by the end of the study in horses receiving drotaverine compared to the saline group. Moreover, RMSSD values decreased highly significant at T90 (P=0.009) in drotaverine group compared to saline.

In the present study, drotaverine caused a highly significant decrease in SDNN at T30 (P=0.008) and the most significant at T60 (P=0.001) compared to saline group.

Table 1. Heart rate variability (HRV) parameters and gastrointestinal motility at the measurement time points (T0, T30, T60, T90, T120, T180) in the two treatment groups

HRV parameter	Group	Recording Time					
		T0	T30	T60	T90	T120	T180
RR interval (ms)	S	1576.05±149.58	1606.7±149.52	1557.25±121.54	1634.25±130.96	1514.15±74.32	1553.85±125.26
	D	1626.9±163.33	1771.55±74.76	1824.25±75.31**	1545.5±37.90	1696.1±175.49	1594.35±286.95
SDNN (ms)	S	152.05±30.72	169.55±3.23	194.25±11.22	166.35±18.78	155.25±31.49	164.25±44.09
	D	163±34.94	113.85±35.43*	94.75±2.79***	179.3±73.72	183.45±20.21	160.35±5.09
Heart rate (beats/min)	S	38.76±3.56	38.12±3.57	39.425±3.06	37.35±2.92	40.21±2.2	38.35±4.02
	D	37.61±3.65	34.08±1.34*	33.035±1.36**	39.215±0.60	36.23±3.91	39.15±7.10
RMSSD (ms)	S	64.8±14.67	147.95±48.36	56.56±12.42	68.35±1.25	83.86±45.61	71.05±3.45
	D	74.9±6.35	63.55±24.48*	45.75±5.31*	80.55±7.50**	104.05±26.67*	131.3±56.52*
LF (ms^2)	S	2574±1191.84	5391.5±594.27	5477.5±4567.46	1363±915.79	6734±3191.03	2485±1289.34
	D	4729±369.16*	2811±2751.75*	342.5±0.54*	3964.5±1446.53**	8157±1146.93*	5339±1940.03*
HF (ms^2)	S	773±281.52	5901±3456.13	2265±1680.41	698.5±164.86	5300.5±1644.81	880.5±163.76
	D	4161.5±2378.75*	522±422.84*	448±283.72*	1078.5±560.32*	3758±3040.96*	1939±1048.34*
LF/HF	S	3.21±0.37	1.34±0.89	2.16±0.41	3.74±0.56	1.55±1.08	2.67±0.96
	D	5.10±3.17	4.03±2.00**	1.14±0.72*	3.99±0.73*	4.32±3.19*	4.23±3.29
SD1 (ms)	S	44.9±8.43	105.05±34.34	66.3±19.82	48.55±0.82	86.75±2.57	50.4±2.40
	D	53.2±4.38	45.15±17.47*	36.15±3.88*	57.15±5.42*	73.9±18.84*	93.25±40.03*
SD2 (ms)	S	209.55±46.39	213.35±11.44	265.8±21.68	229.8±28.59	200.3±50.06	226.4±64.85
	D	209.55±46.39	213.35±11.44	265.8±21.68	229.8±28.59	200.3±50.06	226.4±64.85
GIT motility	S	Normal	Normal	Normal	Normal	Normal	Normal
	D	Normal	Reduced	Reduced	Reduced	Reduced	Normal

Data are expressed as the mean ± SD; *significant P ≤ 0.05; ** highly significant P ≤ 0.01; *** most significant P ≤ 0.001; S, Saline group; D, drotaverine group; GIT, gastrointestinal tract

All frequency domain parameters showed low values after drotaverine administration. Furthermore, in the drotaverine group, HF recorded significantly low values (T0, P=0.050; T30, P=0.042; T60, P=0.012; T90, P=0.032; T120, P=0.021; T180, P=0.042) throughout the study relative to the control group. LF also recorded significantly low values (T0, P=0.050; T30, P=0.026; T60, P=0.030; T120, P=0.050; T180, P=0.048) during the entire study relative to the saline group. Moreover at T90 the difference was highly significant (P=0.010). Over the period T30-T120, the LF/HF ration was significantly lower (T60, P=0.036; T90, P=0.044; T120, P=0.020) in drotaverine group compared with saline. Drotaverine caused a highly significant decrease in LF/HF ration at T30 (P=0.010).

Non linear parameters also showed low values after drotaverine administration. SD1 was significantly suppresed by drotaverine relative to saline by the end of the study (T30, P=0.020; T60,

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P=0.022; T90, P=0.038; T120, P=0.050; T180, P=0.025). The most decreased values was at T60 (36.15±3.88 ms). Drotaverine significantly reduced SD2 compare to saline at T30 (P=0.036) and T60 (P=0.045).

In the present study, drotaverine treatment induced a decrease in gastrointestinal motility at T30, T60, T90 and T120. The most decreased values were one hour after drug administration. Horses receiving drotaverine showed drowsiness as a side effect expressed by holding the head above the withers, weight was sustained on the thoracic limbs and one pelvic limb. The flexed limb was often raised to kick and occasionally stretched caudally appearing as a stretch.

4. Discussion

Heart rate variability analysis has been used to evaluate changes in sympathovagal balance associated with the effects of drotaverine in horses. In the present study all horses received the experimental (drotaverine hydrochloride) and control (saline) treatment, separated by one week allowing for each horse to serve as its own control to account for possible confounding variables such as age, personality, sex and breed. Although previous studies have reported evidence to suggest that HRV in horses exhibits good age stability and a high degree of repeatability when tested over subsequent days [35,36,59], large interindividual variations in the basal values of HRV in horses have been also described [37]. Moreover, in one study, Nagel et al. [60], at no time found no significant differences between morning and afternoon recordings.

The ten horses have been monitored non-invasively with digital ECG system that has been suggested by several authors [37,61,62] to be the gold standard for recording of heart beat activity, specially interbeat intervals (RR intervals). ECG recordings in horse are not totally free from errors. Error sources include disruption of normal electrical activity in the heart, movement of electrode leads, muscle contraction, or problems with the monitoring equipment and environmental electromagnetic interference [63]. Although a single anomaly in short recording intervals may affect HRV parameters, its presence may be without effect in longer segments of data but non-linear analytical methods seem more resistance to the presence of spurious beats [37]. In our study, stable 5 min segments of data for analysis were chosen from a 10 min window before and after saline or drotaverine administration. The Task Force of the ESC and NASPE [47] recommends that RR intervals data sets undergoing HRV analyses should contain at least 5-min of consecutive RR intervals measured during stationary conditions [37]. Previous studies have shown that analyzing 5-min segments of RR intervals data in the time-, frequency- and non-linear domains provides comparable or even better results than analysis of 24-hour data [64,65].

There are three methods for quantifying HRV: time domain, frequency domain and non-linear analysis methods. In horses, changes in time domain HRV variables have been reported in relation to equine activities [53,63,66], foaling [67,68], weaning [69], hot iron branding of foals [70], road transport [71–73] although not air transport [74], or changing the stabling system in adult horses [75]. Horses receiving drotaverine had higher RR values than the control group throughout the duration of the study, with highly significant differences between groups at 1 hour after drotaverine administration which can be related with an increase of parasympathetic activity. Heart rate is a suitable parameter for short-term assessment for studying animal responses to physiological or environmental challenges and to judge the level of stress load on the animals [76,77]. Significantly lower HR values were observed at T30 and highly significant at T60 in horses receiving drotaverine compared to those receiving saline. Throughout the study heart rate values in horses of both groups were within normal limits. Reduced heart rate can occur from increased parasympathetic activity as well as from decreased sympathetic tone or in most cases from a combination of both [37]. In the present study, RMSSD had significantly decreased values by the end of the study in horses receiving drotaverine compared to the saline group indicating a decrease in parasympathetic activity. Authors report that RMSSD reflects alternations in the autonomic nervous system (ANS) that are predomi-

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nantly vagally mediated [64]. In contrast, SDNN is more complex parameter being influenced by both sympathetic and parasympathetic activity [37]. The European Task Force [47] concluded that the SDNN is a very imprecise variable for the characterization of HRV, especially for the short ECG procedure. In the present study SDNN decreased the most significant one hour after drotaverine administration comparing with saline group. The analysis of time-domain parameters is straightforward but usually insufficient to make statements about the sympathetic–vagal balance.

The frequency domain analysis is based on a power spectral analysis using a fast Fourier transformation and the obtained power specters are decomposed in a high frequency (HF) range and a low frequency (LF) range [37,78]. All frequency domain parameters showed low values after drotaverine administration. Furthermore, in drotaverine group, HF recorded significantly low values from the begining to the end of the study indicating a decrease in parasympathetic activity. The authors suggested that the parasympathetic system reacts faster than the sympathetic system, therefore HF variations mainly represent vagal activit [37]. The LF component which mainly measures the sympathetic tone [79], was decreased during the entire study relative to the saline group. Authors have reported that LF is a poor marker for sympathetic drive, as it likely results from the interaction of both vagal and sympathetic activity and probably influences from other physiological pathway [80,81]. Horses receiving drotaverine showed a significant decrease in LF/HF ratio for 120 minutes after drug administration, reflecting a decrease in sympathetic versus parasympathetic activity.

Nonlinear mechanisms are involved in heart rate regulation. It is well known from studies in human medicine that evolution over time of HRV contains nonlinear chaotic components [82–84]. Research in ruminants indicates that the combination of linear (time and frequency domains) and non-linear parameters of HRV can be used as a sensitive indicator of stress [85,86]. Quantitative Poincaré measures were reported to provide useful information on the vagal regulation of cardiac dynamics that is not easily revealed by other domains of HRV analysis. Poincare plot is a two-dimensional graphical representation of the correlation between consecutive RR intervals. It is a quantitative method obtained by adjusting an ellipse to the figure formed by the plot, from which the indices are SD1 (standard deviation of instantaneous inter-beat interval variability measured from axis 1, marker of parasympathetic modulation [49,87]) and SD2 (standard deviation of long-term continuous inter-beat interval variability measured from axis 2, marker of sympathetic modulation [49,88]). The decrease in both parameters, RMSSD and SD1, reflects a markedly reduced parasympathetic tone in reaction to drotaverine administration. SD1 considered as indexe of pain [89] was more sensitive to drotaverine recording low values for 3 hours after administration while SD2 was decreased only one hour. Geometric analysis also showed significant changes for SD2 that are predominantly caused by changes in sympathetic regulation [37].

Drotaverine has a good relaxing effect on intestinal smooth muscle, which helps in alleviating pain and does not have side effects like anticholinergics [3–5]. In the present study, drotaverine treatment induced a decrease in gastrointestinal motility for two hours after administration. This is consistent with findings in previous study [90] which demonstrated that large intestinal motility is restore within 30 minutes while duodenal contractions returned to the normal rate after 120 min of hyoscine-N-butylbromide administration. Other authors [91] found that dipyrone in combination with hyoscine-N-butylbromide relieved pain within 30 seconds after injection and the relief lasted for 50 minutes in ponies, but hyoscine-N-butylbromide alone produced an analgesic effect after injection which lasted for 20 minutes. In spasmodic colic, horses show brief attacks of pain associated with hypermotility and loud sounds of the intestine on auscultation. It can affect both small and large intestines [32]. Studies have recommended drotaverine in persistent colic in horses for the spasmolytic effect that occurs within 30 minutes of the first injection [28] but it is not specified how long the effect lasts. Hyoscine-N-butylbromide and drotaverine hydrochloride were compared for spasmolytics effectiveness and the results obtained determine drotaverine as more appropriate than

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hyoscine in inducing relaxation to provide optimum colonic distension in CT colonography in human [10]. Hyoscine is also a vagolytic drug used in horses with acute abdominal pain [42]. It reduced the high frequency (HF) power in one equine study [33], whereas other studies in humans [92–94] have shown that hyoscine at a low dosage can paradoxically increase the vagal activity. Other authors compared the effects of two spasmolytic and anticholinergic substances (hyoscine-N-butylbromide and propantheline-bromide) and found that both reduced gastrointestinal sounds, the effects of propantheline being longer lasting in horses [33]. In contrast to drotaverine, the two substances increase heart rate in horses. Considering that HR is a key variable used in the management of colic, the potential influence of these drugs on HR raises concerns when choosing this medical pathway as a treatment for colic [33].

The most common adverse drug reaction of drotaverine are dizziness, hypotension and sleep problems [95]. In the present study, all horses receiving drotaverine were standing for the duration of the study but experienced drowsiness as a side effect. Authors identified four stages of vigilance in horses: wakefulness, drowsiness, slow wave sleep and paradoxical sleep (also called REM) [96]. In horses adapted to their environment, wakefulness and drowsiness occur while standing and heart rate decreases to 32-36 beats/min [96]. Drowsiness (relaxed wakefulness) was defined as a period of quiescence, standing or lying still, that followed wakefulness. The slow wave sleep occurs during sternal recumbency and REM is associated with lateral recumbency [96]. In cows, the authors found that HR decreased with sleep depth and REM was associated with increased HRV. Regarding non-REM sleep stages, cows presented more activity of the parasympathetic while sympathetic nervous systems was reduced [97]. In humans, during sleep, RMSSD and HF parameters are reduced, suggesting a decrease in vagal modulation of heart rate [98]. Horses with drowsiness from drotaverine group, showed a decrease of parasimpathetic and sympathetic activities although the drug has no anticholinergic effects. Authors suggested that decreased HRV could be interpreted as a state of autonomic hypervigilance caused by daily stress [99,100]. At rest, horses have a high parasympathetic (vagal) tone and sympathetic activity plays little role in determining heart rate [78]. Activation of the sympathetic branch of the autonomous nervous system and decreased parasympathetic tone are the body immediate physiological reaction to perceived danger [101].

It is difficult to achieve standardized experimental conditions in studies with horses, hence the control of ANS stimulation to achieve high correlations and significant changes in HRV measures remains difficult. It has been proposed that several factors including the behavior, temperament, environment, nutritional status and genotype of the horse play a major role in the large inter-individual variations in basal HRV [37]. The balance between sympathetic and parasympathetic nervous activity has an important role to play in HRV, as a decrease in HRV is an indicator of an autonomic system imbalance.

The limitations of the study are represented by the inhomogeneous age, sex, breed, temperament of the horses group. Studies provide controversial findings on the influence of age or sex on HRV in animals. Regarding the age, a study comparing horses between 4 and 6 years of age and horses >22 years of ages found a higher HR and lower interval RR in the older horses [102]. However, another study did not find any significant influence of age on the HRV in horses [36]. In another study the individual variability was partly explained by the sex and age effects while the breed effect had only a weak influence [103]. In a study authors found that there was no breed difference in HRV concluding that heart rate and heart rate variability values determined in healthy warmblood fetuses can be used as reference values for the Shetland pony [104]. Finally, horse temperament may influence HRV [35,105] but it could not be evaluated in the current study.

5. Conclusions

This study provides evidence of a significant reduction in cardiac vagal modulation of heart rate and gastrointestinal motility following drotaverine administration. Although the horses received the recommended dose of drotaverine, all experienced drowsiness as a side effect of this drug. Thus, future studies on electroencephalography are needed to assess cerebral cortical function and to identify different stages of sleep in horses receiving drotaverine.

Another interesting finding was that horses showed a decrease in parasympathetic and sympathetic activities, although drotaverine has no anticholinergic effects. As a result, the conclusion is that decreased HRV is suggestive for a pervasive state of sympathetic hypervigilance. The marked effect of drotaverine on HRV and GIT function in normal horses should be taken into consideration when evaluating a clinical response to this drug in diseases associated with smooth muscle spasm. However, more studies with homogeneous groups of horses are necessary to shed more light on the effects of drotaverine on HRV and gastrointestinal motility under clinical conditions, as well as to investigate repeated administration of this drug.

6. Patents

Author Contributions: Conceptualization, A.A.; methodology, A.A.; software, A.A.; validation, A.A.; formal analysis, A.A.; investigation, A.A.; resources, A.A.; data curation, A.A.; writing—original draft preparation, A.A.; writing—review and editing, A.A.; visualization, A.A.; supervision, A.A.; project administration, A.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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