Journal of Veterinary Behavior 9 (2014) 248–253

Contents lists available at ScienceDirect

Journal of Veterinary Behavior

journal homepage: www.journalvetbehavior.com

Research

Plasma serotonin, tryptophan, hematological, and functional responses to horse trekking

Giuseppe Bruschetta, Patrizia Di Pietro, Esterina Fazio, Alida M. Ferlazzo*

Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, Messina, Italy

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form
20 May 2014
Accepted 6 June 2014
Available online 13 June 2014

Keywords:
serotonin
tryptophan
hematological variables
functional variables
horse

ABSTRACT

Horse trekking is a noncompetitive physical activity conducted as a recreation and leisure experience, but so far potential effects on physiological changes in horses have not been investigated. The aim of the present research was to study whether trekking would affect plasma serotonin (5-hydroxytryptamine [5-HT]) concentrations in platelet poor plasma samples, together with its precursor tryptophan, and other hematological and functional variables. The study was carried out on 28 clinically healthy horses, habitually used for trekking, of which 18 participated in a 2-day trekking event, and 10 were used as the control group. The results obtained showed a significant effect of exercise on the serotonin, red blood cell, white blood cell, platelet, hematocrit, hemoglobin, heart rate, respiratory rate, and rectal temperature changes. These effects are probably related to a combination of submaximal exercise and some degree of dehydration. Prolonged and aerobic exercise, like that involved in trekking, could affect both the release of 5-HT from the stores in the blood stream as well as hematological and functional adaptations in response to physical effort. Moreover, results confirm a physiological influence of 5-HT in the regulation of the vascular system, induced by exercise. The present findings imply that assessment of plasma 5-HT as well as hematological and functional variables may be proposed as an additional tool to evaluate the physiological adjustment in response to physical activity in trained horses that may influence their performance.

Introduction

Horses provide a means of entertainment because they are involved in a wide variety of sports and leisure activities. Horse trekking involves having horse and rider walking along fields, mountains, hills, and sandy beaches, and it can easily be performed in all seasons. Trekking has become a very popular recreational activity, offering significant positive effects on human health. This physical activity is used to maintain and improve aerobic fitness and involves significant total energy expenditure in horse, as suggested by cortisol and hematochemical changes (Medica et al., 2010). Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter synthesized from the amino acid tryptophan (Linder et al., 2007). It controls a wide variety of processes in brain and peripheral tissues, but, so far, the function of circulating 5-HT has been little investigated in different species. The literature reports 5-HT values measured using different methods applied to humans (Brenner et al., 2007) and horses (Haritou et al., 2008; Lebelt et al., 1998; Torfs et al., 2012). An increase in serum 5-HT concentrations is often related to common horse pathologies as acute laminitis (Ayala et al., 2012). An influence of age (Ferlazzo et al., 2012a) on daily variations of plasma 5-HT levels in 2-year-old mares (Bruschetta et al., 2013) has been observed, as has an effect of diet on plasma tryptophan and serotonin in trained horses (Alberghina et al., 2010a). A significant effect of exercise on plasma and whole blood 5-HT has been reported in show jumping horses (Alberghina et al., 2010b). Equine hematological parameters and functional variables during athletic activity have been well studied because they are indices of performance, helping in planning the training programs and competitive activities (Evans et al., 1995). As limited information is available regarding the effects of trekking on the physiological horse's responses, this study was designed to investigate whether a noncompetitive physical activity as trekking would affect plasma 5-HT concentrations, tryptophan, as well as hematological and functional variables in clinically healthy horses.

* Address for reprint requests and correspondence: Alida M. Ferlazzo, BSc, Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy. Tel: +39-90-3503588; Fax: +39-90-3503975.
E-mail address: alferl@unime.it (A.M. Ferlazzo).

1558-7878/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jveb.2014.06.001

Materials and methods

This study was approved by the Ethical Committee for the Care and Use of Animals of University of Messina, in accordance with the standards recommended by the European Union (EU) Directive 2010/63/EU.

Animals

The research work was carried out on 28 clinically healthy horses, heterogeneous in breed (5 thoroughbreds, 8 Appaloosas, 15 crossbreds), 13 geldings, and 15 mares, aged 10 ± 4 years (mean body weight, 480 ± 50 kg) often used for trekking activities. All experienced and trained horses were randomly divided into 2 groups, paired by gender and age. Group A (18 horses: 3 thoroughbreds, 4 Appaloosas, 11 crossbreds; 8 geldings; and 10 mares) was designed as working animal group, taking part in 2-day trekking tour in the Peloritani mountain district (total trekking distance, 74 km; highest point, 1120 m); group B (10 horses: 2 thoroughbreds, 4 Appaloosas, 4 crossbreds; 5 geldings; and 5 mares) was designed as the control group, represented by animals in resting condition. All horses were stabled in individual boxes (5×4 m^2) with natural lighting, allowing reciprocal visual contact, in the starting place. The animals were individually fed with a mixed diet of silage (50%) and fresh forage (50%). They also had free access to water. Horses were weighed in resting condition and at the end of 2 consecutive days of trekking using an electronic scale.

Blood collection

Blood samples were collected, at the starting place, in May, from the jugular vein using vacutainer tubes (Venoject; Terumo Europe N.V., Leuven, Belgium) with tripotassium-ethylenediaminetetraacetic acid for hematological variables and heparin for high-performance liquid chromatography (HPLC) analysis (5-HT and tryptophan); in group A at 9:00 (T_0, pre-exercise), 14:00 (T_1, post-exercise), and 17:00 (T_2, post-exercise) during the first day of trekking (length of route, 40 km; duration of trekking, 6 hours; mean speed, 7 km/hour), with a pause of 2 hours (from 14:00 to 16:00) and at 9:00 (T_0, pre-exercise) and 14:00 (T_1, post-exercise) during the second day of trekking (length of route, 34 km; duration of trekking, 5 hours; mean of speed, 7 km/hour).

Blood sampling of group B (control group) was carried out at the same time of group A.

Heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were performed before blood sampling. All samples were collected by the same operator under quiet conditions. Environment temperature recorded from start to finish ranged between 20 and 25°C. The typology of route was mainly characterized by hill course, and horses were ridden by the same owner riders during the 2-day trekking tour.

Sample processing

Hematological variables were performed within 1 hour from collection of tripotassium-ethylenediaminetetraacetic acid plasma samples. Afterward, blood samples in heparin were centrifuged at 4°C at 4500×*g* for 10 minutes to obtain a platelet poor plasma (PPP) fraction, devoid of 98% of platelets (mean value ± standard deviation [SD] of remnant platelets, 4 ± 0.7 K/μL). Equal volumes (100 μL) of internal standard represented by *N*-methylserotonin (Chromsystems, München, Germany) and protein precipitation reagent (Chromsystems) were added to 100 μL of heparin PPP. The solutions were vortex mixed for 30 seconds, incubated at 4°C for 10 minutes, and then centrifuged at 4500×*g* for 10 minutes. The resulting supernatants were stored at −20°C for the following HPLC analysis.

Analysis

Separation of PPP 5-HT and tryptophan were carried out by an isocratic reverse phase HPLC method as already reported (Bruschetta et al., 2013). Twenty microliters of previously obtained supernatants were analyzed by HPLC. Qualitative and quantitative analyses of 5-HT and tryptophan were performed by comparison of their peak positions and areas in chromatographic runs with those ones of freshly prepared standard mixtures with known concentrations. The assay sensitivity of HPLC detector was 0.5 ng/mL for 5-HT and 3 ng/mL for tryptophan.

The venous blood samples were analyzed within 1 hour by an automatic photometer Slim for hematological assays (RADIM/SEAC Co., Rome, Italy) measuring red blood cell (RBC), white blood cell (WBC), platelet, hematocrit (Hct), and hemoglobin (Hb).

HR and RR were obtained using a stethophonendoscope (Littman, 3M Group, Pioltello [Milano], Italy). RT was measured with a clinical digital thermometer (Mebby Flexo, Medel Group, Parma, Italy) for 1 minute.

Statistics

Data are presented as mean ± SD in Figures 1 and 2 as well as the Table.

Statistical analyses used a 1-way repeated-measures analysis of variance (1-way RM-ANOVA) to determine whether the exercise had any effect. Significant differences between working animals and animals at rest, respectively, at T_0, T_1, and T_2 were performed using 2-way repeated-measures analysis of variance (2-way RM-ANOVA). The same analyses were used to evaluate significant differences among different breed, gender, and age. Significant

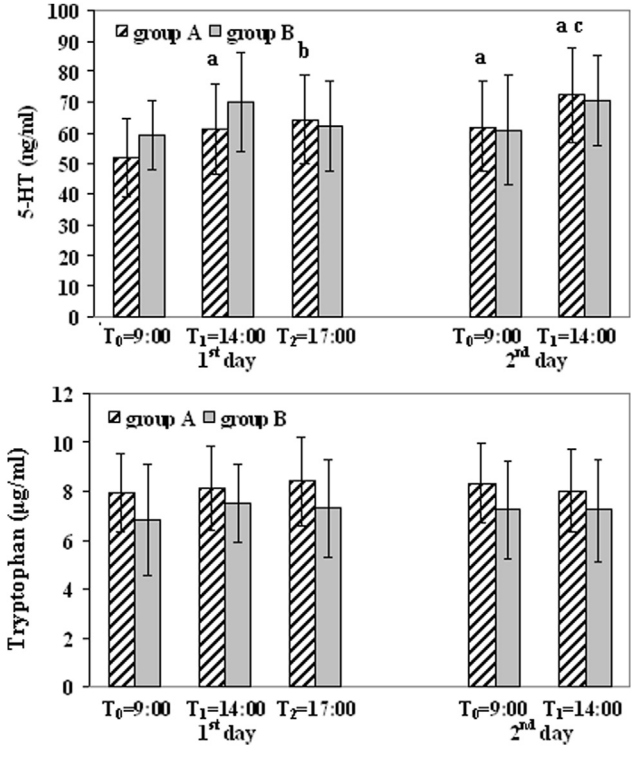

Figure 1. 5-HT and tryptophan levels (mean ± standard deviation) in horses during 2-day trekking tour. Different superscript letters show significant differences between T_0: a = $P < 0.05$, b = $P < 0.01$ and T_1: c = $P < 0.01$. 5-HT, 5-hydroxytryptamine.