

Chapter 2

Effect of Altitude on Plasma Serotonin Levels in Horses

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Abstract The aim of this work was to carry out a preliminary study about the effect of altitude on plasma serotonin (5-HT) and tryptophan levels in horses. Subjects came from a riding school at sea level and 600 m above sea level. To evaluate animal welfare linked to physiological status, cortisol levels, blood cell count and hematochemical variables (glucose, total proteins, albumin, creatinine, urea, aspartate transaminase, creatine kinase, lactate dehydrogenase, triglycerides and total cholesterol) were measured. Comparison of mean plasma levels of 5-HT, tryptophan, and cortisol in horses coming from different altitudes were not significantly different. Plasma 5-HT levels were affected by altitude in a gender-dependent way, showing an opposing trend between mares and geldings, with the highest levels in plasma of geldings coming from farms at sea level. Both 5-HT and tryptophan were higher in mares than in geldings from 600 m above sea level. Plasma cortisol levels, which were significantly higher in mares than in geldings at sea level, were more affected by gender than altitude.

Keywords Horse · Plasma serotonin · Tryptophan · Biochemical variables

Abbreviations

5-HT Serotonin
Try Tryptophan

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2.1 Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter produced independently in the central nervous system and peripheral tissues from two distinct isoforms of the enzyme tryptophan hydroxylase (TPH-1 and TPH-2), which catalyses the rate limiting step of the synthesis process. Its many biological functions include pulmonary arterial smooth muscle cell proliferation, smooth muscle bronchial vasoconstriction and local microthrombosis (MacLean et al. 2000). Indeed, high circulating 5-HT levels were associated with the onset of pulmonary arterial hypertension (PAH) (Hervé et al. 1995). Blood 5-HT levels were increased in hypoxic conditions, even in mice (Callebert et al. 2006). 5-HT is synthesised from the amino acid tryptophan (Try) in the brain, in mast cells and in intestinal enterochromaffin cells, where the gene of the classic isoenzyme TPH-1, which controls the peripheral 5-HT production, is mostly expressed (Walther and Bader 2003). Both TPH-1 and peripheral 5-HT play an essential role in the development of hypoxia-induced increased pulmonary pressure and pulmonary vascular remodelling (Morecroft et al. 2007). Platelets do not synthesise 5-HT, but they are its major site of storage and transport in the peripheral blood (Andres et al. 1993). The 5-HT uptake within them keeps plasma 5-HT concentrations low. In the horse, plasma 5-HT values are reported to be higher than in humans (Bailey and Elliott 1998; Di Pietro et al. 2010; Lebelt et al. 1998), and often an increase of circulating 5-HT is linked to common pathological conditions such as laminitis (Bailey et al. 2009). Considering the clinical importance of this variable, the aim of this study was to evaluate, in horses of different breeds, the effect of horse farm altitude on plasma levels of 5-HT and Try, its precursor amino acid. Moreover, some haematochemical and hormonal parameters were determined to signal the animal welfare status and/or the presence of any stress conditions.

2.2 Materials and Methods

Twenty clinically healthy horses (9 geldings and 11 mares) of various breeds (San Fratellana, Sella Italiana and crossbred) were used. Mean age was 10 ± 6 years. Horses came from farms and riding schools in Messina, Catania and their nearby environs at sea level or at an altitude of 600 m above sea level (asl). All horses housed in Messina, in individual boxes, were fed with fresh forages, concentrates and water. Blood samples were collected from the jugular vein into EDTA tubes at 8:30 a.m., at approximately 25 °C, in the month of May. Blood cell count was performed, and after centrifugation at 2,000 g, the following haematochemical parameters were detected: glucose (Glu), total proteins (TP), albumin (ALB), creatinine (Crea), urea, aspartate transaminase (AST), creatine kinase (CPK), lactate dehydrogenase (LDH), triglycerides (TG), total cholesterol (TCho), by spectrophotometry and serum cortisol levels (in duplicate, by ELISA kit [Radim,

Pomezia, Italy)). The platelet poor plasma (PPP) fraction was obtained by centrifugation at 4,500 g. Equal volumes of plasma, N-methylserotonin (internal standard) and protein precipitation reagent were vigorously vortex mixed, incubated at 4 °C and centrifuged at 4,500 g. The obtained supernatant was used for the detection of plasma serotonin and tryptophan by reverse phase HPLC (Waters 1,525 binary HPLC pump) with electrochemical detector (ESA). Statistical analysis was done by Student’s unpaired *t* test and Pearson’s correlation and linear regression tests.

2.3 Results

The blood cell count and haematochemical parameters were in the physiological range for all animals (Kaneko 1989; Ubaldi et al. 1982) and indicated an overall state of wellness. Preliminary data (Table 2.1) did not show any significant differences of plasma 5-HT, tryptophan and cortisol levels with altitude change, nor significant correlations among them. However, a difference in plasma 5-HT levels based on altitude was observed when the horses were divided by gender (Table 2.2). Plasma tryptophan levels (Table 2.2) were similar in mares and geldings at sea level, but slightly higher in mares at 600 m asl, with a trend comparable to 5-HT. In mares, higher concentrations of cortisol were observed in both groups (Table 2.2), which were significant at sea level and at 600 m asl.

2.4 Discussion

Mares seem to respond to higher altitude with greater 5-HT values compared to those housed at sea level (Table 2.2), consistent with observations in cows (Bruschetta et al. 2010) and rats (Awabdy et al. 2003). At higher altitude, platelet function could be downregulated, producing a decrease of platelet serotonin uptake and increased circulating serotonin levels. On the contrary, in geldings, there was a significant increase in 5-HT at sea level. Moreover, a gender-dependent effect on plasma 5-HT levels was detected in horses at sea level, which was slight in horses at 600 m asl. An analysis using a larger sample size could support these results and clarify the existence of an interaction among different variables that may have opposite effects on plasma 5-HT levels. Further, significant findings could come from the comparison of horses coming from farms or riding schools at sea level with horses farmed at altitudes higher than 1,000 m asl.

Table 2.1 Plasma levels of 5-HT, Try and cortisol (Mean ± S.D.) in horses at different altitudes

Altitude	5-HT (ng/ml)	Try (µg/ml)	Cortisol (ng/ml)
Sea level (<i>n</i> = 12)	55.4 ± 13.2	7.54 ± 1.37	85 ± 21
600 m (<i>n</i> = 8)	49.9 ± 12.1	7.95 ± 1.78	92 ± 15

Table 2.2 Plasma levels of 5-HT, Try and cortisol (Mean \pm S.D.) in horses at different altitudes and separated by gender

Altitude	Sea level	Sea level	600 m	600 m
Gender	Geldings (n. 5)	Mares (n. 7)	Geldings (n. 4)	Mares (n. 4)
5-HT (ng/ml)	64.4 \pm 6.9	49.0 \pm 13.1 ^a	44.3 \pm 10.0 ^a	55.5 \pm 12.6
Try (μ g/ml)	7.52 \pm 1.47	7.55 \pm 1.42	7.09 \pm 1.44	8.80 \pm 1.84
Cortisol (ng/ml)	71.0 \pm 13.8	95.4 \pm 19.8 ^a	84.0 \pm 12.2	99.3 \pm 14.5 ^a

^a vs. geldings at sea level: $p < 0.05$

The major changes in tryptophan values were observed in mares. These data could suggest the existence of sexual dimorphism, which could be deeper investigated using a greater number of horses. The higher concentration of cortisol observed in mares in both groups (Table 2.2) confirms the existence of a regulation of the hypothalamic–pituitary–adrenal axis of horses in a gender-dependent manner, as already observed in humans (Stroud et al. 2011) and rats (Viau et al. 2005). With a larger sample size, this could be of interest for geldings farmed at 600 m asl.

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