

PLASMATIC VARIATION OF VASOACTIVE AMINES IN HORSES UNDER EFFECT OF CARBOHYDRATE OVERLOAD AND NON-STEROIDAL ANTIINFLAMMATORIES

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ABSTRACT

Twenty horses were submitted to carbohydrate overload (CO) and plasmatic concentrations of the bioactive amine serotonin (5-HT), putrescin (PUT) and cadaverin (CAD) were determined by gas chromatography. After 36h of carbohydrate overload, horses were randomly distributed into four groups (n=5) and were submitted to four intravenous treatments every 12 hours. The treatments were as follows: 10ml of saline (GC), ketoprofen 2.2mg/kg (GK), phenylbutazone 4.4mg/kg (GF), and flunixin meglumine 1.1mg/kg (GFM). Blood samples

were collected at regular intervals (0-72h) after the CO. After 6h, there were peaks in the concentrations of 5-HT, which rapidly returned to values above the basal standard. No variations of PUT concentrations were observed; however CAD concentrations increased at 6h, remaining elevated (with some variation) until the end of the observation period. In conclusion, CO induced early (6h) increases in plasmatic concentrations of 5-HT and CAD. Plasmatic concentrations of the amines were not changed by any of the treatments used.

KEYWORDS: equine; NSAIDs; vasoactive amines.

VARIAÇÕES PLASMÁTICAS DE AMINAS VASOATIVAS EM EQUINOS SOB EFEITO DE SOBRECARGA DE CARBOIDRATOS E ANTI-INFLAMATÓRIOS NÃO ESTEROIDAIS

RESUMO

Vinte equinos foram submetidos à sobrecarga de carboidratos (SC) e as variações plasmáticas das concentrações de serotonina (5-HT), putrescina (PUT) e cadaverina (CAD) foram determinadas por cromatografia gasosa. Após 36h da SC, os animais foram divididos em quatro grupos (n=5) e receberam, a cada 12h, por via intravenosa: solução salina 10mL (GC), cetoprofeno 2,2mg/kg (GK), fenilbutazona 4,4 mg/kg (GF) e flunixinina meglumina 1,1 mg/kg (GFM). Amostras de sangue foram coletadas em intervalos regulares após a SC (0 - 72h). As

6h após a SC observou-se um pico nas concentrações de 5-HT, com rápido retorno a valores abaixo dos basais. Não foram observadas variações nas concentrações de PUT; contudo, as concentrações de CAD se elevaram às 6h, assim permanecendo (com algumas variações) até o final do experimento. Conclui-se que a SC induz aumentos precoces (6h) nas concentrações plasmáticas de 5-HT e CAD. As concentrações plasmáticas das aminas estudadas não foram alteradas por nenhum dos tratamentos empregados.

PALAVRAS-CHAVE: aminas vasoativas; anti-inflamatórios não esteroidais; equinos.

INTRODUCTION

When there is provision of large amounts of fermentable substrate, Gram-positive microorganisms in the cecum proliferate and produce lactic acid (GARNER et al., 1978). Decarboxylation of amino acids results in a low pH, thus there is increased production of amines (BEARSON et al., 1997). Some amines produced by the bacteria can induce vasoconstriction due to their structural similarities with endogenous vasoconstrictor amines, such as the catecholamines (RANG & DALE, 1991). The acute laminitis has been associated with changes in the cecum fermentative digestion, resulting in the production of vasoactive substances that affect digital circulation (GARNER et al., 1978).

Bioactive amines (BA) are aliphatic (putrescine, cadaverine, spermine, spermidine), alicyclic (tyramine, phenethylamine) or heterocyclic organic bases (histamine, tryptamine) (SILLA SANTOS, 1996).

Tryptamine, phenylethylamine and tyramine cause *in vitro* vasoconstriction in digital arteries and veins, due to the stimulation of serotonin receptors, the release of noradrenaline or the release of serotonin by platelets (BAILEY et al., 2000; BAILEY et al., 2001). The tryptamine is considered the most potent amine produced in the cecum, causing vasoconstriction, both *in vitro* and *in vivo*, because it directly activates the serotonin receptors, with a clear selectivity in the veins of the digits (ELLIOTT et al., 2003). Previous studies have demonstrated increase of tryptamine, tyramine and phenylethylamine in animals submitted to carbohydrate overload to induce laminitis (BOTTEON et al. 2008).

Putrescine and cadaverine are not very well known; however, it is known that such amines are present in high concentrations in cells with high metabolism and high turnover (LIMA & GLORIA, 1999).

This study aimed to analyze the plasma variation of the amines serotonin (5-HT), putrescine (PUT) and cadaverine (CAD) in horses under the effect of carbohydrate overload and the therapeutic effect of nonsteroidal anti-inflammatory drugs (ketoprofen, phenylbutazone and flunixin meglumine), started 36h after overload induction.

MATERIAL AND METHODS

We used 20 healthy, castrated male horses (*Equus caballus caballus*). The undefined breed animals were aged between 3 and 15 years, weighed

between 264 and 344kg and presented body score 2-3 (SPEIRS, 1997). Before the experiment, the animals were put in quarantine, when they were assessed, weighed and numbered from 01 to 20, being kept in a collective paddock up to seven days before receiving a carbohydrate overload (CO). Water was supplied *ad libitum*, and *coast-cross* hay (*Cynodon dactylon*), concentrate diet¹ and 50 g of mineral salt for horses were offered twice a day, in quantities equivalent to 2.5% and 1% of body weight, respectively. Seven days prior to receiving the CO, the animals were deprived of commercial feed and underwent endo and ectoparasites control, by means of a conjugate base of ivermectin and praziquantel pamoate² and deltamethrin, respectively. After the carbohydrate overload, the animals received electrolyte supplement³ (25g/10L water) added daily to the drinking water.

Animals were randomly distributed into four experimental groups (n = 5), and 36h after receiving the CO they received intravenously 10 ml of saline solution⁴ (BF), 2.2 mg/kg of ketoprofen⁵ (GK), 4.4 mg/kg of phenylbutazone⁶ (GF) and 1.1 mg/kg of flunixin meglumine⁷ (GFM) every 12 hours, in accordance with the group. The left jugular vein was used for administration, preserving the right vein for blood samples collection in vacuum bottles containing EDTA⁸. Samples were taken before carbohydrate overload, 6 hours after CO and every 12 hours until 72 hours evaluation, i.e., samples were taken at 0h, 6h, 12h, 24h, 36h, 48h, 60h and 72h.

After 12-hour feed and water fast, the animals received corn starch⁹ (20g/kg), according to a modification of the experimental model mentioned by WEISS et al. (1998). Each kg of starch was dissolved in one liter of water, and administered through a nasogastric tube (11mm). Immediately after starch administration, the animals were offered hay again.

For the extraction of vasoactive amines, samples of 1.5 ml of plasma were used and the protein precipitation was carried out by adding 100 µl of a trifluoroacetic acid¹⁰ solution at 10 mol. The samples were then centrifuged¹¹ at 12,000 rpm for 15 minutes. An aliquot of 0.2 mL of supernatant was

¹ Nutriage-Guabi (15% protein). Brazil.

² Centurion-Valleé. Brazil.

³ Plusvital-Marcolab. Brazil.

⁴ Saline solution 0.9%- Sanobiol. Brazil.

⁵ Ketofen- Merial. Brazil.

⁶ Equipalazone- Marcolab. Brazil.

⁷ Flumegan- Marcolab. Brazil.

⁸ Vacutainer. Bacton & Dickinson. Brazil.

⁹ Milhena- Nutril. Brazil.

¹⁰ Trichloroacetic acid PA/ACS -Cristal (brand Synth)

¹¹ 5415D Eppendorf

transferred to cryogenic tubes¹², submitted to lyophilization and stored at -20°C. The lyophilized material was resuspended in 100 mL of a 1:1 trifluoroacetic anhydride / acetonitrile solution, which was homogenized in a vortex and heated in the microwave¹³ for 3 min. The acylated derivatives were dried in a nitrogen line and then taken with 50 mL of acetonitrile marked with internal standard¹⁴; 2 µL of this solution were placed in the chromatograph¹⁵ for analysis.

The operating conditions of the gas chromatograph were maintained at 150°C, 1 min, 7°C / min, 220°C, 20°C / min, 270°C, injector and detector at 280°C, Split 1/100, using the capillary column¹⁶. Hydrogen was used as carrier gas.

The obtained serotonin concentrations presented abnormal dispersion and variables transformation was not possible, so the data were analyzed using the Kruskal-Wallis test at a significance level of 5%. Means were submitted to multiple paired comparisons using the procedure of Dunn / bilateral test. On the other hand, the concentrations of putrescine and cadaverine showed normal dispersion and the data were analyzed using analysis of variance and means were submitted to Tukey test.

RESULTS AND DISCUSSION

The amine production by bacteria is influenced by the pH, free amino acids and fermentable carbohydrates concentration (LIMA & GLORIA, 1999). The production of amines is stimulated in acidic media, because decarboxilases present the highest level of activity in these pH conditions. Because of its structural similarities with endogenous amines, many of the amines found in the cecal content of horses, if released, can affect vasculature (RANG & DALE, 1991). Although the pathophysiology of laminitis is not yet fully understood, the digital ischemia is considered one of the events that leads to the occurrence of this disease (HOOD et al., 2001).

Plasma concentration of serotonin (5-HT) showed a peak at time 6 hours in all groups (Table 1). The 5-HT in GK should be especially observed, as its concentration at the time 6h was statistically superior to time 0.

The increase in the serotonin concentration may be due to carbohydrates overload. According to

FERNSTROM & WURTMAN (1971), carbohydrate intake leads to indirect increase in tryptophan concentrations, the serotonin precursor amino acid. Although there is no amino acid in carbohydrates, when they are ingested, a release of insulin causes an increase in tryptophan concentration; therefore, there is increased production of serotonin.

Serotonin, or 5-Hydroxytryptamine (5-HT), is a biogenic amine known primarily for its role as a neurotransmitter. It is a molecule with several effects on the central and peripheral nervous systems, acting as hormone, neurotransmitter, etc. The 5-HT has an important role in the modulation of gastrointestinal motility and it is involved in maintaining peripheral vascular tone, cerebral vascular tone and platelet function, being related to the physiopathology of mood, behavior, appetite, hypertension disorders etc. (KANDEL, 2001).

According to KANDEL (2001), depression, mania, anxiety, behavioral changes, and other types of disorders are associated with the decrease of available 5-HT concentration in the CNS. An increase of 5-HT concentration was observed in this study.

However, the deleterious effects caused by the excessive increase of serotonin in the body should be accounted. It is known that 5-HT has an important role in the amplification of platelet aggregation. Even in the absence of endothelium injury, platelets can adhere and promote aggregation on intact endothelium (ROSENBLUM, 1997; REININGER, et al., 1998) leading to thrombi formation, for example. In a study, PAES LEME et al. (2006) observed that the platelet activation is initiated six hours after carbohydrate administration and the non-steroidal anti-inflammatories reduce platelet activation of horses *in vivo*, and flunixin meglumine presents the best results.

The vasoconstrictor effects of 5-HT are also relevant. According to ELLIOTT et al. (2003), serotonin can be released by activated platelets, inducing vasoconstriction in large arteries, veins and veinlets. Furthermore, it amplifies the action of other substances such as norepinephrine, histamine etc. Amines, such as tryptamine, phenylethylamine and tyramine, also analyzed in this study, cause vasoconstriction in digital arteries and veins *in vitro* by stimulation of serotonin receptors or by the release of serotonin from platelets (BAILEY et al., 2000; BAILEY et al., 2001).

¹² Cryogenic tube with screw cap, sterile (brand Alfa).

¹³ Panasonic Piccolo 600 W.

¹⁴ C18 – Octadecano 0.74 g/L

¹⁵ Gas chromatograph Varian CP-3380

¹⁶ BP10 (14% cyanopropylphenyl dimethyl siloxane) 25 m x 0.22 mm x 0.25 µm (SGE)

Table 1 - Mean plasma concentrations of serotonin, at different times (h), of horses under the influence of carbohydrate overload and non-steroidal anti-inflammatory

Time(h)	GK		GF		GFM		GC	
0	8.2	Ba	35.7	Aa	19.4	Aa	38.8	Aa
6	105.7	Aa	100.5	Aa	67.9	Aa	45.3	Aa
12	1.9	Ca	25.9	Ba	29.7	Ba	77.1	Aa
24	0.4	Da	0.0	Da	0.1	Da	0.3	Ca
36	1.9	Ca	0.5	Da	0.2	Da	0.1	Ca
48	0.0	Da	0.7	Da	0.4	Da	0.1	Ca
60	2.6	Ca	5.3	Ca	1.7	Ca	2.8	Ba
72	4.4	Ba	5.8	Ca	4.2	Ca	4.9	Ba

Means followed by different capital letters (in columns) and lower case letters (lines) differ significantly ($P = 0.0001$) by Kruskal-Wallis test. Plasma levels of 5-HT in all studied times and in relation to anti-inflammatories ketoprofen (Merial-Ketofen, Brazil) (GK) 2.2 mg / kg, phenylbutazone (Equipalazone-Marcobal, Brazil) (GF) 4.4 mg / kg, flunixin meglumine (Flumegan-Marcobal, Brazil) (GFM) 1.1 mg / kg and saline solution (0.9% saline – Sanobiol, Brazil) (GC) 10 ml.

According to BAILEY et al. (2002), in an *in vitro* study, putrescine (PUT) concentration in cecal contents of horses is increased when there is carbohydrate overload. Approximately 1 liter of cecal content of horses was kept under anaerobic conditions, different concentrations of the carbohydrate were added to the medium and the cecal content was analyzed using high performance liquid chromatography (HPLC). Amines, including putrescine, were identified and their concentrations measured. An increase in the concentration of putrescine after 18h incubation was observed. In the

present study, this fact was not verified because there were no variations in the concentrations of PUT in the different groups and times evaluated (Table 2). This difference may be due to methodological differences, as the present study was performed *in vivo* and the method of analysis was gas chromatography.

There was a significant decrease in PUT concentration only in GC at the time 72h. This result may indicate the possible action, not yet understood, of the anti-inflammatory drugs on amine plasma concentrations.

Table 2 - Mean plasma concentrations of putrescine, at different times (h), of horses under the influence of carbohydrate overload and non-steroidal anti-inflammatory

Time(h)	GK	GF	GFM	GC
0	8.7 Aa	4.1 Aa	3.3 Aa	13.1 Aa
6	3.1 Aa	1.9 Aa	2.1 Aa	1.5 Ba
12	3.2 Aa	3.2 Aa	3.6 Aa	5.1 ABa
24	1.6 Aa	1.9 Aa	1.3 Aa	1.4 Ba
36	2.4 Aa	0.5 Aa	5.4 Aa	1.9 Ba
48	2.4 Aa	1.4 Aa	1.5 Aa	0.9 Ba
60	3.2 Aa	4.8 Aa	2.3 Aa	4.6ABa
72	1.6 Aa	0.6 Aa	0.5 Aa	0.5 Ba

Means followed by different capital letters (in columns) are significantly different using analysis of variance ($P = 0.001$) and means followed by different low case letters (lines) do not differ significantly by ANOVA ($P = 0.48$). Plasma variations of PUT were observed at the evaluated times in relation to the anti-inflammatories ketoprofen (Merial-Ketofen, Brazil) (GK) 2.2 mg / kg, phenylbutazone (Equipalazone-Marcobal, Brazil) (GF) 4.4 mg / kg, flunixin meglumine (Flumegan-Marcobal, Brazil) (GFM) 1.1 mg / kg and saline solution (0.9% saline – Sanobiol, Brazil) (GC) 10 ml.

As for cadaverine (DAC), the concentrations were unstable during the evaluated times (Table 3).

An interesting fact was observed by analyzing the GC. Concentration of CAD at time 72h returned

to baseline (time 0h) only in the GC. This may be indicative that there are effects of non-steroidal anti-inflammatory drugs on the plasma concentration of cadaverine. To clarify this finding, more researches

are necessary.

In literature, putrescine and cadaverine are not often mentioned and their function is not well

known; however, it is known that they are involved in the proliferation and maintenance of cell viability (COHEN, 1998; LIMA & GLORIA, 1999).

Table 3 - Mean plasma concentrations of cadaverine, at different times (h), of horses under the influence of carbohydrate overload and non-steroidal anti-inflammatory

Time(h)	GK	GF	GFM	GC
0	1.3 Ba	3.2 Ba	9.3 Ca	1.9 Ba
6	17.2 Aa	20.2 Aa	17.5 Ba	19.8 Aa
12	11.9 Aa	6.7 Ba	65.9 Aa	10.9 Aa
24	3.3 Ba	24.2 Aa	21.7 Ba	21.8 Aa
36	18.1 Aa	2.4 Ba	3.9 Ca	4.7 Ba
48	20.1 Aa	18.7 Aa	19.3 Ba	17.7 Aa
60	0.9 Ba	16.9 Aa	2.1 Ca	11.5 Aa
72	14.4 Aa	14.7 Aa	42.2 Aa	5.0 Ba

CONCLUSION

The carbohydrate overload induces early increases (6h) in plasma concentrations of serotonin (5-HT) and cadaverine (CAD).

Plasma concentrations of the studied amines (serotonin, putrescine and cadaverine) were not altered by any treatment (ketoprofen, phenylbutazone and flunixin meglumine).

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ETHICS COMMITTEE AND BIOSAFETY

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