*GENERATION OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE MEDIATED BY GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE IN NEUTROPHILS OF THOROUGHBRED HORSES**

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SUMMARY: Twenty adult thoroughbred horses were investigated for neutrophil glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase specific activities which were found to be 945 ± 288 mIU mg^{-1} of protein and 375 ± 88 mIU mg^{-1} of protein respectively, per minute at 37 °C.

UNITERMS: NADP; Glucose-6-phosphate dehydrogenase; 6-Phosphogluconate dehydrogenase; Neutrophils; Horses, PSI

* This work was performed in the Instituto de Ciências Biomédicas da USP and in Instituto dos Laboratórios de Investigação Médica, Faculdade de Medicina da USP.

INTRODUCTION

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) plays a key role in microbicidal process carried out by neutrophils, as it is involved in superoxide generation by NADPH oxidase (BABIOR et al. [2], 1981). In resting neutrophils, NADPH oxidase is barely detected, but when they are stimulated for phagocytosis the enzyme activity is considerably increased (BABIOR et al. [1], 1976; MC PHAIL et al. [9], 1976; HOHN; LEHRER [6] 1975; DE CHATELET et al. [4], 1974). This work aimed at assaying in thoroughbred horse neutrophils, the two sequential enzymes of the pentose shunt responsible for the NADP reduction to NADPH, the glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and the 6-phosphogluconate dehydrogenase. Both enzymes keep the NADPH pool at optimal levels in order to supply NADPH to the NADPH oxidase activity during the neutrophil respiratory burst.

MATERIAL AND METHOD

Neutrophils from twenty adult thoroughbred horses from the Jockey Club of São Paulo were studied; 20 ml of blood were collected after the morning exercise in heparin (10 IU ml^{-1} of blood). Soon after drawing the blood, the neutrophils were separated according to standard procedures (FERRANTE; THONG [5], 1980), by using Ficoll 400.000 (Sigma Co.) and Hypaque 90% (Wintrop Products Inc.). The neutrophils (95% of purity-ascertained by examining 200 leucocytes in a Romanowski stained smear) were suspended in 1.0 ml of saline, lysed by freeze-and-thawing (conic tubes with blood immersed in an acetone and dry ice mixture, and defrost at 37 °C), and centrifuged at 16.000 G. The supernatant was employed for enzyme assay, and the protein was measured according to LOWRY et al. [8] (1951).

Glucose-6-phosphate-dehydrogenase activity was determined in a reaction system containing 100 mM TRIS-HCl pH 8.0, 100 mM magnesium chloride, 0.2 mM NADP and 0.6 mM glucose-6-phosphate (G-6-P) (BEUTLER [3], 1984). 6-phosphogluconate dehydrogenase activity was followed in a reagent system containning 100 mM TRIS-HCl pH 8.0, 100 mM magnesium chloride, 0.2 mM NADP and 0.6 mM G-6-P (BEUTLER [3], 1984). Enzyme activities expressed as international units, were calculated as micromoles of NADP reduced to NADPH at 340 nm per minute, per miligram of protein, at 37 °C. A Gilford spectrophotometer model 2400-2 with recorder was employed.

RESULTS AND DISCUSSION

Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6-PGD) activities assayed in 20 horses are shown in Tab. 1. The neutrophil G6PD specific activity exhibited values of 945 ± 288 mIU mg^{-1} of protein per minute at 37 °C, and 375 ± 88 mIU mg^{-1} of protein per minute at 37 °C for 6-PGD, indicating a clearly higher G6PD specific activity. Such an increase of activity has also been found in human neutrophils (492 mIU mg^{-1} of protein for G6PD and 259 mIU mg^{-1} for 6-PGD), according to LANE et al. [7] (1984) and personal communication **.

Although from a different lineage, the erythrocytes may help understanding the neutrophil characteristics. In fact MEDEIROS et al. [10] (1982) found in thoroughbred horse erythrocytes a great imbalance favouring G6PD activity, with a ratio G6PD/6-PGD = 16, whereas in neutrophils this ratio is 2.5 (Tab. 1).

These findings suggest that in erythrocytes there is a limiting role for 6-PGD but in neutrophils a limiting role for 6-PGD does not seem to occur. This is suggestive of a harmonic relationship between both enzymes, which must work together in order to maintain the NADPH generation, the crucial step for keeping the efficiency of phagocytic process.

There is, moreover, a striking increase in enzyme specific in neutrophils as compared to that in erythrocytes, disclosing an overwhelming increase in gene expression for both G6PD and 6-PGD, mostly for the later, wich is 200 times more active in neutrophils than in erythrocytes.

A close correlation between G6PD and 6-PGD specific activities is disclosed by a Fisher's coefficient of 0.75 suggesting that both specific activities vary together.

Both G6PD and 6-PGD are involved in transforming NADP to the reduced state NADPH. It is known that NADPH inhibits G6PD (YOSHIDA [11], 1973). Inasmuch as in neutrophil respiratory burst there is a high NADPH consumption by NADPH oxidase, the decrease of NADPH concentration would allow G6PD, and possibly 6-PGD as well, to work at maximum rate, without the inhibitory effect of NADPH. The maximum rate would be reached depending only upon the availability of its substrate glucose-6-phosphate.

LEITE, A.A.; BARRETTO, O.C. de O.; MEDEIROS, L.F.; MEDEIROS, L.O.; Geração da nicotinamida adenina dinucleotídeo fosfato reduzida mediada pela glicose-6-fosfato desidrogenase e 6-fosfogliconato desidrogenase em neutrófilos de cavalos Puro-Sangue Inglês. *Braz. J. vet. Res. anim. Sci.*, São Paulo, v.28, n.1, p.7-9, 1991.

RESUMO: Em vinte cavalos Puro-Sangue Inglês determinou-se a atividade da glicose-6-fosfato-desidrogenase e da 6-fosfogliconato-desidrogenase de neutrófilos, encontrando-se atividades específicas de 945 ± 288 mUI mg^{-1} de proteína e 375 ± 88 mUI mg^{-1} de proteína, respectivamente, por minuto a 37 °C.

UNITERMOS: NADP; Glicose-6-fosfato desidrogenase; 6-Fosfogliconato desidrogenase; Neutrófilos; Cavalos, PSI

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TABLE 1 - Thoroughbred horse neutrophils glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. São Paulo, 1989.

Enzime activity in mIU mg^{-1} of protein per minute at 37 °C	ERYTHROCYTES (E)*	NEUTROPHILS (N)	N/E
Glucose-6-phosphate dehydrogenase	29.2 ± 4.5	945 ± 288	32
6-phosphogluconate dehydrogenase	1.84 ± 0.2	375 ± 88	208
G6PD/6-PGD	16	2.5	

* MEDEIROS et al. [10] (1982)