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# BOVINE SERUM LIMITATION INCREASES THE ACTIVITY AND EXPRESSION OF ENZYMES INVOLVED ON ATP, ADP AND AMP EXTRACELLULAR HYDROLYSIS IN *TRICHOMONAS VAGINALIS*

Frasson A.P.[1*]; Rosemberg D.B.[2]; Charão M.F.[3]; De Carli G.A.[4]; Bogo M.R.[2]; Garcia S.C.[3]; Tasca T[1].

[1]Laboratório de Pesquisa em Parasitologia, Faculdade de Farmácia, UFRGS; [2]Laboratório de Biologia Genômica e Molecular, PUCRS; [3]Laboratório de Toxicologia, Faculdade de Farmácia, UFRGS; [4]Instituto de Geriatria e Gerontologia, PUCRS

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**Introduction:** The flagellated protozoan *Trichomonas vaginalis* causes trichomonosis, the most common non-viral STD in the world[1]. The infection causes serious impact on public health; therefore, it is important to investigate the biochemical aspects of the parasite. Extracellular nucleotides are released by cells under stress, anoxia or injury[2] and they can be inactivated by hydrolysis via ectonucleotidases. The members of E-NTPDase family hydrolyze nucleoside di- and tri-phosphates and the ecto-5'-nucleotidase is able to hydrolyze nucleoside monophosphate, producing adenosine[3]. Important, *T. vaginalis* lacks the ability to synthesize purines and pyrimidines *de novo*, and the enzymes act on the salvage pathways generating the nucleosides[4].
**Objective:** The aims of this study were to investigate the metabolism of ATP in trophozoites of *T. vaginalis* by enzymatic activities determination and gene expression analysis, under a condition of bovine serum limitation.
**Materials and Methods**: For the assays, the trophozoites from ATCC 30236 and LACH1 isolates were grown in TYM medium supplemented with 1.0% inactivated bovine serum, while control cultures were maintained with 10% serum. The enzymatic tests were performed in the reaction mixture containing 50mM Tris buffer, 5.0mM $CaCl_2$ or $MgCl_2$, trophozoites and substrate (1.0 mM ATP, 1.0mM ADP or 3.0mM AMP), at 37°C. The specific activity was determined by colorimetric method and expressed as nmol Pi released/min/mg protein. The gene expression patterns were carried out by a RT-PCR assay using specific primers for NTPDase A and B; genes responsible for AMP hydrolysis were searched by BLAST function in GenBank Database and primers were designed for SurE (183), SurE(257), SurE(258) and 5`(3`) enzymes. Extracellular adenine nucleotide hydrolysis was estimated by HPLC analysis.
**Results:** The bovine serum limitation causes an increase on the hydrolysis of the nucleotides tested. In ATCC 30236, there was an increase of 154.4%, 176.1% and 302.5% on the hydrolysis of ATP, ADP and AMP, respectively, when compared with control cultures; in the clinical isolate, LACH1, the increase was to 14.4%, 22.5% and 162.5%. Transcript levels of NTPDase A were significantly increased in both isolates, while NTPDase B and SurE(183) mRNA transcript levels increased only on the LACH1 isolate. Extracellular ATP metabolism is increased in limiting serum, leading to higher ATP hydrolysis and formation of ADP and AMP.
**Conclusions:** Our data suggest that under serum limitation condition these *T. vaginalis* enzymes play an important role in the provision of adenosine for parasite growth. Moreover, the enzymes may contribute to escape mechanisms of the parasite by breaking down ATP.

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