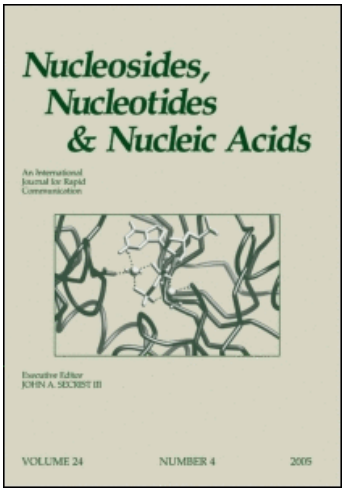

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C. Prior[a]; R. J. Torres[a]; J. G. Puig[b]
[a] Biochemistry Laboratory, La Paz University Hospital, Madrid, Spain [b] Internal Medicine Department, La Paz University Hospital, Madrid, Spain

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HYPOXANTHINE EFFECT ON EQUILIBRATIVE AND CONCENTRATIVE ADENOSINE TRANSPORT IN HUMAN LYMPHOCYTES. IMPLICATIONS IN THE PHATOGENESIS OF LESCH-NYHAN SYNDROME

C. Prior and R. J. Torres □ *Biochemistry Laboratory, La Paz University Hospital, Madrid, Spain*

J. G. Puig □ *Internal Medicine Department, La Paz University Hospital, Madrid, Spain*

□ *We postulated that increased levels of hypoxanthine, a main characteristic of hypoxanthine phosphoribosyltransferase (HPRT) deficiency, may influence adenosine function which could be related to some of the neurological features of the Lesch-Nyhan syndrome. We have examined the effect of hypoxanthine on different adenosine transporters in peripheral blood lymphocytes from control subjects. Increased hypoxanthine concentrations (25 μM) significantly decreased adenosine transport. The equilibrative adenosine transporters (79.6% of the adenosine transport), both NBTI sensitive and NBTI insensitive, were affected significantly. In contrast, the concentrative adenosine transporters were not influenced by hypoxanthine. These results supports the hypothesis that increased hypoxanthine levels influence equilibrative (predominantly NBTI-insensitive type) adenosine transporters.*

Keywords Lesch Nyhan; HPRT; Adenosine; ENT; CNT; Hypoxanthine

INTRODUCTION

Purines are released into the extracellular space where they act as intercellular signalling molecules. The nucleoside adenosine regulates many physiological processes, including neurotransmission, via binding to specific G-protein coupled receptors in the neurone cell surface.[1] Adenosine

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Address correspondence to R. J. Torres, Biochemistry Laboratory, La Paz University Hospital, Paseo de la Castellana 261, 28046 Madrid, Spain. E-mail: rtorres.hulp@salud.madrid.org

effects on the CNS have been implicated in several motor and behavioral changes, including self-injurious behavior.[2]

Lesch-Nyhan syndrome is an inborn error of purine metabolism due to deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity.[3,4] The most prominent characteristic of HPRT deficient cells is the elevated amounts of hypoxanthine into the extracellular medium.[5] We postulated that increased hypoxanthine levels may influence adenosine function, which may have a pathogenetic role to explain the neurological features of Lesch-Nyhan syndrome.

The concentration of adenosine available to the cell surface receptor is regulated by the uptake of adenosine by a nucleoside transporters family.[6] In previous studies,[7] we have documented that hypoxanthine excess causes a significant dose-dependent decrease in adenosine transport in peripheral blood lymphocytes in control (PBL-C) and HPRT-deficient cells (PBL-LN). In addition, hypoxanthine potentiates CGS-21680-stimulated cAMP levels in both PBL types, indicating that hypoxanthine affects adenosine transport and function.[7]

Adenosine is introduced into human cells through equilibrative and concentrative transporters. Equilibrative transporters are not sodium dependent and on the basis of their sensitivity to Nitrobenzylthioinosine (NBTI) they can be classified as: sensitive (es o ENT1, being the substrates adenosine and uridine); and insensitive (ei o ENT2, being the substrates adenosine, uridine, hypoxanthine and uracil).[8] Concentrative adenosine transportation is carried out by high-affinity, active, concentrative, and sodium dependent systems.[9] In PBL at least 2 types have been described: CNT1 (insensitive to NBTI) and CNT5 (sensitive to NBTI).[10]

We hypothesized that the high hypoxanthine levels present in the extra-cellular medium of HPRT deficient cells may predominantly influence NBTI-insensitive transporters. To test this hypothesis we have examined the effect of hypoxanthine on the different adenosine transport activities in control PBL.

MATERIALS AND METHODS

Heparinized blood samples were obtained from control subjects in whom informed consent was obtained according to standard Ficoll-Histopaque centrifugation. [2-3H] Adenosine transport was determined in parallel under basal conditions and after 15 minutes incubation with 25 μM hypoxanthine, using 96 wells Filter Plates Multiscreen (Millipore, Iberica SA, Madrid, Spain) at 25°C, with 1 μM [2-3H] adenosine (25 Ci/mmol) during 2 minutes with: a) medium with 137 mM sodium chloride or b) 137 mM choline chloride, with or without 10 μM NBTI. After incubation

filter plates were rapidly filtrated and the radioactivity retained on the filters was counted in a β-scintillation counter.

Equilibrative transport (sodium independent) is considered as the adenosine transport in choline buffer, and concentrative transport (sodium-dependent) is the result of the transport in sodium-buffer (total transport) minus the equilibrative transport (transport in choline buffer).

RESULTS

We have found 4 types of adenosine transport in PBL : 41.6 ± 9.3% (mean ± SD)Equilibrative transport-NBTI insensitive, 38.0 ± 16.1 % equilibrative transport-NBTI sensitive, 13.0 ± 8.5% concentrative transport-NBTI sensitive, and 7.4 ± 4.0% concentrative transport-NBTI insensitive. Incubation with hypoxanthine (25 μM) markedly decreased total adenosine transport from 0.80 ± 0.09 to 0.59 ± 0.10 pmol/10^6 cells; $p < 0.005$) (Figure 1).

Hypoxanthine significantly decreased equilibrative transport, both NBTI-sensitive (0.22 ± 0.05 versus 0.17 ± 0.04; $p < 0.05$) and NBTI insensitive type (0.38 ± 0.07 versus 0.22 ± 0.04; $p < 0.005$) being affected, although the decrease was more pronounced in the NBTI insensitive type (Figure 1). Concentrative transport (total, NBTI-sensitive or NBTI-insensitive) was not affected significantly by hypoxanthine incubation.

Total NBTI insensitive adenosine transport (equilibrative plus concentrative) was markedly influenced by incubation with hypoxanthine (0.43 ± 0.06 versus 0.27 ± 0.04 pmol/10^6 cells; $p < 0.005$). However, total NBTI-sensitive transport was not significantly affected by hypoxanthine incubation (0.37 ± 0.04 versus 0.32 ± 0.06; NS).

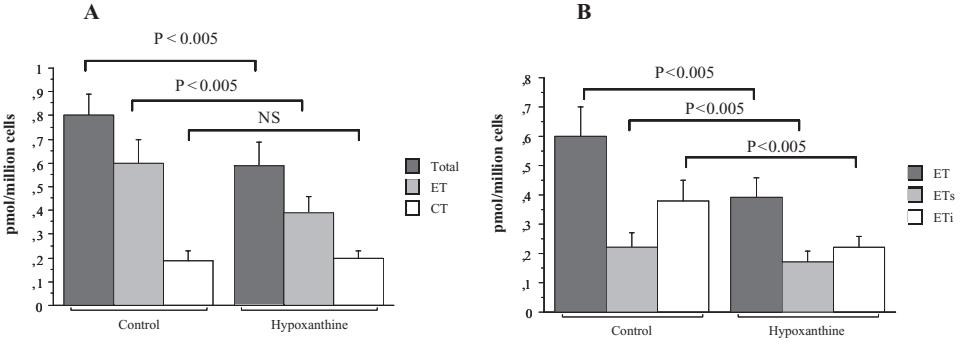

FIGURE 1 Adenosine transport in control PBL under basal conditions (control) and after incubation with 25 μM hypoxanthine (hypoxanthine). A) Bars represent total, equilibrative (ET), and concentrative (CT) adenosine transport. B) Bars represent total equilibrative transport (ET), equilibrative transport NBTI sensitive (ETs), and equilibrative transport NBTI insensitive (ETi).

DISCUSSION

This study shows that adenosine transportation is markedly influenced by increased hypoxanthine concentrations. The reduced adenosine PBL uptake caused by increased hypoxanthine concentrations, could be attributed to dysfunction of the adenosine equilibrative transporters, which are the main adenosine transporters in PBL (79.6% of total transport). Among these, the NBTI insensitive transporters appeared to be predominantly affected (Figure 1B).

These results are in accordance with our previous hypothesis that the high hypoxanthine levels present in the extracellular medium of HPRT deficient patients influence markedly equilibrative NBTI-insensitive transporters.

What are the implications of these results in the pathogenesis of the Lesch-Nyhan syndrome? Clinical, biochemical, experimental, and PET data in Lesch-Nyhan patients support an alteration of the basal ganglia and dopaminergic neurotransmission.[12–15] Although equilibrative nucleoside transporters function bi-directionally, inhibition of adenosine transport, in vivo and in vitro, may increase extra-cellular cerebral adenosine.[16] An increase in the extra-cellular adenosine concentration in the striatum, which may be induced after the systemic administration of NMDA, has been correlated to motor depression, providing evidence that modifications in extracellular adenosine concentrations may influence neuronal functions.[17]

In the basal ganglia, adenosine and dopamine receptors are coupled and adenosine agonist and antagonist can modulate dopaminergic neurotransmission.[18] Consequently, increased hypoxanthine extracellular levels may increase extracellular adenosine which could be implicated in the dopaminergic alterations found in HPRT-deficient animal models and in Lesch-Nyhan patients.

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