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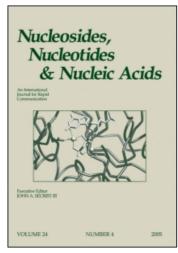
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## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713597286

# Effects of Hypoxanthine on Adenosine Transport in Human Lymphocytes. Implications in the Phatogenesis of Lesch-Nyhan Syndrome

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Online publication date: 27 October 2004

To cite this Article Torres, R. J. , DeAntonio, I. , Prior, C. and Puig, J. G.(2004) 'Effects of Hypoxanthine on Adenosine Transport in Human Lymphocytes. Implications in the Phatogenesis of Lesch-Nyhan Syndrome', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1177-1179

To link to this Article: DOI: 10.1081/NCN-200027444 URL: http://dx.doi.org/10.1081/NCN-200027444

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### NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS Vol. 23, Nos. 8 & 9, pp. 1177–1179, 2004

# Effects of Hypoxanthine on Adenosine Transport in Human Lymphocytes. Implications in the Phatogenesis of Lesch-Nyhan Syndrome

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#### **ABSTRACT**

We have examined the effect of hypoxanthine on adenosine transport and [<sup>3</sup>H] NBTI binding in peripheral blood lymphocytes (PBL) cultures. Pre-incubation with hypoxanthine originates a dose dependent decrease of adenosine transport and [<sup>3</sup>H] NBTI binding sites in PBL.

Key Words: Hypoxanthine; Lesch-Nyhan; HPRT; Adenosine; Nucleoside transport.

#### INTRODUCTION

Hypoxanthine excess could be implicated in the pathogenesis of the neurological symptoms of Lesch-Nyhan patients by altering adenosine transport. We have examined the effect of hypoxanthine on adenosine transport in peripheral blood lymphocytes (PBL) cultures.

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DOI: 10.1081/NCN-200027444 Copyright © 2004 by Marcel Dekker, Inc. 1525-7770 (Print); 1532-2335 (Online) www.dekker.com

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#### **METHODS**

To determine adenosine transport, cells were placed in 96 wells Filter Plates Multiscreen<sup>TM</sup> (Millipore) and incubated at 37°C, 5% CO<sub>2</sub>, with 0,5 μCi/well of [2-<sup>3</sup>H] adenosine, (25 Ci/mmol), and non-labelled adenosine was added to give the required final concentration. [1,2] PBL were incubated at 37°C, 5% CO<sub>2</sub> during 24 h: 1) with different hypoxanthine concentrations ranging from 0 to 50 µM, and then transport was carried out with a final adenosine concentration of 1 µM; and 2) with or without (control) 25 µM hypoxanthine and, then, transport was analysed with final adenosine concentrations ranged from 1 to 10 µM. To carried out [3H] NBTI binding assays, cells were placed in 96 wells Filter Plates Multiscreen<sup>TM</sup> and were incubated at 37°C, 5% CO<sub>2</sub> during 24 h: 1) with different hypoxanthine concentrations ranging from 0 to 50 μM and, then, binding was carried out with a final NBTI concentration of 1 nM; and 2) with or without 25 µM hypoxanthine and, then, binding was analysed with NBTI concentration ranging from 1 to 15 nM. After 24 h-incubation, cells were incubated with 2 IU/ml adenosine deaminase (ADA) during 30 min and then, with the different [<sup>3</sup>H] NBTI concentrations, in the presence or absence of 10 µM NBTI to determine the non-specific binding.<sup>[3]</sup>

#### **RESULTS**

Hypoxanthine at concentrations ranging from 1 to 50  $\mu$ M concentrations caused a significant dose-dependent decrease on 1  $\mu$ M adenosine transport expressed as % of 0  $\mu$ M hypoxanthine transport (1 $\mu$ M = 86.9 ± 1.8 %; 5  $\mu$ M = 83.7 ± 3.7 %; 25  $\mu$ M = 74.7 ± 1.9 %; 50  $\mu$ M = 70.2 ± 2.6 %; F = 25.71; p < 0.0005). Hypoxanthine at 25  $\mu$ M induced a significant decrease of the Vmax for the adenosine transport in PBL cultures versus controls (9 ± 0,11 vs. 19 ± 0,5 pmol/10<sup>6</sup> cells/min; p < 0.001). Hypoxanthine originated a dose-dependent significant reduction in the 1 nM [ $^3$ H] NBTI binding in PBL cultures, expressed as % of 0  $\mu$ M hypoxanthine binding (5  $\mu$ M = 83.9 ± 6.3 %; 10 $\mu$ M = 76.8 ± 6.8 %; 25 $\mu$ M = 61.2 ± 6.9 %; 50  $\mu$ M = 54.9 ± 12.6 %; F = 9.922; p < 0.005). Hypoxanthine at 25  $\mu$ M originated a significant decrease of the Bmax for NBTI with respect to controls in PBL cultures (7,880 ± 322 vs. 9873 ± 404 high affinity sites per cell; p < 0.001).

#### **CONCLUSIONS**

In cultured PBL, pre-incubation with excess of hypoxanthine reduces both the adenosine transport and the [<sup>3</sup>H] NBTI binding sites per cell.

#### **ACKNOWLEDGMENTS**

This work has been supported by grants from Fondo de Investigaciones Sanitarias 00/0350, and G03/054 (REDEMETH), Spain. Carmen Prior is supported by a fellowship from Programa BEFI (Fondo de Investigaciones Sanitarias 02/9272).

#### REFERENCES

- 1. Miras-Portugal, M.T.; Torres, M.; Rotllan, P.; Aunis, D. Adenosine transport in bovine chromaffin cells in culture. J. Biol. Chem. **1986**, *261*, 1712–1719.
- Torres, M.; Bader, M.F.; Aunis, D.; Miras-Portugal, M.T. Nerve growth factor effect on adenosine transport in cultured chromaffin cells. J. Neurochem. 1987, 48, 233– 235
- 3. Torres, M.; Delicado, E.G.; Miras-Portugal, M.T. Adenosine transporters in chromaffin cells: subcellular distribution and characterization. Biochim. Biophys. Acta **1988**, *969*, 111–120.