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Effects of Hypoxanthine on Adenosine Transport in Human Lymphocytes. Implications in the Pathogenesis of Lesch–Nyhan Syndrome

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ABSTRACT

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

To determine adenosine transport, cells were placed in 96 wells Filter Plates MultiscreenTM (Millipore) and incubated at 37°C, 5% CO₂, with 0,5 µCi/well of [2-³H] 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% CO₂ 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 MultiscreenTM and were incubated at 37°C, 5% CO₂ 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 [³H] NBTI concentrations, in the presence or absence of 10 µM NBTI to determine the non-specific binding.^[3]

RESULTS

Hypoxanthine at concentrations ranging from 1 to 50 µM concentrations caused a significant dose-dependent decrease on 1 µM adenosine transport expressed as % of 0 µM hypoxanthine transport (1µM = 86.9 ± 1.8 %; 5 µM = 83.7 ± 3.7 %; 25 µM = 74.7 ± 1.9 %; 50 µM = 70.2 ± 2.6 %; F = 25.71; p < 0.0005). Hypoxanthine at 25 µM induced a significant decrease of the Vmax for the adenosine transport in PBL cultures versus controls (9 ± 0,11 vs. 19 ± 0,5 pmol/10⁶ cells/min; p < 0.001). Hypoxanthine originated a dose-dependent significant reduction in the 1 nM [³H] NBTI binding in PBL cultures, expressed as % of 0 µM hypoxanthine binding (5 µM = 83.9 ± 6.3 %; 10µM = 76.8 ± 6.8 %; 25µM = 61.2 ± 6.9 %; 50 µM = 54.9 ± 12.6 %; F = 9.922; p < 0.005). Hypoxanthine at 25 µM 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 [³H] NBTI binding sites per cell.

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