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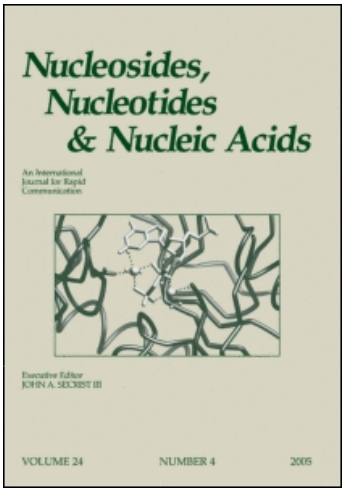

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Adenosine Transport in HPRT Deficient Lymphocytes from Lesch-Nyhan Disease Patients

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ABSTRACT

We have analysed adenosine transport and [^{3}H] NBTI binding in peripheral blood lymphocytes obtained from Lesch-Nyhan patients, in basal conditions and following 24 h incubation with hypoxanthine. We found that adenosine transport and [^{3}H] NBTI Binding were significantly decreased in PBL-LN with respect to PBL-C in basal conditions. Following 25 μM hypoxanthine incubation, adenosine transport is decreased in PBL-LN with respect to basal transport, however, [^{3}H] NBTI binding in PBL-LN was not decreased following hypoxanthine incubation.

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

INTRODUCTION

In previous studies, we have found that elevated concentrations of hypoxanthine reduce adenosine transport and increase cAMP levels in human peripheral blood lymphocytes (PBL-C). The aim of the present study is to analyse adenosine transport in HPRT deficient lymphocytes (PBL-LN) obtained from Lesch-Nyhan patients.

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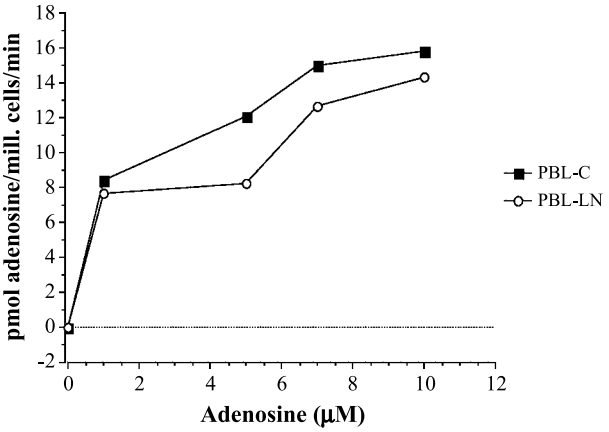

Figure 1. Kinetic representation of adenosine transport on PBL-C and PBL-LN.

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METHODS

[2-^{3}H] Adenosine transport, and [^{3}H] NBTI binding assays were determined in parallel in PBL-C and PBL-LN, in basal conditions and after 24-h incubation with 25 μM hypoxanthine. Adenosine transport was determined according to methods described by Miras-Portugal et al.[1] and Torres et al.[2] Briefly, cells were placed in 96 wells Filter Plates Multiscreen^{TM} (Millipore) and incubated at 37°C, 5% CO_2, with 0.5 μCi/well of [2-^{3}H] adenosine, (25 Ci/mmol), and non-labelled adenosine was added to give the required final concentration. PBL were incubated at 37°C, 5% CO_2 during 24-h with or without 25 μM hypoxanthine and, then, transport was analysed with final adenosine concentrations ranged from 1 to 10 μM. [^{3}H] NBTI binding assays were carried out according the method described by Torres et al.[3] PBL were placed in 96 wells Filter Plates Multiscreen^{TM} and were incubated at 37°C, 5% CO_2 during 24 h with 25 μM hypoxanthine or without hypoxanthine. After 24-h incubation, cells were incubated with 2 IU/ml adenosine deaminasa (ADA) during 30 min and then, binding was analysed with [^{3}H] NBTI concentrations ranged from 1 to 15 nM, in the presence or absence of 10 μM NBTI to determine the non-specific binding.

Table 1. Adenosine transport in PBL-C and PBL-LN.

Adenosine (μM)	PBL_C pmol/10^6cells/min	PBL_{LN} pmol/10^6cells/min	PBL_C vs. PBL_{LN}
1	8.5 ± 0.18	7.5 ± 0.16	$P < 0.005$
5	12.1 ± 0.80	8.1 ± 0.31	$P < 0.005$
7	15.03 ± 0.66	12.99 ± 0.41	$P < 0.05$
10	15.84 ± 0.62	14.81 ± 0.38	NS

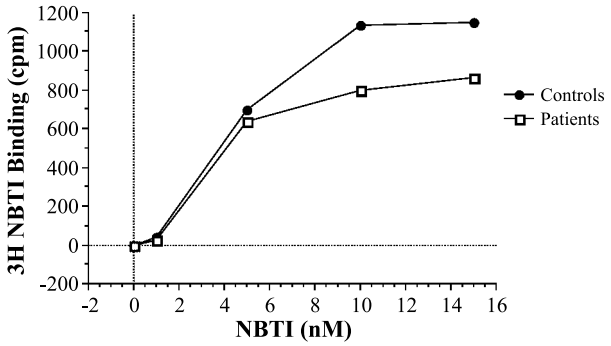

Figure 2. NBTI binding in PBL-C and PBL-LN.

RESULTS

Adenosine transport displays a sigmoid curve in PBL_{LN} versus the hyperbolic normal curve (Fig. 1). Adenosine transport was significantly decreased in PBL_{LN} with respect to PBL_C at adenosine concentrations of 1, 5 and 7 μM (Table 1).

[^{3}H] NBTI Binding was significantly decreased in PBL-LN with respect to PBL-C (6,782 ± 395 high affinity sites per cell (Bmax) in PBL-LN, versus 9,500 ± 9 in PBL-C; $p < 0.05$). A dissociation constant (Kd) of 3.7 ± 0.2 nM in PBL-LN was observed versus a Kd of 1.25 ± 0.11 nM in PBL-C; $p < 0.05$ (Fig. 2).

After 24 h incubation with 25 μM hypoxanthine, adenosine transport is decreased in PBL-LN with respect to basal transport (no hypoxanthine) (Vmax with hypoxanthine = 8.5 ± 0.34 vs. Vmax basal = 14.35 ± 0.81 pmol/10^6 cells/min; $p < 0.001$), and the sigmoidal kinetics changes to Michaelis Menten saturation kinetics (Fig. 3). However, [^{3}H] NBTI binding in PBL-LN was not decreased with hypoxanthine addition (Fig. 4).

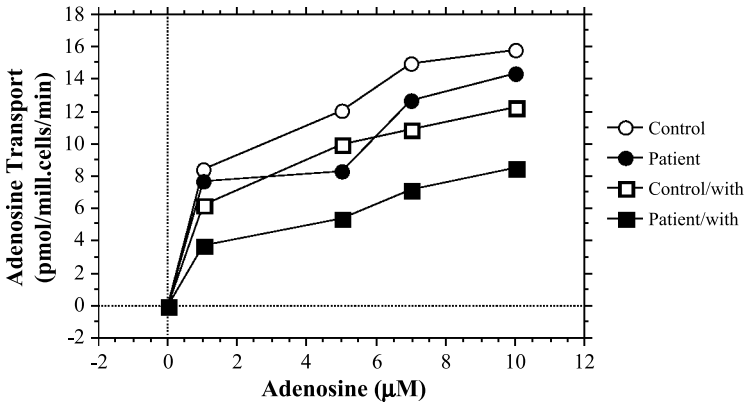

Figure 3. Effect of 25 μM hypoxanthine on adenosine transport in PBL-C and PBL-LN.

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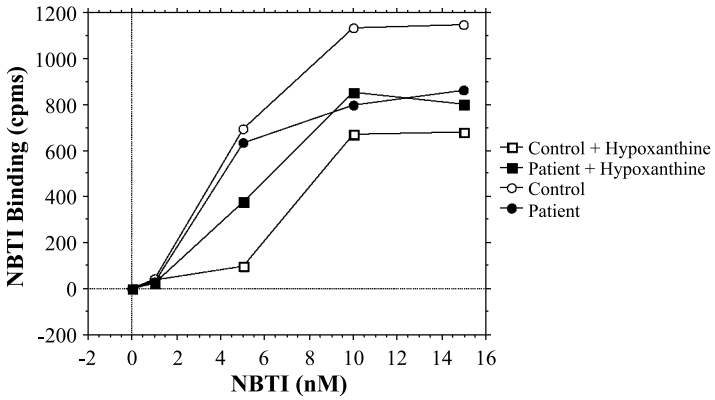

Figure 4. Effect of 25 μM hypoxanthine on NBTI binding in PBL-C and PBL-LN.

CONCLUSIONS

This study shows that adenosine transport is markedly abnormal in HPRT deficient lymphocytes, and that hypoxanthine concentration influences adenosine transport in these cells. Further studies are necessary to fully characterize adenosine transport in HPRT deficient cells.

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