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Abnormal adenosine and dopamine receptor expression in lymphocytes of Lesch–Nyhan patients

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ABSTRACT

Self-injurious behavior is the most outstanding feature of Lesch–Nyhan syndrome and has recently been ascribed to an obsessive–compulsive behavior. Lesch–Nyhan syndrome results from the complete enzyme deficiency of hypoxanthine–guanine phosphoribosyl transferase (HPRT) but the link between abnormal purine metabolism and its neurological and behavioral manifestations remains largely unknown. Previous studies led us to hypothesize that adenosine and dopamine receptor expression could be altered in HPRT-deficient cells. To test this hypothesis, we examined mRNA expressions of adenosine (ADORA2A and ADORA2B) and dopamine receptors (DRD1 and DRD2 like), and dopamine transporter (DAT1) in peripheral blood lymphocytes (PBLs) from Lesch–Nyhan patients. We also examined the influence of hypoxanthine in these expressions. As compared to normal PBLs, both ADORA2A and DRD5 expression were abnormal in PBLs from Lesch–Nyhan patients. In contrast, DAT1 expression was similar to control values in HPRT deficient PBLs. These results indicate an abnormal adenosine and dopamine receptor expression in HPRT-deficient cells and suggest disrupted adenosine and dopamine neurotransmission may have a significant role in the pathogenesis of the neurological manifestations of Lesch–Nyhan syndrome.

1. Introduction

Lesch–Nyhan disease is an inborn disorder of purine metabolism resulting from complete deficiency of the enzyme hypoxanthine–guanine phosphoribosyl transferase (HPRT) (Lesch and Nyhan, 1964; Seegmiller et al., 1967) (OMIM 300322). Affected individuals exhibit over-production of uric acid, along with a characteristic neurobehavioral syndrome that includes severe action dystonia, choreoathetosis, ballismus, cognitive and attention deficit, and self-injurious behavior (Jinnah et al., 2006; Schretlen et al., 2001). Compulsive self-injurious conduct is the most striking feature of Lesch–Nyhan syndrome and has recently been ascribed to an obsessive–compulsive behavior.

The connection between the aberrant purine metabolism and these neurological and behavioral characteristics remains largely unknown. Three facts have been clearly documented in HPRT deficiency: increased hypoxanthine concentration (Lloyd et al., 1981); dopaminergic deficit, together with dopamine hypersensitivity (Watts et al., 1982; Jinnah et al., 2006; Lloyd et al., 1981; Silverstein et al., 1985; Jankovic et al., 1988), and abnormal adenosine transport (Torres et al., 2004). In HPRT-deficient rat neuron cells, increased amounts of hypoxanthine are secreted into the extracellular medium (Zoref-Shani et al., 1993). This is particularly relevant in the rat brain where xanthine oxidase activity is relatively low (Schultz and Lowenstein, 1976). The increase in hypoxanthine concentration has been shown to interfere with adenosine transport in HPRT deficient peripheral blood lymphocytes (PBLs) from Lesch–Nyhan patients (Torres et al., 2004), probably by a competitive mechanism (Prior et al., 2007). Inhibition of adenosine transport probably increases adenosine extracellular levels and adenosine binding to its receptors. Adenosine and dopamine receptors are coupled in the basal ganglia (Fink et al., 1992); thus adenosine agonists and antagonists could influence dopamine neurotransmission (Ferré et al., 1997; Fuxe et al., 2007). *Post-mortem* studies in two Lesch–Nyhan patient brains, by immunohistochemical methods, have shown that dopamine D1 and D2 receptors were increased in the putamen, and less significantly in the caudate nucleus (Saito and Takashima, 2000). Increased expression of adenosine A1 receptors has also been found in the brain tissue of HPRT-deficient mutant mice (Bertelli et al., 2006). These studies led us to hypothesize that adenosine and dopamine receptor expression could be altered in HPRT-deficient cells. We tested this hypothesis in PBLs obtained from Lesch–Nyhan patients. These cells show the metabolic characteristics of HPRT-deficient cells (Brosh et al., 1976) and express both adenosine and dopamine receptors (Moroz et al., 1981; McKenna

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et al., 2002), and the latter is deregulated in different neuropsychiatric disorders (Bondy et al., 1984, 1985; Bondy and Ackenheil, 1987; Ilani et al., 2001; Kwak et al., 2001; Barbanti et al., 2000; Nagai et al., 1996; Rocc et al., 2002). Finally, Positron Emission Tomography studies have documented significant decreases in [^{18}F]fluorodopa uptake and dopamine transporters in the basal ganglia of Lesch–Nyhan patients (Ernst et al., 1996; Wong et al., 1996); thus we have also explored dopamine transporter expression in PBLs.

2. Materials and methods

2.1. Peripheral blood separation and RNA extraction

Heparinized blood samples were collected from 12 Lesch–Nyhan disease patients (age 8.4 ± 6.4 years; range 2–20 years) and from 12 healthy individuals of similar ages (±2 years). Deficiency of HPRT was diagnosed on the basis of all of the following characteristics: clinical symptoms and signs characteristic of the enzyme defect, increased plasma and urinary uric acid, hypoxanthine and xanthine concentrations, markedly decreased HPRT activity in the hemolysate with a simultaneously increased adenine phosphoribosyltransferase, and characterization of the genetic mutation accounting for the deficient enzyme activity (García Puig et al., 2001).

Informed consent was obtained from the parents of the children and/or the patients and from the normal subjects, after the study protocol was approved by the Ethics Committee of La Paz Hospital.

Isolation of mononuclear cells from peripheral blood was performed by the Ficoll density sedimentation method as described by Boyum (1964), employing a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077 ± 0.001 g/ml (Histopaque®-1077, Sigma Diagnostic). The mononuclear cells isolated correspond in 88.8% ± 1.0% to lymphocytes. The lymphocyte layer collected was washed twice in erythrocyte lysis buffer (Qiagen GmbH, d-40724, Hilden, Germany). Total RNA was isolated using the QIAmp RNA Blood Mini Kit (Qiagen GmbH, d-40724, Hilden, Germany).

2.2. Effect of hypoxanthine excess

Heparinized blood samples were collected from 13 healthy individuals. The lymphocyte layer, isolated as described before, was washed twice in medium containing RPMI 1640 (Sigma Diagnostic) and 10% (v/v) fetal bovine serum (FBS) (Gibco, Barcelona, Spain). Then cells were transferred to RPMI 1640 medium containing 10% (v/v) FBS, streptomycin (50 μg/ml) and amphoterycin B (2.5 μg/ml) (Sigma Diagnostic) and incubated at 37 °C in a humid atmosphere of 5% CO_2. After 24 h, cells were divided into two aliquots and cultured in medium containing RPMI 1640, 1% (v/v) FBS, streptomycin (50 μg/ml) and amphoterycin B (2.5 μg/ml) with (C-PBLHx) or without (C-PBLw) 25 μM hypoxanthine at 37 °C in a humid atmosphere of 5% CO_2. After 72 h of incubation, cell pellets were obtained by centrifugation and washed twice in erythrocyte lysis buffer (Qiagen GmbH, d-40724, Hilden, Germany) and the total RNA was isolated using the QIAmp RNA Blood Mini Kit (Qiagen GmbH, d-40724, Hilden, Germany).

2.3. DAT1, adenosine receptors A2A and A2B and dopamine receptors DRD2, DRD3, DRD4 and DRD5 mRNA quantification by real-time PCR

Total RNA was reverse transcribed into a first-strand cDNA template using the ImProm-II™ Reverse Transcriptase system (Promega, Promega Corporation, WI, USA) and oligo (dT) 15 mer as primer for RT-PCR. Expression was quantified by real-time PCR with the use of a relative quantification method (Rasmussen, 2001). We employed a housekeeping gene such as β-actin as a reference gene and the results were expressed as a relative ratio of target gene expression to reference gene expression measured in the same sample material.

To obtain the concentration of these parameters, a standard curve for each target was used. A control RNA was reverse transcribed and the cDNA obtained was employed as calibrator. A standard curve for human β-actin and for each target was constructed using serial dilutions of this calibrator. The calibrator sample was assigned a value of 100.

Quantification of β-actin expression was determined by means of real-time PCR performed in a Roche LightCycler using LC Fast Start DNA Master SYBR Green I (Roche). A melting curve analysis was used to determine the melting temperature (T_m) of the amplified products so as to ensure its specificity.

Quantification of DAT1, ADORA2A, ADORA2B and dopamine receptors DRD2, DRD3 and DRD4 mRNA was carried out by means of a Taq Man detection method with probes from the Universal Probe Library (Roche Applied Science). These probes are single hydrolysis probes of only 8 or 9 bases pre-labeled with a reporter fluorophore (FAM) and a dark quencher dye. The high probe-melting temperatures characteristic of longer probes are retained by using Locked Nucleic Acid (LNA) nucleotide chemistry in these shorter probes. Because probes are only 8 or 9 bases long, each probe can hybridize to over 7000 transcripts. Primers for DAT1, ADORA2A, ADORA2B and dopamine receptors DRD2, DRD3 and DRD4 were designed with the ProbeFinder version 2.40 for human software (Roche Diagnostics) and the probe is chosen according to these primers (Table 1).

We used TaKaRa Premix Ex Taq™ (Perfect Real Time) (TaKaRa Bio Europe, France), designed for qPCR using TaqMan® probe detection method. This premix includes TaKaRa's high-performance Ex Taq™ Hot Start DNA Polymerase, which uses antibody-mediated hot start technology to prevent non-specific amplification.

No adequate probe could be employed from the Universal Probe Library for DRD5 expression quantification so we designed primers and a TaqMan probe (http://biotools.umassmed.edu) (Table 1). As the genetic architecture of the DRD5 receptor does not allow the design of an adequate intron spanning PCR assay, we treated the RNA with DNAse (Ambion, Applied Biosystems) to avoid genomic contamination.

Primers, probes and amplicon lengths for DAT1, ADORA2A, ADORA2B and dopamine receptors DRD2, DRD3, DRD4 and DRD5 and for housekeeping gene β-actin, are shown in Table 1.

2.4. Data analysis

Analysis of quantification data was made with LightCycler software. This software only considers fluorescence values measured in the exponential growing phase of the PCR amplification process. The crossing point (Cp) is defined as the cycle numbers where fluorescence levels of all samples are the same, just above background. The Cp is automatically calculated by the LightCycler software by the "Second Derivative Maximum Method". This is achieved by a software algorithm that identified the first turning point of the fluorescence curve. This turning point corresponds to the first maximum of the second derivative curve. A standard curve is generated by plotting the Cp versus the logarithm of the concentrations for each dilution of the calibrator sample or the standard. The software calculated a linear regression line through the data point and this allows interpolating the Cp of any sample and calculating the respective concentration (Rasmussen, 2001).

Data was expressed as a gene target concentration/β-actin concentration ratio. Data are presented as means ± SD. DAT1, ADORA2A, ADORA2B and dopamine receptors DRD2, DRD3,

Table 1
Primers, probes and amplicon lengths for DAT1, adenosine receptors ADORA2A and ADORA2B and dopamine receptors DRD3, DRD4 and DRD5 and for housekeeping gene β-actin.

Gene	Primer sequence	Universal Probe Library	Amplicon length (bp)	RefSeq code	Proposed function of the protein
DAT1 (SLC6A3)	5′-CTCTGCGAGGCGTCTGTT 5′-AGCTGGAGAAGGCGATCAG	7	89	NM_001044.3	Dopamine transporter mediates the active reuptake of dopamine from the synapse. It is a principal regulator of dopaminergic neurotransmission
ADORA2A	5′-CTACATTGCCATCCGCATC 5′-AACCTAGCATGGGAGTCAGG	3	122	NM_000675.4	This protein is one of several receptor subtypes for adenosine. Its activity is mediated by G proteins which activate adenylate cyclase. The encoded protein is abundant in basal ganglia, vasculature and platelets and it is a major target of caffeine
ADORA2B	5′-TCTGTGTCCCGCTCAGGT 5′-GATGCCAAAGGCAAGGAC	56	89	NM_000676.2	This protein is an adenosine receptor subtype, member of the G protein-coupled receptor superfamily, which stimulates adenylate cyclase activity in the presence of adenosine. It also interacts with netrin-1, which is involved in axon elongation
DRD2	5′-AGACCATGAGCCGTAGGAAG 5′-GCAGCCAGCAGATGATGA	3	96	NM_000795.3	The D2 subtype of the dopamine receptor is a G-protein-coupled receptor inhibits adenylate cyclase activity. A missense mutation in this gene causes myoclonus dystonia
DRD3	5′-GGCAGATTATCGACATCTTTGA 5′-GCAGCCAGCAGACAATGA	7	121	NM_000796.3	Dopamine receptor subtype receptor. Its activity is mediated by G proteins which inhibit adenylate cyclase. Genetic variation in this gene may be associated with susceptibility to hereditary essential tremor 1
DRD4	5′-GCTCTTCGTCTACTCCGAGGT 5′-CGCACAGGTTGAAGATGGA	66	116	NM_000797.2	The D4 subtype of the dopamine receptor is a G-protein-coupled receptor which inhibits adenylate cyclase. Mutations in this gene have been associated with various behavioral phenotypes, including autonomic nervous system dysfunction, attention deficit/hyperactivity disorder, and the personality trait of novelty seeking
DRD5	5′-CGACGTGAATGCAGAGAACT 5′-GTAGATGCGCGTGTAGGTCA Probe 5′-CTTCCTCGCTCATCAGCTTCT		118	NM_000798.4	The D5 subtype is a G-protein-coupled receptor which stimulates adenylate cyclase. It has a 10-fold higher affinity for dopamine than the D1 subtype
β-Actin (ACTB)	5′-GAGCGGGAAATCGTGCGTGACATT 5′-GAAGGTAGTTTCGTGGATGCC		76	NM_001101.3	It is one of six different actin proteins that are involved in cell motility, structure, and integrity. This actin is a major constituent of the contractile apparatus and one of the two non-muscle cytoskeletal actins

DRD4 and DRD5 mRNA expression differences between PBLs from control subjects (C-PBL) and PBLs from Lesch–Nyhan patients (LN-PBL) were tested using the *t*-Test for unpaired variables. DAT1, ADORA2A, ADORA2B and dopamine receptors DRD2, DRD3, DRD4 and DRD5 mRNA expression differences between cultured PBL in the presence (C-PBLHx) or absence (C-PBLw) of hypoxanthine 25 μM were tested using the *t*-Test for paired variables. Statistical tests were performed using the Statview® software package (SAS Institute, Inc., USA). $P < 0.05$ was considered significant. In the figures the data are presented as Box-Plot. The Box-Plot displays: the upper and lower quartiles, the median and the minimum and maximum data values. The "box" itself represents the middle 50% of the data or the interquartile range.

3. Results

Appropriate standard curves were constructed for DAT1 (slope 2.493, $r = 0.99$); ADORA2A (slope 2.792, $r = 1$); ADORA2B (slope 3.852, $r = 0.99$); DRD3 (slope 2.064, $r = 0.99$); DRD4 (slope 2.818, $r = 0.98$); DRD5 (slope 2.765, $r = 1$) and for β-actin (slope 3.191, $r = 1$). Although expression was detected, no appropriate standard curve could be constructed for the dopamine receptor DRD2. Thus, DRD2 expression was not quantified.

3.1. Quantification of DAT1 mRNA expression

DAT1 mRNA expression was not significantly different between C-PBL and LN-PBL (means ± SD 0.918 ± 0.539 vs. 0.609 ± 0.318; $P = 0.122$) (Fig. 1A). Incubation of PBLs for 72 h resulted in a significant sevenfold increase of mean DAT1 expression in control cells ($P < 0.0001$), with a non-significant difference between C-PBLw and C-PBLHx (mean ± SD 6.763 ± 2.060; vs. 8.464 ± 6.086; $P = 0.439$) (Fig. 1 B).

3.2. Quantification of adenosine receptors ADORA2A and ADORA2B mRNA expression

ADORA2B and ADORA2A expression was not significantly different between C-PBL and LN-PBL (Fig. 2A) (means ± SD; ADORA2A; 1.261 ± 0.133 vs. 1.188 ± 1.160; $P = 0.8302$; ADORA2B; 1.575 ± 0.564 vs. 1.215 ± 0.375; $P = 0.176$). ADORA2A expression in C-PBLs showed a range of 1.029 to 1.398 and no value of ADORA2A expression in LN-PBL was included in the control range (Fig. 2B). Thus we divided LN-PBLs into two groups according to their ADORA2A expression levels: High, higher than control range (>1.389) and Low, lower than control range (<1.029). A significant difference was found between C-PBL and High LN-PBL (mean ± SD 2.536 ± 1.098; $P < 0.05$; $n = 4$) and between C-PBL and Low LN-PBL (mean ± SD 0.514 ± 0.204; $P < 0.0001$; $n = 8$) (Fig. 2B). Differences between patients with high and low ADORA2A expression were analyzed. There was no clinical, biochemical, enzymatic, molecular or treatment-related difference between both groups. We compared variables that may differentiate Lesch–Nyhan patients as: age at the moment of the RNA extraction, grade of self-injurious behavior (SIB 0–4), and HPRT mRNA expression in patients with high and low levels of ADORA2A expression and we did not found any correlation among these variables and ADORA2A expression. Incubation of control PBLs for 72 h resulted in a significant twofold increase of mean ADORA2A ($P < 0.001$) and ADORA2B ($P < 0.05$)

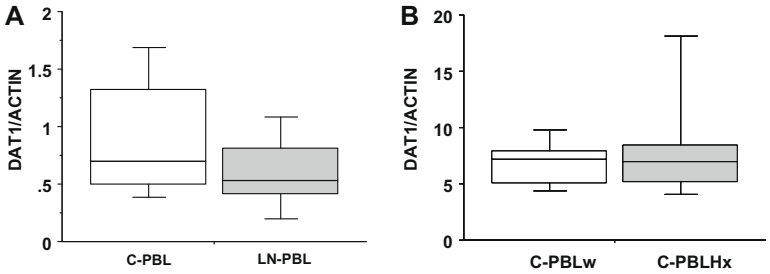

Fig. 1. Box plot showing: (A) DAT1 mRNA expression in C-PBLs compared to LN-PBLs. (B) DAT1 mRNA expression in C-PBLHx vs. C-PBLw. Data were expressed as DAT1 concentration/β-actin concentration ratio. The Box-Plot displays: the upper and lower quartiles, the median, and the minimum and maximum data values. The "box" itself represents the middle 50% of the data. The upper boundary of the box locates the 75th percentile of the data set while the lower boundary indicates the 25th percentile. The area between these two boundaries is the "inter-quartile range". The line in the box indicates the median. The "whiskers" of the Box-Plot (the vertical lines of the plot extending from the box), indicate the minimum and maximum values in the dataset.

adenosine receptors expression in control cells (Fig. 2C). There were non-significant differences in the mRNA ADORA2A or ADORA2B expression between C-PBLw and C-PBLHx (ADORA2B: 2.802 ± 0.863 vs. 2.641 ± 0.752; P = 0.680; ADORA2A: 2.929 ± 1.602; vs. 2.966 ± 1.504; P = 0.960).

3.3. Quantification of dopamine receptors DRD3, DRD4 and DRD5 mRNA expression

DRD3 and DRD4 expression was not significantly different from C-PBL and LN-PBL (DRD3: mean ± SD 0.693 ± 0.409 vs. 0.854 ± 0.844; $P = 0.595$; DRD4: 0.569 ± 0.491 vs. 1.191 ± 1.131; $P = 0.1098$ (Fig. 3A). However, DRD5 mRNA levels were significantly higher in LN-PBLs than in C-PBLs (9.779 ± 7.510 vs. 4.011 ± 1.555; $P < 0.05$) (Fig. 3A).

Incubation of control PBLs caused a significant eightfold increase of mean DRD3 expression ($P < 0.0005$), a non-significant threefold decrease of mean DRD4 expression ($P = 0.051$), and a significant fourfold decrease of mean DRD5 expression ($P < 0.0001$). No differences in DRD3 (5.847 ± 3.690 vs. 5.353 ± 3.644; $P = 0.779$), DRD4 (0.183 ± 0.163 vs. 0.137 ± 0.098; $P = 0.48$) and DRD5 (1.033 ± 0.583 vs. 0.796 ± 0.320; $P = 0.332$) mRNA expression were found when control PBLs were cultured with and without hypoxanthine 25 μM (Fig. 3B).

4. Discussion

In this study, we examined mRNA expressions of adenosine (ADORA2A and ADORA2B) and dopamine receptors (DRD1 and DRD2 like), and dopamine transporter (DAT1) in PBLs from Lesch–Nyhan patients, and the influence of hypoxanthine in these expressions. Our results show that both ADORA2A and DRD5 expression are abnormal in PBLs from Lesch–Nyhan patients. In contrast, DAT1 expression was similar to control PBLs in HPRT deficient PBLs.

The primary mechanism through which dopamine is cleared from synapses is provided by DAT1. Because DAT1 terminates the dopamine signal, it is implicated in a number of dopamine-related disorders, including attention deficit hyperactivity disorder (Yang et al., 2007), bipolar disorder (Greenwood et al., 2001), clinical depression (Laasonen-Balk et al., 1999) and alcoholism (Sander et al., 1997; Ueno et al., 1999). Although PET studies have suggested that dopamine transporters are reduced in the brain of Lesch–Nyhan patients (Wong et al., 1996), we did not find a statistical significant difference between DAT1 expression in C-PBLs and LN-PBLs. Nevertheless, functional studies that investigate dopamine transport in HPRT-deficient cells will be necessary to rule out a possible abnormality.

Both adenosine and dopamine have been implicated in the pathogenesis of the neurological dysfunction in Lesch–Nyhan patients. The presence and function of adenosine A2A receptors in

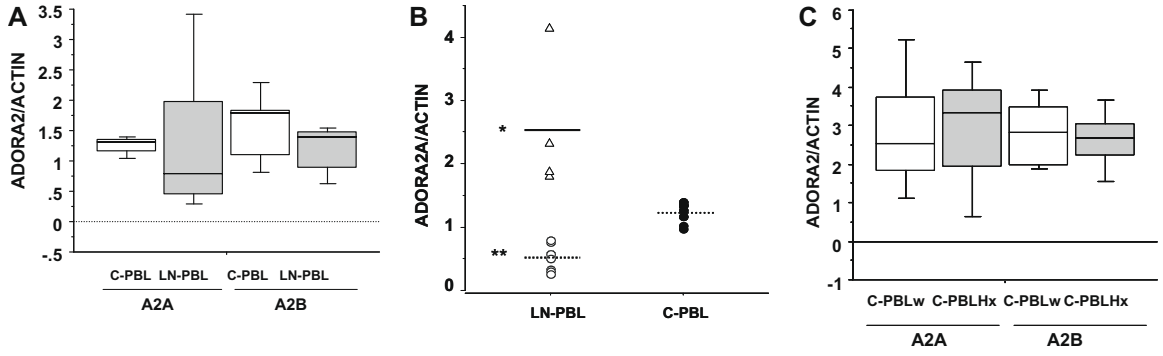

Fig. 2. (A) Box plot showing ADORA2A (A2A) mRNA expression and ADORA2B(A2B) mRNA expression in C-PBLs compared to LN-PBLs; (B) individual ADORA2A mRNA expression from controls (filled circles) and Lesch–Nyhan patients. Patients have been divided into two subgroups: LN HIGH group represent Lesch–Nyhan patients with a mRNA expression of ADORA2A >1389 (triangles); LN LOW group represent Lesch–Nyhan patients with a mRNA expression of ADORA2A receptor <1029 (empty circles). Horizontal lines represent the mean value for controls, LN HIGH and LN LOW groups. *C-PBLs vs. LN HIGH $P < 0.05$; **C-PBLs vs. LN LOW $P < 0.0001$.; (C) Box plot showing ADORA2B and ADORA2A expression in C-PBLHx vs. C-PBLw. Data were expressed as ADORA2A or ADORA2B concentration/β-actin concentration ratio. The Box-Plot displays: the upper and lower quartiles, the median, and the minimum and maximum data values. The "box" itself represents the middle 50% of the data. The upper boundary of the box locates the 75th percentile of the data set while the lower boundary indicates the 25th percentile. The area between these two boundaries is the "inter-quartile range". The line in the box indicates the median. The "whiskers" of the box-plot (the vertical lines of the plot extending from the box), indicate the minimum and maximum values in the dataset.

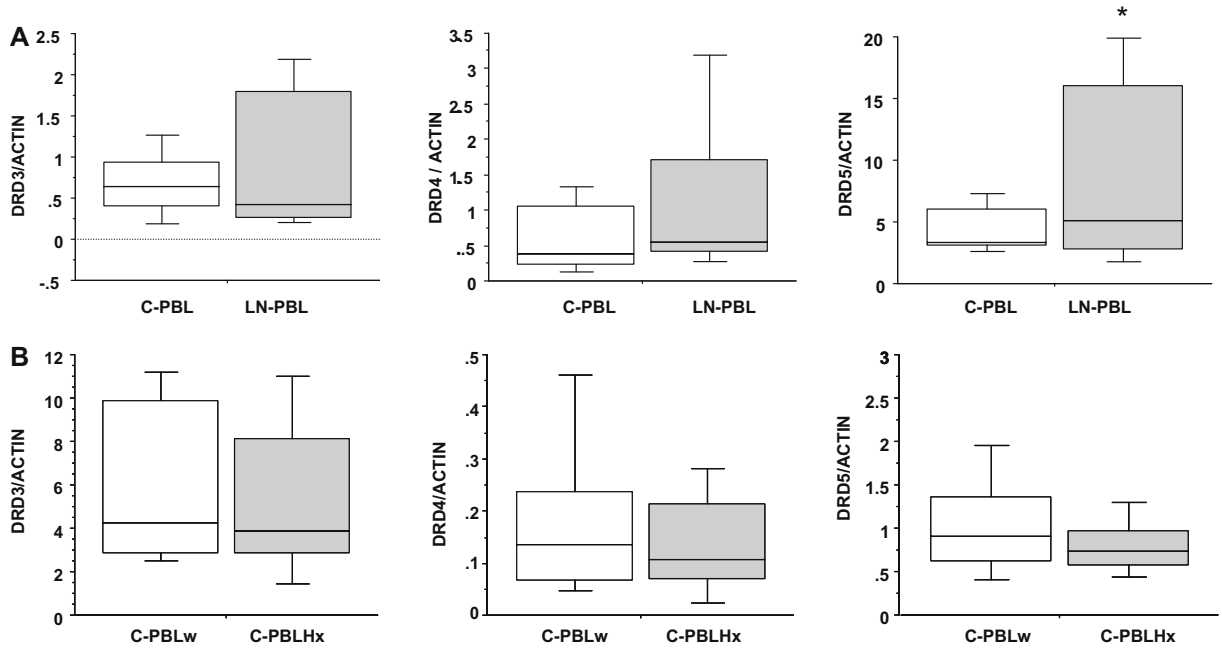

Fig. 3. Box plot showing. (A) Dopamine receptors DRD3, DRD4 and DRD5 expression in C-PBLs vs. LN-PBLs. (1) Dopamine receptor DRD3 mRNA expression, (2) dopamine receptor DRD4 mRNA expression and (3) dopamine receptor DRD5 mRNA expression. (B) Dopamine receptors DRD3, DRD4 and DRD5 expression in C-PBLHx vs. C-PBLw. Data were expressed as DRD3, DRD4 or DRD5 concentration/β-actin concentration ratio. *C-PBLs vs. LN-PBLs $P < 0.05$. The Box-Plot displays: the upper and lower quartiles, the median, and the minimum and maximum data values. The "box" itself represents the middle 50% of the data. The upper boundary of the box locates the 75th percentile of the data set while the lower boundary indicates the 25th percentile. The area between these two boundaries is the "inter-quartile range". The line in the box indicates the median. The "whiskers" of the Box-Plot (the vertical lines of the plot extending from the box), indicate the minimum and maximum values in the dataset.

PBLs have been well characterized, but no previous studies have quantified their mRNA expression using real-time PCR. The expression of A2A receptor in human lymphocytes has been detected both by functional assays (cAMP accumulation) and by flow cytometry (Koshiba et al., 1999). ADORA2B expression in PBLs has been detected by immunocytochemical studies (Mirabet et al., 1999) and by selective antagonist radioligand assay (Gessi et al., 2005). In this study, we confirmed the presence of both receptors by real-time PCR technology. The expression of dopamine receptors in PBLs has been investigated by laser flowcytometry (McKenna et al., 2002) and real-time PCR (Ostadali et al., 2004; Kirillova et al., 2008). In this study, we have detected the expression of all DRD2-like receptors and the DRD5 receptor, belonging to DRD1-like family. However, DRD2 expression levels were very low and we could not accurately quantify them.

We have found that the expression of ADORA2B in C-PBLs and LN-PBLs were not significantly different. However, ADORA2A expression was abnormal in LN-PBLs with high and low levels. Bertelli et al. (2006) examined the expression of ADORA1, ADORA2A and ADORA2B in the brain of a murine model of Lesch–Nyhan disease by real-time PCR. As compared to control mice, HPRT deficient knockout mice showed a 95% increase in ADORA1 expression, a 15% decrease in ADORA2A expression, and no change in ADORA2B expression. Unfortunately, ADORA1 receptors are not expressed in PBLs. However, our results in PBLs confirm the abnormality of ADORA2A expression found in the animal model. Most patients (67%) in our study presented a decreased ADORA2A expression; with a 39% mean decrease as compared to controls, which is consistent with previous results. However, we haven't an explanation for the high levels of ADORA2A expression found in some of our patients of patients. We did not found any clinical, biochemical, enzymatic, molecular or treatment-related difference between both groups, HPRT deficient patients with high and low ADORA2A expression. Further studies will be necessary to clarify this fact. We previously reported that hypoxanthine 25 μM increases cAMP levels in PBLs by means of a receptor A2-type effect (Torres et al., 2004). In the present study, we did not find a significant effect of hypoxanthine incubation in ADORA2A or ADORA2B expression in control PBLs. Thus, the increase in cAMP levels should be ascribed to the effect of hypoxanthine on adenosine transport, resulting in an increase of extracellular adenosine levels.

The finding that DRD5 expression is significantly higher in PBLs from Lesch–Nyhan patients than in control PBLs is in agreement with the increase in dopamine receptors demonstrated by immunohistochemical methods in the *post-mortem* analysis of the putamen, and the caudate nucleus of two Lesch–Nyhan patients (Saito and Takashima, 2000). This is the first report that supports the hypothesis of dopaminergic hypersensitivity in HPRT deficiency by using cells from living patients.

Ferrari et al. have recently reported, using also real-time PCR, that dopaminergic receptor DRD5 mRNA expression is increased in PBLs from Tourette syndrome patients, (Ferrari et al., 2008) and DRD5 expression was correlated with the severity of this compulsive disorder. This syndrome shares many features with obsessive compulsive disorder, attention deficit hyperactivity disorder and impulse control disorder with which it is also commonly associated (Carter et al., 1994; Kurlan et al., 2002; Spencer et al., 2001). Evidence derived from both pharmacological trials and imaging studies suggests that alterations of the dopaminergic and serotonergic neurotransmitter systems play a key role in the pathogenesis of Tourette syndrome (Albin and Mink, 2006). Thus, we may speculate on a relationship between DRD5 over expression in PBLs and compulsive and attention deficit disorders.

The main limitation of our study is the cell type used to assess receptor expression. Unfortunately, animal models do not reproduce the motor and behavioral manifestations of the disease, and cells from patient's brain are not available. However, according to the peripheral biomarker hypothesis, neurotransmitter receptor expression in peripheral immune cells could reflect expression of these receptors in the brain. This hypothesis is supported by sev-

eral results: in Parkinson's disease reduced mRNA expression of the dopamine receptor DRD3 has been found in PBLs and DRD3 expression correlates with the clinical severity (Nagai et al., 1996). Dopamine receptor DRD3 mRNA expression in human lymphocytes is negatively correlated with the personality trait of persistence (Czermak et al., 2004) and expression levels of DRD3 and DRD4, assessed by real-time PCR, was found to be similar in PBLs and in the brain (Kirillova et al., 2008). Padín et al. (2006) demonstrated the peripheral biomarker hypothesis in rat platelets. In that study, olanzapine-mediated regulation of serotonin 5-HT_{2A} and dopamine D3 receptors were studied in parallel in rat CNS and blood cells, after acute (24 and 48 h) and sub chronic (16 days) drug treatment. This study demonstrated the parallel olanzapine-induced regulation of 5-HT_{2A} and mRNA D3 receptors in CNS and peripheral blood cells. These facts should stimulate further studies to confirm the validity of the peripheral immune cells receptors as biomarkers in the pharmacology of mental diseases.

In summary, the results of this study show an altered adenosine and dopamine receptor expression in HPRT-deficient cells. These results emphasize a pivotal role for disrupted adenosine and dopamine neurotransmission in the pathogenesis of neurological manifestations of Lesch–Nyhan syndrome and encourage further studies to lead to a better understanding of the adenosine–dopamine interaction in HPRT deficiency.

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