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Research paper

# Altered histamine neurotransmission in HPRT-deficient mice

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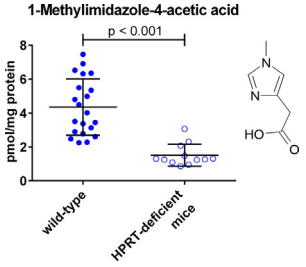
## HIGHLIGHTS

- HPRT knockout mice show decreased neuronal histamine activity.
- Altered histamine neurotransmission may contribute to Lesch–Nyhan syndrome.
- Modulation of histaminergic activity might be of therapeutic relevance.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Lesch–Nyhan syndrome (LNS) is an X-chromosomal disorder with congenital deficiency of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) as underlying defect. We determined the concentrations of dopamine, histamine and their metabolites in brains of HPRT knockout mice, which serve as an animal model for LNS, and compared the results to those obtained from wild-type controls. Analyses were performed by high performance liquid chromatography (HPLC)-coupled tandem mass spectrometry (MS/MS). Besides a decrease of dopamine and 3-methoxytyramine (3-MT) concentrations in the cerebral hemisphere, HPRT-deficient mice also exhibited significantly reduced 1-methylhistamine (1-MH) and 1-methylimidazole-4-acetic acid (1-MI4AA) concentrations in the brain hemisphere and medulla. Moreover, the amount of 1-MI4AA was significantly decreased in the cerebellum. Our findings show that neuronal perturbations caused by HPRT deficiency are not restricted to the dopamine system but also affect histaminergic neurotransmission. These new insights into the brain metabolism of an LNS mouse model may help to find new therapeutic strategies to improve the quality of life of LNS patients.



*Abbreviations:* 1-MH, 1-methylhistamine; 1-MI4AA, 1-methylimidazole-4-acetic acid; 3-MT, 3-methoxytyramine; d4-dopamine, 2-(3,4-dihydroxyphenyl)ethyl-1,1,2,2-d4-amine HCl; d4-histamine, histamine-d4 diHCl; d5-DOPAC, 3,4-dihydroxyphenylacetic acid (ring-d3,2,2-d2); d6-norepinephrine, (±)-norepinephrine-2,5,6,α,β,β-d6 HCl; DOPAC, 3,4-dihydroxyphenylacetic acid; HDC, L-histidine decarboxylase; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; HPRT, hypoxanthine-guanine phosphoribosyltransferase; HPRT$^-$, HPRT-deficient mice; HVA, homovanillic acid; I4AA, imidazole-4-acetic acid; LLOQ, lower limit of quantification; LNS, Lesch–Nyhan syndrome; MS/MS, tandem mass spectrometry; QC, quality control; SD, standard deviation; SNP, single-nucleotide polymorphism; TMN, tuberomamillary nucleus; TS, Tourette syndrome; WT, wild-type mice.

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## 1. Introduction

Lesch–Nyhan syndrome (LNS) is an X-chromosomal recessively inherited disorder caused by mutation of the *HPRT1* gene and subsequent deficiency of the purine-salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT), leading to accumulation of uric acid with hyperuricemia and gout development [1]. Moreover, the patients suffer from severe motor handicap characterized by dystonia often combined with choreoathetosis or spasticity [2] and exhibit neuropsychiatric perturbations such as cognitive impairment [3] and recurrent compulsive self-injurious behavior [4]. The mechanisms by which missing HPRT enzyme activity leads to those neurological and neuropsychiatric symptoms are still unknown and no pharmacological therapies are established so far.

Impaired basal ganglia function is considered an important factor in LNS pathology [5], and a reduction of neuronal dopamine was revealed in post-mortem studies with brain tissue from LNS patients [6]. This was confirmed in brains of HPRT-deficient mice [7–9], which serve as an animal model for LNS but do not show self-injurious behavior [10,11]. However, unlike dopamine, histamine and histamine metabolites have never been analyzed in HPRT-deficient brains. This is very surprising because the release of dopamine is modulated by the histaminergic system, e.g. by the inhibitory action of presynaptic histamine $H_3$ receptors [12]. Moreover, impaired histaminergic neurotransmission may cause some forms of Tourette syndrome (TS), which is also a basal ganglia-related disorder [13], involving compulsive behavioral features like recurrent movement or vocalization tics [14]. For some TS patients, a congenital L-histidine decarboxylase (HDC) deficiency has been reported [15] and HDC-deficient mice exhibit behavioral abnormalities interpreted as TS-like phenotype [16,17].

The lack of information about an involvement of histamine in LNS prompted us to study histamine and histamine metabolites in brains of HPRT-deficient mice. The pathways of dopamine and histamine metabolism are illustrated in Supplementary Figs. S1 and S2, respectively. We quantified dopamine and its metabolites 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as histamine and the histamine metabolites 1-methylhistamine (1-MH), 1-methylimidazole-4-acetic acid (1-MI4AA) and imidazole-4-acetic acid (I4AA) in brains of HPRT knockout mice [18] in comparison to wild-type controls. Quantification was performed by high performance liquid chromatography (HPLC)-coupled tandem mass spectrometry (MS/MS).

## 2. Materials and methods

### 2.1. HPRT knockout mice

The mice that show a deletion in the *Hprt* gene were first described by Hooper et al. [18] and were backcrossed from C57BL/6 (Jackson) to the C57BL/6JRj background from Janvier (Le Genest, Saint-Isle, France) for seven to ten generations. PCR genotyping was performed with DNA from tail samples isolated at an age of 9–12 days. The primers used for genotyping were previously described [19]. Genetic background was additionally analyzed by testing for 39 single-nucleotide polymorphisms (SNPs). After more than seven backcrossings, only a SNP on chromosome 12 (rs13481634) and another one on chromosome X (rs13483883 merged into rs6275359) was left in some animals, which presumably originates from the original Jackson C57BL/6 strain. Males carrying the defective *Hprt* gene and the wild-type controls emerged from the same colony. Mice were bred and maintained at the animal laboratory of Hannover Medical School (Hannover, Germany) and kept at a twelve hours light–dark cycle with free access to food and water. Mice were euthanized with $CO_2$ at the age of 3.5–5 months in order to collect brain tissue. The age of 3.5–5 months was chosen to ensure complete brain development. Moreover, we assumed that a higher age might result in increased pathological alterations of the brain, enhancing visibility of the phenotype. Experimental procedures were performed according to the German Animal Welfare Act (Tierschutzgesetz, § 4) and approved by the Local Institutional Animal Care and Research Advisory Committee of the Hannover Medical School and the Lower Saxony State Office for Consumer Protection and Food Safety (Approval ID: 2012/8 and 33.12-42502-04-12/1040).

### 2.2. Preparation of mouse brains

Directly after euthanasia, brains were removed, placed on a piece of wet filter paper and, after removal of the olfactory bulb, cerebellum and medulla were isolated. The remainder of the brain was cut sagitally to separate the two hemispheres. The whole procedure is depicted in Supplementary Fig. S3. After preparation, brain hemispheres, cerebellum and medulla were transferred into 1.5 mL reaction tubes, snap-frozen in liquid nitrogen and kept at −80 °C until analysis. Only the right hemispheres were used for neurotransmitter analysis.

### 2.3. Extraction of dopamine, histamine and their metabolites from murine brain tissue

FastPrep® Tubes (MB Biomedicals, Santa Ana, California, USA) were filled with 200 mg of Garnet Matrix A Bulk (MP Biomedicals) and one ¼″ Ceramic Sphere (MP Biomedicals). Tubes then were weighed and placed on ice. Each right brain hemisphere, cerebellum and medulla was transferred into one of the prepared tubes. Filled with brain tissue, tubes were weighed again and immediately placed back on ice. To each tube, 800 μL ice-cold extraction solvent were added, consisting of acetonitrile/water (4/1, v/v) (water was classified as HPLC gradient grade, acetonitrile as ultra gradient HPLC grade; both from J.T. Baker, Deventer, The Netherlands) supplemented with 0.2% (v/v) formic acid (Merck, Darmstadt, Germany) and the following internal standards: 0.2 μM 2-(3,4-dihydroxyphenyl) ethyl-1,1,2,2-d4-amine HCl (d4-dopamine) (CDN Isotopes, Pointe-Claire, Quebec, Canada), 0.2 μM (±)-norepinephrine-2,5,6,α,β,β-d6HCl (d6-norepinephrine) (CDN Isotopes), 0.2 μM histamine-d4 diHCl (d4-histamine) (TLC PharmaChem, Vaughan, Ontario, Canada) and 0.5 μM 3,4-dihydroxyphenylacetic acid (ring-d3, 2,2-d2) (d5-DOPAC) (Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA). Samples were homogenized twice at level 5 for 30 s using a FastPrep®—24 instrument (MP Biomedicals). Afterwards, samples were centrifuged for 10 min at 20,800 × *g* and 4 °C. 600 μL of the supernatants was transferred into 1.5 mL reaction tubes and stored at −20 °C until analysis (at least overnight in order to precipitate residual protein). Pellets were dried for subsequent protein determination. After mixing thoroughly, samples were once again centrifuged as described above and supernatants were transferred into new reaction tubes. Sample volumes not needed for analysis were stored at −20 °C. As quality control (QC) for intra-experimental reproducibility, two additional right brain hemispheres were extracted together with the samples. QC extracts were pooled after the last centrifugation in order to have a greater volume for several injections within the measurements.

### 2.4. Protein assay

Protein determination was performed with the Pierce® BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts,