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Neurotransmitter and their metabolite concentrations in different areas of the HPRT knockout mouse brain

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ABSTRACT

Lesch–Nyhan syndrome (LNS) is characterized by uric acid overproduction and severe neurobehavioral symptoms, such as recurrent self-mutilative behavior. To learn more about the pathophysiology of the disease, we quantified neurotransmitters and their metabolites in the cerebral hemisphere, cerebellum and the medulla oblongata of HPRT knockout mice, an animal model for LNS, in comparison to the corresponding wild-type. Our analyses included L-glutamate, 4-aminobutanoic acid (GABA), acetylcholine, serotonin, 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine, L-normetanephrine, epinephrine and L-metanephrine and were conducted via high performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS). Among these neurotransmitter systems, we did not find any abnormalities in the HPRT knockout mouse brains. On one side, this might indicate that HPRT deficiency most severely affects dopamine signaling, while brain functioning based on other neurotransmitters is more or less spared. On the other hand, our findings may reflect a compensating mechanism for impaired purine salvage that protects the brain in HPRT-deficient mice but not in LNS patients.

1. Introduction

Lesch–Nyhan syndrome (LNS) is an orphan disease caused by an X-linked congenital deficiency of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) [1]. Beside marked overproduction of uric acid [1], symptoms include severe motor disabilities mainly presenting as dystonia [2] and neuropsychological impairments, such as mental disabilities [3] and, as the predominant symptom, recurrent self-mutilation [4]. While uric acid overproduction can be well explained [1], the mechanism, by which HPRT deficiency leads to those severe neurological and neurobehavioral disturbances, remains enigmatic. To further elucidate the pathophysiology of LNS, HPRT knockout mice [5,6] have been established as an animal model for LNS and have extensively been studied in the past (e.g. [7–17]). Since LNS is associated with basal ganglia dopamine loss [18–20], previous neurotransmitter quantification studies investigated the basal ganglia dopamine system of HPRT-deficient mice [7,8,10,13–15,17]. Some investigators have also reported data for serotonin [7,8,13,15,17], norepinephrine [7,8,13,15,17], epinephrine [8] and some of their metabolites [8,13,17] in brains from HPRT-deficient mice. In a recent study, we have as well determined dopamine and dopamine metabolite concentrations, including the whole HPRT knockout mouse brain which we segmented into cerebral hemisphere, cerebellum and medulla oblongata with a part of the pons [21]. Additionally, we have analyzed histamine and its metabolite contents in the same brain regions [21]. In this study, we quantified the neurotransmitters L-glutamate, 4-aminobutanoic acid (GABA), acetylcholine, serotonin (and its metabolite 5-hydroxyindoleacetic acid, 5-HIAA) and norepinephrine (and its metabolite L-normetanephrine) in the right cerebral hemisphere, cerebellum and the medulla oblongata together with a part of the pons of HPRT knockout and wild-type mice. Moreover, we have analyzed the occurrence of epinephrine and its metabolite L-metanephrine. Measurements were based on high performance liquid chromatography (HPLC) in combination with tandem mass spectrometry (MS/MS). The connecting links between neurotransmitters and their respective metabolites are shown in Fig. 1.

To our knowledge, L-glutamate, GABA, acetylcholine and the norepinephrine and epinephrine metabolites L-normetanephrine and L-

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 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; DHPG, 3,4-dihydroxyphenylethyleneglycol; GABA, 4-aminobutanoic acid; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; HPRT, hypoxanthine-guanine phosphoribosyltransferase; HPRT⁻, HPRT knockout mice; LLOQ, lower limit of quantification; LNS, Lesch–Nyhan syndrome; MS/MS, tandem mass spectrometry; QC, quality control; SD, standard deviation; WT, wild-type mice.

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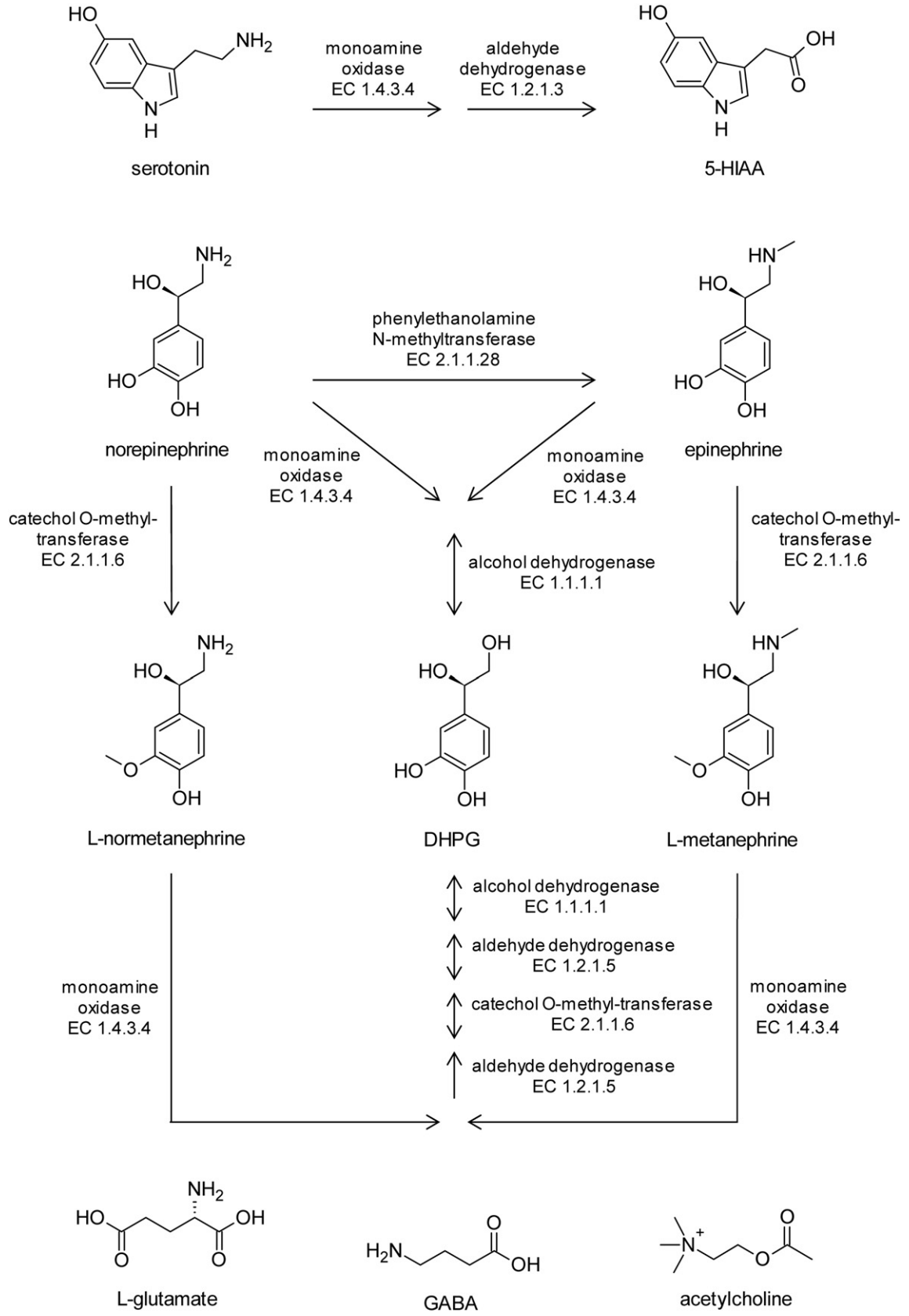

Fig. 1. The connecting links between neurotransmitters and their metabolites. Information on reaction pathways was taken from the Kyoto Encyclopedia of Genes and Genomes (http://www.kegg.jp; [22]) and the BioCyc Database Collection (http://www.biocyc.org; [23]). 5-HIAA: 5-hydroxyindoleacetic acid, DHPG: 3,4-dihydroxyphenylethyleneglycol, GABA: 4-aminobutanoic acid.