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Review

Life without brain serotonin: Reevaluation of serotonin function with mice deficient in brain serotonin synthesis

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HIGHLIGHTS

- *Tph2*$^{-/-}$ mouse is an animal model depleted of the neurotransmitter serotonin.
- In this review we critically discuss the recent data obtained from *Tph2*$^{-/-}$ mice.
- We highlight the most robust phenotypes of this mouse model.
- We suggest explanations for inconsistencies observed between different studies.

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ABSTRACT

Tryptophan hydroxylase (TPH) is a rate limiting enzyme in the synthesis of serotonin (5-HT), a monoamine which works as an autacoid in the periphery and as a neurotransmitter in the central nervous system.

In 2003 we have discovered the existence of a second *Tph* gene, which is expressed exclusively in the brain, and, therefore, is responsible for the 5-HT synthesis in the central nervous system. In the following years several research groups have independently generated *Tph2*-deficient mice. In this review we will summarize the data gained from the existing mouse models with constitutive or conditional deletion of the *Tph2* gene, focusing on biochemical, developmental, and behavioral consequences of *Tph2*-deficiency.

Contents

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxy-tryptamine (serotonin); 5-HTP, 5-hydroxytryptophan; 8-OH-DPAT, (±)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide; AADC, aromatic amino acid decarboxylase; ADHD, at tention deficit hyperactivity disorder; BH4, tetrahydrobiopterin; BLA, basolateral amygdala; CNS, central nervous system; DA, dopamine; DOI, 2.5-dimethoxy-4-iodoamphetamine; DOPAC, 3.4-dihydroxyphenylacetic acid; DREADD, designer receptor exclusively activated by a designer drug; eGFP, enhanced green fluorescent protein; E, embryonic day; EPM, elevated plus maze; FC, frontal cortex; FST, forced swim test; GABA, gamma-aminobutyric acid; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; IHC, immunohistochemistry; ISH, *in situ* hybridization; LC, locus coeruleus; L-DOPA, L-3.4-dihydroxyphenylalanine; NE, norepinephrine; NM, normetanephrine; NTS, nucleus tractus solitarius; P, day post partum; PAH, phenylalanine hydroxylase; *p*CPA, *para*chlorophenylalanine; PFC, prefrontal cortex; SERT, serotonin transporter; SNRI, serotonin–norepinephrine reuptake inhibitors; SSRI, selective serotonin reuptake inhibitor; SU, subunit; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; Trp, tryptophan; TST, tail suspension test; VMAT2, vesicular monoamine transporter 2; WT, wild type.

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1. Tryptophan hydroxylase 2 (TPH2): Serotonin-synthesizing enzyme in the brain

The biosynthesis of serotonin (5-hydroxytryptamine, 5-HT) is a two-step process. In the first step the essential amino acid L-tryptophan (Trp) is metabolized to 5-hydroxytryptophan (5-HTP) by the rate-limiting enzyme, Tryptophan hydroxylase (TPH). Next, 5-HTP is decarboxylated to 5-HT by aromatic amino acid decarboxylase (AADC). This enzyme is involved as well in the synthesis of dopamine from L-3,4-dihydroxyphenylalanine (L-DOPA) [1], tyramine from tyrosine [2], and tryptamine from Trp [3], and its distribution and functions are not restricted just to the serotonergic system.

TPH belongs to the superfamily of aromatic amino acid hydroxylases that also includes tyrosine hydroxylase (TH) and phenylalanine hydroxylase (PAH). These are iron (Fe^{2+})- and tetrahydrobiopterin (BH4)-dependent monooxygenases with substantial structural similarities in their catalytic mechanism [4]. All aromatic amino acid hydroxylases are composed of three functional domains, a regulatory N-terminal domain, a catalytic domain, and a C-terminal oligomerization domain [4,5]. Since the discovery of the 5-HT synthesis pathway, the existence of only one gene coding for a TPH enzyme in vertebrates has been supposed [6]. However, in 2003 the existence of a second gene, *Tph2*, was unraveled by genetic ablation of the by that time only known *Tph* gene, now called *Tph1* [7,8]. This discovery revealed the existence of two independent 5-HT systems in vertebrates—central, driven by TPH2, and peripheral, driven by TPH1.

The expression pattern of *Tph2* was extensively studied during the last years. In the developing brain of mice, *Tph2* is first detected at E10.5 at the RNA level [9] and at E11.25 with TPH2-specific antibodies [10]. Using mice with a *LacZ* gene inserted into the *Tph2* locus it was shown that *Tph2* expression starts at E12.5 in neurons of the dorsal (B4, B6, B7) and median (B5, B8, B9) raphe nuclei, and at E14.5, 15.5, and 18.5 in neurons of the caudal raphe nuclei B1, B2, and B3, respectively, whereas the LacZ signal was undetectable in peripheral embryonic organs [11]. Embryonic *Tph2* expression increases exponentially and peaks at birth (P0.5) [10]. In adult vertebrates, highest expression of *Tph2* is detected in raphe nuclei (mouse) and pons (human), but also at lower levels in other brain regions such as cortex, striatum, hippocampus (in both mouse and human), and putamen, caudal nuclei, and cerebellum (in human) [10–12]. In peripheral organs, such as lung, heart, kidney, or liver, *Tph2* expression was not detected [8,10,11,13,14], but it is present in enteric neurons in the gut (reviewed in [15]).

There are several studies which suggest a contribution of TPH1 to the synthesis of 5-HT in the central nervous system (CNS). Some reports show detectable levels of *Tph1* in the mouse or rat raphe nuclei by *in situ* hybridization (ISH) [12,16–19] or in postmortem human brain by qRT-PCR [14]. However, a thorough qRT-PCR analysis of *Tph1* and *Tph2* mRNA levels revealed that *Tph1* expression in the brain is extremely low or undetectable in different mouse and human brain regions [10,11] (our unpublished data). The false positive detection of *Tph1* in the brain in former studies might be caused by the high homology in the nucleotide sequences of the *Tph1* and *Tph2* genes making it hard to distinguish the two isoforms. If evaluated by RNase protection assay, ISH, immunohistochemistry (IHC), or western blot no specific signal for *Tph1* mRNA or protein is detected in different parts of the brain [8,10,13], while it is abundantly expressed in the pineal gland [10,13,20]. Accordingly, brain 5-HT content is not changed in *Tph1*-deficient mice [8]. Moreover, there is no compensatory upregulation of *Tph1* in the brain of $Tph2^{-/-}$ mice [10,11,21]. Thus, a vast body of evidence has accumulated over the last years confirming independent regulation and mutual exclusive expression of *Tph1* and *Tph2* in adult vertebrates.

2. Genetically modified animal models lacking *Tph2*

Since the discovery of *TPH2*, mouse models genetically depleted of this enzyme were created by several laboratories [11,20,22–27] and shed new light on known and revealed previously unknown functions of 5-HT in the CNS. Table 1 summarizes these models with central 5-HT deficiency including the genetic background, on which they were generated, since this may have influenced the observed phenotypes. Most of these mouse lines represent a constitutive *Tph2* knockout with complete inactivation of endogenous *Tph2* expression [11,20,22–24,26,27]. These mice show a complete lack of the enzyme, as shown at mRNA and protein levels, and as a consequence are unable to synthesize 5-HT in the brain throughout their whole life starting with embryonic development (see below and Table 2).

Also a conditional knockout using the Cre–Lox system was generated by two laboratories [21,28], which allows *Tph2* inactivation in a tissue or time-specific manner. So far $Tph2^{flox/flox}$ mice were bred to three different mouse lines expressing Cre recombinase: $Tph2^{Nes\text{-}cre}$ (expressing Cre under the control of the panneuronal nestin promoter), $Tph2^{Pet1\text{-}cre}$ (expressing Cre under the control of the serotonergic neuron-specific Pet1 promoter), and $Tph2^{Pet1\text{-}icre}$ (expressing a tamoxifen-inducible Cre recombinase under the control of the Pet1 promoter). All three models showed a marked reduction in TPH2-positive cells. $Tph2^{Pet1\text{-}cre}$ mouse exhibited loss of TPH2 in both rostral and caudal raphe nuclei and, thus, represent a neuron-specific knockout of *Tph2*. In the $Tph2^{Nes\text{-}cre}$ model some TPH2-positive cells were still detected in these regions. The $Tph2^{Pet1\text{-}iCre}$ allows silencing of *Tph2* only in adulthood, thus preventing induction of compensatory mechanisms arising from the lack of 5-HT during development. However, $Tph2^{Pet1\text{-}iCre}$ mice treated with tamoxifen at the age of 3 or 5 months also showed a mosaic pattern of *Tph2* inactivation [21]. Thus, both $Tph2^{Nes\text{-}Cre}$ and $Tph2^{Pet1\text{-}iCre}$ exhibited incomplete *Tph2* inactivation (about 80% reduction in *Tph2* expression by real time PCR) in the targeted cell types.

Moreover, in *Tph2*-floxed mice the injection of Cre-expressing viruses into different parts of the raphe region or into areas of serotonergic projections can be used to inactivate the gene