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Spatio-temporal expression of tryptophan hydroxylase isoforms in murine and human brain: Convergent data from *Tph2* knockout mice

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Abstract

Dysregulation of tryptophan hydroxylase (TPH)-dependent serotonin (5-HT) synthesis, has been implicated in various neuropsychiatric disorders, although the differential expression pattern of the two isoforms is controversial. Here, we report a comprehensive spatio-temporal isoform-specific analysis of TPH1 and TPH2 expression during pre- and postnatal development of mouse brain and in adult human brain. TPH2 expression was consistently detected in the raphe nuclei, as well as in fibers in the deep pineal gland and in small intestine. Although TPH1 expression was found in these peripheral tissues, no significant TPH1 expression was detected in the brain, neither during murine development, nor in mouse and human adult brain. In support of TPH2 specificity in brain 5-HT synthesis, raphe neurons of *Tph2* knockout mice were completely devoid of 5-HT, with no compensatory activation of Tph1 expression. In conclusion, our findings indicate that brain 5-HT synthesis across the lifespan is exclusively maintained by TPH2.

1. Introduction

The brain serotonin (5-hydroxytryptamine, 5-HT) system has been implicated in both the pathophysiology of syndromal dimensions of a wide spectrum of neuropsychiatric conditions, such as depression, suicide, anxiety, bipolar disorder, schizophrenia, autism, obsessive–compulsive disorder (OCD) and attention-deficit/hyperactivity disorder (ADHD) as well as the mechanism of action of numerous psychoactive compounds including antidepressants, anxiolytics, antipsychotics, stimulants,

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and drugs of abuse. Although most of the 5-HT can be found in the periphery, it is also widely distributed in the central nervous system and, as a neuromodulator and neurotransmitter, plays a role in basic physiological functions ranging from early development to complex behaviors. Several *in vitro* studies showed a morphogenic effect of 5-HT on proliferation, differentiation, migration, and survival of neural cells (Gaspar et al., 2003; Di Pino et al., 2004). During ontogeny, 5-HT appears long before maturation of raphe serotonergic neurons suggesting a fundamental role in embryonic and brain development. Although *in vivo* studies generally underscore this notion (Gaspar et al., 2003; Vitalis et al., 2007), conditional *Lmx1b* knockout mice which are largely deficient in brain serotonergic neurons (Ding et al., 2003; Zhao et al., 2006) or *Tph2* knockout mice which lack 5-HT synthesis in raphe neurons (Gutknecht et al., 2008) are viable without apparent developmental abnormalities.

5-HT is synthesized from L-tryptophan in two steps of which tryptophan hydroxylase (TPH, EC 1.14.16.4) catalyses the first reaction and therefore is able to regulate 5-HT availability in peripheral tissues and central neurotransmission. Based on the generation of knockout mice for *Tph1*, a second isoform, *Tph2*, has been discovered and characterized (Côté et al., 2003; Walther and Bader, 2003; Walther et al., 2003; Carkaci-Salli et al., 2006). The genes encoding Tph1 and Tph2 are located on chromosome 7B5 and 10D1 in the mouse; in humans the two isoforms were mapped to chromosome 11p15.1 and 12q21.1, respectively. Human variants of the two genes, *TPH1* and *TPH2*, have already been extensively investigated for association with personality and behavioral traits as well as with various clinical cohorts characterized by emotional dysregulation. *TPH1* variations have long been suggested to influence the risk for depression and suicide (for review see Bondy et al., 2006). More recently single nucleotide polymorphism (SNP) and haplotype analyses of the *TPH2* revealed evidence for association of *TPH2* variants with depression (Zill et al., 2004a; Van Den Bogaert et al., 2006), suicide (Zill et al., 2004b), and bipolar affective disorder (Harvey et al., 2004; Lopez et al., 2007), although negative findings were also reported (Mann et al., 2008).

Our group reported transmission disequilibrium of variants in the transcriptional control region of *TPH2* in ADHD (Walitza et al., 2005) and showed preferential transmission of a haplotype of *TPH2* in early-onset OCD (Mossner et al., 2006) as well as association of potentially functional *TPH2* variants (Scheuch et al., 2007; Chen et al., 2008) with anxiety-related personality traits and personality disorders of cluster B and C, suggesting a biological link between negative emotionality and associated disorders (Gutknecht et al., 2007). In support of this notion, functional magnetic resonance imaging (fMRI) provided evidence that acute tryptophan depletion, which results in a transient reduction of brain 5-HT, as well as a potentially functional variant in the upstream regulatory region of *TPH2* (SNP G-703T, rs4570625) biases the responsiveness of the amygdala, a structure critically involved in the modulation of emotional behaviors (Brown et al., 2005; Canli et al., 2005).

Zhou et al. (2005) demonstrated a haplotype-based association with major depression and anxiety disorders. The frequency of the same haplotype, which was predictive of lower 5-hydroxyindoleacetic acid (5-HIAA) concentrations in cerebrospinal fluid, was also increased in suicide attempters. Although higher *TPH2* mRNA concentrations were found in the dorsolateral prefrontal cortex (Brodmann Area 46) of post-mortem brain from 23 individuals with completed suicide compared to controls, this difference was not significant (De Luca et al., 2006). Investigation of the same brain region from samples of bipolar and schizophrenic patients revealed higher concentrations of *TPH2* mRNA in the bipolar group in comparison with controls (De Luca et al., 2005). While these findings have to be interpreted on the basis that *TPH2* is specifically transcribed in the somatodendritic segment of 5-HT neurons and a variable fraction of *TPH2* mRNA is transported to terminal fields, measurement of *TPH2* mRNA in the brainstem of depressed patients who had committed suicide demonstrated greater expression in the caudal dorsal and median raphe nuclei, compared to controls (Bach-Mizrachi et al., 2008).

The amino acid sequence of TPH1 and TPH2 isoforms are highly homologous with an overall identity of 68% in mouse and 71% in humans, and have a similar three-dimensional structure regarding active site geometry or position of substrate binding amino acid residues (Walther and Bader, 2003; McKinney et al., 2005). Although TPH2 has a higher molecular weight due to an elongated N-terminal domain (theoretical value of 56 kDa versus 51 kDa for TPH1), different kinetic properties (including a higher specificity for L-tryptophan but a lower catalytic efficiency) and an additional phosphorylation site, the most striking difference in their physiologic effects appears to be related to tissue-specific expression. In this regard, the investigations on tissue specificity of TPH2 in comparison with TPH1 have yielded contradictory results. Initial studies resulting in the discovery of Tph2 indicated expression of both isoforms as mutually exclusive with Tph1 being the non-neuronal and peripheral form, very abundant in the pineal gland and enterochromaffin cells of the gut, and with Tph2 being defined as the neuronal form, highly expressed in the serotonergic neurons of the raphe nuclei and tightly regulating the 5-HT synthesis in brain (Côté et al., 2003; Walther and Bader, 2003; Walther et al., 2003; Zhang et al., 2004). However, several subsequent differential expression studies reported *Tph1* expression in the raphe nuclei of different species. In the rat brain, Patel et al. (2004) and Malek et al. (2005) detected a very low level, as compared to *Tph2*, of *Tph1* mRNA in dorsal and median raphe with radiolabeled riboprobes. In a study focussing on the effect of estrogens on *Tph1* expression, Gundlah et al. (2005) also detected *Tph1* mRNA signal in the dorsal raphe of mice using *in situ* hybridization which was increased by estradiol treatment. By means of quantitative real time PCR in human post-mortem brain, Zill et al. (2007) observed that nearly 20% of *TPH* expressed in the raphe nuclei containing region was actually *TPH1* mRNA and that all other brain regions showed equal or higher expression levels of *TPH1* than *TPH2*. Studying two postnatal developmental stages and adult brain of mice, Nakamura et al. (2006) found a preferential expression of *Tph1* mRNA at the postnatal day 21 (P21) in the raphe using semiquantitative reverse transcription PCR and *in situ* hybridization. *Tph1* mRNA was not detected at P7 and the signal was very weak in 2 month old animals, suggesting that *Tph1* may play a role specifically during late developmental stages of the brain.

Given the importance of 5-HT and thus TPH isoforms in brain development and function, the present study was undertaken