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Tryptophan Hydroxylase as Novel Target for the Treatment of Depressive Disorders

Susann Matthes Valentina Mosienko Saleh Bashammakh Natalia Alenina
Michael Bader

Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany

Key Words
Serotonin · Depression · Schizophrenia · Antidepressants

Abstract
Serotonin (5-HT) is a monoamine implicated in a variety of physiological processes that functions either as a neurotransmitter or as a peripheral hormone. Pharmacological and genetic studies in humans and experimental animals have shown that 5-HT is important for the pathophysiology of depressive disorders. The 5-HT system is thus already a main target for the therapy of these diseases. The peripheral and cerebral biosynthesis of 5-HT is initiated by two distinct tryptophan hydroxylases: TPH1 and TPH2. This duality of the serotonergic system and the existence of a brain-specific TPH isoform provide a promising new target for pharmacological intervention with higher selectivity and specificity and, therefore, possibly with reduced side effects and increased efficiency. This paper summarizes the data which support TPH2 as novel drug target and discusses strategies for its pharmacological exploitation.

Introduction

Since its discovery as a vasoconstrictor substance in the blood [1], serotonin (5-hydroxytryptamine, 5-HT) has been revealed to be of importance in a broad range of physiological processes as it has been shown to be involved in the control of smooth muscle tone and vascular function [2–4], hemostasis and platelet function [5, 6], hepatitis and liver regeneration [7–10], mammary gland plasticity [11], insulin secretion [12], development and wiring of neurons [13], as well as regulating sleep/wakefulness, appetite, gastrointestinal motility, temperature, pain sensation and nociception, mood, stress, maternal or sexual behavior and aggression [14–16].

The biosynthesis of the monoamine 5-HT starts with the essential amino acid tryptophan, which is metabolized to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH) in an initial, rate-limiting step; 5-HTP is then further decarboxylated to 5-HT by aromatic amino acid decarboxylase (fig. 1). TPH belongs to the superfamily of aromatic amino acid hydroxylases which also includes tyrosine hydroxylase and phenylalanine hydroxylase (PAH). These are iron (Fe^{2+})- and tetrahydrobiopterin-dependent monooxygenases with substantial similarities in structure and catalytic mechanism [17].

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Prof. Dr. Michael Bader
Max Delbrück Center for Molecular Medicine
Robert-Rössle-Strasse 10
DE–13125 Berlin-Buch (Germany)
Tel. +49 30 9406 2193, Fax +49 30 9406 2110, E-Mail mbader@mdc-berlin.de

All aromatic amino acid hydroxylases are composed of 3 functional domains, a regulatory N-terminal domain, a catalytic domain and a C-terminal oligomerization domain [17, 18].

The identification of a second *Tph* gene in 2003, named *Tph2*, unravelled the existence of two independent 5-HT systems in vertebrates (fig. 2). Whereas TPH2 is specifically expressed in the brain, TPH1 is responsible for 5-HT synthesis in peripheral tissues [19]. Within the last years, different studies of mRNA and protein levels in rodent and human tissues confirmed TPH2 to be the central isoform, predominantly expressed in the neurons of raphe nuclei in the brain stem [20–22] and in peripheral myenteric neurons in the gut [23, 24] but not in peripheral organs (lung, heart, kidney or liver) [25, 26]. On the other hand, TPH1 is mainly expressed in the enterochromaffin cells of the gut and also in the pineal gland, where it produces 5-HT as a precursor of melatonin synthesis [19, 27] (fig. 1).

5-HT System and Depression

Based on its functions as a neurotransmitter influencing various neurological and behavioral processes in the central nervous system, 5-HT has also been suggested to play a role in different diseases of the central nervous system [15, 28]. The biosynthesis and release of neuronal 5-HT depend on circulating levels of free tryptophan in the blood and brain. Experimental tryptophan depletion was associated with increased symptom severity in depressed patients [29–31] and lowered 5-HT and/or 5-hydroxyindoleacetic acid, its degradation product (fig. 1), have been found in the cerebrospinal fluid of suicidees and depressed suicide attempters [32, 33]. Disturbed social behavior, increased irritability or lack of impulse control could also be observed in inflammatory and other diseases that are accompanied by low tryptophan levels [34]. The fact that the 5-HT system also became an efficient target for antidepressant therapies confirms that alterations in the 5-HT system may be related to the development and pathophysiology of affective disorders [15, 35–38]. Affective disorders are a group of psychiatric conditions among which major (unipolar) depression and bipolar disorder (major depression plus mania) are the most prevalent types [39, 40]. Patients with major depression suffer predominately from mood disturbances, but also from psychomotor retardation, sleep disorders, fatigue or thoughts of death [41]. This paper intends to give an overview on recent genetic studies in humans and animals investigating a possible connection of TPH and psychiatric disorders, with particular emphasis on affective disorders.

Genetic Studies on TPH in Humans

In recent years, there has been a dramatic increase in the number of publications dealing with molecular variants of genes that are involved in 5-HT synthesis (TPH), 5-HT neurotransmission (5-HT receptors and 5-HT transporter, SERT) and 5-HT catabolism (monoamine oxidases) (fig. 1) to identify alterations that may contribute to a dysfunction within the serotonergic system. A huge amount of data has accumulated; in the following we will only focus on genetic studies on *TPH* (table 1).

It has been shown that the prevalence of affective disorders is affected by gender, age and genetic background. Depressive disorders have been found to be more frequent in women, and twin studies suggest a heritability of up to 50% [42, 43]. But at present the results on *TPH* polymorphisms remain inconclusive. Several single nucleotide polymorphisms (SNPs) in the *TPH2* gene have been found to be associated with depression [44–49], suicidal behavior [46, 47, 50–55] and bipolar disorder [48, 50–56]. On the other hand, a number of publications found no association between *TPH2* SNPs and major depression or suicide [57–62]. These discrepant results may reflect the heterogeneous nature of neuropsychiatric diseases and populations as well as methodological differences, sample size and statistical power.

In particular, the nonsynonymous SNP (G1463A) published by Zhang et al. [44] in 2005 seemed to be a promising step towards identifying a link between TPH*2* and depressive disorders. The resulting missense mutation in the TPH2 protein (R441H) that they found in an elderly cohort of unipolar depressed patients showed an 80% decrease in 5-HT production in PC12 cells. The fact that the mutation is located within the part of the oligomerization domain that has previously been shown to be pivotal for TPH*2* activity [63] together with the description of a corresponding severe pathogenic mutation in PAH (R408W) led to the expectation that the first loss-of-function mutation in human *TPH2* had been identified [64]. However, this functional polymorphism could not be confirmed in any other study [62, 65–72]. Thus it is either a rare mutation within a very unique cohort with unipolar depression or the result of a genotyping error.

As the coding sequence of *TPH2* represents less than 2% of the gene, coding sequence variants are expected to

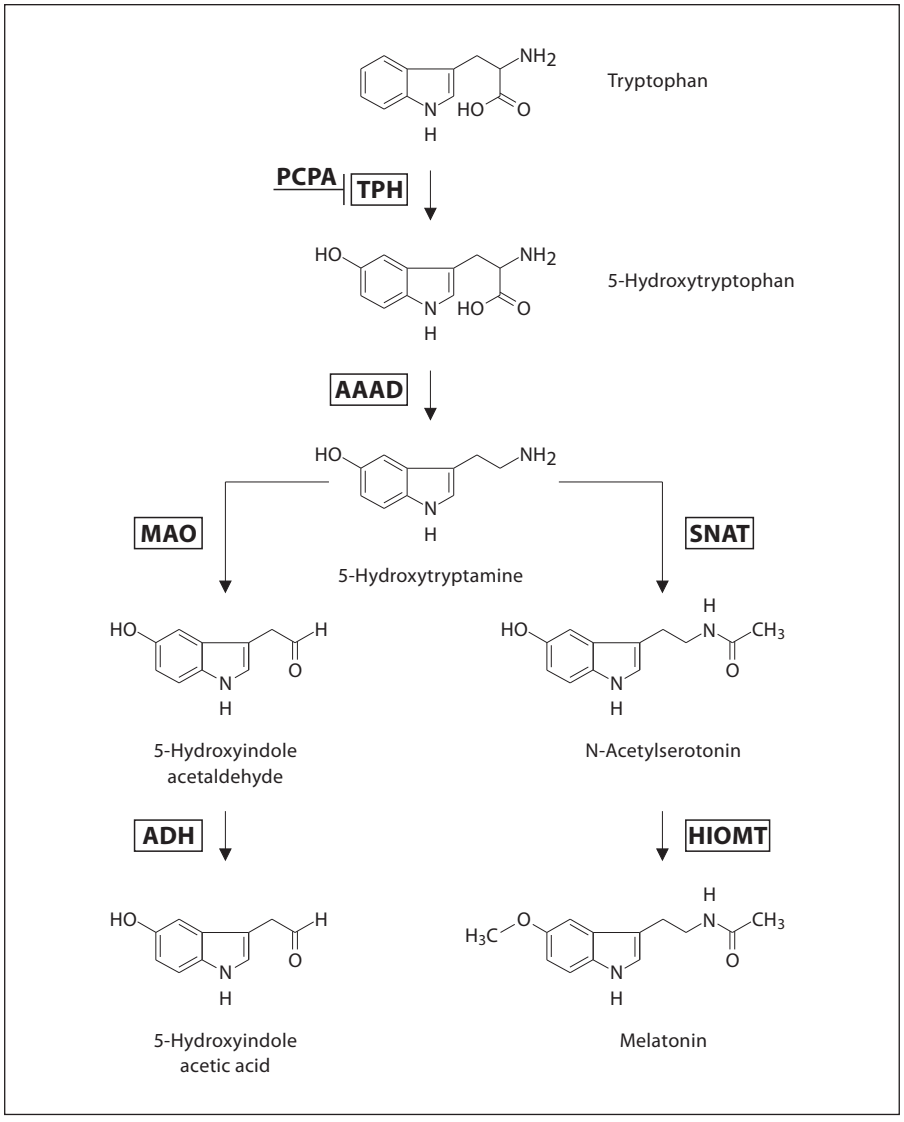

Fig. 1. 5-HT metabolism. AAAD = Aromatic amino acid decarboxylase; SNAT = serotonin N-acetyltransferase; HIOMT = hydroxy-indole-O-methyl transferase; MAO = monoamine oxidase; ADH = alcohol dehydrogenase. The other abbreviations are explained in the text.

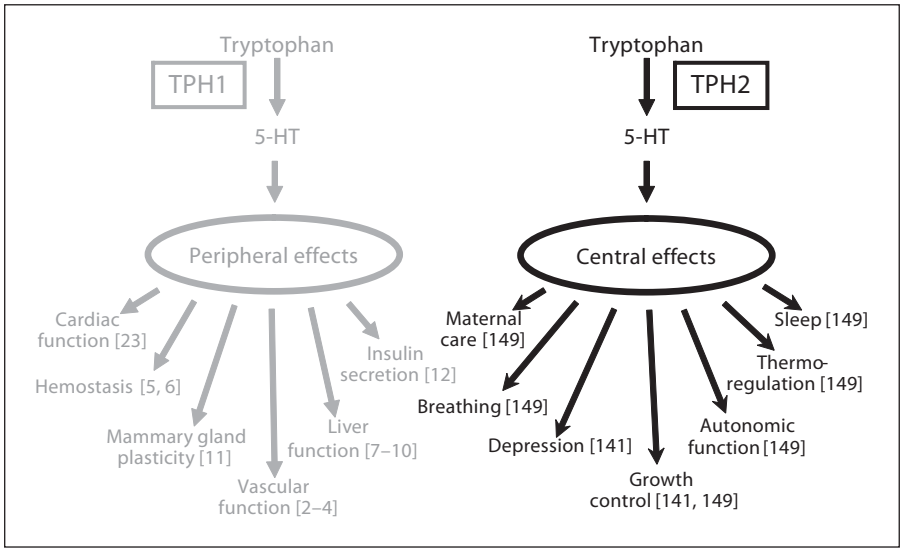

Fig. 2. Duality of the 5-HT system. Functions of peripheral (TPH1-derived) and central (TPH2-derived) 5-HT that are confirmed in *Tph1*- and *Tph2*-deficient animals are shown.

Table 1. TPH2 SNPs and association with affective disorders

SNP	Location	Function	Allele	Protein residue	Disorder	Association	Reference
G1463A	exon 11	missense	G/A	Arg/His	major depression	yes	[44]
rs1386494	intron 5		A/G	–	major depression	yes	[45][1, 2]
rs17110747	3′ UTR		A/G	–	major depression	yes	[49][2]
rs17110563	exon 6	missense	C/T	Ser/Pro	major depression	yes	[46][1]
rs11179003	intron 4		C/T	–	suicidal behavior		
rs1386494	intron 5		A/G	–			
rs1007023	intron 8		G/T	–			
rs1473473	intron 8		A/G	–			
rs17110747	3′ UTR		A/G	–			
g.22879	exon 6[3]/intron 5[4]		A/G	–	major depression	yes	[47][1, 2]
rs1386493	intron 5		C/T	–	suicidal behavior		
rs1386494	intron 5		A/G	–			
rs11178997	5′ near gene	synonymous	A/T	–	major depression	yes	[48][1, 2]
rs4131348	upstream 5′ region		C/T	–	bipolar disorder		
rs4290270	exon 9	synonymous	A/T	–	bipolar disorder	yes	[51][1]
rs17110563	exon 6	missense	C/T	Ser/Pro	bipolar disorder	yes	[50][1, 2]
rs11178997	5′ near gene	synonymous	A/T	–			
rs1386482	intron 8		A/C	–	bipolar disorder	yes	[54][1]
rs1386486	intron 8		C/T	–			
rs7305115	exon 7	synonymous	A/G	–	bipolar disorder	yes	[52][1]
rs17840794	intron 7		C/T	–			
rs1007023	intron 8		G/T	–			
rs11615016	intron 8		A/G	–			
rs4290270	exon 9	synonymous	A/T	–			
rs1386494	intron 5		A/G	–	bipolar disorder	yes	[53][1]
rs1007023	intron 8		G/T	–	suicidal behavior		
rs9325202	intron 8		A/G	–			
rs7305115	exon 7	synonymous	A/G	–	suicidal behavoir	yes	[191]
rs4448731	5′ near gene		C/T	–	suicidal behavoir	yes	[192][1]
rs4641527	intron 1		G/T	–			
rs1386494	intron 5		A/G	–	suicidal behavoir	yes	[55][1, 2]
rs11178997	5′ near gene	synonymous	A/T	–	suicidal behavoir	yes	[193]
rs4570625	5′ near gene		G/T	–	suicidal behavoir	yes	[194]
rs1843809	intron 5		G/T	–	ADHD	yes	[84][1, 2]
rs4570625	5′ near gene		G/T	–	ADHD	yes	[85][1]
rs11178997	5′ near gene	synonymous	A/T	–			
rs4341581	intron 1		G/T	–	autism	yes	[86][1]
rs11179000	intron 4		A/T	–			
rs4570625	5′ near gene		G/T	–	OCD	yes	[87][1, 2]
rs4565946	intron 2		C/T	–			
rs4570625	5′ near gene		G/T	–	panic disorder	yes	[88][2]
G1463A	exon 11	missense	G/A	Arg/His	major depression	no	[62, 65–72]

Table 1 (continued)

SNP	Location	Function	Allele	Protein residue	Disorder	Association	Reference
rs7488262	exon 11	missense	T/G	–	major depression	no	[62]
rs1386494	intron 5		A/G	–	major depression	no	[60]
rs10748185	intron 2		A/G	–	major depression	no	[57][1, 2]
rs2129575	intron 4		G/T	–			
rs1386495	intron 5		C/T	–			
rs1386494	intron 5		A/G	–			
rs7305115	exon 7	synonymous	A/G	–			
rs4131347	upstream 5′ region		C/G	–	major depression bipolar disorder suicidal behavior	no	[61]
rs1487280	intron 9		A/G	–	bipolar disorder	no	[58][1, 2]
rs4760816	intron 6		C/T	–	suicidal behavior		
rs10784941	intron 6		A/G	–			
rs6582071	5′ near gene		A/G	–	suicidal behavior	no	[59][1, 2]
rs4570625	5′ near gene		G/T	–			
rs11178997	5′ near gene	synonymous	A/T	–			
rs11178998	5′ UTR		A/G	–			
rs11178999	intron 1		A/G	–			
rs1386494	intron 5		A/G	–			
rs1386493	intron 5		C/T	–			
rs1386491	intron 5		C/G	–			
rs7305115	exon 7	synonymous	A/G	–			
rs1386498	intron 8		A/G	–			
rs1487278	intron 8		C/T	–			
rs11179044	intron 8		C/T	–			
rs4290270	exon 9	synonymous	A/T	–			
rs11179064	intron 9		A/G	–			
rs17110747	3′ UTR		G/A	–			
rs4570625	5′ near gene		G/T	–	panic disorder	no	[90][1, 2]
rs4565946	intron 2		C/T	–			
rs11178997	5′ near gene	synonymous	A/T	–	suicidal behavior	no	[89][1, 2]
rs10784941	intron 2		A/G	–	schizophrenia		
rs11178997	5′ near gene	synonymous	A/T	–	suicidal behavior	no	[91]
rs4131347	upstream 5′ region		C/G	–			

[1] Haplotype analysis. [2] Single SNP analysis. [3] TPH2 short transcript isoform (ENST00000266669). [4] TPH2 long transcript isoform (ENST00000333850). ADHD = Attention-deficit hyperactivity disorder, OCD = obsessive-compulsive disorder.

be rather rare [73]. Therefore, most of the *TPH2* SNPs known so far are located in introns and promoter regions. Although they are not likely to be of importance for protein function, they could affect gene expression on the transcriptional level, e.g. via mRNA stability or splicing processes [74]. The T allele of the *TPH2* promotor polymorphism rs4570625 (–703G/T) has been shown to be involved in increased prefrontal brain activity [75], anxiety-related personality disorders [76] and amygdala reactivity [77, 78] and therefore might show an impact on heightened stress responsivity and anxiety due to alterations in TPH2 expression [79]. Another intronic *TPH2* SNP has been reported to reduce promoter activity by reduced binding of transcription factor POU3F2 [56, 80]. Evidence for an inhibitory effect of the *TPH2* 5′-UTR on gene expression has been derived from studies on com-

mon polymorphisms and haplotypes in this region [81]. From the *TPH2* missense mutations known so far, 4 have been reported in patients with clinical symptoms [82]. Nevertheless, there is still a great demand for functional and clinical data to define the role of *TPH2* polymorphisms in particular phenotypes of depressive disorders [83].

Furthermore, discrepant results have been published on other types of affective disorders. Some studies showed *TPH2* polymorphisms to be related to schizophrenia, obsessive-compulsive disorder, attention-deficit hyperactivity disorder, and autism or panic disorder [84–88] while others did not observe any association [89–91].

Concordant response rates from several studies on relatives suggest that antidepressant treatment response is also an inheritable trait possibly influenced by *TPH2* polymorphisms [92, 93]. However, whereas some studies show a significant link between a *TPH2* SNP haplotype and specific responses to 5-HT reuptake inhibitors (SSRIs) [49, 94, 95], others, here again, do not confirm any association between this trait and *TPH2* SNPs in various ethnic groups [60, 62, 72].

Although TPH1 is responsible for peripheral 5-HT synthesis, there are several studies on the influence of *TPH1* polymorphisms A218C and A779C in affective disorders, with contrasting outcomes. Both SNPs are proposed to be involved in suicidal behavior [16, 96–100], depression [101–103], bipolar disorder [104–106] or altered antidepressant drug response [107–109]. But other studies could not find any association of *TPH1* SNPs and suicidal behavior [110–114], major depression [113, 115–118], bipolar disorder [113, 119] or antidepressant treatment response [115, 120]. It is not clear whether 5-HT synthesis through TPH1 in the brain just serves for melatonin synthesis or whether there might be some contribution to the neurotransmitter pool [121]. Effects of TPH1 on central 5-HT synthesis can be caused by altered enzyme activity during ontogeny as an impact of TPH1 on the development and maturation of 5-HT neurons has been suggested by studies in mice [122–124], which was contradicted by others, however [125].

Affective disorders are known to be complex and heterogeneous disorders characterized by polygenic influences, different clinical profiles and different responsiveness to drugs [28, 126]. It is also known from studies on multifactorial disorders that different mutations within a single gene may be linked to a spectrum of clinical phenotypes [82]. As the 5-HT system exhibits an extensive network of different genes involved in the development, function and plasticity of 5-HT neurons as well as several 5-HT receptors and 5-HT transporter-associated proteins, contributions from many points in this network in a disease as complex as depression should be expected [68]. In order to improve the success of genetic studies of depression and optimize the phenotypic definition of depression, there is a need to dissect possible psychopathological and biological endophenotypes [127].

Studies in Mouse Models

Validity of Mouse Models of Depression

Studies in genetically modified laboratory animals have an important impact on our understanding of depressive disorders and are necessary to test new pharmacological tools that could be further used in treating these diseases in humans. The mouse – a species in which human mutations can be easily mimicked by genetic modifications of its genome – is especially valuable in this respect. However, the usefulness and validity of mouse models in evaluating human depression has always been a subject of debate. As there are differences in brain anatomy and capacity for processing complex psychological concepts between humans and mice, it is impossible to model certain aspects of disease symptoms, such as low self-esteem, suicidal ideation or 'fear of going crazy' in mice. On the other hand, the brains of mammals have a common structural organization, similar circuits connecting these structures, and conserved physiological and behavioral responses. Therefore, to a certain extent, the mouse can be used as a model for understanding human behavior and disease, but the results of such studies must be interpreted with caution [128].

Current models gauge an animal's 'depression-related' responses to acute or chronic inescapable stress. These include the forced swimming test (FST) which quantifies immobility in a water bath, and the tail suspension test (TST), which measures periods of agitation and immobility of a mouse suspended by the tail; these periods of agitation and immobility resemble 'behavioral despair' observed in depressions. Other assays include measurement of social interaction (model of social estrangement in depression-related conditions), the learned-helplessness test (measures the passive responses to inescapable foot shock), and intracranial self-stimulation (evaluation of the animal's effort to stimulate the cerebral reward circuit electrically) [129–132]. These assays are usually accompanied by a battery of tests evaluating activity and anxiety, such as open field and elevated plus maze to exclude the misinterpretation of results.

Pharmacological Mouse Models of Depression

The first studies to investigate the role of TPH in depressive disorders were pharmacological models in which enzyme activity was specifically blocked with *p*-chlorophenylalanine (PCPA), an irreversible TPH inhibitor (fig. 1) [133]. Mice prenatally exposed to PCPA show increased depression-related behavior in FST and TST, decreased anxiety and increased activity in open field [134–136]. Another pharmacological tool to study the relation between depressive behavior and TPH is local intracerebroventricular application of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), which selectively kills serotonergic neurons, and was shown to cause significant depletion of 5-HT levels in the brains of mice [137, 138]. Due to the complicated procedure of intracerebroventricular injection, most of the studies with neurotoxins were done on rats; they showed that 5,7-DHT treatment induces a depressive-like behavior [139, 140]. The few studies in mice demonstrated that 5,7-DHT injections in the striatum significantly decrease locomotor activity [138]. However, these pharmacological models have a number of limitations: (1) PCPA is not specific for a certain form of TPH; therefore it is difficult to distinguish effects caused by TPH1 or TPH2 depletion; (2) 5,7-DHT is a neurotoxin that kills serotonergic neurons; hence, this model does not exclude behavioral abnormalities aroused by the absence of other components of the serotonergic system and cotransmitters of these cells; (3) PCPA and 5,7-DHT induce partial but not complete 5-HT reductions in brain regions, and these approaches do not tell whether the residual amount of 5-HT is enough for normal brain functioning. Therefore, it is more appropriate to use genetically modified mouse models to investigate the role of TPH isoenzymes in depressive disorders.

Genetic Mouse Models of Depression: Role of Tph1

Although several studies showed the discrete expression pattern of *Tph* genes, with neuronal localization of *Tph2* in dorsal raphe and myenteric neurons and non-neuronal localization of *Tph1* in pineal gland and duodenum, the presence and role of *Tph1* transcripts in the raphe region remain a subject of controversy [123, 125]. According to Nakamura et al. [123], there is a peak of *Tph1* expression in the brain stem at weaning in mice whereas in adulthood its level drops to nearly zero. Moreover, in these young mice both the affinity for tryptophan and enzymatic activity of TPH1 are higher than those of TPH2, partially due to the low tetrahydrobiopterin concentration in the developing brain stem and, therefore, TPH1 was considered as a main 5-HT-producing enzyme in the mouse brain at weaning [123]. In the search for the linkage between *Tph1* and murine behavior, two polymorphisms – in the promoter region and in the 3′ UTR of the *Tph1* gene – were recently identified in New Zealand white and SWR mice [123]. Due to these mutations, both strains were shown to have lower *Tph1* expression in the brain stem at 21 days of age in comparison to other laboratory mouse lines and, as a consequence, decreased brain 5-HT levels at this time point, but normal 5-HT levels in adulthood. Interestingly, both strains have shown an increased depression-like behavior in FST and TST tests. However, further studies, on congenic lines carrying the above-mentioned mutations, as well as evaluation of this behavior in *Tph1*-deficient mice, have to be conducted in order to clarify whether impairment in *Tph1* expression can contribute to depressive disorders.

Moreover, other studies did not find considerable TPH1 activity in the mouse raphe nuclei during embryogenesis, postnatal development and in adulthood [19, 125]. Furthermore, *Tph1* remained undetectable even in raphe of *Tph2*-deficient mice, in which compensatory upregulation may have been expected [125]. In addition, studies in *Tph1*-knockout mice did not reveal any changes in 5-HT-related behavior in anxiety tests [19, 141], rendering the contribution of TPH1-produced 5-HT to 5-HT signaling in the brain unlikely.

A second way by which TPH1-derived 5-HT can influence behavior is via its transformation to melatonin in the pineal gland. Melatonin mediates photoperiodic effects on reproduction and may influence a variety of circadian activities. However, most laboratory mouse strains cannot synthesize melatonin due to a lack of 5-HT-N-acetyltransferase (SNAT) that converts 5-HT to an intermediate precursor of melatonin, N-acetylserotonin [121] (fig. 1). Thus, at least in laboratory mice, *Tph1* cannot influence behavior via melatonin.

Genetic Mouse Models of Depression: Role of Tph2

Tph2 Polymorphism

It is known that certain mouse strains markedly differ in depression-like behavior. Zhang et al. [142] first surmised that such a difference between 129X1/SvJ and BALB/cJ mice may be caused by altered TPH2 activity. Indeed, sequencing analysis revealed an (C1473G) SNP in the coding region of *Tph2* between these two lines resulting in the substitution of a highly conserved proline residue with an arginine at position 447 and, as a consequence, lowering of enzyme activity in in vitro experiments [142, 143]. Afterwards, it was found that the 5-HT content in the brain was reduced by approximately 50%

in the mouse strains carrying only the 1473G allele in comparison to mice homozygous for the 1473C allele; an observation that was also confirmed in an F2 intercross [142, 144, 145]. This led to the hypothesis of a direct link between the *Tph2* polymorphism and the development of a depressive state in those mice. However, attempts to create congenic lines only differing in the $mTPH2_{1473}$ polymorphic allele yielded controversial results and put this hypothesis into question. Osipova et al. [144] showed that transfer of the 1473G allele into the C57BL/6J genome over 3 generations [B6–1473G (G/G)] significantly decreased TPH2 activity in the brain and led to shorter immobility time in the FST. However, we created two congenic lines carrying homozygous 1473G or 1473C alleles obtained from breeding the 1473G allele from DBA/2 mice over 8 generations to the C57BL/6 background and did not find any differences in the 5-HT content of brain regions or changes in depression-related behavior [146], indicating that not *Tph2*, but other variations in the genetic background are responsible for the interstrain differences. Moreover, no association was found between the murine *Tph2* C1473G polymorphism and depressive state in TST [147]. This discrepancy in the results from different investigators can be explained by different experimental protocols or by the degree of genetic background homogeneity of the mice. Furthermore, it cannot be ruled out that *Tph2* is closely linked to some other genes that directly influence FST immobility in mice.

Tph2 Knockin

Beaulieu et al. [148] generated knockin mice expressing a mutant form of TPH2 that contains the rare human mutation R441H identified in few individuals with unipolar depression (see above). TPH2 activity in R439H *Tph2*-knockin mice was reduced by 80% in the brain while the expression of SERT, a target of most antidepressants, was unchanged. Expression of the mutant *Tph2* results in increased anxiety, depression-related behavior in TST and enhanced intermale aggression. The pharmacological or genetic inhibition of GSK3β prevented the behavioral changes, suggesting that drugs that enhance 5-HT transmission may exert some of their actions through GSK3β.

Tph2 Knockout

Several groups recently reported the generation of *Tph2*-knockout mice [22, 141, 149]. We showed increased aggressive behavior in *Tph2*-deficient female mice that cannibalized their pups in contrast to wild-type mice that never ate the whole litter [149]. Female aggression is directly linked to anxiety and depressive disorders [150]. Surprisingly, female *Tph2*-deficient mice behaved normally in the FST in contrast to the TST in which they showed longer immobility times [141]. *Tph2*-deficient males did not show any significant behavioral changes in a standard TST; however, decreased immobility times were recorded in the FST [141]. Different results obtained on the TST and FST are likely due to the different neurochemical and neuroanatomical pathways involved in the modulation of the response to different stress stimuli [132]. Testing *Tph2*-deficient mice for the anxiety-related phenotypes also yielded inconsistent results: open field did not reveal any linkage between TPH2 and anxiety while the buried-marble test showed an increased level of anxiety in these mice [141]. However, it cannot be excluded that increased marble burying is an indication of obsessive/compulsive behavior rather than anxiety-like behavior in these animals [151]. Interestingly, differences observed in depression- and anxiety-like behavior were more pronounced in *Tph2/Tph1* double-knockout mice than in *Tph2*-deficient mice, which may be due to peripheral and developmental discrepancies caused by the additional absence of *Tph1* [141].

Pah-Deficient Mice

PAH is a hepatic enzyme which metabolizes phenylalanine to tyrosine. Deficiency in this enzyme leads to the accumulation of phenylalanine and its conversion into phenylpyruvate and causes a severe disease in humans: phenylketonuria, which is accompanied by postnatal brain damage and mental retardation. *Pah*-deficient mice ($Pah^{Enu2-/-}$ mice [152]) exhibit behavioral alterations [153–155]; however, no evaluation of depression-like behavior was made. Interestingly, it was recently shown that PAH ablation in the mouse causes 70% reduction in 5-HT production in the brain without a loss of the 5-HT precursor tryptophan [156]. Nonetheless, a dramatic reduction in brain 5-HTP levels as well as in the 5-HTP/tryptophan ratio was observed in $Pah^{Enu2-/-}$ mice, suggesting that accumulation of phenylalanine leads to the inhibition of Tph2 activity in vivo, corresponding to its effect on TPH2 activity in vitro [156, 157]. Therefore, the behavioral phenotype observed in *Pah*-deficient mice can be partially attributed to lower TPH2 activity in the brain. Interestingly, administration of 5-HTP to *Pah*-deficient mice restored the cortical release of several monoamines in a model of restraint stress [157].

For the same reason, a lack of PAH in *Drosophila melanogaster*, Henna, leads to a dramatic decrease in 5-HT. Therefore, for a long time Henna has been considered to

be the PAH and the TPH of insects until the main TPH, CG9122, was discovered [158].

Serotonergic Neuron Ablation

Ablation of TPH2-producing neurons is another possibility to study the association between TPH2 and depressive disorders. Hendricks et al. [159] demonstrated that expression of the transcription factor Pet-1 is restricted to serotonergic neurons and its disruption in mice leads to abnormal development and loss of these cells but does not influence brain morphology and other monoamine systems [159]. *Pet-1* null male mice show dramatically increased anxiety and intermale aggressive behavior supporting the link between aggression and serotonergic hypofunction. Moreover, a profound deficit in offspring survival born from *Pet-1*$^{-/-}$ dams was reported as a result of deficient maternal behavior [160]. However, the level of female anxiety and aggressiveness that could contribute to offspring mortality was not assessed in these studies.

Another mouse model, in which *Lmx1b* (LIM homeobox transcription factor 1β) was only deleted in *Pet-1*-expressing cells, i.e. Lmx1b(f/f/p) mice, lack nearly all serotonergic neurons but do not show any alteration in other monoamine systems [161]. These animals display normal locomotion in Rotarod and open field tests [161], but show reduced basal sensitivity to mechanic stimuli and exhibit enhanced inflammatory pain response [162]. Moreover, this model was used to study the mechanism of analgesic actions of antidepressants, including SSRIs, 5-HT-norepinephrine reuptake inhibitors, and tricyclic antidepressants [162]. The results revealed that the analgesic effect of antidepressant drugs, including those affecting both norepinephrine and 5-HT levels, was abolished in the acute thermal pain model.

Pharmacological Targeting of TPH2

The actual treatment strategies for depressive disorders enhance the general serotonergic tone either by inhibition of the 5-HT transporter by SSRIs, or tricyclic antidepressants or by prevention of 5-HT degradation using monoamine oxidase inhibitors [15, 35]. Also the intermediate product in 5-HT synthesis, 5-HTP (fig. 1), has been successfully used for antidepressive therapy [163–165]. Upon oral administration 5-HTP can reach the brain due to its ability to cross the blood-brain barrier and effectively increase central 5-HT synthesis. The onset of any antidepressant drug effect usually takes up to 4 weeks. Therefore, it is suggested that not just neurochemical but also structural changes, like the stimulation of neurogenesis in the hippocampus [166] or other long-term adaptive changes in 5-HT neurotransmission, may be responsible for behavioral effects of chronic antidepressant treatment [167].

All of the currently applied antidepressant drug therapies show wide variability among patients in treatment response [49]. While 30–40% of patients with major depression do not respond to SSRI treatment [95], just about one third of patients gains full remission after antidepressant therapy [168]. The nonspecific inhibition of the 5-HT transporter can cause peripheral side effects, like a high risk of bleeding [169, 170] or skeletal changes [171]. Further adverse effects, such as weight gain, insomnia and sexual dysfunction, are another problematic issue in humans and have been reported to be the main reason for noncompliance, discontinuation or premature termination of treatment by depressed patients [172]; these adverse effects stress the need for better treatment strategies. Therefore, the duality of the serotonergic system and the existence of a brain-specific TPH isoform constitute a promising new target for pharmacological intervention in the treatment of neuropsychiatric diseases. Instead of acting on already produced 5-HT, the opportunity to specifically influence the initial enzyme of 5-HT synthesis, TPH2 might enable selective manipulation of central 5-HT synthesis with less side effects and a better response rate.

Inhibitors of both TPH enzymes, such as PCPA (fig. 1), have been known for a long time and others have recently been published; however, they act only on *Tph1* due to permeability barriers [173, 174], and the treatment of central diseases needs specific activation of TPH2.

Despite their 70% sequence homology, TPH1 and TPH2 show some remarkable differences which might allow specific modulation of the two isoenzymes. The in vitro expression of recombinant fusion proteins indicated that TPH2 is more soluble than TPH1, and exhibits less substrate inhibition by tryptophan and tetrahydrobiopterin [175, 176]. In addition, these enzymes seem to differ in their substrate specificities: TPH2 showed lower catalytic efficiency towards phenylalanine in vitro [175, 177, 178]. The most important difference is an additional N-terminal regulatory domain in TPH2 consisting of 44 amino acids, which is absent in TPH1 [179]. We have shown that this domain is associated with an inhibitory effect on TPH2 activity [63]. Other studies reported a negative effect of this domain on translational efficiency, stability and solubility of TPH2 compared to tyrosine hydroxylase and PAH [180–183].

Aromatic amino acid hydroxylases share several conserved phosphorylation sites. However, the serin 19 residue in the N-terminal domain of TPH2 is a unique phosphorylation target for protein kinase A and calcium/calmodulin-dependent protein kinase II [184, 185]. This phosphorylation seems to stabilize the protein [184, 186, 187] and is also thought to be relevant for enzyme activation and 14-3-3 protein binding [184, 188]. However, recent studies did not confirm an alteration in the catalytic function of TPH2 upon phosphorylation [185] or binding to the 14-3-3 protein BMH1 [175].

Its unique structural properties, in particular its inhibitory N-terminal domain, may allow specific activation of TPH2 by low molecular-weight substances. Such drugs can be tested either by fluorescence-based activity assays in test tubes [189] or in a cell-based system, for example using the suicide pro-drug 7-hydroxytryptophan, which kills cells depending on their intrinsic TPH activity [190]. Furthermore, identifying the still missing X-ray structure of TPH2 will be another important step in understanding the catalytic mechanism of the enzyme in order to develop novel drugs by virtual design.

Conclusions

In this review, we have shown that variations in the genes for TPH1 and TPH2, the rate-limiting enzymes in 5-HT synthesis, were linked to neuropsychiatric diseases, such as bipolar disorder and major depression in humans, and to depression-like behavior in mice. However, these data are not always reproducible and in particular it is not obvious how alterations in the peripheral enzyme TPH1 may influence functions of the central nervous system. Nevertheless, genetically altered animal models with changes in TPH2 expression support a role of this enzyme in brain function and depression-like behavior. Thus, activation of TPH2 may open new perspectives in the treatment of neurological and psychiatric disorders caused by alterations in brain 5-HT levels. Since TPH2 carries an internal inhibitory domain, activation by small molecules should be feasible. The discrimination between TPH1 and TPH2 is a major task in the search for such compounds since some peripheral actions of 5-HT are deleterious and should not be stimulated. TPH2-activating drugs may exhibit higher efficiency and specificity with less side effects than existing therapies for the treatment of depressive disorders, which are increasingly relevant for public health.

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