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Tryptophan Hydroxylase-2: An Emerging Therapeutic Target for Stress Disorders

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Abstract

Serotonin (5-HT) has been long recognized to modulate the stress response, and dysfunction of 5-HT has been implicated in numerous stress disorders. Accordingly, the 5-HT system has been targeted for the treatment of stress disorders. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in 5-HT synthesis, and the recent identification of a second, neuron-specific TPH isoform (TPH2) opened up a new area of research. With a decade of extensive investigation, it is now recognized that: 1) TPH2 exhibits a highly flexible gene expression that is modulated by an increasing number of internal and external environmental factors including the biological clock, stressors, endogenous hormones, and antidepressant therapies; and 2) genetically determined TPH2 activity is linked to a growing body of stress-related neuronal correlates and behavioral traits. These findings reveal an active role of TPH2 in the stress response and provide new insights into the long recognized but not yet fully understood 5-HT-stress interaction. As a major modulator of 5-HT neurotransmission and the stress response, TPH2 is of both pathophysiological and pharmacological significance, and is emerging as a new therapeutic target for the treatment of stress disorders. Given that numerous antidepressant therapies influence TPH2 gene expression, TPH2 is already inadvertently targeted for the treatment of stress disorders. With increased understanding of the regulation of TPH2 activity we can now purposely utilize TPH2 as a target to develop new or optimize current therapies, which are expected to greatly improve the prevention and treatment of a wide variety of stress disorders.

Keywords

Tryptophan hydroxylase-2; serotonin; stress response; therapeutic target; gene expression; hypothalamus-pituitary-adrenal axis; sympathetic nervous system

1. Introduction

The stress response, which is essentially a crosstalk between the organism and environment, renders the organism to maintain a homeostasis by adapting its biological functions to environmental changes. Dysfunction of the stress response is involved in a wide variety of mental and somatic disorders, such as depression, anxiety and chronic fatigue syndrome

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Conflict of Interest Statement

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(CFS) [1]. The stress response is mediated by the stress system, a complex neuroendocrine, cellular and molecular infrastructure which primarily comprises the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). It has been well established that serotonin (5-hydroxytryptamine, 5-HT), a major neurotransmitter implicated in many brain functions, modulates the stress response by interacting with the HPA axis and SNS. In accordance, dysregulation of 5-HT neurotransmission has been implicated in numerous stress disorders, and the 5-HT system is a major therapeutic target for treating stress disorders [2]. For example, the selective serotonin reuptake inhibitors (SSRIs) (e.g. fluoxetine and citalopram) and the monoamine oxidase inhibitors (MAOIs) (e.g. isocarboxazid and phenelzine), which primarily target 5-HT re-uptake and metabolism, respectively, are widely used for the treatment of depression and anxiety. Recently, the synthesis of 5-HT has received much attention due to the discovery of tryptophan hydroxylase-2 (TPH2) – a second, neuron-specific isoform of the rate-limiting enzyme TPH in the synthesis of 5-HT.

2. Discovery of TPH2 as a neuron-specific TPH isoform encoded by a novel gene

TPH catalyzes the first, rate-limiting step in 5-HT synthesis – the hydroxylation of the essential amino-acid tryptophan to 5-hydroxytryptophan (5-HTP), which is further decarboxylated to 5-HT by the aromatic L-amino-acid decarboxylase (AADC). Although the existence of TPH isoforms has been long suggested, it had been thought that the potential TPH isoforms are derived from a single gene (now referred to as *TPH1*) until in 2003, it was found that *Tph1* knockout (KO) mice showed loss of 5-HT in the periphery but unchanged amounts of 5-HT in the brain. This finding led to the identification of TPH2, a second, neuron-specific TPH isoform-encoded by a novel *TPH2* gene with distinct chromosomal localization from *TPH1* [3, 4].

The human TPH1 and TPH2 proteins, which contains 444 and 490 amino acids, respectively, share an overall 71% amino-acid identity and a similar three-dimensional structure, but differ in some properties including solubility, binding affinity to the substrate tryptophan and efficiency of 5-HTP formation [4, 5]; however, the most striking difference between the two isoforms lies in their tissue distribution. While TPH1 is predominantly expressed in the periphery (especially in the enterochromaffin cells of the gut) and the pineal gland, TPH2 is primarily expressed in the brain and enteric neurons [3–7]. Since its identification, TPH2 has been extensively investigated for its involvement in neuropsychiatric disorders, with the past decade having seen an explosion of hundreds of publications on TPH2.

3. *TPH2* gene structure and its spatiotemporal and daily rhythmic expression

3.1 *TPH2* gene structure and major cis-elements involved in *TPH2* gene expression

The human *TPH2* (*hTPH2*) gene locates on chromosome 12, and it contains 11 exons and 10 introns spanning about 95 kb [3, 4]. The core promoter of *hTPH2* locates between nt -107 and 7 (the transcription start site designated as 1) [8], which contains a calcium-responsive element that mediates cell-type specific induction of *TPH2* transcription by calcium mobilization [9].

It is noteworthy that the 5'-untranslated region (5'-UTR) of *hTPH2* contains a binding motif (repressor element-1, RE1 or NRSE) for the repressor element-1 silencing transcription factor (REST, also known as NRSF for neuron-restrictive silencer factor), and recent data

suggests that this motif functions as an “on-off” switch for *TPH2* transcription [8, 10]. Originally identified as a repressor of neuronal genes in non-neuronal cells, REST is now recognized as a coordinate transcriptional and epigenetic regulator that orchestrates the cellular epigenome by recruiting numerous transcriptional and epigenetic cofactors to widely distributed genomic regulatory sequences including RE1, which is harbored by thousands of genes involved in many biological processes. Mechanisms by which REST controls the repression/activation of *TPH2* gene expression under distinct pathophysiological conditions (e.g. stress response) are still unknown; however, it was reported that tissue-specific induction of gene expression by glucocorticoids (GC), a major class of stress hormones, was mediated by REST splice variants [11]. Specifically, the full-length REST represses GC induction of gene expression in non-neuronal cells, whereas a truncated splice variant (REST4) which lacks the C-terminal repression domain enhances GC induction of gene expression in neuronal cells [11]. Notably, neurogenesis is accompanied by increased REST4 but decreased full-length REST expression, such that neuron genes are expressed in matured neurons [12]. In agreement, increased *TPH2* expression, which is accompanied by decreased *REST* mRNA expression, were observed during differentiation of the neural cell line A1 mes-c-myc [13]. In support of the emerging role of pre-mRNA splicing in adaptive responses [14], we recently found that *REST* undergoes extensive alternative splicing that is highly vulnerable to environmental factors (unpublished data).

In addition, *hTPH2* 5'-UTR contains a putative motif for the CCCTC-binding factor (CTCF) – the only chromatin insulator protein in mammals, and indeed this region shows an enriched signal of CTCF and other epigenetic marks (e.g. nucleosome positioning and histone modifications) as indicated by ENCODE Open Chromatin and Histone Mark/Modifications views of the UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>) [2]. Furthermore, *hTPH2* 5'-UTR harbors an asymmetric bidirectional promoter that may influence *TPH2* gene expression [15]. Accordingly, the 5'-UTR of *hTPH2* is a critical locus that mediates the epigenetic regulation of *TPH2* gene expression.

TPH2 gene expression is also regulated by the transcription factor POU3F2, whose effect can be differentiated by the single nucleotide polymorphisms (SNPs) –703G>T and –473T>A in *hTPH2* promoter [16].

3.2 Spatiotemporal expression of TPH2

TPH2 expression commences at early developmental stage and is maintained across the lifespan. For example, *Tph2* gene expression in mouse brain was detectable as early as embryonic day 11.25 (E11.25), increased exponentially until the end of gestation and peaked at birth, then maintained at intermediate to high levels throughout postnatal development and adult life [17]. In catfish, *tph2* mRNA expression in the whole brain was evident from 5 days post hatch (dph), and increased during the period of gonadal sex differentiation, which begins around 40–50 dph [18].

Initially identified as a neuron-specific TPH isoform, *TPH2* was consistently detected in different brain regions, especially in raphe nuclei where the majority of 5-HT neurons reside [6, 17, 19, 20]. However, *TPH2* was also expressed in enteric neurons of the gut and several specialized neuroepithelial cells including the taste receptor cells of the taste buds [21], cholangiocytes of the bile duct [22] and the retinal pigment epithelium [23], while a ubiquitous expression of *tph2* mRNA was detected in catfish brain and peripheral tissues [18]. In addition, it was reported that placental lactogens during pregnancy and high fat diet (HFD) potentially induced mouse *tph2* expression in the islets of Langerhans (regions of the pancreas that contain the endocrine beta cells) and adipose tissues, respectively [24, 25]. Thus, besides its major expression in the brain, *TPH2* is also expressed in some specific

peripheral tissues, and under some pathophysiological conditions it can be induced in some peripheral tissues that normally exhibit no *TPH2* gene expression. These findings suggest that *TPH2* gene expression regulation contributes not only to central 5-HT neurotransmission, but also to peripheral 5-HT signaling, especially in metabolism which is a main target of the stress response. Accordingly, TPH2 may constitute a component of mechanisms underlying the close link between neuropsychiatric and metabolic diseases [26, 27], and manipulation of 5-HT synthesis by modulating TPH2 gene expression/function has implications for both mental and somatic disorders.

3.3 Daily rhythmic expression of TPH2

A significant variation in *Tph2* mRNA expression during the light/dark (LD) cycle was observed in rat retina [23] and raphe [19]. Particularly, the daily rhythmic *Tph2* mRNA expression in rat raphe was correlated with circadian variations in levels of TPH protein and 5-HT synthesis in serotonergic neurons projecting to the hypothalamic suprachiasmatic nuclei (SCN) and thalamic intergeniculate leaflets (IGL) – both are involved in the entrainment of the biological clock. Similarly, daily variation in *Tph2* mRNA and TPH protein levels was also observed in hamster raphe [28]. In accordance with 5-HT regulation of circadian rhythm [29], these findings suggest a reciprocal interaction between *TPH2* gene expression and the daily biological clock, which actually represents the adaptive response of the organism to the environmental LD cycle (or day/night transition). Accordingly, TPH2 might be targeted for the correction of a disturbed circadian rhythm, which may underlie an altered stress response and stress-related disorders [30].

The circadian rhythmic *TPH2* gene expression presumably contributes to the daily fluctuation of 5-HT levels [31], which may result in a time-dependent effect of medications targeting 5-HT neurotransmission, suggesting that timing of medication might be an important factor affecting the efficacy of drugs targeting the 5-HT system and should thus be considered for medications for stress disorders.

4. Reciprocal interaction between TPH2 and the stress response

The role of TPH2 in the stress response can be inferred by the daily rhythmic TPH2 expression in the retina and midbrain neurons that project to the circadian system (SCN and IGL), which controls the circadian rhythm of the HPA axis and SNS. Indeed, there is now ample and robust evidence supporting a reciprocal interaction between TPH2 and the stress response, which sheds light on the long recognized but not yet fully understood 5-HT-stress interaction.

4.1 Regulation of TPH2 gene expression by stressors and stress hormones

4.1.1 Regulation of TPH2 gene expression by stressors—Owing to the inaccessibility of tissues for *in vivo* human research, there is no data available on stressor regulation of *hTPH2* gene expression; however, numerous studies in discrete animal models indicate that *TPH2* gene expression is modulated by various environmental stressors. For example, hypotensive haemorrhage triggered a significant increase of *tph2* expression in rat caudal midline medulla [32], just in accordance with the involvement of 5-HT neurons of the caudal raphe nuclei in hypotensive hemorrhage [33], whereas hypoxia led to a significant decline of *tph2* expression in the hypothalami of the Atlantic croaker [34]. In addition, exposure of rats [35] and mice [36] to repeated forced swim and chronic variable stress, respectively, resulted in elevated *tph2* expression in the midbrain, while early-life experience (maternal separation) and adulthood stressful events (social defeat) interacted to affect *Tph2* mRNA expression in rat raphe [37]. Moreover, exposure of mice to lipopolysaccharide (LPS), which represents an immune challenge, increased *tph2* expression

in the dorsal raphe nucleus ventrolateral part/ventrolateral periaqueductal gray region (DRV/L/VLPAG) and hippocampus [38, 39], while it increases extracellular concentrations of 5-HT in rat hippocampus and other brain regions [40]. Accordingly, stressor regulation *TPH2* gene expression partly contributes to the 5-HT-stress interaction and gene-environment interactions involved in the stress response, and drugs or other interventions that alleviate stress may affect the 5-HT system through the modulation of *TPH2* gene expression.

4.1.2 Regulation of TPH2 gene expression by GC—Upon activation by stressors, the HPA axis and SNS release a number of stress hormones including GC (primarily cortisol in primates and corticosterone in rodents) and catecholamines, which act in concert to mediate the stress response. Particularly, GC is a major family of stress hormones which affect a wide variety of biological processes including metabolism, cardiovascular function, the inflammatory/immune response, as well as behavior and cognition, primarily by modulating the expression of an array of genes. Meanwhile, GC exerts negative feedback on the HPA axis, such that the magnitude and duration of the stress response are finely tuned. It is now well established that *TPH2* is one of the many genes that are modulated by GC. For example, exposure of mice to repeated administration of the synthetic GC dexamethasone led to a significant decrease of raphe *tph2* expression, which was abolished by co-administration of mifepristone - an antagonist of GC receptor (GR) [20]. In accordance, adrenalectomy which deprives GC production resulted in an elevation of mouse raphe *tph2* expression which was reversed by GC replacement [41], supporting GC suppression of *tph2* expression in mice. By contrast, GC up-regulated *tph2* expression in rat [42], suggesting a species-dependent effect of GC on *TPH2* expression.

Since GC is released upon activation of all types of stressors, GC regulation of *TPH2* expression presumably contributes to the above-mentioned stressor regulation of *TPH2* expression, and it underlies the daily rhythmic *TPH2* expression, which is induced by the daily fluctuation of GC production [28, 42]. In both rat and hamster, adrenalectomy abolished the daily rhythmic *tph2* expression in raphe nucleus, while restoration of GC daily variation in adrenalectomized animals induced a *tph2* rhythmic pattern *de novo* [42, 43]. Collectively, these studies on GC regulation of *TPH2* expression uncover a new mechanism underlying the 5-HT-stress interaction, and further support the concept that GC may have implications for the treatment of neuropsychiatric disorders related to 5-HT dysfunction. It is yet to be determined whether GC regulation of *TPH2* expression is neuron-specific or not; however, given that metabolism is a major target of GC and is closely associated with *TPH2* gene regulation as mentioned above, it is possible that GC also modulates *TPH2* expression in peripheral tissues.

4.2 Genetically determined TPH2 expression/function and the stress response

Naturally occurring genetic polymorphisms and genetically manipulated animals render it possible to evaluate the effect of altered *TPH2* expression/function on stress-related neurobiology and phenotypic traits. With a decade of extensive research, a growing body of stress-related endophenotypes (e.g. amygdala reactivity measured by functional MRI) and behavioral traits (e.g. depression and anxiety) has been linked to genetically determined *TPH2* expression/function.

4.2.1 TPH2 polymorphisms and stress-related endophenotypes and behavioral traits in humans—The SNP -703G>T in the promoter of *hTPH2*, which affects gene expression *in vitro*, differentiates the responsiveness of the amygdala [44], a group of nuclei crucial for emotional and cognitive processing. Innervated by 5-HT fibers derived from the midbrain, the amygdala is activated during the stress response. In

accordance, -703G>T has been linked to a wide variety of neural correlates and behavioral traits related to emotional and cognitive processing, including attention [45] and attention deficit hyperactivity disorder (ADHD) [46], executive control functions [47], depression [48], anxiety [49], aggression [50], suicidal behavior [51], and early-onset obsessive-compulsive disorder [52]. Besides -703G>T, a number of other polymorphisms or haplotypes of *hTPH2*, have been linked to various stress-related neural correlates and behavioral traits [53]. These findings add to the contribution of genetic predisposition in the 5-HT system to stress-related behavioral traits and disorders, and suggest that *hTPH2* genetic variance should be taken into account (i.e. individualized based on *hTPH2* genetic predisposition) for medications targeting TPH2.

4.2.2 TPH2 polymorphisms and the stress response in nonhuman primates—

Nonhuman primates are important biomedical research models not only because of their high similarity to humans, but also because their genetic variations tend to functionally mimic human genetic variations, and in this regard *TPH2* genetic variance has been extensively in rhesus monkeys [54–57]. Of particular interest, a SNP (2051A>C) in the 3'-UTR of rhesus monkey *TPH2* (*rhTPH2*) affects gene expression and differentiates HPA axis function and stress-related behaviors [54]. Specifically, the mutant 2051C allele, which is predictive of reduced TPH2 expression, was linked to a striking increase of cortisol production in the early morning (circadian-related) and in response to the adrenocorticotrophic hormone (ACTH) stimulation when the adrenal gland is activated, but not in the afternoon when the adrenal gland is not activated. By contrast, 2051A>C exerted no effect on plasma ACTH and cerebrospinal fluid corticotropin-releasing hormone (CRH). Accordingly, 2051A>C regulation of cortisol is dependent on the status of adrenal gland, and it is primarily mediated by altered adrenal response to ACTH but not CRH/ACTH secretion. These findings, in line with the disassociation of GC and ACTH [58], suggest the existence of an ACTH-independent pathway that controls GC production. Indeed, the neuronal SCN-SNS-adrenal pathway is emerging as an important player in circadian-induced GC production. The adrenal gland is innervated by nerve fibers from the SNS, which is controlled by the SCN – a light-entrainable circadian oscillator critical for the adaptation of biological functions to the environmental LD cycle. Emerging evidence indicates that light modulates adrenal response to ACTH through the SCN-SNS-adrenal pathway, and this neuronal modulation of adrenal response to ACTH, rather than hormonal regulation of CRH/ACTH production, contributes to the circadian GC rhythm [59]. Notably, it was reported that SCN receives a dense 5-HT fiber innervation arising from midbrain, and 5-HT acts as an inhibitory neurotransmitter that modulates light effects on circadian rhythm [60]. Accordingly, 5-HT may negatively modulate the circadian-induced morning cortisol surge through the SCN-SNS-adrenal pathway. Considering GC regulation of TPH2 expression and adrenal status-dependent 2051A>C regulation of cortisol, we speculate that upon activation of the adrenal gland, the burst of cortisol induces TPH2 expression that is differentiated by 2051A>C, and the resultant *de novo* 5-HT synthesis in turn inhibits cortisol secretion through the neuronal modulation of adrenal response to ACTH. Thus, the higher levels of circadian- and ACTH-induced cortisol production linked to the 2051C allele (predictive of lower TPH2 expression) might be caused by a deficiency of 5-HT-mediated negative feedback. Accordingly, 2051A>C regulation of cortisol suggests the involvement of GC-induced TPH2 expression and *de novo* 5-HT synthesis in the feedback regulation of cortisol production, shedding new light on the 5-HT-stress interaction [2].

While the inductive TPH2 expression (induced by high GC secretion and differentiated by 2051A>C) primarily exerts its effect through modulation of the neuronal SNS, the constitutive TPH2 expression (during low GC secretion and primarily differentiated by *rhTPH2* promoter polymorphisms) preferably interacts with the hormonal HPA axis. In other words, the 5'- and 3'-regulatory polymorphisms of *rhTPH2* exert differential effects

on the stress response, presumably due to the differential roles of 5'- and 3'-regulatory regions in the regulation of gene expression [2]. Again, these findings from rhesus monkeys suggest that GC status-dependent *TPH2* gene expression, as well as the differential pathways involved in 5-HT-regulation of the stress system, should be considered for medication-targeting of TPH2. Unlike *rhTPH2*, the human and rodent *TPH2* contain no known common functional 3'-UTR polymorphisms, such that rhesus monkeys may serve as a particular genetic model to examine the effect of inductive TPH2 expression on the neuronal SNS pathway involved in 5-HT-stress interactions, and to develop specific TPH2 therapeutics.

4.2.3 Genetically determined TPH2 activity and the stress response in rodents

—A non-synonymous SNP (1473C>G or 447P>R), which results in lowered tph2 enzymatic activity and 5-HT synthesis without affecting *Tph2* gene expression, was identified in mouse *Tph2* with a strain-specific expression [7]. The 1473C>G polymorphism is linked to aggression and depressive-like behavior [61], also it affects the antidepressant effect of SSRIs [62]. Notably, the 1473G allele (predictive of reduced 5-HT synthesis) was only observed in some inbred strains of mice was not detected in wild mice, probably due to the forces of natural selection [63].

Tph2-KO(−/−) mice with 5-HT deficiency in the brain showed growth retardation, dysfunction of autonomic control, increased lethality in early life and altered serotonergic neuronal circuitry formation [64–66], as well as changes in stress-related behavioral traits including aggression, anxiety, and social interaction and communication [67, 68]. Similarly, *tph2* His⁴³⁹ knockin mice with impaired tph2 activity and reduced 5-HT levels in the brain exhibited changes in stress-related phenotypic traits including depression, anxiety and aggression [69]. In addition, over-expression or knockdown of *Tph2* in rat brain led to altered anxiety behavior depending on the status of estrogen [70]. These findings provide further evidence for the relevance of TPH2 to the stress response and stress-related behavioral traits, and support the feasibility of treating stress disorders by genetic therapies targeting TPH2.

5. TPH2 as an emerging therapeutic target for stress disorders

As a major modulator of 5-HT neurotransmission, 5-HT synthesis can be targeted for the treatment of stress disorders. For example, the intermediate product 5-HTP is sold over the counter in the United States as a dietary supplement for use as an antidepressant, appetite suppressant and sleep aid, and is also marketed in many European countries for the indication of major depression. There is no doubt that the discovery of TPH2 provides a new strategy for the manipulation of 5-HT neurotransmission at the synthesis level. Owing to its role in brain 5-HT synthesis and its implication in stress-related endophenotypes and phenotypic traits, TPH2 is emerging as a new target for the treatment of stress disorders.

Since 5-HT can not readily cross the blood-brain barrier (BBB), the total 5-HT in the body can be divided into two pools (peripheral and central) which differentially contribute to normal physiology and disease etiology. The central 5-HT, rather than the peripheral 5-HT, plays an important role in the stress response and stress disorders. Notably, the peripheral and central 5-HT pools utilize distinct rate-limiting enzymes (TPH1 and TPH2) for the synthesis but share common components (5-HT transporter and MAO) for the re-uptake and degradation processes. Accordingly, current medications which target 5-HT re-uptake (SSRIs) or degradation (MAOI) for stress disorders affect not only the central 5-HT, but also the peripheral 5-HT, such that side effects related to peripheral 5-HT will be a major problem. However, medications that selectively modulate TPH2 will primarily target the

central 5-HT, such that side effects related to peripheral 5-HT can be greatly minimized. Basically, TPH2 can be targeted by modulating its gene expression or enzymatic activity.

5.1 TPH2 gene expression as a therapeutic target

Although mechanisms for the regulation of *TPH2* gene expression are still not fully understood, it is now recognized that in addition to the above-mentioned stressors and stress hormone glucocorticoids, a growing body of environmental factors can affect (mostly induce) *TPH2* gene expression, implying a ubiquitous and critical role of TPH2 in the stress response. Literature on the effect of various environmental factors on *TPH2* gene expression was briefly summarized in Table 1. Undoubtedly, the flexible and inducible expression of TPH2 is not only of pathophysiological importance, but also of pharmacological significance [2]. Particularly, *TPH2* gene expression is induced by antidepressant drugs or other therapies and contributes to the antidepressant effects, suggesting that before its identification in 2003, TPH2 had been unintentionally targeted for the treatment of stress disorders for many years. With the knowledge of *TPH2* gene expression regulation, it is now possible for us to deliberately use TPH2 as a therapeutic target to develop new drugs or optimize the current treatment for stress disorders.

5.1.1 Endogenous hormones and growth factors affecting TPH2 gene expression

—It has been repeatedly demonstrated that estrogen, a family of sex hormones exhibiting an antidepressant-like action, induces *TPH2* gene expression [71, 72]. Accordingly, estrogen regulation of TPH2 expression may contribute, at least in part, to its antidepressant-like action, as well as sex-related differences in TPH2 expression [18] and stress-related behavioral traits [73]. Indeed, estrogen-induced *tph2* expression in rat midbrain was associated with anxiety behavior [71], while over-expression or knockdown of rat *tph2* exerted an estrogen-dependent effect on anxiety behavior [70]. Notably, exogenous and endogenous estrogen may exert opposite effects on TPH2 expression, which may account for the differential effects of exogenous (anxiolytic) and endogenous (panicolytic) estrogen on anxiety [72].

TPH2 gene expression may also be influenced by other hormones or growth factors, including the methyl-testosterone [18], placental lactogens [24], thyroid hormone [74], and the transforming growth factor beta 1 (TGF- β 1) [22]. Particularly, thyroid hormone modulation of TPH and 5-HT in the midbrain was suggested to contribute to the early-life programming of GR expression and HPA axis plasticity [75]. In line with the regulation of TPH2 expression by early-life stressful events, these findings suggest that TPH2 may be targeted for pharmacological intervention to reverse the effects of early-life adverse experience on the development of stress disorders in later life.

5.1.2 Exogenous compounds affecting TPH2 gene expression—*TPH2* gene expression is influenced by a number of antidepressants such as fluoxetine [76], lithium [77], and electroconvulsive seizure [78]. Particularly, fluoxetine-induced *tph2* expression in rat brain correlated with its antidepressant effect [76], while *hTPH2* genetic variance has been linked to the antidepressant response to SSRIs and electroconvulsive therapy [79, 80]. In addition, it was reported that the Indian herb *Bacopa monniera* leaf extract which has an antidepressant effect up-regulates *Tph2* gene expression in rat hindbrain [81]. These findings highly suggest that the pharmacological action of the antidepressants is presumably attributable, at least in part, to their effect on *TPH2* gene expression, i.e., *TPH2* gene expression is targeted by a number of antidepressant drugs.

5.1.3 Diet/nutrition and exercise affect TPH2 gene expression—It has been reported that a high-fat diet (HFD) potentially induced *Tph2* mRNA expression in mouse

adipose tissues [25], and maternal consumption of HFD during pregnancy led to a significant increase of TPH2 expression in the rostral raphe of nonhuman primate offsprings [82]. In addition, omega-3 fatty acid (primarily DHA) deficiency during perinatal development led to a significant reduction in midbrain *Tph2* mRNA expression in adult female rats [83], while it was reported that bipolar depressive symptoms might be improved by adjunctive use of omega-3 fatty acid [84]. More recently, it was reported that voluntary exercise, which is known to have an antidepressant effect, resulted in an increase of *Tph2* gene expression and 5-HT levels in rodents [42, 78]. Thus, TPH2 is also the target for the treatment of stress disorders by diet supplements or exercise.

5.2 TPH2 enzymatic activity as a therapeutic target

A high-throughput screen of approximately 200,000 compounds using purified human TPH1 and radio-labeled tryptophan has identified several classes of 5-HT synthesis inhibitors, of which a small molecule LP533401 inhibited both human TPH1 and TPH2 *in vitro* with an IC50 (concentration causing half of maximal inhibition) value of 0.7 μ M [85]. Oral administration of LP533401 selectively lowered 5-HT levels in the gut without affecting brain 5-HT levels, presumably because it does not cross the blood-brain barrier. LP533401 is currently under development and has recently been tested in rodents as a treatment of osteoporosis [86]; however, this compound might be alternatively administered so as to target TPH2 without affecting the peripheral TPH1. In addition, TPH2 can be inhibited by some other compounds including p-chlorophenylalanine, which is currently employed as a drug tool for the study of 5-HT synthesis [87].

It is noteworthy that a C-terminal truncated TPH2 splice variant (TPH2-TR), which lacks TPH2 enzyme activity, dominant-negatively affects the full-length TPH2 function and results in reduced 5-HT synthesis [88]. Accordingly, another strategy for the modulation of TPH2 activity is to modify the alternative splicing of *TPH2* pre-mRNA.

6. Summary

5-HT interacts with the HPA axis and SNS to modulate the stress response, and dysfunction of 5-HT neurotransmission has been implicated in a wide variety of stress disorders. Accordingly, the 5-HT system is a major therapeutic target for the treatment of stress disorders. The recent identification of TPH2, a second TPH isoform responsible for the synthesis of neuronal 5-HT, has been extensively studied for its involvement in neuropsychiatric disorders. It is now established that TPH2 exhibits a flexible, context-dependent gene expression under the control of numerous internal and external environmental factors including the biological clock, stressors, hormones, growth factors and exogenous chemicals, of which some exhibit antidepressant actions. In addition, genetically determined TPH2 expression or activity has been linked to a growing number of stress-related neuronal correlates, behavioral traits and disorders. These findings provide robust evidence for a crucial role of TPH2 in the pathophysiology of the stress response, and shed new light on the long-recognized but not yet fully understood 5-HT-stress interaction.

TPH2 functions as a critical modulator of 5-HT neurotransmission and is emerging as a therapeutic target for the treatment of stress disorders. Considering the effects of numerous currently used antidepressant therapies on *TPH2* gene expression, it is likely that TPH2 has already been unintentionally targeted for the treatment of stress disorders. With the knowledge of the regulation of TPH2 activity, it is now possible for us to deliberately utilize TPH2 as a target to develop new or optimize current therapies for the treatment of stress disorder. We anticipate that therapies targeting TPH2 will be developed in the near future and will have implications in the prevention and treatment of a wide variety of stress disorders.

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Table 1Environmental factors reported to affect *TPH2* gene expression

Factor	Species	Tissue/cell	TPH2 expression	Reference
Stressor				
Hypotensive haemorrhage	Rat	Caudal midline medulla	mRNA↑	33
Hypoxia	Atlantic croaker	Hypothalami	mRNA↓	34
Forced swim	Rat	Midbrain	mRNA↑	35
Chronic variable stress	Mouse	Midbrain	mRNA↑	36
Early-life adverse experience	Rat	Raphe	mRNA↑	37
Hormone/Growth factor				
Dexamethasone	Mouse	Raphe	mRNA & Protein↓	20
Corticosterone	Rat	Raphe	mRNA↑	42
Placental lactogens	Mouse	Islets of Langerhans/beta cells	mRNA↑	24
Estrogen	Rat	Raphe	mRNA↑	70, 71
Methyltestosterone	Catfish	Preoptic area–hypothalamus	mRNA↑	18
TGF-β1	Rat	Cholangiocytes	mRNA↓	22
Antidepressant drug/therapy				
Fluoxetine	Rat	Midbrain	mRNA↑	76
Lithium	Rat	primary raphe neurons	mRNA & protein↓	77
Bacopa monniera leaf extract	Rat	hindbrain	mRNA↑	81
Electroconvulsive seizure	Mouse	Hippocampus	mRNA↑	78
Diet/Nutrition				
High fat diet	Mouse	Adipose tissue	mRNA↑	25
Maternal consumption of HFD	Macaque	Rostral raphe	mRNA↑	82
Omega-3 fatty acid deficiency	Rat	Midbrain	mRNA↓	83
Excise				
Voluntary exercise	Mouse	Hippocampus	mRNA↑	78
Enhanced locomotor activity	Rat	Raphe	mRNA↑	42