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Commentary
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A unique central tryptophan hydroxylase isoform

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Abstract

Serotonin (5-hydroxytryptophan, 5-HT) is a neurotransmitter synthesized in the raphe nuclei of the brain stem and involved in the central control of food intake, sleep, and mood. Accordingly, dysfunction of the serotonin system has been implicated in the pathogenesis of psychiatric diseases. At the same time, serotonin is a peripheral hormone produced mainly by enterochromaffin cells in the intestine and stored in platelets, where it is involved in vasoconstriction, haemostasis, and the control of immune responses. Moreover, serotonin is a precursor for melatonin and is therefore synthesized in high amounts in the pineal gland. Tryptophan hydroxylase (TPH) catalyzes the rate limiting step in 5-HT synthesis. Until recently, only one gene encoding TPH was described for vertebrates. By gene targeting, we functionally ablated this gene in mice. To our surprise, the resulting animals, although being deficient for serotonin in the periphery and in the pineal gland, exhibited close to normal levels of 5-HT in the brain stem. This led us to the detection of a second TPH gene in the genome of humans, mice, and rats, called *TPH2*. This gene is predominantly expressed in the brain stem, while the classical TPH gene, now called *TPH1*, is expressed in the gut, pineal gland, spleen, and thymus. These findings clarify puzzling data, which have been collected over the last decades about partially purified TPH proteins with different characteristics and justify a new concept of the serotonin system. In fact, there are two serotonin systems in vertebrates, independently regulated and with distinct functions.

1. Introduction

Serotonin is a monoaminergic neurotransmitter involved in a wide variety of brain functions such as mood control, the regulation of sleep and body temperature, anxiety, drug abuse, food intake, and sexual behavior [1–6] with tryptophan hydroxylase (TPH; EC 1.14.16.4) as the first-step and rate-limiting enzyme in its biosynthesis [7–9]. TPH uses Fe^{2+} as cofactor and O_2 and tetrahydrobiopterin (BH4) as co-substrates to hydroxylate tryptophan generating 5-hydroxytryptophan (5-HT) (Fig. 1). This metabolite is decarboxylated by aromatic amino acid decarboxylase (AADC) to 5-HT or serotonin.

The serotonergic projection system is the most extensive monoaminergic system in the brain of vertebrates, but it is also the most difficult to study. The roots of this system are confined to a handful of selectively 5-HT-synthesizing neurons within the midbrain, pons, and medulla oblongata, which altogether constitute the several groups of raphe nuclei B1–B9 [10]. Furthermore, serotonin represents an intermediate product in melatonin synthesis and, therefore, the pineal gland expresses highest amounts of TPH under circadian control with maximal activity in the dark period [11]. Projections from the pineal gland to several brain regions have been described [12,13], in which the rate-limiting enzyme in the biosynthesis of melatonin, serotonin *N*-acetyltransferase (AANAT) is present [14], suggesting local conversion of 5-HT to melatonin in the projection areas.

Besides the brain and the pineal gland, TPH has been found in enteric neurons [15], in preimplantation embryos [16], mast cells [17], and most prominently in enterochromaffin cells of the gastrointestinal tract [18]. These cells are supposed to be the source of 5-HT in the blood where it is almost exclusively located in the dense core storage vesicles of thrombocytes [19]. 5-HT has been implicated in different processes in peripheral tissues, such as regulation of vascular tone (“serotonin” [20,21]) and intestinal motility [22], primary haemostasis [23], and T cell-mediated immune responses [24].

The enzyme TPH belongs to a superfamily of aromatic amino acid hydroxylases (AAAH), together with phenylalanine (PAH) and tyrosine hydroxylase (TH) [25,26]. While the other family members have been studied in great

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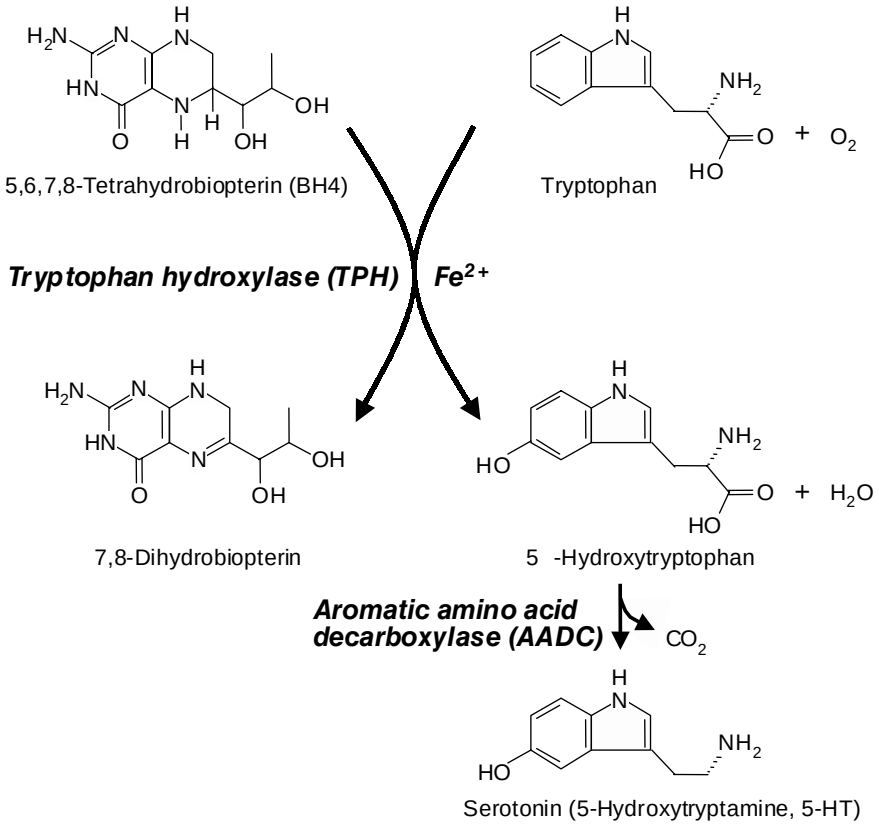

Fig. 1. Catalytic mechanisms in serotonin synthesis. Tryptophan hydroxylase (TPH) is the rate limiting enzyme in serotonin synthesis and catalyzes the hydroxylation of tryptophan using the cofactor Fe^{2+} and the co-substrates O_2 and tetrahydrobiopterin (BH4). Aromatic amino acid decarboxylase (AADC) decarboxylates the resulting 5-hydroxytryptophan to yield 5-hydroxytryptamine (5-HT) or serotonin.

detail concerning structure, characteristics, and regulation [27], TPH has been left behind, due to the extremely low abundance of TPH mRNA in the CNS and the difficulties to purify the protein [28], as well as to the lack of serotonin-producing neuronal cell lines suitable for *in vitro* studies [29].

TPH-cDNAs have been cloned from different mammalian species (rabbit: [30]; mouse: [31]; rat: [32]; human: [33]). The first TPH gene to be characterized contained 11 exons and was located on human chromosome 11 and mouse chromosome 7 [31,34–37]. For more than a decade this gene has been thought to be the only TPH gene in the vertebrate genome [38]. By targeted ablation of this TPH gene (now called *Tph1*) in mice, we recently discovered the existence of a second TPH isoform, TPH2, encoded by an additional gene on human chromosome 12 and mouse chromosome 10 [39]. TPH1 is mainly present in pineal gland, thymus, spleen, and gut while TPH2 predominates in brain stem. This commentary will summarize the history and the relevance of this discovery and the characteristics of TPH2.

2. Historical evidence for two TPH isoforms

Since more than thirty years there is evidence in the literature for the existence of isoforms of TPH [28,29]. In purification procedures, two peaks of activity with different isoelectric points were detected in total brain protein preparations probably including brain stem and pineal gland [40]. Moreover, partially purified TPH enzymes with different biochemical properties were described, depending on the analyzed tissues [41–45]. Furthermore, the first generation of antibodies against TPH purified from a murine mastocytoma cell line (P815), detected the enzyme in the gut but not in the brain [46]. Recently developed monoclonal antibodies could also distinguish between TPH from brain stem and from pineal gland or detected products of different sizes in both tissues [47,48]. However, the widely used commercially available antibodies cross-react with TPH1 and TPH2 and therefore detect the enzyme in central and peripheral sites. Until recently, these differences between TPH from brain stem and pineal gland or mastocytoma cells were explained by distinct posttranslational modifications of TPH in different tissues, e.g. phosphorylation [25,28].

Further evidence for TPH isoforms came from the different mRNA to protein levels in the pineal gland and the raphe nuclei [49–51]. Comparable TPH protein amounts were present in pineal gland and raphe nuclei while the mRNA levels were up to 150 times lower in brain stem. Due to the lack of any other explanation, the divergence in protein/mRNA ratios was attributed to different

translational efficiencies of TPH1 mRNAs differentially spliced in the 5′-untranslated region. However, at this time mainly TPH1 mRNA was detected, since the probes used may have only partially crossreacted with TPH2 mRNA, and, indeed, TPH1 is expressed at a more than 100-fold lower level than TPH2 in brain stem [39].

Moreover, early transgenic studies using the *Tph1* promoter suggested the existence of an additional TPH gene. Several genes were expressed in a tissue-specific manner in transgenic mice using the 6.1 kb 5′-flanking region of the mouse *Tph1* gene [52,53]. Highest and in some lines even exclusive expression of the *lacZ* reporter gene was found in the pineal gland [52], a fact that remained unexplained. Furthermore, in some lines, the reporter gene was also expressed in brain regions that normally do not express TPH at detectable levels, such as the superior colliculus, the cerebellum, and the dentate gyrus [52], but are known to express AANAT [14]. Since 5-HT has been shown to be largely distributed in the cell soma and in CNS-invading processes of the pineal gland [54], it is conceivable, that the presence of *lacZ* in central brain regions of the highly expressing mouse lines was due to pineal projections. Further support for this explanation comes from the known expression of AANAT, the rate-limiting enzyme in the biosynthesis of melatonin, in hippocampus, midbrain, and brain stem besides other brain regions [14], suggesting local melatonin synthesis from 5-HT in possible pineal projections [12].

Concordantly, targeted tumorigenesis in transgenic mice using the 6.1 kb 5′-upstream region of the mouse *Tph1* gene fused to the SV40 T-antigen led only to pineal tumors, not to tumors of major serotonergic brain areas [53]. Interestingly, the invasion of the pineal tumor could be observed in sagittal brain sections through the hippocampus [53], in our opinion, following pineal projections.

Taken together, all efforts undertaken to identify TPH isoforms were unfruitful due to the lack of differentiating molecular probes or antibodies. Nonetheless, a strong sensibility remained for the existence of possible TPH isoforms, as can be deduced from several recent reports [28,48,55].

3. Mice deficient in TPH1

To elucidate the physiological impact of the loss of 5-HT synthesis, we generated mice genetically deficient for TPH1 [39]. Although we had expected a lethal phenotype of this genetic manipulation, as has been found for TH knockout mice [56], surprisingly, we obtained viable homozygous *Tph1*-deficient (*Tph1*−/−) mice. These mice lack 5-HT in the periphery, in particular in the gut, in the blood and in the pineal gland (Fig. 2), and, therefore, they allow to uncover the actions of 5-HT in cardiovascular regulation, in primary haemostasis and in the immune system. Unexpectedly however, there was only a minor

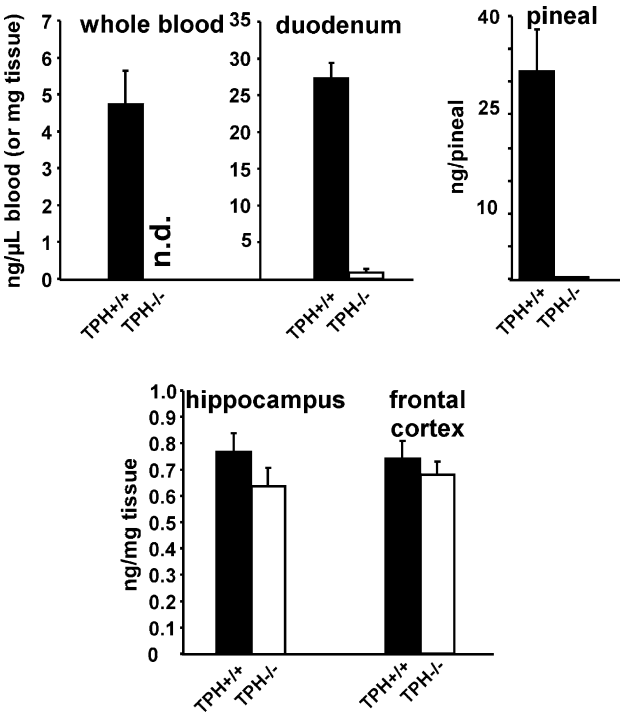

Fig. 2. Serotonin content of whole blood, duodenum, pineal gland, hippocampus, and frontal cortex in *Tph1*-deficient mice (TPH−/−) compared to wild-type (TPH+/+). While duodenum, blood and pineal gland are almost depleted from serotonin, 5-HT is only slightly diminished in hippocampus and frontal cortex of *Tph1*-deficient mice (modified after [39]).

reduction of steady-state 5-HT levels in serotonergic brain regions of *Tph1*−/− mice (Fig. 2) suggesting the existence of a second *Tph* gene not affected by the gene targeting. Therefore, we screened the Human Genome Database and detected a homolog to *TPH1* called *TPH2*. TPH2 cDNAs were isolated from mouse, rat, and humans and expression of TPH2 cDNA in cell culture enabled the expressing cells to synthesize 5-hydroxytryptophan, proving the identity of TPH2 as a real tryptophan hydroxylase [39]. The expression of both isoforms is mutually exclusive. TPH2 is predominantly expressed in brain stem and TPH1 mRNA is present in peripheral organs such as the intestine, spleen, and thymus of mice [39]. Furthermore, the very active 5-HT synthesis in the pineal gland, which is considered to be a peripheral tissue since it is separated from the brain by the blood–brain barrier, is completely dependent on the presence of TPH1 (Fig. 2). Therefore, the slightly reduced 5-HT levels in the hippocampus (Fig. 2) could simply reflect the loss of its synthesis in pinealocytes and concomitantly in their processes. Accordingly, a significant 5-HT reduction has been found in hippocampus and midbrain of pinealectomized rats [57].

4. Predicted characteristics of TPH2

TPH1 and TPH2 are highly homologous proteins exhibiting 71% of amino acid identity in humans (Fig. 3). All

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hTPH1:   1                  M------------------------IE----------------    3
                            M                        E
hTPH2:   1                  MQPAMMMFSSKYWARRGFSLDSAVPEEHQLLGSSTLNKPNSGKN  44

                                                                  14-3-
             BH4                hydrophobic interaction          CamKII/PKA
hTPH1:   4  DNKENKDHS-----LERGRASLIFSLKNEVGGLIKALKIFQEKHVNLLHIESRKSKRRNS  58
            D+K NK  S       E G+ +++FSLKNEVGGL+KAL++FQEK VN++HIESRKS+RR+S
hTPH2:  45  DDKGNKGSSKREAATESGKTAVVFSLKNEVGGLVKALRLFQEKRVNMVHIESRKSRRRSS 104

            3                                                 border
hTPH1:  59  EFEIFVDCDINREQLNDIFHLLKSHTNVLSVNLPDNFTLKEDGMETVPWFPKKISDLDHC 118
            E EIFVDC+  + + N++  LLK  T ++++N P+N   +E+ +E VPWFP+KIS+LD C
hTPH2: 105  EVEIFVDCECGKTEFNELIQLLKFQTTIVTLNPPENIWTEEEELEDVPWFPRKISELDKC 164

                BH4
hTPH1: 119  ANRVLMYGSELDADHPGFKDNVYRKRRKYFADLAMNYKHGDPIPKVEFTEEEIKTWGTVF 178
            ++RVLMYGSELDADHPGFKDNVYR+RRKYF D+AM YK+G PIP+VE+TEEE KTWG VF
hTPH2: 165  SHRVLMYGSELDADHPGFKDNVYRQRRKYFVDVAMGYKYGQPIPRVEYTEEETKTWGVVF 224

                                                                BH4
                                                                Trp
hTPH1: 179  QELNKLYPTHACREYLKNLPLLSKYCGYREDNIPQLEDVSNFLKERTGFSIRPVAGYLSP 238
            +EL+KLYPTHACREYLKN PLL+KYCGYREDN+PQLEDVS FLKER+GF++RPVAGYLSP
hTPH2: 225  RELSKLYPTHACREYLKNFPLLTKYCGYREDNVPQLEDVSMFLKERSGFTVRPVAGYLSP 284

                                          BH4
                          Trp             Trp
            BH4       Fe    CamKII        Fe   Fe
hTPH1: 239  RDFLSGLAFRVFHCTQYVRHSSDPFYTPEPDTCHELLGHVPLLAEPSFAQFSQEIGLASL 298
            RDFL+GLA+RVFHCTQY+RH SDP YTPEPDTCHELLGHVPLLA+P FAQFSQEIGLASL
hTPH2: 285  RDFLAGLAYRVFHCTQYIRHGSDPLYTPEPDTCHELLGHVPLLADPKFAQFSQEIGLASL 344

                     BH4   Fe
                BH4 Trp    Trp              Trp
hTPH1: 299  GASEEAVQKLATCYFFTVEFGLCKQDGQLRVFGAGLLSSISELKHALSGHAKVKPFDPKI 358
            GAS+E VQKLATCYFFT+EFGLCKQ+GQLR +GAGLLSSI ELKHALS  A VK FDPK
hTPH2: 345  GASDEDVQKLATCYFFTIEFGLCKQEGQLRAYGAGLLSSIGELKHALSDKACVKAFDPKT 404

hTPH1: 359  TCKQECLITTFQDVYFVSESFEDAKEKMREFTKTIKRPFGVKYNPYTRSIQILKDTKSIT 418
            TC QECLITTFQ+ YFVSESFE+AKEKMR+F K+I RPF V +NPYT+SI+ILKDT+SI
hTPH2: 405  TCLQECLITTFQEAYFVSESFEEAKEKMRDFAKSITRPFSVYFNPYTQSIEILKDTRSIE 464

              Leucine zipper
hTPH1: 419  SAMNELQHDLDVVSDALAKVSRKPSI 444
            + + +L+ DL+ V DAL K+++   I
hTPH2: 465  NVVQDLRSDLNTVCDALNKMNQYLGI 490
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Fig. 3. Comparison of human TPH1 and TPH2. The central line indicates identical and similar (+) amino acid residues. Functionally important residues of TPH1 are marked. Fe: iron (Fe^{2+}) binding site, Trp, tryptophan binding site, BH4, co-substrate binding site, 14-3-3, binding site for 14-3-3 proteins, PKA: protein kinase A phosphorylation site; CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II phosphorylation site; also, the hydrophobic interaction domain and the leucine zipper involved in multimerization and the border between the regulatory and the catalytic domains are shown.

residues which have been detected to be important for the structural and functional properties of TPH1 are conserved in TPH2 [58–62]. Therefore, most of the features of TPH1 should also be present in TPH2. In fact, by using TPH preparations from brain stem, previous reports have already unwittingly supported this notion. It has been shown that brain stem TPH (TPH2) can be phosphorylated by Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and

protein kinase A (PKA) [63–65]. The phosphorylation sites for CaMKII have been mapped to serine 58 and 260 and for PKA to serine 58 in recombinant TPH1 [58,66–68]. Both are conserved in TPH2 suggesting that these are also the phosphorylation sites in the newly discovered isoform. After phosphorylation, a 14-3-3 protein binds probably to the phosphoserine residue 58 in TPH1, increases the activity of the enzyme, and inhibits its dephosphorylation [66, 69–74]. This may be of functional importance since the activation of both kinases has been implicated in the regulation of 5-HT synthesis and release in the brain [75,76]. On the other hand, it has recently been shown that phosphorylation of TPH1 by CaMKII triggers its degradation by the proteasome [55]. It may be speculated that binding of a 14-3-3 protein to TPH competes with proteasome degradation and occurs only in some tissues or only with one of the two isoforms *in vivo* explaining the strikingly different stabilities described for TPH of different sources [77,78].

Recently, comparison with X-ray structures of PAH and TH and the first X-ray structural analysis of TPH1 itself has defined several amino acids involved in the binding of tryptophan, iron and the cofactor BH4 [58–60,62]. As shown in Fig. 3 all these residues are identical in TPH2 and may therefore fulfill the same functions in this isoform. Furthermore, the leucine zipper motif at the C-terminus and the hydrophobic interaction domain at the N-terminus of TPH1 responsible for tetramerization of the protein [68,79–81] are conserved in TPH2 indicating that TPH2 also exists as multimeric complex.

However, there are also molecular differences between the two isoforms which may be responsible for the described biochemical and functional differences between purified TPH preparations from brain stem and peripheral sources. In particular, the N-termini of the proteins which contain the regulatory domains are quite divergent. Probably as a consequence, the K_m values for tryptophan of the purified and recombinant enzymes from carcinoid tumors and pineal gland (TPH1) have been reported to be between 13 and 23 μM, while the brain stem enzyme (TPH2) exhibits a value of 142 μM [74].

Nevertheless, all these predicted characteristics of TPH2 have to be verified by experiments with defined isoform preparations.

5. Clinical implications

As expected from the biochemical and molecular biological findings in *Tph1−/−* mice showing normal serotonin levels in the brain, no behavioral alterations were detectable, although the peripheral 5-HT pools were almost depleted [39]. Therefore, the behavioral effects of 5-HT are fully uncoupled from 5-HT and its metabolites in peripheral tissues. This fact is particularly important, since many efforts have been undertaken to find diagnostically useful correlations between peripheral levels of 5-HT metabolites and 5-HT function in the CNS of human patients suffering from 5-HT-related psychiatric disorders [82,83]. Furthermore, numerous studies have tried to link polymorphisms in the *TPH1* gene to such diseases [84–89]. The negative outcome of most of these studies is not surprising in light of the fact that *TPH1* is not expressed in brain stem at considerable level. However, an influence on behavior and psychiatric diseases via the synthesis of melatonin, for which TPH1 is essential, cannot be completely excluded.

On the other hand, *TPH2* is now a good candidate gene for 5-HT related psychiatric diseases such as bipolar affective disorder. Indeed, the chromosomal locus of the

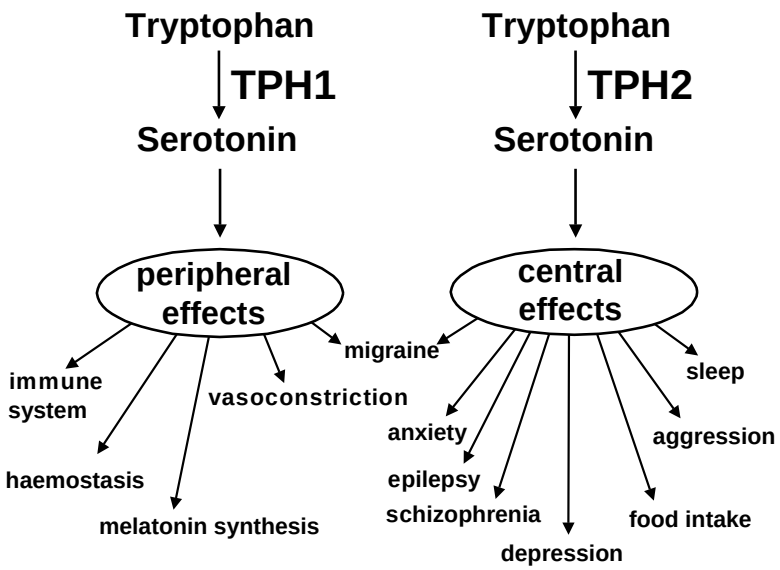

Fig. 4. Schematic representation of the duality of the serotonergic system. Serotonin is synthetized by two distinct TPH enzymes in the brain (TPH2) and in the periphery (TPH1). Thus, the indicated peripheral and central functions of serotonin are differentially regulated and can be targeted independently. Only in the pathogenesis of migraine, serotonin from both sources may be involved.

human gene, 12q15, has recently been detected with a high LOD score by a genetic linkage study of families affected with this disease [90].

The two distinct serotonin systems in the brain and in the periphery (Fig. 4) allow novel therapeutic approaches targeting peripheral and central 5-HT synthesis independently. Thus, it may be helpful to inhibit peripheral serotonin synthesis in order to interfere with haemostasis or immune responses without risking unwanted central side effects. Additionally, drugs could be designed which stimulate central serotonin synthesis without altering haemostasis, in contrast to classical antidepressants which inhibit serotonin reuptake into cells and thereby sporadically induce bleeding episodes by depleting platelets from the hormone [91–93].

6. Conclusions

The discovery of TPH2 explains the previously puzzling data compiled in the past thirty years about divergent protein/mRNA ratios and biochemical characteristics of TPH from peripheral sources and from the CNS. More importantly, it justifies a change in the concept of the serotonin system. In fact, there are two serotonin systems in vertebrates with independent regulation and distinct functions defined by the two TPH isoforms, TPH1 and TPH2 (Fig. 4). Consequently, both systems can be targeted independently by pharmacological agents and new therapeutical options are available for psychiatric diseases on one side and disorders of haemostasis and the immune system on the other side.

References

[1] Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang Feng T, Chang AS, Ganapathy V, Blakely RD. Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. Proc Natl Acad Sci USA 1993;90: 2542–6.

[2] Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC, De Maeyer E. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 1995;268:1763–6.

[3] Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D. Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature 1995;374:542–6.

[4] Rocha BA, Scearce Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, Hen R. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 1998;393:175–8.

[5] Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci USA 1998;95:10734–9.

[6] Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 1999;283:397–401.

[7] Grahame-Smith DG. Tryptophan hydroxylation in brain. Biochem Biophys Res Commun 1964;16:586–92.

[8] Lovenberg W, Jequier E, Sjoerdsma A. Tryptophan hydroxylation: measurement in pineal gland, brainstem, and carcinoid tumor. Science 1967;155:217–9.

[9] Jequier E, Robinson DS, Lovenberg W, Sjoerdsma A. Further studies on tryptophan hydroxylase in rat brainstem and beef pineal. Biochem Pharmacol 1969;18:1071–81.

[10] Dahlström A, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol Scand Suppl 1964;232:1–55.

[11] Reuss S. Components and connections of the circadian timing system in mammals. Cell Tissue Res 1996;285:353–78.

[12] Sallanon M, Claustrat B, Touret M. Presence of melatonin in various cat brainstem nuclei determined by radioimmunoassay. Acta Endocrinol (Copenh) 1982;101:161–5.

[13] Miguez JM, Martin FJ, Aldegunde M. Melatonin effects on serotonin synthesis and metabolism in the striatum, nucleus accumbens, and dorsal and median raphe nuclei of rats. Neurochem Res 1997;22:87–92.

[14] Coon SL, Mazuruk K, Bernard M, Roseboom PH, Klein DC, Rodriguez IR. The human serotonin *N*-acetyltransferase (EC 2.3.1.87) gene (AANAT): structure, chromosomal localization, and tissue expression. Genomics 1996;34:76–84.

[15] Fiorica-Howells E, Maroteaux L, Gershon MD. Serotonin and the 5-HT(2B) receptor in the development of enteric neurons. J Neurosci 2000;20:294–305.

[16] Walther DJ, Bader M. Serotonin synthesis in murine embryonic stem cells. Brain Res Mol Brain Res 1999;68:55–63.

[17] Finocchiaro LM, Arzt ES, Fernandez-Castelo S, Criscuolo M, Finkielman S, Nahmod VE. Serotonin and melatonin synthesis in peripheral blood mononuclear cells: stimulation by interferon-gamma as part of an immunomodulatory pathway. J Interferon Res 1988;8:705–16.

[18] Weber LJ, Horita A. A study of 5-hydroxytryptamine formation from L-tryptophan in the brain and other tissues. Biochem Pharmacol 1965; 14:1141–9.

[19] Champier J, Claustrat B, Besancon R, Eymin C, Killer C, Jouvet A, Chamba G, Fevre-Montange M. Evidence for tryptophan hydroxylase and hydroxy-indol-*O*-methyl-transferase mRNAs in human blood platelets. Life Sci 1997;60:2191–7.

[20] Rapport MM, Green AA, Page IH. Crystalline serotonin. Science 1948;108:329–30.

[21] Chester AH, Martin GR, Bodelsson M, Arneklo-Nobin B, Tadjkarimi S, Tornebrandt K, Yacoub MH. 5-Hydroxytryptamine receptor profile in healthy and diseased human epicardial coronary arteries. Cardiovasc Res 1990;24:932–7.

[22] Hixson EJ, Lehrmann GV, Maickel RP. Contractile responses to tryptamine analogues in isolated smooth muscle. Arch Int Pharmacodyn Ther 1977;229:4–14.

[23] Holland JM. Serotonin deficiency and prolonged bleeding in beige mice. Proc Soc Exp Biol Med 1976;151:32–9.

[24] Geba GP, Ptak W, Anderson GM, Paliwal V, Ratzlaff RE, Levin J, Askenase PW. Delayed-type hypersensitivity in mast cell-deficient mice: dependence on platelets for expression of contact sensitivity. J Immunol 1996;157:557–65.

[25] Hufton SE, Jennings IG, Cotton RG. Structure and function of the aromatic amino acid hydroxylases. Biochem J 1995;311(Pt 2):353–66.

[26] Fitzpatrick PF. Tetrahydropterin-dependent amino acid hydroxylases. Annu Rev Biochem 1999;68:355–81.

[27] Kobe B, Jennings IG, House CM, Michell BJ, Goodwill KE, Santarsiero BD, Stevens RC, Cotton RG, Kemp BE. Structural basis of autoregulation of phenylalanine hydroxylase. Nat Struct Biol 1999;6: 442–8.

[28] Cash CD. Why tryptophan hydroxylase is difficult to purify: a reactive oxygen-derived species-mediated phenomenon that may be implicated in human pathology. Gen Pharmacol 1998;30:569–74.

[29] Mockus SM, Vrana KE. Advances in the molecular characterization of tryptophan hydroxylase. J Mol Neurosci 1998;10:163–79.

[30] Grenett HE, Ledley FD, Reed LL, Woo SL. Full-length cDNA for rabbit tryptophan hydroxylase: functional domains and evolution of aromatic amino acid hydroxylases. Proc Natl Acad Sci USA 1987;84: 5530–4.
[31] Stoll J, Kozak CA, Goldman D. Characterization and chromosomal mapping of a cDNA encoding tryptophan hydroxylase from a mouse mastocytoma cell line. Genomics 1990;7:88–96.
[32] Darmon MC, Guibert B, Leviel V, Ehret M, Maitre M, Mallet J. Sequence of two mRNAs encoding active rat tryptophan hydroxylase. J Neurochem 1988;51:312–6.
[33] Boularand S, Darmon MC, Ganem Y, Launay JM, Mallet J. Complete coding sequence of human tryptophan hydroxylase. Nucleic Acids Res 1990;18:4257.
[34] Stoll J, Goldman D. Isolation and structural characterization of the murine tryptophan hydroxylase gene. J Neurosci Res 1991;28: 457–65.
[35] Ledley FD, Grenett HE, Bartos DP, van Tuinen P, Ledbetter DH, Woo SL. Assignment of human tryptophan hydroxylase locus to chromosome 11: gene duplication and translocation in evolution of aromatic amino acid hydroxylases. Somat Cell Mol Genet 1987;13: 575–80.
[36] Craig SP, Boularand S, Darmon MC, Mallet J, Craig IW. Localization of human tryptophan hydroxylase (TPH) to chromosome 11p15.3-p14 by *in situ* hybridization. Cytogenet Cell Genet 1991;56:157–9.
[37] Nielsen DA, Dean M, Goldman D. Genetic mapping of the human tryptophan hydroxylase gene on chromosome 11, using an intronic conformational polymorphism. Am J Hum Genet 1992;51:1366–71.
[38] Kim KS, Wessel TC, Stone DM, Carver CH, Joh TH, Park DH. Molecular cloning and characterization of cDNA encoding tryptophan hydroxylase from rat central serotonergic neurons. Brain Res Mol Brain Res 1991;9:277–83.
[39] Walther DJ, Peter JU, Bashamakh S, Hörtnagl H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 2003;299:76.
[40] Cash CD, Vayer P, Mandel P, Maitre M. Tryptophan 5-hydroxylase. Rapid purification from whole brain and production of a specific antiserum. Eur J Biochem 1985;149:239–45.
[41] Koe BK. Tryptophan hydroxylase inhibitors. Fed Proc 1971;30: 886–96.
[42] Nakata H, Fujisawa H. Tryptophan 5-monooxygenase from mouse mastocytoma P815. A simple purification and general properties. Eur J Biochem 1982;124:595–601.
[43] Nakata H, Fujisawa H. Purification and properties of tryptophan 5-monooxygenase from rat brain-stem. Eur J Biochem 1982;122:41–7.
[44] Kuhn DM, Meyer MA, Lovenberg W. Comparisons of tryptophan hydroxylase from a malignant murine mast cell tumor and rat mesencephalic tegmentum. Arch Biochem Biophys 1980;199:355–61.
[45] Yang XJ, Kaufman S. High-level expression and deletion mutagenesis of human tryptophan hydroxylase. Proc Natl Acad Sci USA 1994;91:6659–63.
[46] Hasegawa H, Yanagisawa M, Inoue F, Yanaihara N, Ichiyama A. Demonstration of non-neural tryptophan 5-mono-oxygenase in mouse intestinal mucosa. Biochem J 1987;248:501–9.
[47] Chung YI, Park DH, Kim M, Baker H, Joh TH. Immunochemical characterization of brain and pineal tryptophan hydroxylase. J Korean Med Sci 2001;16:489–97.
[48] Haycock JW, Kumer SC, Lewis DA, Vrana KE, Stockmeier CA. A monoclonal antibody to tryptophan hydroxylase: applications and identification of the epitope. J Neurosci Methods 2002;114:205–12.
[49] Dumas S, Darmon MC, Delort J, Mallet J. Differential control of tryptophan hydroxylase expression in raphe and in pineal gland: evidence for a role of translation efficiency. J Neurosci Res 1989; 24:537–47.
[50] Hart RP, Yang R, Riley LA, Green TL. Post-transcriptional control of tryptophan hydroxylase gene expression in rat brain stem and pineal gland. Mol Cell Neurosci 1991;2:71–7.
[51] Austin MC, O'Donnell SM. Regional distribution and cellular expression of tryptophan hydroxylase messenger RNA in postmortem human brainstem and pineal gland. J Neurochem 1999;72: 2065–73.
[52] Huh SO, Park DH, Cho JY, Joh TH, Son JH. A 6.1 kb 5′ upstream region of the mouse tryptophan hydroxylase gene directs expression of *E. coli* lacZ to major serotonergic brain regions and pineal gland in transgenic mice. Mol Brain Res 1994;24:145–52.
[53] Son JH, Chung JH, Huh SO, Park DH, Peng C, Rosenblum MG, Chung YI, Joh TH. Immortalization of neuroendocrine pinealocytes from transgenic mice by targeted tumorigenesis using the tryptophan hydroxylase promoter. Mol Brain Res 1996;37:32–40.
[54] Juillard MT, Collin JP. Pools of serotonin in the pineal gland of the mouse: the mammalian pinealocyte as a component of the diffuse neuroendocrine system. Cell Tissue Res 1980;213:273–91.
[55] Iida Y, Sawabe K, Kojima M, Oguro K, Nakanishi N, Hasegawa H. Proteasome-driven turnover of tryptophan hydroxylase is triggered by phosphorylation in RBL2H3 cells, a serotonin producing mast cell line. Eur J Biochem 2002;269:4780–8.
[56] Zhou QY, Quaife CJ, Palmiter RD. Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. Nature 1995;374:640–3.
[57] Aldegunde M, Miguez I, Veira J. Effects of pinealectomy on regional brain serotonin metabolism. Int J Neurosci 1985;26:9–13.
[58] Jiang GC, Yohrling GJ, Schmitt JD, Vrana KE. Identification of substrate orienting and phosphorylation sites within tryptophan hydroxylase using homology-based molecular modeling. J Mol Biol 2000;302:1005–17.
[59] McKinney J, Teigen K, Froystein NA, Salaun C, Knappskog PM, Haavik J, Martinez A. Conformation of the substrate and pterin cofactor bound to human tryptophan hydroxylase. Important role of Phe313 in substrate specificity. Biochemistry 2001;40:15591–601.
[60] Martinez A, Knappskog PM, Haavik J. A structural approach into human tryptophan hydroxylase and its implications for the regulation of serotonin biosynthesis. Curr Med Chem 2001;8:1077–91.
[61] Daubner SC, Moran GR, Fitzpatrick PF. Role of tryptophan hydroxylase phe313 in determining substrate specificity. Biochem Biophys Res Commun 2002;292:639–41.
[62] Wang L, Erlandsen H, Haavik J, Knappskog PM, Stevens RC. Three-dimensional structure of human tryptophan hydroxylase and its implications for the biosynthesis of the neurotransmitters serotonin and melatonin. Biochemistry 2002;41:12569–74.
[63] Boadle-Biber MC. Activation of tryptophan hydroxylase from slices of rat brain stem incubates with N6, 02′-dibutyryl adenosine-3′:5′-cyclic monophosphate. Biochem Pharmacol 1980;29:669–72.
[64] Ehret M, Cash CD, Hamon M, Maitre M. Formal demonstration of the phosphorylation of rat brain tryptophan hydroxylase by Ca^{2+}/calmodulin-dependent protein kinase. J Neurochem 1989; 52:1886–91.
[65] Johansen PA, Jennings I, Cotton RG, Kuhn DM. Phosphorylation and activation of tryptophan hydroxylase by exogenous protein kinase A. J Neurochem 1996;66:817–23.
[66] Kuhn DM, Arthur Jr R, States JC. Phosphorylation and activation of brain tryptophan hydroxylase: identification of serine-58 as a substrate site for protein kinase A. J Neurochem 1997;68:2220–3.
[67] Kumer SC, Mockus SM, Rucker PJ, Vrana KE. Amino-terminal analysis of tryptophan hydroxylase: protein kinase phosphorylation occurs at serine-58. J Neurochem 1997;69:1738–45.
[68] Yohrling GJ, Jiang GC, Mockus SM, Vrana KE. Intersubunit binding domains within tyrosine hydroxylase and tryptophan hydroxylase. J Neurosci Res 2000;61:313–20.
[69] Yamauchi T, Nakata H, Fujisawa H. A new activator protein that activates tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of Ca^{2+}/calmodulin-dependent protein kinase. Purification and characterization. J Biol Chem 1981;256: 5404–9.

[70] Ichimura T, Isobe T, Okuyama T, Takahashi N, Araki K, Kuwano R, Takahashi Y. Molecular cloning of cDNA coding for brain-specific 14-3-3 protein, a protein kinase-dependent activator of tyrosine and tryptophan hydroxylases. Proc Natl Acad Sci USA 1988;85:7084–8.
[71] Makita Y, Okuno S, Fujisawa H. Involvement of activator protein in the activation of tryptophan hydroxylase by cAMP-dependent protein kinase. FEBS Lett 1990;268:185–8.
[72] Furukawa Y, Ikuta N, Omata S, Yamauchi T, Isobe T, Ichimura T. Demonstration of the phosphorylation-dependent interaction of tryptophan hydroxylase with the 14-3-3 protein. Biochem Biophys Res Commun 1993;194:144–9.
[73] Banik U, Wang GA, Wagner PD, Kaufman S. Interaction of phosphorylated tryptophan hydroxylase with 14-3-3 proteins. J Biol Chem 1997;272:26219–25.
[74] Kowlessur D, Kaufman S. Cloning and expression of recombinant human pineal tryptophan hydroxylase in *Escherichia coli*: purification and characterization of the cloned enzyme. Biochim Biophys Acta 1999;1434:317–30.
[75] Stenfors C, Ross SB. Evidence for involvement of protein kinases in the regulation of serotonin synthesis and turnover in the mouse brain *in vivo*. J Neural Transmembr 2002;109:1353–63.
[76] Chen C, Rainnie DG, Greene RW, Tonegawa S. Abnormal fear response and aggressive behavior in mutant mice deficient for alpha-calcium-calmodulin kinase II. Science 1994;266:291–4.
[77] Meek JL, Neff NH. Tryptophan 5-hydroxylase: approximation of half-life and rate of axonal transport. J Neurochem 1972;19:1519–25.
[78] Sitaram BR, Lees GJ. Diurnal rhythm and turnover of tryptophan hydroxylase in the pineal gland of the rat. J Neurochem 1978;31: 1021–6.
[79] Mockus SM, Kumer SC, Vrana KE. Carboxyl terminal deletion analysis of tryptophan hydroxylase. Biochim Biophys Acta 1997; 1342:132–40.
[80] Yohrling GJ, Mockus SM, Vrana KE. Identification of amino-terminal sequences contributing to tryptophan hydroxylase tetramer formation. J Mol Neurosci 1999;12:23–34.
[81] Moran GR, Daubner SC, Fitzpatrick PF. Expression and characterization of the catalytic core of tryptophan hydroxylase. J Biol Chem 1998;273:12259–66.
[82] Bailly D, Vignau J, Racadot N, Beuscart R, Servant D, Parquet PJ. Platelet serotonin levels in alcoholic patients: changes related to physiological and pathological factors. Psychiatry Res 1993;47:57–88.
[83] Moffitt TE, Brammer GL, Caspi A, Fawcett JP, Raleigh M, Yuwiler A, Silva P. Whole blood serotonin relates to violence in an epidemiological study. Biol Psychiatry 1998;43:446–57.
[84] Nielsen DA, Goldman D, Virkkunen M, Tokola R, Rawlings R, Linnoila M. Suicidality and 5-hydroxyindoleacetic acid concentration associated with a tryptophan hydroxylase polymorphism. Arch Gen Psychiatry 1994;51:34–8.
[85] Mann JJ, Malone KM, Nielsen DA, Goldman D, Erdos J, Gelernter J. Possible association of a polymorphism of the tryptophan hydroxylase gene with suicidal behavior in depressed patients. Am J Psychiatry 1997;154:1451–3.
[86] Lalovic A, Turecki G. Meta-analysis of the association between tryptophan hydroxylase and suicidal behavior. Am J Med Genet 2002;114:533–40.
[87] Rietschel M, Schorr A, Albus M, Franzek E, Kreiner R, Held T, Knapp M, Muller DJ, Schulze TG, Propping P, Maier W, Nothen MM. Association study of the tryptophan hydroxylase gene and bipolar affective disorder using family-based internal controls. Am J Med Genet 2000;96:310–1.
[88] Souery D, Van Gestel S, Massat I, Blairy S, Adolfsson R, Blackwood D, Del Favero J, Dikeos D, Jakovljevic M, Kaneva R, Lattuada E, Lerer B, Lilli R, Milanova V, Muir W, Nothen M, Oruc L, Papadimitriou G, Propping P, Schulze T, Serretti A, Shapira B, Smeraldi E, Stefanis C, Thomson M, Van Broeckhoven C, Mendlewicz J. Tryptophan hydroxylase polymorphism and suicidality in unipolar and bipolar affective disorders: a multicenter association study. Biol Psychiatry 2001;49:405–9.
[89] Tang G, Ren D, Xin R, Qian Y, Wang D, Jiang S. Lack of association between the tryptophan hydroxylase gene A218C polymorphism and attention-deficit hyperactivity disorder in Chinese Han population. Am J Med Genet 2001;105:485–8.
[90] Morissette J, Villeneuve A, Bordeleau L, Rochette D, Laberge C, Gagne B, Laprise C, Bouchard G, Plante M, Gobeil L, Shink E, Weissenbach J, Barden N. Genome-wide search for linkage of bipolar affective disorders in a very large pedigree derived from a homogeneous population in Quebec points to a locus of major effect on chromosome 12q23-q24. Am J Med Genet 1999;88:567–87.
[91] Vandel P, Vandel S, Kantelip JP. SSRI-induced bleeding: two case reports. Therapie 2001;56:445–7.
[92] Layton D, Clark DW, Pearce GL, Shakir SA. Is there an association between selective serotonin reuptake inhibitors and risk of abnormal bleeding? Results from a cohort study based on prescription event monitoring in England. Eur J Clin Pharmacol 2001;57:167–76.
[93] Dalton SO, Johansen C, Mellemkjaer L, Norgard B, Sorensen HT, Olsen JH. Use of selective serotonin reuptake inhibitors and risk of upper gastrointestinal tract bleeding: a population-based cohort study. Arch Int Med 2003;163:59–64.