

Late Developmental Stage-Specific Role of Tryptophan Hydroxylase 1 in Brain Serotonin Levels

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Serotonin [5-hydroxytryptamine (5-HT)] is a major therapeutic target of psychiatric disorders. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting reaction in the biosynthesis of 5-HT. Two isoforms (TPH1 and TPH2) having tryptophan hydroxylating activity were identified. Association studies have revealed possible TPH1 involvement in psychiatric conditions and behavioral traits. However, *TPH1* mRNA was reported to be mainly expressed in the pineal gland and the periphery and to be barely detected in the brain. Therefore, contribution of TPH1 to brain 5-HT levels is not known, and the mechanisms how TPH1 possibly contributes to the pathogenesis of psychiatric disorders are not understood. Here, we show an unexpected role of TPH1 in the developing brain. We found that TPH1 is expressed preferentially during the late developmental stage in the mouse brain. TPH1 showed higher affinity to tryptophan and stronger enzyme activity than TPH2 in a condition reflecting that of the developing brainstem. Low 5-HT contents in the raphe nucleus were seen during development in New Zealand white (NZW) and SWR mice having common functional polymorphisms in the *TPH1* gene. However, the 5-HT contents in these mice were not reduced in adulthood. In adult NZW and SWR mice, depression-related behavior was observed. Considering an involvement of developmental brain disturbance in psychiatric disorders, TPH1 may act specifically on development of 5-HT neurons, and thereby influence behavior later in life.

Key words: behavior; depression; RAPHE; serotonin; tryptophan hydroxylase; development

Introduction

Serotonin [5-hydroxytryptamine (5-HT)] has been implicated in a variety of physiological and pathological functions in CNSs (Lucki, 1998; Lesch, 2004). Numerous studies have suggested associations between various neuropsychiatric disorders and genes that modulate central serotonergic neurotransmission, such as the 5-HT transporter (Lesch et al., 1996), 5-HT receptors (Lucki, 1998), and monoamine oxidases (Shih et al., 1999). Therefore, the brain 5-HT system is a major target for several psychiatric disorders such as tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and psychostimulants (Lucki, 1998; Gainetdinov and Caron, 2003).

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in 5-HT synthesis, which catalyzes the hydroxylation of L-tryptophan. Two isoforms (TPH1 and TPH2) having tryptophan hydroxylating activity were identified (Kuhn et al., 1980;

Hasegawa and Ichiyama, 1987; Walther et al., 2003; Zhang et al., 2004). *TPH2* mRNA was reported to be preferentially expressed in the brain (Walther et al., 2003). In contrast, *TPH1* mRNA was reported to be barely detected in the brain and instead to be mainly expressed in the pineal gland and the periphery (Walther et al., 2003). Thus, the role of TPH1 in serotonin synthesis in the brain is not explored.

Nevertheless, *TPH1* gene has been the subject of intensive study regarding its possible involvement in many psychiatric and behavioral traits. Polymorphisms within the *TPH1* gene have been reported to be associated with suicidal behavior (Mann et al., 1997; Nielsen et al., 1998; Rujescu et al., 2003; Bellivier et al., 2004). Furthermore, the polymorphisms are closely implicated in antidepressant response in patients with unipolar major depression (Peters et al., 2004).

Considering subtle *TPH1* expression in the brain (Walther et al., 2003), it seems to be difficult to elucidate the functional link between *TPH1* polymorphisms and psychiatric disorders. However, based on the observation in rats that the *TPH1* mRNA level peaked at postnatal day 22 (P22) and then declined between P22 and P61 in the dorsal raphe nucleus, which correlated well with tryptophan hydroxylase enzyme activities in raphe (Rind et al., 2000), TPH1 may affect 5-HT levels specifically in the developing brain. In addition to its action as a crucial neurotransmitter in various regions in the adult brain, 5-HT is essential for development of 5-HT neurons in an autocrine manner (Gaspar et al.,

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2003). Thus, changes in brain 5-HT contents during development could disturb development of 5-HT neurons, which might cause permanent changes in adult behavior.

In the present investigation, we sought to ask the hypothesis by estimating kinetic properties of TPH1 in a condition mimicking that of the developing brain. 5-HT contents *in vivo* were also studied using brains from several mouse strains. Finally, we assessed depression-related behavior of mice showing low TPH1 expression during development.

Materials and Methods

Animals. SWR/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Other mice were obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan). Animals were maintained according to the guidelines of Juntendo University.

Reverse transcription-PCR and *in situ* hybridization. Reverse transcription-PCR was done using total RNA derived from brain and primers: *TPH1*, 5'-GCTCTCAAATACTGTGGCTATCGG-3' and 5'-AACCTGAACACTCTGTGTGTACGG-3'; *TPH2*, 5'-CACTGCTCTCAGCACCAGGGTCTGGACA-3' and 5'-GGAACCACGGCACA-TCCCTCGAGATCTTCTT-3'; *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)*, 5'-CGACTGCCAGAACATCATCCCTGC-3' and 5'-TTAGTGGGCCCTGGATGCCAGCTT-3'. Because 27 PCR cycles yielded products that were in the linear range of logarithm, we chose 27 and 40 cycles. *GAPDH* was used for internal control. Relative product densities for multiple experiments were estimated. For *in situ* hybridization, sense and antisense digoxigenin-labeled cRNA probes specific to *TPH1* were designed according to the study by Côté et al. (2003). *In situ* hybridization was done using coronal brain sections from C57BL/6 mice, 250 μ m in thickness, essentially as described previously (Ravassard et al., 1997). Signals were visualized with anti-digoxigenin-conjugated alkaline phosphatase and bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate (Vector Laboratories, Burlingame, CA). The sense control probe gave no signals (data not shown).

Sequencing and luciferase reporter assay. The 5'-flanking sequences (-430 to +117 relative to the transcription start site) and a 209 bp fragment from the 3'-untranslated regions (UTRs) of the *TPH1* gene were generated by PCR from genomic DNA of C57BL/6, C57BL/10, C3H, New Zealand black (NZB), New Zealand white (NZW), and SWR mice, using appropriate primers. Sequencing analysis was performed using the fragments.

To yield luciferase constructs, the PCR product of the promoter region from NZB or NZW mice was inserted into upstream of the luciferase coding region of the pGL3-Basic vector (Promega, Madison, WI), and those of the 3'UTRs were inserted into downstream of the luciferase coding region of the pGL3-Promoter vector having the cytomegalovirus promoter (Promega). Differentiated RN46A cells (1×10^6) were transiently transfected with 5 μ g of each luciferase construct by electroporation using a Gene pulser (300 V/950 μ F; Bio-Rad, Hercules, CA). Two days after electroporation, luciferase activity was measured for a 20 s time course with the use of a dual system (Promega) and a luminometer (Lumat LB 9507; Berthold, Wildbad, Germany). The relative luciferase activities were expressed based on the activity of the construct without the promoter (pGL3-Basic vector) or that without the 3'UTR of *TPH1* (pGL3-Promoter vector).

Neurochemical analysis. To examine TPH1 and TPH2 enzyme activities, the coding region of the *TPH1* or *TPH2* gene was inserted into the *NotI*-*Apal* fragment of pMT/V5-HisB (Invitrogen, Carlsband, CA) to yield pMTTPH1His or pMTTPH2His. The *NotI*-*PmeI* fragment from pMTTPH1His or pMTTPH2His, including the coding region of each gene, and the histidine hexamer region was blunted and ligated with *NotI*-digested and blunted pUC-CAGGS (Niwa et al., 1991). Then, 5×10^6 Cos 7 cells grown to subconfluence were transiently transfected with 20 μ g of each expression plasmid (pCAGTPH1His or pCAGTPH2His) by electroporation (300 V/950 μ F). Two days after transfection, cells were harvested, and the amount of TPH1 or TPH2 protein fused to the histidine hexamer was evaluated by Western blot analysis using anti-

histidine-hexamer antibody (Sigma, St. Louis, MO). Then, the TPH activity was determined through a three-step procedure (Iida et al., 2002).

For the quantitation of tissue tryptophan, the brainstem of male mice at P21 was dissected under anesthesia with diethyl ether and frozen at -80°C until analysis. Tissues were homogenized in 0.1 N HCl then deproteinized in 1 M perchloric acid. Determinations were made using an HPLC system equipped with a fluorescence monitor (model FP920; JASCO, Tokyo, Japan) set at 290 and 350 nm for excitation and emission, respectively. The solid phase was octadecyl silica (4.6×250 mm; Jasco Finepak SIL-C18T5), the mobile phase was a 100:5:10 mixture of 40 mM sodium acetate (adjusted to pH 3.5 with formic acid), acetonitrile, and methanol, and the flow rate was 1 ml/min.

The determination of tissue tetrahydrobiopterin was determined by a differential oxidation procedure as described previously (Sawabe et al., 2004).

Immunohistochemistry. Animals were deeply anesthetized by injection of pentobarbital-Na (5 mg/animal, i.p.) and then perfused transcardially with saline followed by a fixative solution [4% PFA in 0.1 M phosphate buffer (PB)]. The brains were dissected out from the skull and postfixed overnight at 4°C in the same fixative solution. Then, brains were immersed in 0.1 M PB containing 30% sucrose until they sank. For the immunohistochemistry of 5-HT, the sections (50 μ m in thickness) were incubated with anti-5-HT antibody (Chemicon, Temecula, CA) and visualized with 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.01% H_2O_2 in 50 mM Tris-HCl, pH 7.5. The intensities of signals were quantitatively estimated using NIH Image.

Behavioral tests. For the forced swim test, a cylindrical container (height, 50 cm; 20 cm in diameter) with 30 cm of water maintained at 22°C was used. Behavior in the water was categorized into immobility or swimming, and immobility time was counted. Immobility time was assessed using two different schedules. The test was done during 15 min on the first day. After 24 h, mice were placed in the water for 5 min again. In addition, to assess the effects of antidepressants, the test was done during 5 min. Paroxetine (8 mg/kg, s.c.; GlaxoSmithKline, Uxbridge, UK) or imipramine (30 mg/kg, i.p.; Sigma) was injected 30 min before the test. Paroxetine and imipramine were dissolved in water and saline, respectively.

For the tail suspension test, mice were fastened with adhesive tape by the tip (2 cm) of the tail to a flat steel bar placed horizontally at the height of 30 cm above the ground in a white Plexiglas box that isolated the mouse from visual distractions while permitting observation of behavior. The presence or absence of immobility, defined as the absence of limb movement, was examined during a 5 min test session.

Statistics. Statistical significance was set at $p < 0.05$ using Student's *t* test.

Results

To explore the possible role of TPH1 in the brain, we first assessed *TPH1* mRNA expression levels in the C57BL/6 mouse brainstem at P7, P21, and 2 months by semiquantitative RT-PCR using specific primers. The *TPH1* expression was highest at P21 (Fig. 1A). The expression was not detected at P7. Similarly, *TPH1* mRNA expression was detected in the raphe nucleus of C57BL/6 mice at P21 by *in situ* hybridization using a digoxigenin-labeled *TPH1*-specific riboprobe (Fig. 1B). However, only faint signal was detected in adulthood. In contrast, apparent difference was not found in *TPH2* mRNA levels between P21 and adult stage (Fig. 1A). Our observation was in line with a previous report in rats that the *TPH1* mRNA level peaked at P22 and then declined between P22 and P61 in the dorsal raphe nucleus of rats (Rind et al., 2000).

Sophisticated analysis using human TPH1 or TPH2 fusion protein elucidated different kinetic properties between the two enzymes (McKinney et al., 2005), in which fusion proteins from *Escherichia coli* and *in vitro* transcription/translation system were assessed in various tryptophan and 6R-*L*-erythro-5,6,7,8-tetrahydrobiopterin (BH4) concentrations. However, the kinetic

properties were not determined at tryptophan and BH4 concentrations reflecting those in the brain. We extended the analysis in a condition mimicking the brainstem of a P21 mouse. Histidine hexamer-tagged mouse TPH1 or TPH2 protein was expressed in Cos7 cells, and the dependence of the enzyme activities of TPH1 and TPH2 on concentrations of the substrate tryptophan and the cosubstrate BH4 were compared with the respective cell extracts (Fig. 1C). The expression level of TPH1 or TPH2 fusion protein was estimated by Western blot analysis using anti-histidine hexamer antibody. The efficiency of the 5-HT precursor, 5-hydroxytryptophan (5-HTP), formation of TPH1 was much higher than that of TPH2 at all concentrations of BH4 and tryptophan. Apparent K_m values for tryptophan at 400 μM BH4 were 16.6 and 19.2 μM in TPH1 and TPH2, respectively. However, the affinity was much higher in TPH1 at a lower concentration of BH4; the K_m was 7.5 μM for tryptophan at 4 μM BH4, whereas that of TPH2 was constant (19.2 μM), suggesting that TPH1 sustains enzyme activity even with lowered tryptophan levels under possible BH4-limiting conditions.

We determined tissue BH4 and tryptophan contents in the brainstem of various mouse strains at P21. BH4 content was ~ 0.9 nmol/g. Assuming an even distribution of BH4 in the tissue, ~ 1 μM could be estimated, which was far less than the K_m values of both TPH1 and TPH2 for BH4 (~ 20 μM). Because tryptophan content was between 15 and 30 nmol/g wet tissue, the K_m of TPH2 for tryptophan (19.2 μM) was around the tissue tryptophan level, whereas the K_m of TPH1 for tryptophan (7.5 μM) was several-fold lower than the tissue level at the BH4 concentration (4 μM), essentially reflecting that in the brainstem of P21 mouse. Under the conditions mimicking the brainstem of a P21 mouse, higher affinity to tryptophan of TPH1 was apparent, and higher efficiency of 5-HTP formation of TPH1 was also observed in our condition.

Then, we determined the contribution of TPH1 to brain 5-HT contents *in vivo*. Because constitutive genetic deletions of serotonergic molecules can lead to compensatory changes (Ramboz et al., 1998; Dulawa et al., 2000), we used several strains of laboratory mice. Promoter and 3'UTRs of genes affect the expression level. We isolated the two regions of *TPH1* gene from C57BL/6, C57BL/10, C3H, NZB, NZW, and SWR mice and performed sequencing analysis. We found polymorphisms in the promoter region (Fig. 2A, top). Notably, NZW and SWR mice had common polymorphisms, including putative Sp1 binding site. To ask whether the polymorphisms shared by NZW and SWR mice affect TPH1 ex-

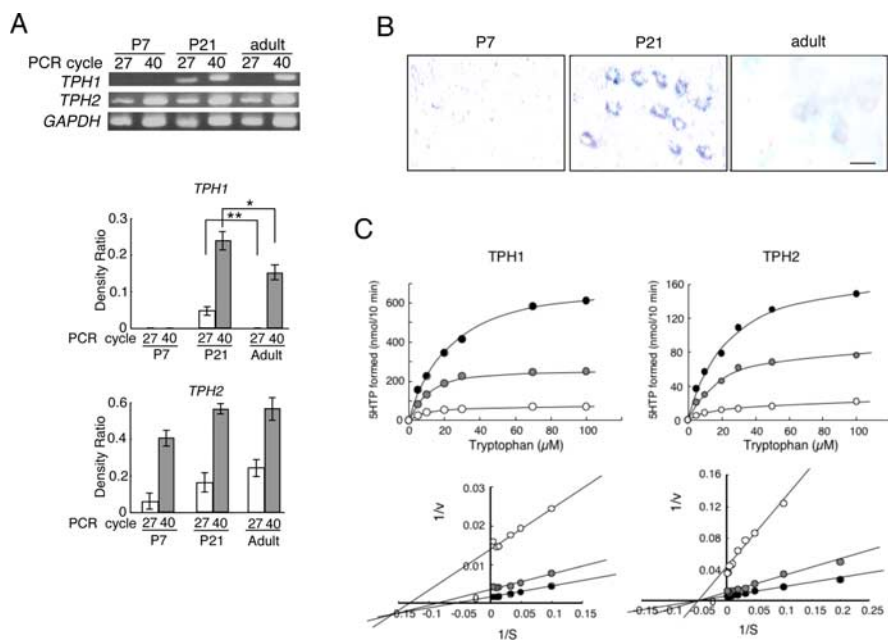


Figure 1. Characterization of TPH1 and TPH2. **A, B**, Developmental change in the expression of *TPH1* and *TPH2* mRNAs in the brainstem. Brains of C57BL/6 mice at P7, P21, and adult stage were studied using semiquantitative RT-PCR with 27 or 40 cycles (*TPH1* and *TPH2*) (**A**) or *in situ* hybridization (*TPH1*) (**B**). Plots of ratio of *TPH1* and *TPH2* product densities to those of *GAPDH* are also shown ($n = 4$, each) (**A**). Scale bar, 20 μm . Error bars represent mean \pm SEM. $*p < 0.05$; $**p < 0.01$. **C**, Comparison of the kinetics between TPH1 and TPH2. The top panels are the Michaelis–Menten plots of tryptophan hydroxylase activity (TPH1/left vs TPH2/right) versus tryptophan concentrations at three different concentrations of BH4 (4 μM , open circle; 40 μM , gray circle; 400 μM , closed circle). The bottom panels are corresponding double reciprocal plots. K_m values estimated at the extrapolated cross points for TPH1 were 16.6, 11.4, and 7.5 μM at 400, 40, and 4 μM BH4, respectively, and 19.2 μM at all BH4 concentrations for TPH2.

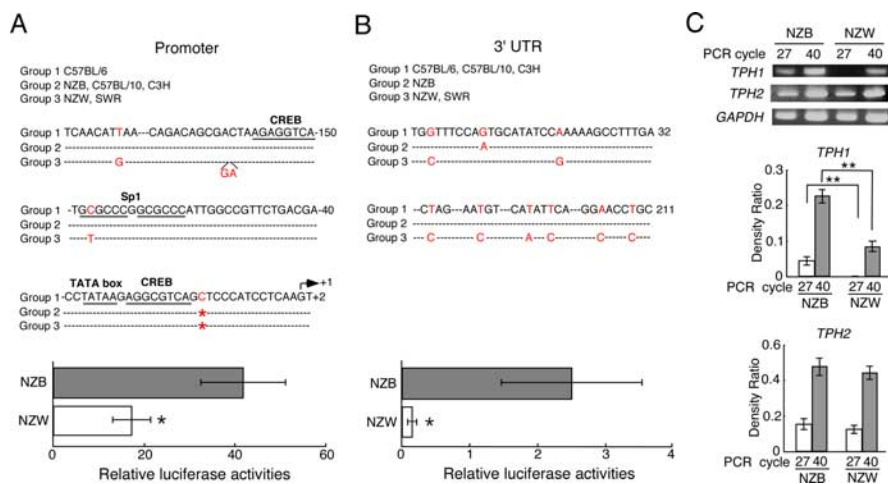


Figure 2. Functional polymorphisms in the *TPH1* gene in NZW and SWR mice. **A, B**, Sequences of the promoter (**A**) and 3' UTR (**B**) of *TPH1* from several strains (top) and luciferase activities using sequences from NZB and NZW mice (bottom). The sequence is numbered relative to the transcription initiation site (+1) (**A**) or the 3' UTR initiation site (**B**). Identity and a single base pair deletion are indicated by — and *, respectively. Mismatch is indicated by the corresponding nucleotides. Putative transcription factor binding sites are underlined according to the study by Stoll and Goldman (1991). **C**, Comparison of *TPH1* and *TPH2* mRNA levels between NZB and NZW mouse brains at P21 using semiquantitative RT-PCR with 27 or 40 cycles. Plots of ratio of *TPH1* and *TPH2* product densities to those of *GAPDH* are also shown ($n = 4$, each). Error bars represent mean \pm SEM. $**p < 0.01$.

pression level, we did luciferase reporter assay. The *TPH1* gene fragment of the promoter region from NZB or NZW mice was linked to the luciferase coding region, and each construct was transfected into RN46A cells, a serotonergic cell line established from embryonic raphe (White et al., 1994). The relative luciferase activity of the promoter region from NZW mice was significantly lower than that of the NZB mice (Fig. 2A, bottom).

We also found common polymorphisms in the 3' UTR of

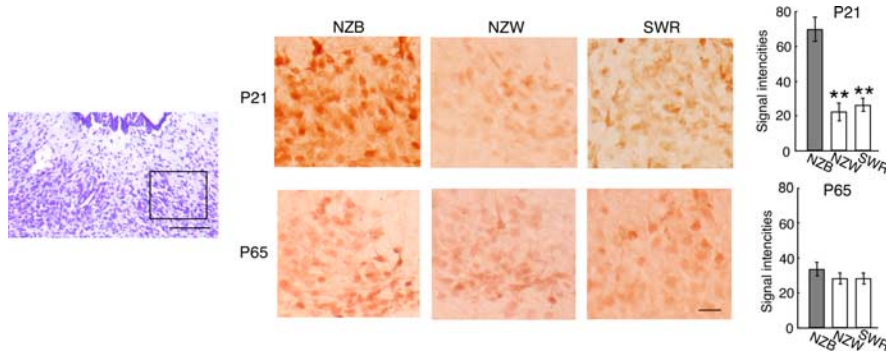


Figure 3. Decreased 5-HT levels in the developing raphe nucleus of NZW and SWR mice. Lateral parts of dorsal raphe nucleus, indicated by the open square in the Nissl-stained coronal sections of the brainstem (left), were stained with anti-5-HT antibody in NZB, NZW, and SWR mice at P21 and P65. The intensities of signals were quantitatively shown (right). Scale bars: left, 200 μ m; middle, 50 μ m. Error bars represent mean \pm SEM. ** p < 0.01.

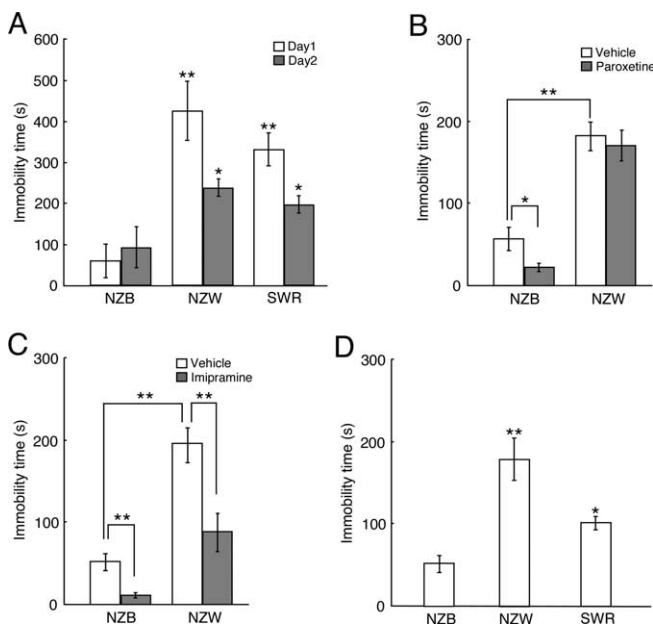


Figure 4. Depression-related behavior. **A–C**, Forced swim test. Immobility time was measured during 15 min on day 1 and 5 min on day 2 in NZB ($n = 6$), NZW ($n = 12$), and SWR ($n = 8$) mice (**A**). To assess the effects of antidepressants, immobility time of paroxetine-treated NZB ($n = 6$) or NZW ($n = 7$) mice was compared with that of vehicle-treated mice ($n = 7$, each) during 5 min (**B**). Imipramine-treated NZB ($n = 7$) or NZW ($n = 7$) mice were also compared with vehicle-treated mice during 5 min ($n = 7$ and 8, respectively) (**C**). **D**, Tail suspension test. Immobility time was measured during 5 min in NZB ($n = 7$), NZW ($n = 7$), and SWR ($n = 8$) mice. Error bars represent mean \pm SEM. * p < 0.05, ** p < 0.01.

NZW and SWR mice (Fig. 2*B*, top). 3'UTR regulates mRNA stability and translation. The luciferase activity of the construct having the *TPH1* 3'UTR from NZW mice was weaker than that of NZB mice (Fig. 2*B*, bottom). Thus, the polymorphisms in both the promoter and 3'UTR of the *TPH1* gene proved to affect *TPH1* expression. Consistently, *TPH1* mRNA expression level in the brainstem of NZW mice at P21 was lower than that of NZB mice (Fig. 2*C*).

Remarkably, immunohistochemistry revealed that 5-HT levels in the dorsal raphe nucleus were lower in NZW and SWR mice than NZB mice at P21 (Fig. 3), which was also the case in the median raphe nucleus (data not shown). However, essentially no difference was found in adulthood.

TPH2 mRNA levels were comparable between NZB and NZW

mice at P21 (Fig. 2*C*), and no polymorphisms were found in the coding region of the *TPH2* gene from NZW and SWR mice (data not shown). In addition, we did not detect the differences in the expression of *monoamine oxidase A* and *serotonin transporter* between NZB and NZW mice (data not shown). Thus, the lower 5-HT levels in the raphe nucleus of NZW mice at 3 weeks of age seems to be attributable to low *TPH1* expression.

We then sought to determine the effect of the low *TPH1* expression in the developing brain on adult behavior using adult NZW and SWR mice. Because the polymorphisms in the *TPH1* gene showed significant association when response to fluoxetine is compared with nonresponse in

patients with unipolar major depression (Peters et al., 2004), we initially chose the forced swim test, a behavioral test for the animal model of depression (Porsolt et al., 1977). On the first day, the mouse was placed in the water for 15 min. The mouse showed swimming or immobility. The immobility time was counted as an index of depression-related behavior. On the next day, the mouse was again introduced in the water and was tested for 5 min. As shown in Figure 4*A*, longer immobility times were observed in NZW and SWR mice than in NZB mice on both days 1 and 2.

We then examined whether antidepressants could overcome the longer immobility time in NZW mice. NZB or NZW mice treated with vehicle or selective serotonin reuptake inhibitor paroxetine were subjected to the forced swim test for 5 min (Fig. 4*B*). Again, the vehicle-treated NZW mice showed longer immobility time than the vehicle-treated NZB mice. Paroxetine treatment decreased immobility in NZB mice but not in NZW mice, suggesting that low 5-HT levels during development in NZW mice might induce functional alterations in 5-HT system. In contrast, imipramine treatment partially decreased immobility in NZW mice (Fig. 4*C*). Likewise, NZW and SWR mice showed longer immobility time than NZB mice in the tail suspension test (Fig. 4*D*), another animal model of depression (Steru et al., 1985). Because motor performance in the runway task was indistinguishable between NZB and NZW mice (K. Nakamura, S. Hirose, unpublished data), the longer immobility time in the two tests seems not to be caused by impaired general motor ability. Thus, adult NZW and SWR mice showed depression-like behavior in the two tests.

Discussion

We explored the developmental stage-specific role of *TPH1*. Of particular importance is that our inspection of kinetic comparison between *TPH1* and *TPH2* was assessed in a condition reflecting that of the developing brainstem, that is, low BH4 concentration (4 μ M). In such a condition, higher affinity to tryptophan of *TPH1* and higher enzyme activity of *TPH1* than of *TPH2* were apparent. The K_m of *TPH2* for tryptophan was around the tissue tryptophan level, whereas the K_m of *TPH1* for tryptophan was several-fold lower than the tissue level. These data likely suggest that *TPH1* uses tryptophan to synthesize 5-HT more efficiently than *TPH2*, and *TPH1* exerts most of its full activity with respect to tryptophan dependence. Because *TPH1* was expressed predominantly during the late developmental stage, 5-HT contents during the period might be affected by *TPH1* expression level. Indeed, NZW and SWR mice having low *TPH1* expression

showed low brain 5-HT contents only during development *in vivo*. Thus, TPH1 is involved in 5-HT levels in the brain during late developmental stage but not in the adult.

5-HT acts as a crucial neurotransmitter in various regions in the adult brain and is closely implicated in psychiatric disorders (Lesch et al., 1996; Lucki, 1998; Shih et al., 1999; Gainetdinov and Caron, 2003; Lesch, 2004). Because 5-HT synthesis in the adult brain is dependent on TPH2 (Walther et al., 2003), polymorphisms in TPH2 might affect 5-HT levels in the adult. Numerous genetic association analyses will be needed to determine whether TPH2 is involved in psychiatric conditions.

The human *TPH1* gene represents candidate genes for psychiatric conditions. The *TPH1* intron 7 A779C polymorphism has been reported to be associated with suicidal behavior in depressed patients (Mann et al., 1997) and in alcoholic offenders (Nielsen et al., 1998). Meta-analyses also provided evidence for an association of suicide-related behavior with TPH1 polymorphism (Rujescu et al., 2003; Bellivier et al., 2004). TPH1 may contribute to psychiatric conditions without affecting brain 5-HT levels in the adult. 5-HT has an additional role during development. 5-HT is essential for the development of 5-HT neurons such as neurogenesis, apoptosis, axon branching, dendritogenesis, and the fine wiring of brain connections in an auto-crine manner (Gaspar et al., 2003). Targeted deletions of molecules involved in 5-HT metabolism or transport in mice affected dendritic maturation during development (Gaspar et al., 2003). However, gross abnormalities were not found in these mice. *TPH1* expression could not be detected using RT-PCR at P7. Instead, *TPH1* was expressed at later developmental stage. Thus, TPH1 is likely involved in the fine-tuning or maturation of 5-HT neurons, which might cause permanent changes in adult behavior. We showed depression-related behavior in adult NZW and SWR mice using the forced swim test and tail suspension test. Both mice are suitable strains to study the roles of TPH1 during childhood in behavior in the adult. Altered response to paroxetine treatment in NZW mice may be caused by functional changes in 5-HT neuronal circuit or 5-HT receptors. Considering neurodevelopmental models of psychiatric disorders (Luna and Sweeney, 2001), the relationship between the polymorphisms in the *TPH1* gene and functional neurodevelopmental abnormalities might be considered in patients with psychiatric disorders. Our study opens a new avenue for understanding of temporally distinct contribution of each TPH to 5-HT neuronal functions associated with psychiatric disorders.

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