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Expression of PTPH1, a rat protein tyrosine phosphatase, is restricted to the derivatives of a specific diencephalic segment

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ABSTRACT **Studies to date have identified only a few proteins that are expressed in a segment-specific manner within the mammalian brain. Here we report that a nonreceptor protein tyrosine phosphatase, PTPH1, is selectively expressed in the adult thalamus. Expression of PTPH1 mRNA is detected in most, but not all, thalamic nuclei. Nuclei that are derived embryonically from the dorsal thalamus and project to the neocortex express this gene, whereas those derived from the ventral thalamus do not. PTPH1 mRNA expression is also restricted to the dorsal thalamus during development and, thus, can serve as a specific marker for the dorsal thalamic nuclei. Since the subcellular localization of PTPH1 protein is not known, its functional role is not clear. However, the restriction of its expression to the thalamic nuclei that have thalamocortical connections suggests that PTPH1 may play a role in the maintenance of these connections or in determining the physiological properties of thalamic relay nuclei.**

The mature brain of all mammals can be divided into different regions on the basis of distinct cellular and biochemical composition, patterns of connectivity, and electrophysiological properties. Although the regional organization of the mammalian brain is well established, the molecular mechanisms by which regional differences develop are largely unknown (1). One approach to understanding the mechanisms by which regional differences are achieved is to identify genes or gene products that are expressed in discrete regions. This approach has resulted in the discovery of a small number of region-specific markers, including the limbic-system-associated membrane protein (LAMP) (2), the striatal phosphoprotein DARPP-32 (3), and the cerebellar Purkinje cell protein L7 (4). LAMP is a surface glycoprotein thought to mediate the innervation of the limbic cortex by specific afferent fibers (5), and DARPP-32 is a cytoplasmic protein that acts as an inhibitor of serine/threonine phosphatases (6). Less is known about the cellular function of L7 in Purkinje cells.

Another region-specific marker, striatum-enriched phosphatase (STEP), is a protein tyrosine phosphatase (PTPase) expressed at very high levels in the rat caudate and putamen (7). In a number of systems, PTPases have been shown to play important roles in cellular differentiation (8, 9). The hallmark of the PTPase family is the presence of a conserved catalytic domain that preferentially dephosphorylates proteins on tyrosine residues. Within the family of PTPases, two subfamilies have been characterized: low molecular weight nonreceptor PTPases and high molecular weight receptor PTPases. The STEP gene encodes a nonreceptor PTPase and gives rise to at least two alternatively spliced transcripts. A 4.4-kb mRNA is present in many brain areas and a 3.0-kb mRNA is dramatically enriched in the striatum. Within the striatum, STEP is expressed by dopaminoceptive neurons (10), and it has been suggested (10) that STEP may play a role in determining neuronal properties specific to the striatum.

The possibility that PTPases may play a role in region-specific neuronal functions is also indicated from studies in *Drosophila*. In the *Drosophila* nervous system, three receptor PTPases are expressed by subsets of neurons only during development (11–13). Two of these, DLAR and DPTP99A, are relatively uniformly expressed in all of the developing axonal tracts, while the third, DPTP10D, is enriched in the longitudinal connectives and anterior commissures. After completion of axonogenesis, mRNAs for all three PTPases are down-regulated.

Given the possible involvement of PTPases in neuronal differentiation and regional specification, we have been studying the expression of PTPase genes in the developing and mature mammalian central nervous system (14, 15). In this paper, we report that a nonreceptor PTPase, PTPH1, is expressed in a regionally specific pattern in the rat brain. PTPH1 was originally cloned from HeLa cells and has homology to the cytoskeleton-associated proteins band 4.1, ezrin, and talin (16). Here, we show that PTPH1 expression is markedly enriched in the thalamus of the adult rat brain. Within the thalamus, PTPH1 mRNA is expressed in most but not all nuclei. PTPH1 is expressed in those thalamic nuclei that are embryonically derived from the dorsal thalamus but not in those that are derived from the ventral thalamus. During development, PTPH1 gene expression is specific to the dorsal thalamus as early as embryonic day 19 (E19) and maintains this regional specificity throughout postnatal life. These observations suggest that PTPH1 is an early and specific marker for the dorsal thalamic nuclei.

MATERIALS AND METHODS

Nucleic Acid Probes. For Northern blot and *in situ* hybridization, we used a partial cDNA probe (D3) corresponding to bp 1337–1586 of the published PTPH1 sequence (16). This cDNA was subcloned into the Bluescript KS vector (Stratagene). The probe for Northern blot hybridization was PCR-generated from this cDNA clone followed by random labeling using [^{32}P]dCTP (Amersham) and NEBlot kit (New England Biolabs). For *in situ* hybridization, ^{35}S-labeled CTP (NEN)-labeled complementary RNA probes were generated by using the T7 or T3 promoters in Bluescript vector and the Riboprobe system (Promega). RPTPγ antisense (15) and PTPH1 sense probes were used as positive and negative controls, respectively.

Northern Blot Hybridization. For the analysis of tissue distribution, Northern blots of RNA from various regions of adult Sprague–Dawley rat brains were generously supplied by Tony Ciaberra (Yale University). Total RNA was extracted from rat brain by using RNAzol B (Cinna/Biotecx Laboratories, Friendswood, TX), based on the method of Chomczynski

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Abbreviations: STEP, striatum-enriched phosphatase; PTPase, protein tyrosine phosphatase; E, embryonic day.

and Sacchi (17). Total RNA (20 μg per lane) was separated on a 1% agarose/formaldehyde gel and transferred to Biotrans(+) nylon membrane (ICN). Equality of loading levels was confirmed by adding ethidium bromide to each RNA sample and examining the 28S and 18S rRNAs under UV illumination. Northern blot hybridization was carried out in 7% (wt/vol) SDS/1% bovine serum albumin/0.5 M sodium phosphate, pH 6.8 (PB)/1 mM EDTA for 18 h at 65°C. Hybridization solution contained the D3 probe (1.2×10^6 cpm/ml) made by random primed labeling. After hybridization, the blot was washed for 15-min periods twice in 5% SDS/0.5% bovine serum albumin/40 mM PB/1 mM EDTA and once in 1% SDS/40 mM PB/1 mM EDTA at 65°C. RNA molecular weight standards (GIBCO/BRL) were used to estimate the sizes of the transcripts. The blot was exposed to Amersham Hyperfilm between two intensifying screens for 7 days.

***In Situ* Hybridization.** *In situ* hybridization was as described (15). Brains from Sprague–Dawley rats taken on E19 and postnatal days 0, 8, and 15 and from adults were frozen on dry ice. Frozen sections (12 μm thick) were thaw-mounted onto gelatin-coated slides and postfixed in 0.1 M sodium phosphate-buffered (pH 7.4) 4% (wt/vol) paraformaldehyde. Sections were rinsed in PBS (137 mM NaCl/2.7 mM KCl/10 mM Na_2HPO_4)/1.8 mM KH_2PO_4/2× SSC and acetylated with 0.5% acetic anhydride in 0.1 M triethanolamine (pH 8.0). Sections were then rinsed in 2× SSC/PBS, dehydrated in ethanols, and delipidated in chloroform. Sections were prehybridized in 2× SSC/50% (vol/vol) formamide at 50°C for 1 h and then hybridized in 50% formamide/1× Denhardt's solution/0.75 M NaCl/10% (wt/vol) dextran sulfate/30 mM dithiothreitol/10 mM Tris·HCl, pH 7.5/1 mM EDTA/tRNA (0.5 mg/ml)/salmon sperm DNA (100 μg/ml)/probe (1×10^6 cpm) at 50°C for 12–15 h. After hybridization, slides were washed in 2× SSC/50% formamide/0.1% 2-mercaptoethanol at 50°C, treated with RNase A (20 μg/ml), washed for 30-min periods in 2× SSC/50% formamide/0.1% 2-mercaptoethanol at 58°C and in 0.1× SSC/0.1% 2-mercaptoethanol at 65°C.

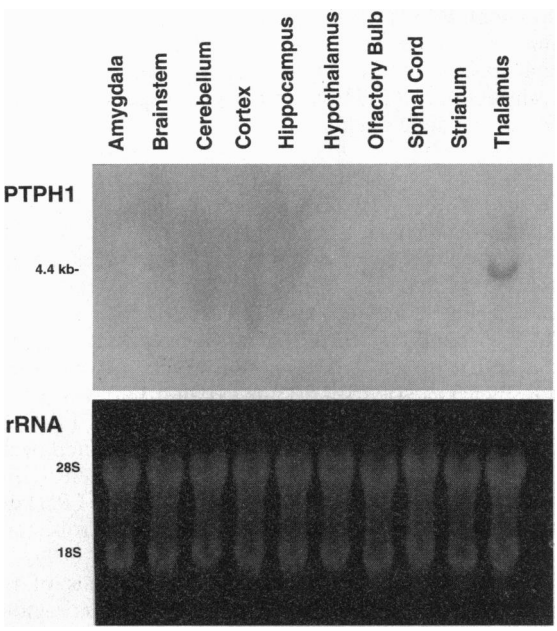

FIG. 1. Northern blot hybridization demonstrates that PTPH1 mRNA is enriched in the thalamus. (*Upper*) Total RNA extracted from different regions of the adult central nervous system was hybridized with probe D3. Only the lane containing RNA from the thalamus shows a band of 4.4 kb. (*Lower*) Equal loading of the lanes was confirmed by ethidium bromide staining and photographing the gel. The rRNA visualized in this manner is shown.

For initial localization of the probe, slides were exposed to film (Hyperfilm, Amersham) for 3–14 days. Autoradiograms were used as negatives for prints. For higher-resolution analysis of probe distribution, slides were dipped in NTB-2 emulsion (Kodak), developed after 35 days, and counterstained with cresyl violet.

RESULTS

PTPH1 Is Encoded by a Single Transcript in the Adult Rat Brain. We used a cloned exon probe, D3 (bp 1337–1586), to

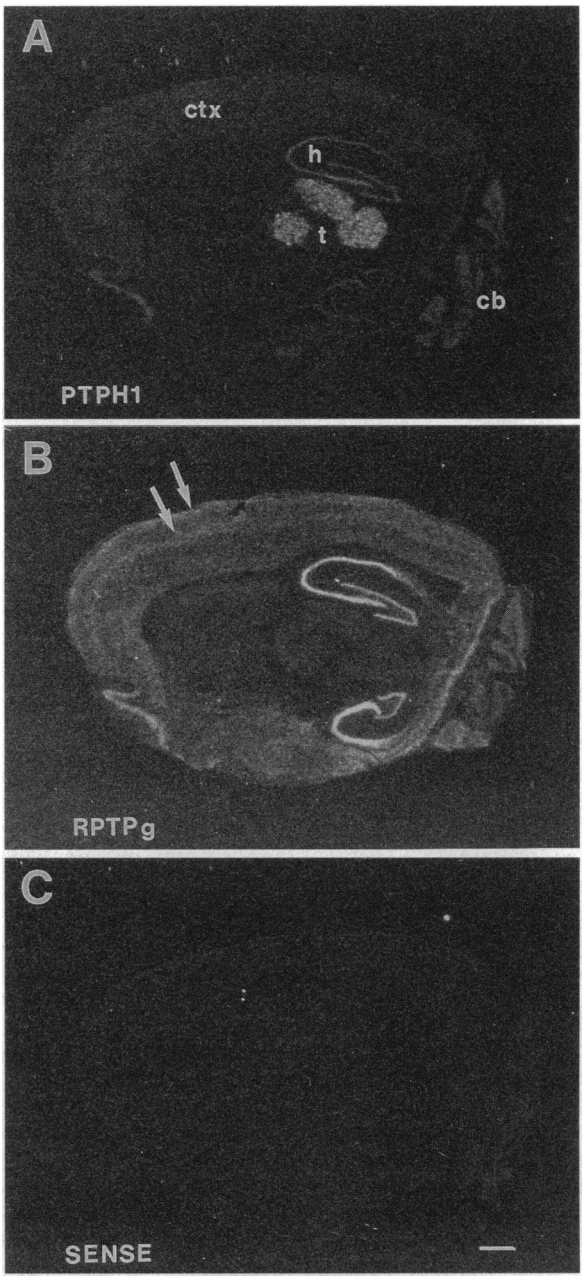

FIG. 2. *In situ* hybridization demonstrates that PTPH1 mRNA is largely restricted to the thalamus. Near-adjacent parasagittal sections of adult brains were hybridized with radiolabeled antisense RNA probes for PTPH1 (*A*) and RPTPγ (*B*) and sense probes for PTPH1 as a negative control (*C*). (*A*) PTPH1 RNA is greatly enriched in the thalamus (t). Low levels of signal are seen in the hippocampus (h) and cerebellum (cb). (*B*) RPTPγ RNA expression is highest in the hippocampus and is also present in the neocortex (ctx), particularly in the middle layers (arrow), and in the cerebellum. No signal is seen in the thalamus. (*C*) Sense probe for PTPH1 shows no signal in the brain. (Bar = 100 μm.)

determine the tissue distribution of PTPH1 mRNA (18). Clone D3 was chosen for this analysis because it lies outside of the conserved PTPase and band 4.1 homology domains of PTPH1 and does not show homology to other genes in GenBank (25).

The expression of PTPH1 in different regions of the adult rat brain was determined initially by Northern blot hybridization (Fig. 1). Hybridization with clone D3 revealed a single band of 4.4 kb, which is very close in size to the transcript detected in HeLa cells (16). This band was detected only in RNA prepared from the adult thalamus. The RNA from other regions of the adult brain including amygdala, brainstem, cerebellum, cortex, hippocampus, hypothalamus, olfactory bulb, spinal cord, and striatum did not produce a signal at an exposure time sufficient to give a strong signal in thalamus RNA. After much longer exposure times (30 days), very faint bands of the same molecular size were detected in the RNA from hippocampus and cerebellum (data not shown). These data indicate that the antisense PTPH1 probe is specific for a single RNA species on Northern blots and that the expression of this transcript is greatly enriched in the rat thalamus.

PTPH1 Expression in the Adult Thalamus. To confirm the thalamus-enriched expression of PTPH1, *in situ* hybridization was performed on adult brain sections. Parasagittal sections of the whole brain were hybridized with an antisense D3 probe. PTPH1 labeling was almost completely restricted to the thalamus (Fig. 2*A*). Within the thalamus, the labeling was not uniform and was most intense in a subset of nuclei, including the medial geniculate nucleus, the lateral geniculate nucleus, and the ventral basal complex. The hippocampus, piriform cortex, cerebellum, and neocortex also showed hybridization signal, but at much lower levels. As a control, a probe for RPTPγ, a receptor PTPase that is expressed in the thalamus during early postnatal ages, was used (15, 19). Hybridization with RPTPγ was highest in the adult cortex, especially in layer 4, and in the hippocampus (Fig. 2*B*). No signal was seen in the adult thalamus. Hybridization with the sense probe for D3 produced no signal (Fig. 2*C*). These experiments show that the hybridization of D3 to the thalamus is specific and is different from other PTPases that are more widely expressed in the brain.

To identify the individual diencephalic nuclei that contain PTPH1 RNA, a series of coronal sections of the adult rat brain were studied. To distinguish among the thalamic nuclei, emulsion-dipped slides were counterstained with cresyl violet. Autoradiograms and high magnification micrographs are shown in Fig. 3 and summarized in Table 1. In the most anterior sections examined, PTPH1 was expressed in the ventral medial, ventral posterior, central median, mediodorsal, and lateral dorsal thalamic nucleic (Fig. 3*A*). Labeling was absent from the reticular thalamic nuclei, zona incerta, subthalamus, and habenula. In more posterior sections, PTPH1 labeling was again detected in the ventral posterior thalamic nuclei, the dorsal lateral geniculate, and posterior thalamic nuclei (Fig. 3*B*). The superior thalamic radiation accounts for the gap between the ventral posterior and dorsal lateral geniculate nuclei. The ventral lateral geniculate nuclei and zona incerta also showed no labeling. Further to the posterior in the thalamus, the medial geniculate nuclei were the only labeled diencephalic structures (Fig. 3*C*). Both ventral and dorsal medial geniculate nuclei contained PTPH1 labeling. In addition, the pretectum and superior colliculi did not show any PTPH1 signal. In all these sections, pyramidal cells in fields CA1–3 of the hippocampus and granular cells of the dentate gyrus showed low hybridization. Adjacent sections probed with antisense RPTPγ and sense PTPH1 probes exhibited no signal in the diencephalon (data not shown).

PTPH1 Is Restricted to the Dorsal Thalamus During Development. We next examined PTPH1 expression during development by *in situ* hybridization with sections of brain

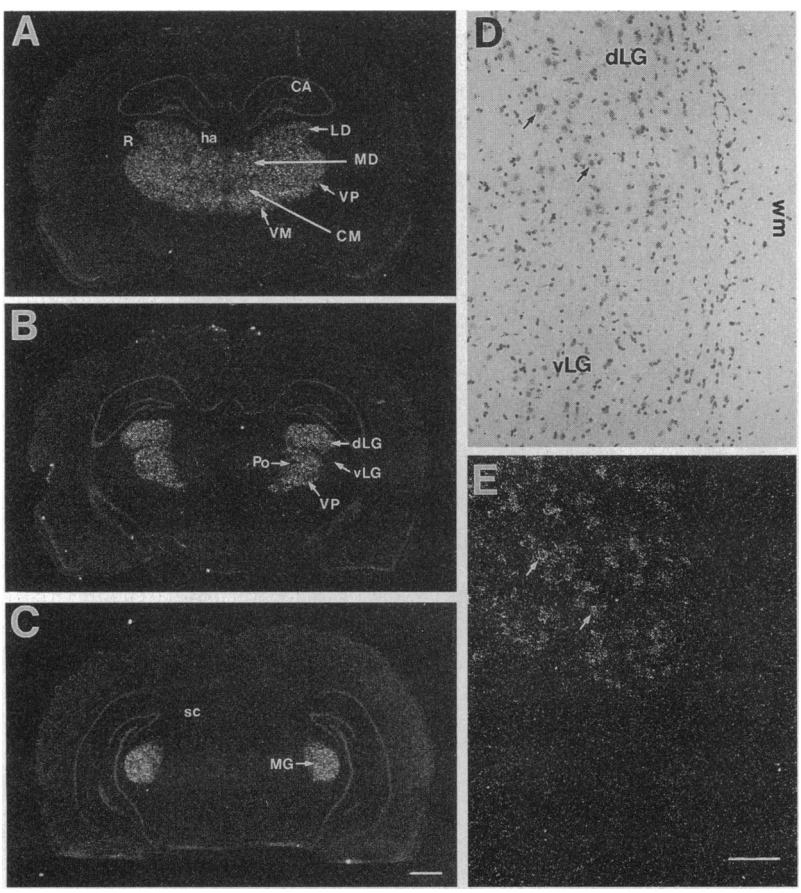

FIG. 3. PTPH1 is expressed in most, but not all, thalamic nuclei. Serial coronal sections of the adult thalamus were hybridized with radiolabeled antisense PTPH1 probe. (*A*) PTPH1 is detected in the ventral medial (VM), ventral posterior (VP), central median (CM), mediodorsal (MD), and lateral dorsal (LD) thalamic nuclei. Labeling is absent from the reticular thalamic nuclei (R) and habenula (ha). (*B*) PTPH1 is expressed in the VP and the dorsal lateral geniculate (dLG) and posterior (Po) thalamic nuclei. (*C*) In more caudal sections, the medial geniculate nuclei (MG) are intensely labeled. The superior colliculi (sc) do not show any PTPH1 signal. In all these sections, CA fields of the hippocampus (CA) and the dentate gyrus show low hybridization. (*D* and *E*) Emulsion-dipped slide of a section near-adjacent to *B* was counterstained with cresyl violet and photographed under bright-field optics for cell localization (*D*) and under dark-field optics for visualization of the silver grains generated by PTPH1 probe (*E*). PTPH1 signal is very high over neurons (arrows) in dorsal lateral geniculate (dLG) nucleus. In contrast, neurons in the neighboring ventral lateral geniculate (vLG) nucleus contain no labeling. White matter (wm) similarly shows no labeling. (Bars: *A–C*, 1.5 mm; *D–E*, 25 μm.)

Table 1. Expression of PTPH1 mRNA in diencephalic nuclei

Nucleus	PTPH1 RNA
Dorsal thalamus	
Anterodorsal nucleus	+
Anteroventral nucleus	+
Anteromedial nucleus	+
Ventral medial nucleus	+
Ventral posterior nucleus	+
Central median nucleus	+
Mediodorsal nucleus	+
Lateral dorsal nucleus	+
Dorsal lateral geniculate nucleus	+
Posterior nucleus	+
Dorsal medial geniculate nucleus	+
Ventral medial geniculate nucleus	+
Ventral thalamus	
Reticular nucleus	−
Ventral lateral geniculate nucleus	−
Zona inserta	−
Epithalamus	
Habenula	−

+, PTPH1 mRNA detected; −, not detected.

starting at E19. PTPH1 RNA was detected as early as E19 in the diencephalon in horizontal sections of the head (Fig. 4*A*). At this stage, hybridization was seen in the analog of the dorsal thalamus and was absent from ventral thalamus (Fig. 4 *D* and *E*) (20). In addition to the diencephalic labeling, PTPH1 mRNA was also detected at high levels in the olfactory epithelium at E19. The expression in the olfactory epithelium is consistent with a previous study that used PCR to show PTPH1 expression in this tissue (21). At E19, PTPH1 mRNA was present at very low levels in the cortex and hippocampus. By postnatal day 8, PTPH1 signal was most intense in the thalamus compared to the rest of the brain (Fig. 4*B*). The neocortex and hippocampus expressed PTPH1 mRNA at low levels throughout the postnatal period. The thalamus-enriched expression of PTPH1 persisted during the first 2 weeks of development (Fig. 4*C*). Thus, PTPH1 is expressed in a thalamus-enriched pattern throughout postnatal life.

DISCUSSION

The nonreceptor PTPase PTPH1 is highly enriched in the adult thalamus compared to other parts of the brain. Within the thalamus, the expression of PTPH1 is widespread, excluding only the ventral lateral geniculate and reticular thalamic nuclei. The thalamus-enriched pattern of expression is established as early as E19 and is retained throughout postnatal development. STEP, another nonreceptor PTPase, also shows a region-specific pattern of expression, being largely restricted to the striatum. In contrast, other nonreceptor PTPases, such as PTPase 1 (22), P19-PTP (14, 23–25), and syp (26–29), are widely expressed throughout the developing central nervous system (15, 27).

PTPH1 Expression and Segmentation of the Diencephalon. The diencephalon can be divided into four compartments: epithalamus, dorsal thalamus, ventral thalamus, and hypothalamus (30). Three lines of evidence from recent experiments indicate that the developing diencephalon is a segmented structure composed of several neuromeres, similar to the organization of the developing hindbrain. (*i*) Clones of cells generated from a single precursor are restricted to one neuromere (31). (*ii*) Neighboring groups of cells in adjacent neuromeres do not mix (31). (*iii*) The expression patterns of several transcription factors and signaling proteins respect neuromeric borders. For instance, Dlx-1 and Dlx-2 are preferentially expressed in the ventral thalamus during embryonic development, whereas Wnt-3 and Gbx-2 are expressed in the dorsal thalamus (32, 33). The diencephalic neuromeres thus identified have been designated D1–D4 by Figdor and Stern (31) or P1–P3 by Rubenstein *et al.* (34). In this discussion, we will use the numbering system from the Rubenstein and Puelles neuromeric model (34).

PTPH1, a PTPase gene, is expressed in a segment-specific pattern in the diencephalon. Its expression in the adult thalamus includes all thalamic nuclei except for the ventral lateral geniculate and reticular nuclei. These two nuclei differ from the rest of the thalamus in two major ways: both are embry-

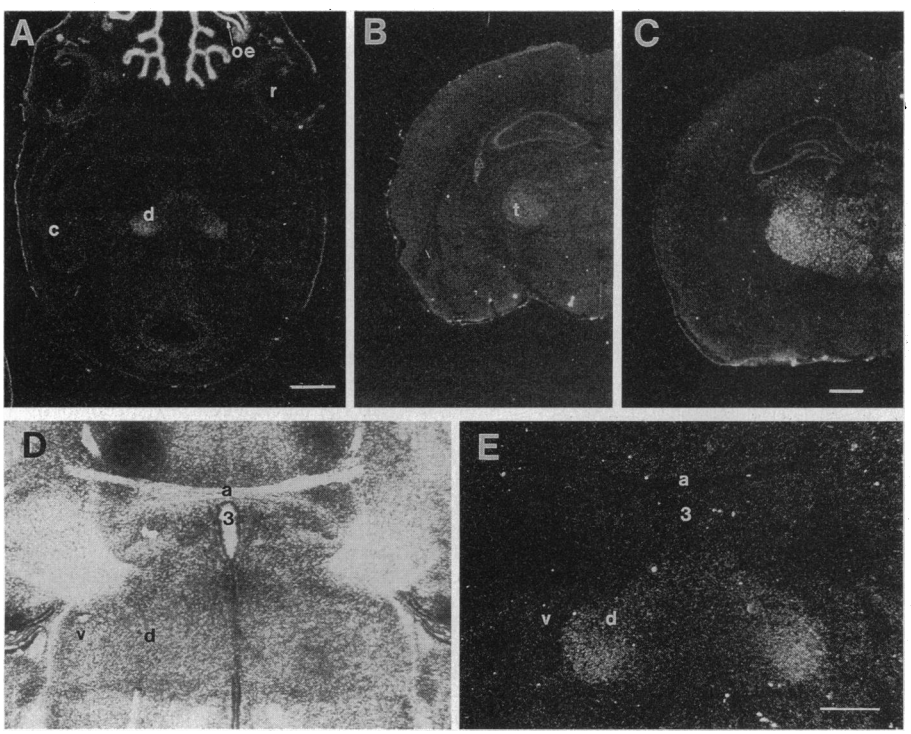

FIG. 4. PTPH1 expression is restricted to the dorsal thalamus throughout development. Transverse section of E19 head (*A*) and coronal section of postnatal days 8 (*B*) and 15 (*C*) and adult (*D*) brains are hybridized with the antisense probe for PTPH1. (*A*) Within the E19 brain, the diencephalon (d) shows labeling for PTPH1 RNA. A much lower level of signal is detected in the cortex (c). Outside of the brain, the olfactory epithelium (oe) also exhibits intense PTPH1 signal. There is no labeling in the retina (r). (*B*) At postnatal day 8, PTPH1 mRNA is detected in the thalamus (t) and hippocampus. (*C*) By postnatal day 15, PTPH1 labeling is most intense in the thalamus. The hippocampus and entorhinal cortex show low levels of labeling. (*D* and *E*) Emulsion-dipped and counterstained slide of a section near-adjacent to that in *A* was photographed under dark-field illumination (*E*) for PTPH1 silver grain visualization and under bright-field illumination (*D*) for cellular localization. PTPH1 labeling is detected in the dorsal thalamus (d) but not in the ventral thalamus (v). Anterior commissure (a) and third ventricle (3) are shown for orientation. (Bars: *A*, 100 μm; *B* and *C*, 100 μm; *D* and *E*, 40 μm.)

onically derived from the ventral thalamus and neither projects to the cortex (20). The other nuclei that make up the adult thalamus are derived from the embryonic dorsal thalamus and almost all have thalamocortical connections. Therefore, PTPH1 is expressed in all adult thalamic nuclei generated from the dorsal thalamus and is absent from the nuclei generated from the ventral thalamus. PTPH1 mRNA is not detected in the zona limitans, which forms the border between the dorsal and ventral thalamus. The expression pattern of PTPH1 most closely corresponds to the location of the diencephalic neuromere P2. However, as currently described, P2 includes both the dorsal thalamus and the epithalamus, and PTPH1 is not expressed in the epithalamus or habenula. This indicates that PTPH1 is restricted to a subsegment of neuromere P2.

Products of two other genes, Wnt-3 and Gbx-2, are also restricted to the P2 segment of the diencephalon (32, 33). Wnt-3 belongs to a family of secreted glycoproteins that are required for pattern formation in *Drosophila* and mammalian embryogenesis. Transgenic mice with null mutations of the *Wnt-1* gene lack a midbrain (35). Gbx-2 is a transcription factor that belongs to the homeobox family (33, 36). While Wnt-3 is expressed in the epithalamus and the dorsal thalamus, Gbx-2 is restricted to the dorsal thalamus. Thus, the pattern of PTPH1 expression overlaps closely with the pattern of expression of the homeobox gene *Gbx-2*.

PTPases are expressed in restricted patterns in both the *Drosophila* and mammalian brains; however, there are differences between the expression in the two systems. (*i*) Unlike STEP and PTPH1, axon-pathway-specific PTPases in the *Drosophila* are receptor PTPases. (*ii*) Subsequent to axonogenesis, the mRNAs for the *Drosophila* PTPases are downregulated, strongly suggesting that they play a role in establishing, rather than maintaining, axon pathways. In contrast, the expression of STEP and PTPH1 is maintained in the adult in a region-specific manner. Like PTPH1, Wnt-3 expression is also retained in the adult thalamus (32).

The subcellular localization of the protein product(s) generated by PTPH1 gene and its substrates are not yet known, so the functional role of this PTPase in the thalamus is far from clear. However, based on its sequence homology and expression pattern, several possibilities can be considered. (*i*) Both the Wnt family of glycoproteins and tyrosine phosphorylation can regulate cell–cell adhesion by modulating cadherin/catenin function (37), suggesting that PTPH1 may be a part of the effector pathway for Wnt-3 in the dorsal thalamus. (*ii*) The restricted expression of PTPH1 to the thalamic nuclei that send axons to the neocortex would be consistent with a structural or regulatory role specific to these axons. The PTPH1 gene has high sequence homology to members of the band 4.1 superfamily, which are thought to mediate the linkage of actin filaments to the plasma membrane (38). Some members of this family are found in membrane extensions such as growth cones and others are located at focal adhesion plaques. Thus, the PTPH1 protein could play a role in the maintenance of thalamocortical connections by regulating cytoskeleton–membrane interactions. (*iii*) PTPH1 function may give rise to some of the physiological properties that are selective to thalamocortical neurons, perhaps through the dephosphorylation of transmembrane proteins, such as ion channels. Accumulating evidence suggests that tyrosine phosphorylation of neurotransmitter- or voltage-gated ion channels can regulate electrical activity and neuronal modulation (39). The thalamus is a relay station for sensory information to the neocortex, and PTPH1 may participate in the phosphorylation/dephosphorylation cascades that regulate coordinated activity of thalamocortical neurons (40).

In summary, we report a PTPase that is expressed in a segment-specific pattern in the diencephalon. The expression pattern of PTPH1 demonstrated in this study provides further support for the neuromeric theories of forebrain development and suggests that PTPases may play a role in the region-specific functions of the nervous system.

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