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PTP NE-6: A Brain-Enriched Receptor-Type Protein Tyrosine Phosphatase with a Divergent Catalytic Domain

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Abstract: A receptor-type protein tyrosine phosphatase, PTP NE-6, was identified from rat olfactory epithelial cDNA and cloned from a rat brain cDNA library. PTP NE-6 mRNA is abundant in brain and expressed at lower levels in olfactory tissue and adrenal gland. In situ hybridization demonstrates that PTP NE-6 mRNA is expressed throughout the brain, with highest levels in the medial habenula and at intermediate levels in layer IV of cortex, medial geniculate nucleus, inferior colliculus, hypothalamus, and thalamus. The predicted amino acid sequence demonstrates that PTP NE-6 contains a single catalytic domain that diverges from the consensus protein tyrosine phosphatase catalytic domain by expressing an aspartate instead of the conserved alanine residue in the catalytic site. Recombinantly expressed PTP NE-6 does not exhibit detectable phosphatase activity. Upon mutation of the aspartate to the consensus alanine, phosphatase activity toward *p*-nitrophenyl phosphate is observable with a k_{cat} value of 3.7 s^{-1} and a K_m of 980 μM. These data demonstrate that the inactivity of native PTP NE-6 toward *p*-nitrophenyl phosphate is due to the divergent aspartate in the catalytic site and not to variant amino acids within the phosphatase domain. **Key Words:** Olfactory—Adrenal—Rat—Protein tyrosine phosphatase.
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Protein tyrosine phosphorylation plays a crucial role in regulating a variety of signal transduction pathways by altering the activity of enzymes and controlling protein interactions. Much emphasis has been placed on elucidating the role of protein tyrosine kinases (PTKs) in signal transduction pathways. By comparison, less is known about the functions of protein tyrosine phosphatases (PTPs). The PTPs are classified as either intracellular or transmembrane and are most similar at the PTP catalytic domain. The intracellular PTPs usually contain only one PTP domain and flanking sequences that are important in protein interactions or intracellular targeting, whereas the transmembrane phosphatases usually have two PTP domains, a single hydrophobic membrane-spanning domain, and an extracellular domain often containing adhesion molecule-like motifs. The transmembrane PTPs are also called receptor-type PTPs (rPTPs) because of their potential to transmit signals across the plasma membrane, although this concept has yet to be widely demonstrated.

Evidence suggesting a role for protein phosphorylation in regulating axonal guidance and neural development is accumulating. In *Drosophila,* neuronal pathway selection is altered in mutants of the PTK derailed (*drl*) (Callahan et al., 1995) and in double mutants involving the PTK *abl* (Elkins et al., 1990). As counterparts to the PTKs, the PTPs are also thought to be important in mediating similar processes. Recently, the *Drosophila* PTPs, DLAR, DPTP69D, and DPTP99D, have been shown by genetic mutation analysis to be necessary for motor neuron axonal pathfinding, establishing rPTPs as important regulators of neural development (Desai et al., 1996; Krueger et al., 1996). Some of the PTPs have been localized to neurons (Lombroso et al., 1993; Pan et al., 1993; Boulanger et al., 1995) and, in some cases, have been shown to be specifically expressed in the axonal growth cone (Tian et al., 1991). This pattern of expression suggests a role for PTPs in neural development. The extracellular domains of many rPTPs contain adhesion molecule-like motifs (Canoll et al., 1993; Walton et al., 1993; Yan et al., 1993; Zhang and Longo, 1995), which are associated with axonal growth, further substantiating the putative role of PTPs in axonal guidance and neural development.

To understand better the evolving role of PTPs in

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The Genbank accession number for the nucleotide sequence reported in this article is U73458.

Abbreviations used: EGF, epidermal growth factor; pNPP, *p*-nitrophenyl phosphate; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; RACE, rapid amplification of cDNA ends; rPTP, receptor-type PTP; SDS, sodium dodecyl sulfate; SSC, saline sodium citrate buffer; UTR, untranslated region.

neural function, it was of interest to identify novel members of this family. We chose the olfactory system as a source of novel PTPs, because this tissue is an exceptional model for the study of neuronal guidance and development. Unlike most adult mammalian nervous tissue, which contains terminally differentiated neurons, olfactory neuroepithelium consists of a receptor neuron population that continually regenerates throughout life. Newly formed primary olfactory neurons, which are derived from a stem cell population, continually reinnervate the olfactory bulb as mature neurons degenerate (Graziadei and Monti-Graziadei, 1979; Caggiano et al., 1994). An extremely high degree of fidelity is required for an axonal process to fasciculate along the olfactory nerve and form a synapse on a precise and distinct subset of the olfactory bulb neurons of the CNS. Potentially, PTPs may be involved in this process, and such PTPs may be expressed in olfactory receptor neurons to confer these unique properties of regeneration in the olfactory system.

To discover PTPs in this system, we performed PCR using degenerate primers to the conserved phosphatase catalytic region of PTPs. We have identified a cDNA encoding a rPTP, PTP NE-6, and have shown that PTP NE-6 mRNA is abundantly expressed in regions of the CNS. Unlike most receptor phosphatases, which contain two catalytic domains, PTP NE-6 contains a single catalytic domain. This domain diverges from the consensus catalytic site by expressing an aspartate instead of the conserved alanine residue. Phosphatase catalytic assays show that recombinantly expressed PTP NE-6 is inactive toward the substrate *p*-nitrophenyl phosphate (pNPP). Analysis of the catalytic site by site-directed mutagenesis reveals that the alanine-to-aspartate divergence is responsible for its lack of activity. The similarity of the extracellular domain and unusual catalytic site of PTP NE-6 to another described rPTP (Lu et al., 1994; Rabin et al., 1994; Kambayashi et al., 1995; Magistrelli et al., 1995; Passini et al., 1995) may be evidence of an emerging new class of rPTPs.

EXPERIMENTAL PROCEDURES

PCR

Degenerate PCR was performed as described previously (Walton et al., 1993). In brief, first strand cDNA was prepared with RNA from rat olfactory epithelium and oligo(dT) primer. The cDNA was amplified by PCR using degenerate primers based upon the conserved phosphatase catalytic domains. The primers, which contained restriction sites for the enzymes *Bam*HI and *Xba*I, were as follows: 5′-CAGTGGATCCAA(A/G)TG(C/T)(C/G)(A/C)N(C/G)A(A/G)TA(C/T)TGGCC-3′ and 5′-CTAGTCTAGACCNA(T/C)(T/A/G)CCNGC(A/G)CT(A/G)CA(A/G)TG-3′.

Library screen, 5′ RACE (rapid amplification of cDNA ends), and sequencing

Approximately 1×10^6 plaques from a rat whole brain λ-zap cDNA library (Stratagene) were plated and immobilized on Protran nitrocellulose filters (Schleicher and Schuell). The novel PTP NE-6 PCR fragment was labeled by random priming and used as a probe for both the primary and secondary screens. Filters were hybridized with 1×10^6 cpm/ml of probe in 6 × saline sodium citrate buffer (SSC), 0.25% dry milk, and 100 μg/ml salmon sperm DNA at 60°C overnight. The filters were washed in 2 × SSC and 0.1% sodium dodecyl sulfate (SDS) and then in 0.5 × SSC and 0.1% SDS at 60°C. 5′ RACE was performed to obtain the 5′ end of the clone by using the 5′ RACE kit (GibcoBRL). A 5′ specific primer (5′-AGAGCGCGAGGCAGTGGCGGC-3′) was used for the reverse transcription of rat whole brain RNA. A nested 5′ specific primer (5′-AGCAGCAGCAGCCAAAAGCGGGAGC-3′) along with the anchor primer supplied in the kit was used for PCR. A band of ~200 bp was ligated into the vector PCR2.1 (Invitrogen). Sequencing was performed by dye terminator cycle sequencing (Perkin–Elmer Applied Biosystems).

Expression of PTP NE-6 intracellular domain

A mutation in PTP NE-6 was created by the Transformer site-directed mutagenesis kit (Clontech) to change the aspartate (D936) in the catalytic site to an alanine. The primer, 5′-CGCCGGCACTGCAATGGACAATTATC-3′, was used to introduce the alanine residue into the catalytic site. The primer, 5′-CACTAGTTCAAGAGCGGCCGCCAC-3′, was used to eliminate the unique restriction site for *Xba*I from pBluescript, and an *Xba*I digestion was used to select for plasmids carrying the desired mutations. Fragments of both PTP NE-6 and D936A mutant DNA that included the coding sequence for the entire cytoplasmic domains from amino acids 629–1,004 were amplified by PCR. The primers were 5′-CATGCGGAATTCGCCACAACTCACACTAC-3′ and 5′-TGATACCTCGAGCTACTGGGGAAGGGCCTTC-3′ and included restriction sites for *Eco*RI and *Xho*I, respectively. The PCR products were digested with the restriction enzymes *Eco*RI and *Xho*I and cloned into pGEX-KG (Guan and Dixon, 1991*b*). *Escherichia coli* BL21 cells (Novagen) were transformed with the constructs and cultured at 30°C. Expression of protein was induced with 0.2 m*M* isopropyl β-D-thiogalactopyranoside, and protein purification and thrombin cleavage were performed according to Guan and Dixon (1991*b*).

Phosphatase catalytic activity assay

The catalytic activities of PTP NE-6, D936A mutation, and LAR were determined by using the artificial substrate pNPP. The assay was performed at 37°C for 15 min in a 200-μl reaction volume containing 20 m*M* Tris-HCl (pH 5.6), 100 m*M* NaCl, 1 m*M* dithiothreitol, 1 m*M* EDTA, and 3.5 μg of PTP protein. The reaction was terminated by addition of 800 μl of 0.2 *M* NaOH, and the amount of product formed was determined by absorbance measured at 410 nm.

Northern blot

Total RNA was isolated from rat brain, adrenal gland, and olfactory neuroepithelium with TRIzol reagent (GibcoBRL), and poly(A)$^+$ RNA was isolated by using the PolyAtract kit (Promega). Five micrograms of poly(A)$^+$ RNA was size-fractionated on a 0.66 *M* formaldehyde/agarose gel at 80 V for 4 h and transferred to a nitrocellulose filter (MSI). PTP NE-6 cDNA was labeled by random priming, and hybridization was performed in 50% formamide, 4 × SSC, 20 m*M* Tris-HCl (pH 8.0), 1 × Denhardt's solution, 0.1% SDS, 200 μg/ml salmon sperm DNA at 42°C, and

washed at high stringency (0.2 × SSC, 0.1% SDS, 65°C). A multiple rat tissue northern blot (Clontech) containing 2 μg of poly(A)$^+$ RNA from heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis was also hybridized according to the manufacturer's protocol.

In situ hybridization analysis

Coronal and sagittal sections (14 μm) of frozen rat brain were cut on a cryostat, mounted on RNase-free slides, and stored at −80°C. Prior to hybridization, the sections were incubated at room temperature for 10 min in 4% formaldehyde, washed for 2 min in phosphate-buffered saline, incubated 15 min in 0.25% acetic anhydride in 0.1 *M* triethanolamine (pH 8.0), and washed for 2 min in phosphate-buffered saline. Sections were then dehydrated in an ethanol series (2 min each in 30, 70, and 100% ethanol) and air-dried. Two riboprobes corresponding to the 3′UTR (untranslated region) of PTP NE-6 were synthesized with [^{33}P]CTP using T7 RNA polymerase. The riboprobes were hybridized overnight at 60°C in 50% formamide, 0.6 *M* NaCl, 10 m*M* Tris-HCl (pH 7.5), 1 × Denhardt's solution, 250 μg/ml tRNA, and 10% dextran sulfate. Following hybridization, sections were rinsed in 2 × SSC for 2 min, treated with RNase A (20 μg/ml) for 30 min at room temperature, washed in 2 × SSC for 30 min, washed in 0.2 × SSC for 1 h at 55°C, and finally air-dried and exposed to film.

RESULTS

Cloning of a novel PTP

A degenerate PCR strategy was used to find cDNAs encoding novel PTPs. First strand cDNA was synthesized from mRNA extracted from rat olfactory neuroepithelium. Degenerate primers corresponding to the conserved amino acid sequence of the phosphatase catalytic domain were used for PCR to amplify putative PTP sequences. Six PCR fragments of ~280 bp encoded novel PTPs (Walton et al., 1993). One of these PTP sequences, PTP NE-6, was characterized by cloning a full-length cDNA.

Northern blot analysis was performed on poly(A)$^+$ RNA from rat brain and olfactory neuroepithelium to determine the size of the transcript and the abundance of the clone in these tissues. The major transcript was ~5.1 kb and was more abundant in brain, so we chose to screen a rat whole brain cDNA library (Stratagene). We identified 23 overlapping clones by screening ~1 × 10^6 plaques using the radiolabeled PCR fragment as a probe. The largest clone was 5,051 bases, consistent with the approximate size of the hybridization signal on the northern blot. The putative initiating methionine was not preceded by an in-frame stop, indicating that additional coding region was possibly present in the 5′ direction. However, 85 bp of new 5′ sequence obtained by 5′ RACE contained an in-frame stop preceding the originally identified putative start site.

Sequence analysis of PTP NE-6

The nucleotide sequence of PTP NE-6 contains an open reading frame from nucleotides 112–3,126 and encodes a predicted protein of 1,004 amino acids (Fig. 1). Immediately surrounding the putative ATG initiation site, the nucleotide sequence corresponds at nine of 13 nucleotides with the consensus start site described by Kozak (1991). The sequence following the putative initiation site resembles a signal peptide (Gierasch, 1989). Although signal peptide sequences are diverse, the general structure features a positively charged amino acid within 10 amino acids after the methionine, followed by seven to 15 primarily hydrophobic residues, and ending with three to seven polar residues. The putative signal sequence of PTP NE-6 does not contain a charged amino acid, but does have a long stretch of hydrophobic residues followed by polar residues. Cleavage of the peptide often occurs at an alanine residue 20–25 amino acids after the methionine. Three alanine residues are present 20–25 amino acids from the putative methionine. A hydrophobic stretch of 25 amino acids is present at positions 604–625, indicating a probable transmembrane domain. The long 3′UTR of 2,009 bp contains a polyadenylation signal (AATAAA) at nucleotides 5,120–5125.

Sequence homology searches of Genbank and EMBL databases revealed that PTP NE-6 is identical to the recently published rat PTP, phogrin (Wasmeier and Hutton, 1996), which was isolated from insulinoma cells. Two mouse homologues, IA-2β (Lu et al., 1996) and PTP NP (Chiang and Flanagan, 1996), have also been reported recently. PTP NE-6 is also similar to the rat ICA105 gene (Passini et al., 1995), a putative tyrosine phosphatase, which is also known as ICA512 (Rabin et al., 1994), IA-2 (Lu et al., 1994; Lan et al., 1996), PTP35 (Magistrelli et al., 1995), and PTP LP (Kambayashi et al., 1995). PTP NE-6 and ICA105 have ~73% amino acid identity within the intracellular domain and a 25% amino acid identity within the extracellular domain, including a conserved cysteine repeat in the N-terminal domain.

Unlike most rPTPs, which contain two catalytic domains, PTP NE-6 and ICA105 contain a single phosphatase domain (Fig. 2). The C-terminal catalytic site contained in the phosphatase domain of both these clones is divergent by one amino acid. Instead of the conserved alanine residue in the consensus sequence of HCXAGXXRS/TG, an aspartate residue is present. This change has been shown previously to eliminate the phosphatase activity of the PTP LAR toward a variety of substrates (Streuli et al., 1990).

Expression of the intracellular domain of PTP NE-6

To determine if PTP NE-6 possesses phosphatase catalytic activity, a chimeric gene encoding a fusion protein of glutathione *S*-transferase at the N-terminus with the intracellular domain of PTP NE-6 was constructed. In addition, a similar chimeric gene containing a single-point mutation of the catalytic domain was also generated that converted the divergent aspartate (D936) to the consensus alanine. As shown in Fig. 3, recombinantly expressed PTP NE-6 lacks activity, whereas the

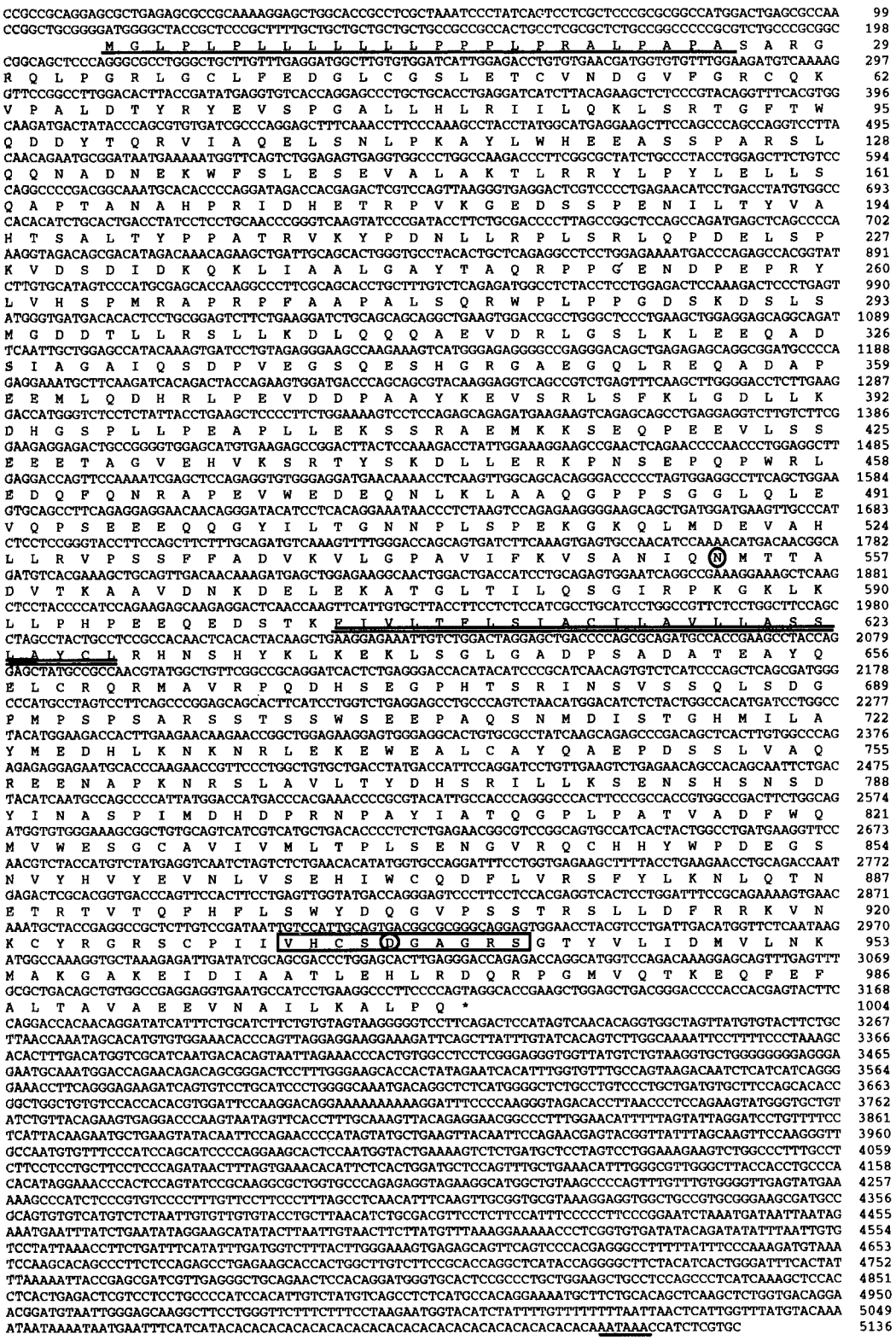

FIG. 1. Complete nucleotide and predicted amino acid sequence of PTP NE-6. The deduced amino acid sequence of PTP NE-6 is shown directly below the cDNA sequence. The putative signal sequence is *underlined.* A potential *N*-glycosylation site is *circled.* The transmembrane domain is *double-underlined* and was determined based upon a hydrophobicity plot (not shown). The putative phosphatase catalytic site is *boxed,* and the divergent aspartate (D) is *circled.* Polyadenylation signal is *underlined.*

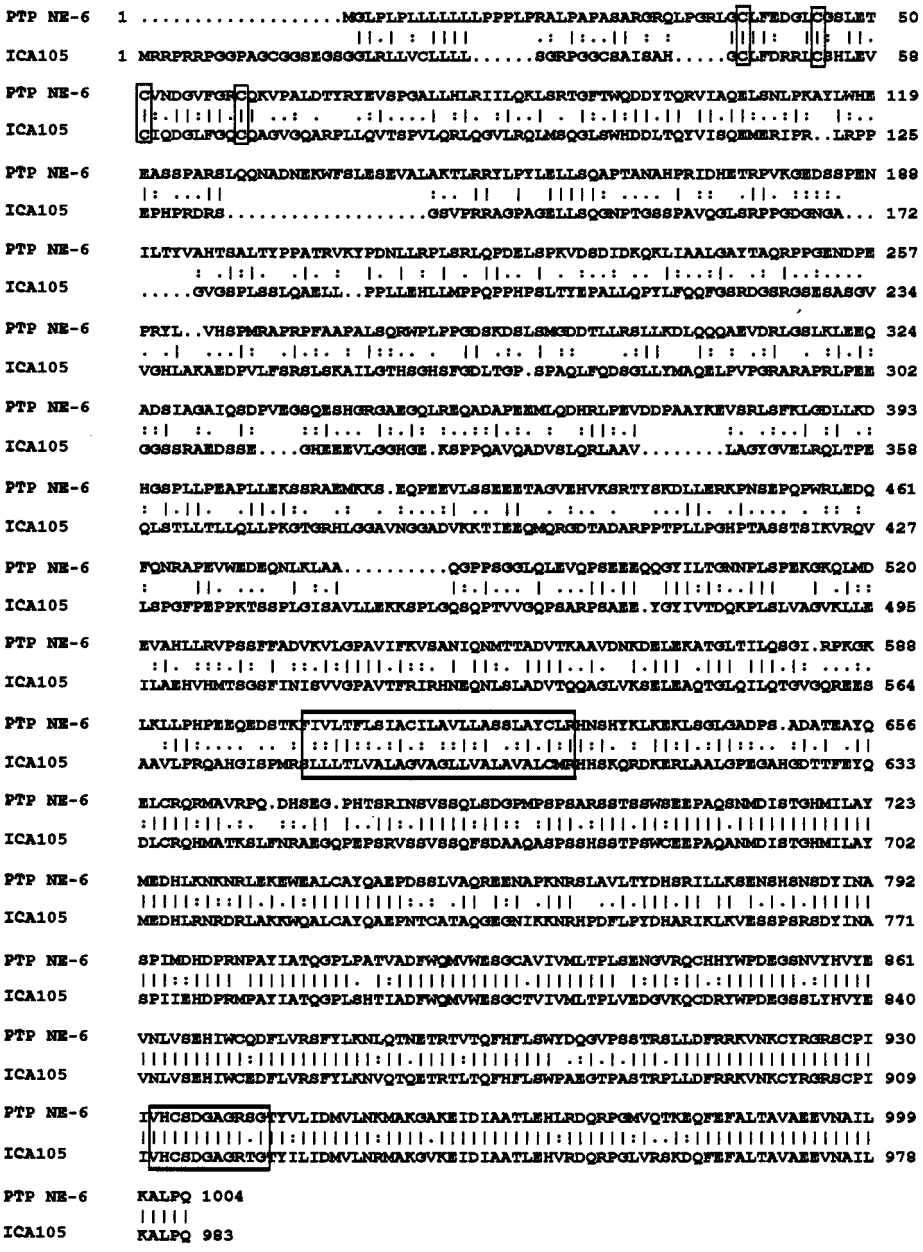

FIG. 2. PTP NE-6 is similar to the rat tyrosine phosphatase ICA105. The amino acid sequence of PTP NE-6 is aligned with that of ICA105. The four cysteines that comprise the N-terminal cysteine repeat are individually *boxed.* The transmembrane sequence and catalytic site are also *boxed.* Identical sequences are connected by *vertical lines,* conserved sequences are connected by *two dots,* and semiconserved sequences are connected by *one dot.*

D936A mutant and LAR demonstrate phosphatase activity that is dependent on the concentration of pNPP substrate. Michaelis–Menten plots of the data show that the D936A mutant has a k_{cat} value of 3.7 s^{-1} and a K_m value of 980 μM. In comparison, LAR has a k_{cat} value of 3.3 s^{-1} and a K_m value of 390 μM. These values are consistent with previously reported analyses of LAR, which demonstrated a K_m of 0.4 mM and a k_{cat} of 6.1 s^{-1} (Pot et al., 1991). Characteristic of PTPs, the phosphatase activity of D936A mutant was inhibited by 1 mM vanadate (Fig. 4).

Tissue distribution of PTP NE-6

To examine the tissue distribution of PTP NE-6, northern blot analysis was done on poly(A)$^+$ RNA from heart, spleen, lung, liver, skeletal muscle, kidney, testis, brain, olfactory neuroepithelium, and adrenal gland. As shown in Fig. 5, a 5.1-kb transcript is expressed abundantly in brain and at detectable levels in olfactory epithelium and adrenal gland. No hybridization signals were detectable in spleen, lung, liver, skeletal muscle, kidney, and testis with a 2-week exposure (data not shown). The weaker PTP NE-6 hybridizing bands, 9.5 kb and 3.7 kb, observed with brain mRNA were not reported for phogrin (Wasmeier and Hutton, 1996) or PTP NP (Chiang and Flanagan, 1996). These transcripts may represent cross-hybridization to other PTPs from the same family. The 3.7-kb transcript may correspond to rat ICA105 mRNA based upon its size and expression in brain. However, at the level of hy-

bridization stringency used in these experiments, we would not expect cross-hybridization to occur. Thus, these transcripts likely represent splice variants of PTP NE-6.

As the northern blot revealed a high level of expression in whole brain, in situ hybridization was performed to detect distinct brain regions expressing PTP NE-6 mRNA. The highest density of PTP NE-6 mRNA was observed in the habenula (Fig. 6). High expression levels were also observed in layer IV of the cortex, medial geniculate nucleus, inferior colliculus, thalamus, and discrete ventral aspects near or within the hypothalamus. Lower expression levels were found in striatum, cerebellum, and the CA1 and CA3 regions of the hippocampus.

DISCUSSION

As accumulating evidence suggests that PTPs play an important role in neural development, it was of interest to identify novel members of this gene family. Using a degenerate PCR cloning approach, we examined the olfactory epithelium for PTPs that may be involved in the unique properties of neuronal regeneration exhibited by the olfactory system. We originally identified six PCR fragments that corresponded to novel PTPs and previously characterized one of these, PTP NE-3 (Walton et al., 1993). The other PCR fragments corresponding to PTP NE-1, PTP NE-2, PTP NE-4, and PTP NE-5 have since been characterized as PTP RL-10 (Higashitsuji et al., 1995), PTP BR-7 (Hendriks et al., 1995*b*; Ogata et al., 1995; Sharma and Lombroso, 1995), PTP BL (Hendriks et al., 1995*a*), and rat PTP 1D (Mei et al., 1994), respectively. We now report the cloning and characterization of PTP NE-6, which contains a hydrophobic transmembrane domain characteristic of a rPTP. Unlike most receptor-type phosphatases, which contain two PTP catalytic domains, PTP NE-6 contains only one domain. Other receptor-type phosphatases with one PTP catalytic domain include PTP BR7 (Ogata et al., 1995), ICA105 (Passini et al., 1995), SAP1 (Matozaki et al., 1994), HPTPb (Krueger et al., 1990), and DPTP10D (Tian et al., 1991; Yang et al., 1991).

Database searches revealed that PTP NE-6 is identical to a recently published rat insulinoma putative PTP, phogrin (Wasmeier and Hutton, 1996), 92% similar to the mouse homologue, PTP NP (Chiang and Flanagan, 1996), and also similar to the partial amino acid sequence of mouse IA-2β (Lu et al., 1996). The phosphatase activity of these clones, however, was not determined. PTP NE-6 is also similar to the pancreatic ICA105 gene (Passini et al., 1995), a putative tyrosine phosphatase, which is also known as ICA512 (Rabin et al., 1994), IA-2 (Lu et al., 1994; Lan et al., 1996), PTP35 (Magistrelli et al., 1995), and PTP LP (Kambayashi et al., 1995). PTP NE-6 and ICA105 possess presumptive transmembrane domains placing them in the receptor-type class of PTPs, yet both contain a

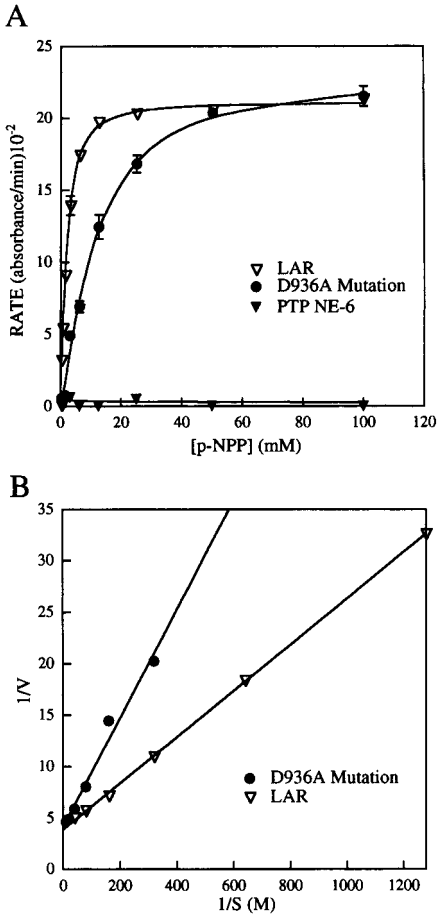

FIG. 3. Comparison of phosphatase activity of PTP NE-6, D936A mutant, and LAR. Fusion proteins containing the catalytic domains of each phosphatase were affinity-purified and assayed for phosphatase activity using pNPP as substrate. The D936A mutant contains an alanine at amino acid position 936 instead of the native aspartate that is encoded by PTP NE-6. **A:** The effect of increasing concentrations of pNPP on the rate of phosphatase activity. **B:** A double-reciprocal plot of enzyme kinetics to determine the K_m and k_{cat} values of the phosphatases.

single PTP catalytic domain typical for cytoplasmic PTPs instead of two domains common for rPTPs. Both PTPs also diverge from the typical tyrosine phosphatase by expressing an aspartate instead of the conserved alanine residue in the catalytic site. The consensus catalytic site for PTPs is defined as HCXAGXXR(S/T)G. Although some mutations at the conserved alanine reduce activity, replacement of an aspartate for alanine has been shown to eliminate the phosphatase catalytic activity of the rPTP LAR on the substrates pNPP and raytide (Streuli et al., 1990). These data are consistent with the lack of phosphatase activity we found with the native PTP NE-6 catalytic domain and with the reported lack of activity previously shown for the related PTP, PTP LP (Kambayashi et al., 1995). Upon mutation of the aspartate in PTP NE-6 to the consensus alanine, PTP NE-6 becomes catalytically

active toward pNPP with a k_{cat} of 3.7 s^{-1} and a K_m of 980 μM. These values fall within the expected range for most phosphatases, demonstrating that the aspartate is solely responsible for the lack of activity seen in the phosphatase assay. Moreover, these data show that the inactivity is not due to other variant amino acid residues within the intracellular domain that are important for binding to substrate.

One explanation for the lack of activity of PTP NE-6 is that it functions as a natural dominant-negative PTP by sequestering substrates from catalytically active PTPs. PTP NE-6 contains the catalytically essential amino acids cysteine and arginine in the catalytic site. During the catalytic reaction, the cysteine forms a thiol-phosphate intermediate, which is stabilized by a salt bridge to the arginine (Guan and Dixon, 1991*a*; Barford et al., 1994). Both these residues are shared by tyrosine-specific phosphatases, as well as dual-specificity phosphatases, such as VH1 (Guan et al., 1991) and PAC1 (Rohan et al., 1993). Other residues within the catalytic site, including alanine, are important for hydrogen bonding to the phosphate group. For PTP NE-6, it is conceivable that substrate binding affinity may be diminished by introduction of the larger and charged aspartate residue. Modeling studies that replace the alanine with an aspartate residue in the catalytic site of *Yersinia* show aspartate will sterically hinder the binding of substrate (J. A. Stuckey and J. E. Dixon, personal communication). In this model, binding may occur if a substrate exists that can be oriented in a different manner. This could create a PTP with restricted substrate specificity. Thus, the inactivity of PTP NE-6 toward pNPP may be due to the choice of substrate used for the phosphatase assay. To show that PTP NE-6 is an active tyrosine phosphatase may require the identification of an endogenous substrate.

Many of the rPTPs exhibit an extracellular domain containing immunoglobulin-like domains or fibronectin type III repeats. These features are characteristic of cell adhesion molecules, which are associated with axonal growth and guidance, suggesting that rPTPs also function in these processes. Analysis of the N-terminal domain of PTP NE-6 did not identify any cell adhesion molecule-like motifs or other known domains. The related PTP, ICA105, which has 25% amino acid identity with PTP NE-6 in the extracellular domain, also did not contain any known motifs. However, both PTPs have a conserved N-terminal cysteine repeat containing four cysteine residues spaced every six to eight residues, which may be important in secondary structure, homodimerization, or other protein interactions. Based on the extracellular and intracellular similarities of these two PTPs, this newly developing class of PTPs may have related ligands and substrates and perform similar functions. To date, however, there is little evidence that binding to the extracellular domain can transduce a signal to the phosphatase domain. Most PTP domains have high activity when expressed without the extracellular domain, and thus it is conceivable that ligand may bind in order to inactivate the PTP. One study examined this issue by constructing a chimeric phosphatase that was responsive to epidermal growth factor (EGF). The chimeric phosphatase consisted of the extracellular and transmembrane domains of the EGF receptor and the intracellular domain of the PTP CD45 (Desai et al., 1993). The response of the chimeric phosphatase to EGF was an inactivation of the tyrosine phosphatase activity. Whether this type of regulation occurs with native phosphatases is not known. The opposite mode of regulation may occur for PTP NE-6, i.e., it is possible that PTP NE-6 is inactive until induced by ligand.

Although we originally isolated PTP NE-6 from olfactory neuroepithelium, we found that PTP NE-6 mRNA is expressed more highly in brain. The highest density of PTP NE-6 mRNA is found in the medial habenula. These nuclei, which are part of the limbic

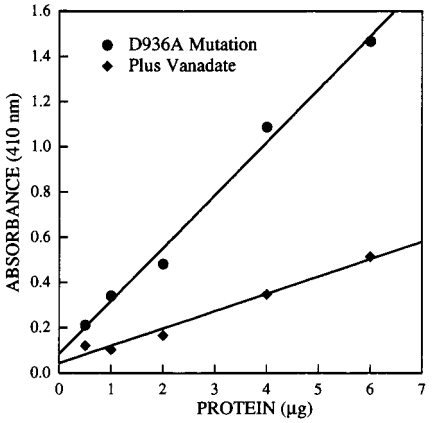

FIG. 4. Phosphatase activity of the D936A mutant is inhibited by vanadate. Phosphatase activity of D936A mutant was determined using 5 m*M* pNPP as substrate and 1 m*M* sodium vanadate.

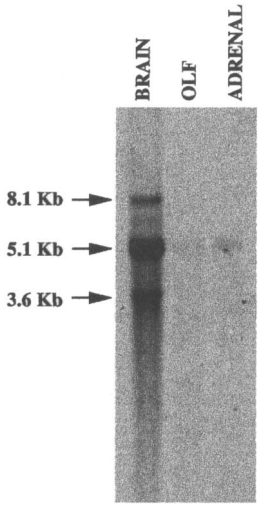

FIG. 5. Tissue-specific expression of PTP NE-6 mRNA. Northern blot analysis was performed on poly(A)$^+$ RNA (5 μg) from rat tissues: brain, olfactory epithelial tissue (OLF), and adrenal gland. The blot was hybridized with probe that was labeled with [^{33}P]dCTP by random priming using the entire PTP NE-6 cDNA as template. The approximate sizes of the hybridization signals are indicated in kilobases and are based on estimations from RNA standards.

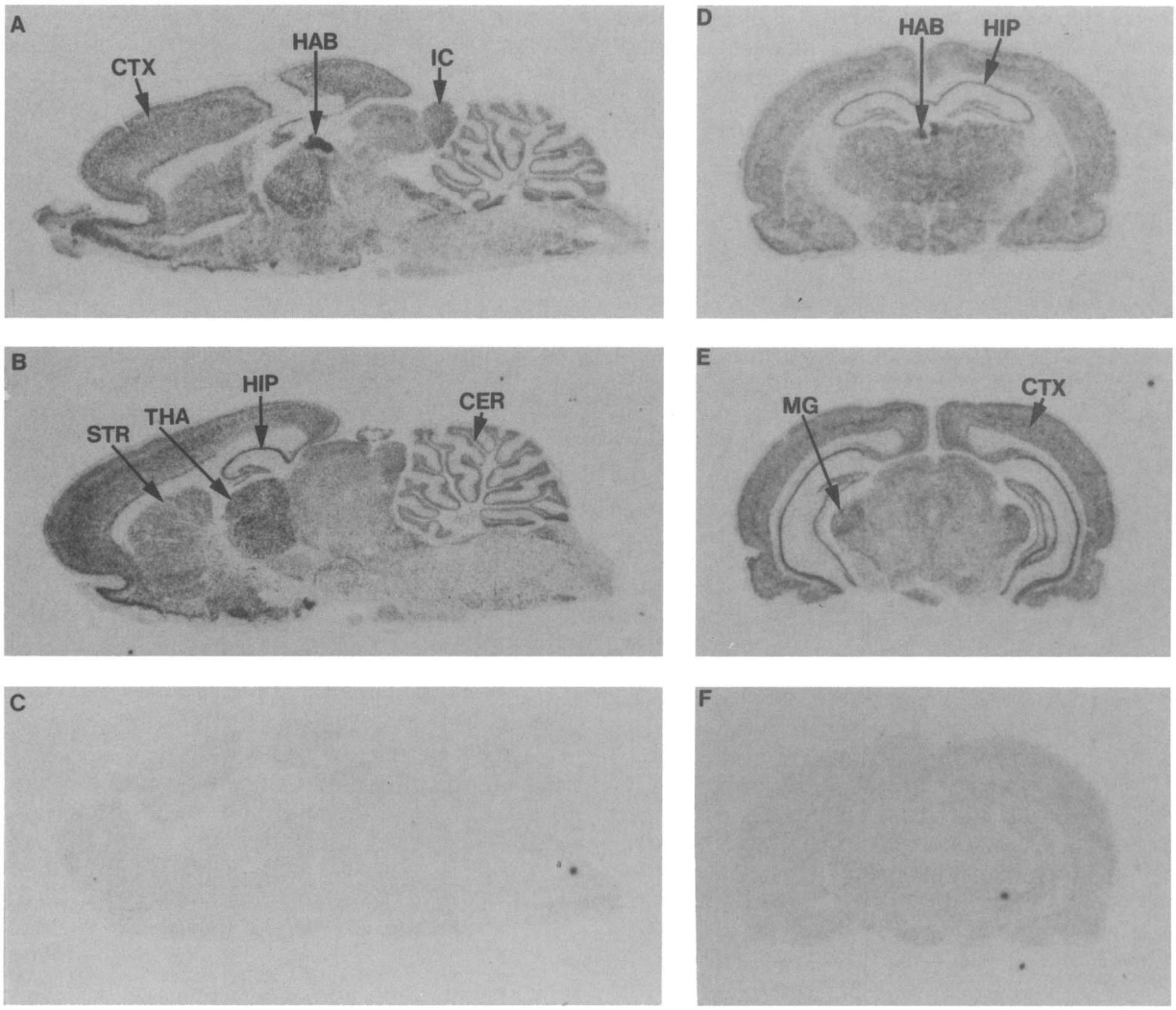

FIG. 6. PTP NE-6 mRNA is expressed abundantly throughout the rat brain. In situ hybridization histochemistry was performed on sagittal (**A–C**) and coronal (**D–F**) sections with two ^{33}P-labeled riboprobes corresponding to the 3′UTR of PTP NE-6. Sections were exposed to Kodak Biomax-MR film for 2 days. The highest density was observed in medial habenula (HAB) (A and D). High expression levels are also shown in cerebellum (CER), cortex (CTX), hippocampus (HIP), inferior colliculus (IF), medial geniculate (MG), striatum (STR), and thalamus (THA) (A, B, D, and E). RNase-pretreated control sections (C and F) give a completely blank hybridization signal.

system, project by way of the fasciculus retroflexus to the interpeduncular nucleus and have been shown to be important in aspects of autonomic and endocrine control, as well as sexual, consummatory, and defensive behavior (Herkenham and Nauta, 1979). Other distinct brain regions, such as intermediate layers of the cortex, medial geniculate nucleus, inferior colliculus, thalamus, and discrete ventral aspects near or within the hypothalamus also express high levels of PTP NE-6 mRNA. PTP NE-6 is likely to serve a role in neural function as indicated by its overall abundance in brain.

IA-2β, the mouse homologue of PTP NE-6, and IA-2, a related PTP, have been shown to be major autoantigens in insulin-dependent diabetes mellitis and thus may play a pathogenic role in this disease process (Lan et al., 1996; Solimena et al., 1996). Both of these putative phosphatases are intrinsic membrane proteins of secretory granules and have been localized to peptide-secreting endocrine cells, such as insulinoma cells (Solimena et al., 1996; Wasmeier and Hutton, 1996). In the brain, PTP NE-6 did not show high levels of mRNA localized selectively to regions that are rich in neurosecretory granules. Instead, levels of mRNA were detected throughout the brain and in other tissues, such as adrenal gland and olfactory neuroepithelium. This broad localization in brain is characteristic of synaptic vesicle markers, such as synapsin I (Walaas et al., 1988). Given the presence of tyrosine-phosphorylated proteins associated with synaptic vesicles, such as synaptophysin and c-*src*, it would be of interest to determine the subcellular localization of PTP NE-6 in brain.

Although the function of PTP NE-6 in brain remains unclear, it is apparent that PTPs are important in neural development. A number of studies have shown that PTPs are expressed in a developmentally regulated manner (Sahin et al., 1995). In addition, localization of PTPs to growth cones suggests that they may be involved in axonal guidance and growth. Recent studies have shown that the receptor-type PTPs, DLAR, DPTP69D, and DPTP99D are necessary for motor neuron axonal pathfinding, further demonstrating that PTPs are regulators of neural development (Desai et al., 1996; Krueger et al., 1996). We have observed that expression of PTP NE-6 mRNA in PC12 cells is increased two- to threefold by nerve growth factor treatment and subsequent differentiation, suggesting that this PTP is induced during periods of neural growth and differentiation.

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