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NEURONAL LOCALIZATION OF THE 25-kDa SPECIFIC THIAMINE TRIPHOSPHATASE IN RODENT BRAIN

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Abstract—Thiamine triphosphate (ThTP) is found in small amounts in most organisms from bacteria to mammals, but little is known about its physiological role. In vertebrate tissues, ThTP may act as a phosphate donor for the phosphorylation of certain proteins; this may be part of a new signal transduction pathway. We have recently characterized a highly specific 25-kDa thiamine triphosphatase (ThTPase) that is expressed in most mammalian tissues. The role of this enzyme may be the control of intracellular concentrations of ThTP. As the latter has been considered to be a neuroactive form of thiamine, we have studied the distribution of ThTPase mRNA and protein in rodent brain using *in situ* hybridization and immunohistochemistry. With both methods, we found the strongest staining in hippocampal pyramidal neurons, as well as cerebellar granule cells and Purkinje cells. Some interneurons were also labeled and many ThTPase mRNA-positive and immunoreactive cells were distributed throughout cerebral cortical gray matter and the thalamus. White matter was not significantly labeled. ThTPase immunoreactivity seems to be located mainly in the cytoplasm of neuronal perikarya. Immunocytochemical data using dissociated cultured cells from hippocampal and cerebellum showed that the staining was more intense in neurons than in astrocytes. The protein was rather uniformly located in the perikarya and dendrites, suggesting that ThTP and ThTPase may play a general role in neuronal metabolism rather than a specific role in excitability. There was no apparent correlation between ThTPase expression and selective vulnerability of certain brain regions to thiamine deficiency. **© 2004 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: thiamine triphosphate, immunohistochemistry, *in situ* hybridization, hippocampus, cerebellum, neurons.

Thiamine triphosphate (ThTP) has been proposed to be a neuroactive form of vitamin B1 (Cooper and Pincus, 1979). ThTP is abundant in electric organ (Eder and Dunant, 1980; Bettendorff et al., 1987) and is more concentrated in neuronal than in glial cells (Bettendorff et al., 1991). As nerve activity is accompanied by a dephosphorylation shift in thiamine phosphate derivatives (Itokawa et al., 1969; Bettendorff et al., 1989), ThTP hydrolysis was thought to be involved in nerve activity (for review see Bettendorff, 1994a). We have also shown that ThTP activates a large conductance anion channel in excised inside-out patches of neuroblastoma cells (Bettendorff et al., 1993a). As the channel remains activated even after removal of ThTP, we have suggested that ThTP might act through phosphorylation of a protein. More recently, it was shown that in *Torpedo* electric organ, [γ-^{32}P]ThTP can indeed phosphorylate rapsyn (Nghiêm et al., 2000), a protein associated with nicotinic acetylcholine receptors and required for the clustering of the receptors at the neuromuscular junction (Gautam et al., 1995). An interesting observation is that phosphorylation of rapsyn by ThTP occurs on a histidyl residue. Histidyl phosphorylation is the rule in prokaryotes (West and Stock, 2001), but is much less documented in mammalian cells (Besant et al., 2003). ThTP also phosphorylates other unidentified proteins in rodent brain (Nghiêm et al., 2000).

While the above results may suggest a role of ThTP in the differentiation of cholinergic synapses, ThTP has been found in practically all organisms studied so far, from bacteria to mammals (Makarchikov et al., 2003). In plant leaves and bacteria, ThTP is synthesized in response to cellular stress, suggesting that this compound may play a more general biological role. Protein phosphorylation by ThTP might be part of a new cellular signaling mechanism. Until now, the only compound known to phosphorylate proteins in animal cells was ATP.

In rodent brain and cultured neuroblastoma cells, ThTP has a relatively rapid turnover (Bettendorff, 1994b; Bettendorff et al., 1994), but its cellular concentration remains low (0.1–1 μM), presumably because of continuous enzymatic hydrolysis (Bettendorff et al., 1993b).

In a recent study, we reported the molecular characterization of a highly specific thiamine triphosphatase (ThTPase) from mammalian brain (Lakaye et al., 2002). It is a soluble 25-kDa monomer with a high catalytic efficiency ($k_{cat}/K_m = 6\times10^6\ s^{-1}\ M^{-1}$). The 25-kDa ThTPase is absent in non-mammalian tissues and has no sequence homology with any other mammalian protein. However, Iyer and Aravind (2002) recently suggested that ThTPase and CyaB-like adenylate cyclases define the novel CYTH superfamily of domains that bind organic phosphates. The mammalian 25-kDa ThTPase may represent a relatively

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Abbreviations: DIG, digoxygenin; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; TBS-T, Tris-buffered saline containing 0.05% (v/v) Tween 20; ThDP, thiamine diphosphate; ThTP, thiamine triphosphate; ThTPase, thiamine triphosphatase.

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recent divergent acquisition of a new catalytic activity in this protein family.

The mRNA for this ThTPase was found to be widely expressed in human tissues (Lakaye et al., 2002), but no data on cell-type specific expression were available so far. The present study is the first to show the distribution of a specific ThTPase using *in situ* hybridization, immunohistochemistry and immunocytochemistry. Our results suggest that, in rodent brain, the 25-kDa ThTPase is mainly expressed in neuronal perikarya and dendrites.

EXPERIMENTAL PROCEDURES

Production of a polyclonal antibody against human recombinant ThTPase

Human recombinant ThTPase, purified as described earlier (Lakaye et al., 2004), was used to raise anti-ThTPase antibodies in a chicken by four injections of 100 μg each (Eurogentec, Seraing, Belgium). Egg yolk immunoglobulins IgY, the chicken IgG homologue, were purified using the EGGstract IgY Purification System (Promega Corporation, Madison, WI, USA). The antibodies were stored at −80 °C at a protein concentration of 2 mg/ml.

All experiments were carried out in accordance with the directives of the European Community Council under the supervision of the Animal Care and Use Committee of the University of Liege. All efforts were made to minimize the number of animals used and their suffering.

Localization of ThTPase by *in situ* hybridization

A 644 bp cDNA fragment was amplified from rat brain mRNA by RT-PCR using forward (5′-CTTGAACTGCTGGGGTCTGG-3′) and reverse (5′-AATCAATGGAGGCTGCTTAGCG-3′) primers. It was cloned into pCRII-TOPO (Invitrogen Life Technologies, Carlsbad, CA, USA) and its sequence confirmed. To produce riboprobes, an *Sph*I/*Sac*I fragment of 430 bp encompassing 134 bp upstream and 224 bp downstream of the stop codon was subcloned into pSP73. For the synthesis of sense and antisense non-radioactive riboprobes with the digoxigenin (DIG)-RNA labeling kit (Roche Diagnostics, Vilvoorde, Belgium), the plasmid was linearized by *Eco*RI or *Hin*dIII respectively.

Male Wistar rats were killed by cervical dislocation and the organs were removed immediately, frozen on dry ice and stored at −80 °C until used. Frozen, 20 μm thick tissue sections were cut with care to avoid RNase contamination of the cryostat knife blade and thaw-mounted onto Menzel Superfrost Plus glass slides (VWR International, Leuven, Belgium). The sections were fixed with 4% paraformaldehyde and acetylated with 0.25% acetic anhydride. Hybridization was carried out at 42 °C with the DIG-labeled riboprobes and the sections were washed in 0.1× SSC at 55 °C (sodium saline citrate, SSC 20×=3 M NaCl and 0.3 M Na citrate). Hybrids were detected by alkaline phosphatase-labeled anti-DIG antibodies and NBT/BCIP (Roche Diagnostics) staining.

Immunohistochemical studies

Adult mice were deeply anesthetized by injection of Nembutal 0.5 mg per 10 g of body weight and perfused transcardially with 0.9% NaCl (0.15 M NaCl) followed by 50 ml of 4% paraformaldehyde dissolved in 130 mM Na-phosphate buffer pH 7.4. Tissues were dissected and postfixed in the same fixative for 1 h and immersed overnight in 30% sucrose cryoprotectant solution in phosphate-buffered saline pH 7.4 (PBS; 140 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4). Then, tissues were frozen on dry ice and cut into 30 μm sections using a cryostat. The free-floating sections were collected in PBS in 24-well plates filled with antifreeze solution (PBS supplemented with 1% polyvinylpyrrolidone, 30% sucrose and ethylene glycol) and stored at −20 °C until use. For immunohistochemical studies, the sections were first treated with 0.6% H_2O_2 in Tris-buffered saline (TBS; 50 mM Tris–HCl pH 7.4, 150 mM NaCl) for 20 min, to block endogenous peroxidase activity. After three washes with TBS, sections were incubated with 1% (v/v) Triton X-100 in TBS for 5 min and then in TBS-T [TBS containing 0.05% (v/v) Tween 20] containing 0.2% (w/v) bovine serum albumin and 3% normal donkey serum for 1 h at room temperature. Chicken anti-ThTPase antibodies diluted 1:250 in TBS-T containing 1.5% (v/v) normal donkey serum were added to sections and incubated overnight at 4 °C. The primary antibodies were amplified by conjugation with a biotinylated donkey anti-chicken IgY, F(ab′)2 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at 1:1000 dilution (1 h incubation at room temperature). After three washes with TBS, sections were incubated with ABComplex Vectastatine Elite Kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions (1:200 dilution in TBS). The immunoreactivity was visualized by the peroxidase reaction after addition of 1.8 mM 3,3′-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) and 0.01% (v/v) H_2O_2 during 5 min. Finally, after three washes in TBS, sections were mounted on slides, air-dried and coverslipped. Controls were done by omitting the primary antibodies. In some control experiments, the primary IgY antibody solution was preabsorbed with recombinant hThTPase (400 μg/l) for 90 min at 37 °C prior to incubation with tissue sections. The antibody solution was then used as described above.

Cell cultures

Hippocampal neurons and cerebellar granule cells were obtained, respectively, from 16 day-old mouse embryos and from 4 day-old mouse pups using methods described previously (Lefèbvre et al., 1987; Leprince et al., 1989). Briefly, hippocampi or cerebella were carefully dissected, freed of meninges and cut in small fragments that were incubated with trypsin (0.25%) and deoxyribonuclease (0.1%) in Ca^{2+}-Mg^{2+}-free salt solution for 25 min at 37 °C. They were then washed with their culture medium which consisted, for hippocampal neurons, of Dulbecco's modified minimum essential medium (Invitrogen Life Technologies) supplemented with glucose (6 g/l, final concentration), 5% (v/v) fetal calf serum (Invitrogen Life Technologies), 10% (v/v) horse serum (Invitrogen Life Technologies) and the N-1 supplement (insulin 5 μg/ml; transferrin 5 μg/ml; progesterone 20 nM; putrescine 100 μM, Na selenite 30 nM; Bottenstein and Sato, 1979) and, for cerebellar granule cells, of minimum essential medium (with potassium concentration raised to 25 mM and sodium concentration decreased in equimolar amount) supplemented with glucose (6 g/l, final concentration), pyruvate (1 mM), bovine insulin (5 μg/ml) and 10% (v/v) horse serum. Dissociation was obtained by up and down aspirations through the large tip of a 5 ml plastic pipette put on the bottom of a conical glass tube. The resulting cell suspension was filtered through a 15 μm and a 40 μm nylon sieve, respectively for cerebellar granule cells and for hippocampal neurons. Fifty microliters of the cell suspension were seeded on poly-ornithine (0.1 mg/ml in distilled water) coated glass coverslips deposited in a 24-wells plate (NUNC, Roskilde, Denmark) at a concentration of 2.5×10^6 cerebellar and 1.25×10^6 hippocampal cells per ml. The medium was renewed once weekly. Cerebellar granule cells and hippocampal neuron preparations were used after respectively 8 and 9 days in culture.

Immunocytochemistry

Immunocytochemical staining was performed as previously described (Chanas-Sacré et al., 2000). Dissociated cell cultures were fixed with 4% paraformaldehyde in PBS for 15 min, followed by three rinses with TBS. The cells were then permeabilized with 0.1% (v/v) Triton X-100 in TBS for 10 min. After three further washes with TBS, the cells were incubated for 1 h with reconstituted nonfat dry milk (15 mg/ml) in TBS. Primary antibodies were then added to this blocking solution and incubated overnight at 4 °C. After three washes with TBS, fluorescently labeled secondary antibodies were added to the blocking solution for 2 h at room temperature. The same washing procedure was repeated, and the preparations were mounted in Fluoprep (bioMérieux SA, Marcy l'Etoile, France) and observed under a MCR-1024 confocal microscope (Bio-Rad, Hertfordshire, UK). Controls for fluorescent labeling were done by omitting the primary antibodies and did not show any nonspecific staining. Among primary antibodies, chicken anti-ThTPase was used at a 1:250 dilution, mouse anti-MAP2ab (Sigma) was used at 1:100 dilution and rabbit anti-GFAP (DakoCytomation A/S, Glostrup, Danmark) was used at 1:1500. Among secondary antibodies, FITC-conjugated donkey anti-chicken IgY was used at 1:150 dilution; Cy5-conjugated donkey anti-mouse and anti-rabbit IgG (all from Jackson Immunoresearch Laboratories) were used at a dilution of 1:500. Cell nuclei were labeled by incubation with 0.2 μM ethidium homodimer-1 (Molecular Probes, Eugene, OR, USA) in TBS for 1 min.

RESULTS

Regional expression of ThTPase mRNA in rat brain by *in situ* hybridization

In cerebral cortex, individual cells were specifically labeled with the antisense probe (Fig. 1A and 1B). No significant labeling was observed in the superficial molecular layer (containing few perikarya but many myelinated fibers); however the external granule cell layer was strongly stained. In the hippocampus, pyramidal neurons (CA1–CA3 fields) and granule cells of the dentate gyrus were highly stained (Fig. 1C). Many ThTPase-

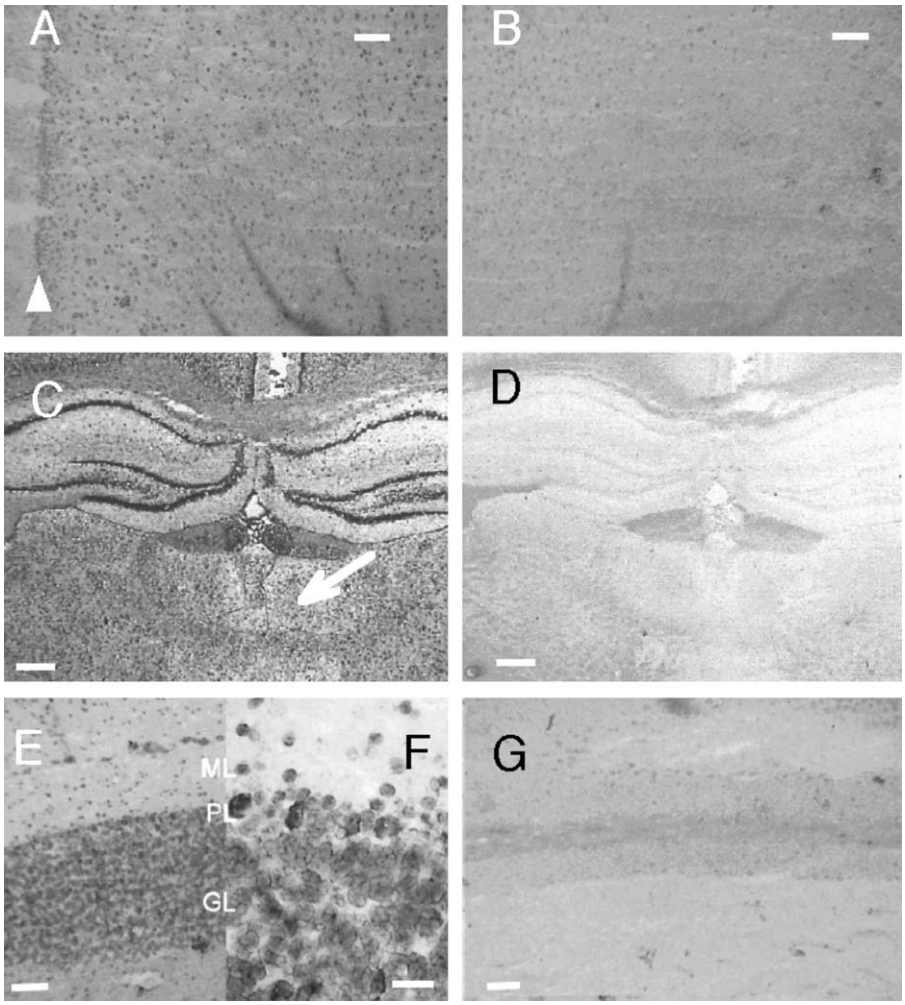

Fig. 1. *In situ* hybridization study of ThTPase mRNA distribution in the rat brain. Specific staining was obtained using an antisense riboprobe (A, C, E and F) and the corresponding non-specific staining obtained using a sense riboprobe (B, D and G). (A, B) Neocortex; the external granule layer is indicated by the arrowhead. (C, D) Hippocampal formation and thalamus; the arrow in C indicates the location of the mediodorsal thalamic nucleus. (E, F, G): Cerebellar cortex (ML, molecular layer; PL, Purkinje cell layer; GL, granule cell layer). Scale bars = 200 μm (A, B, E, G), 500 μm (C, D), 50 μm (F).

mRNA positive cells, probably interneurons, were also distributed in other regions of the hippocampal formation (in particular the stratum lacunosom-moleculare). Many positively stained cells were also observed throughout the thalamus (Fig. 1C). The mediodorsal thalamic nucleus appears somewhat less stained than the rest of the thalamus. In the cerebellum, the neurons of the granular layer (granule cells and probably some Golgi cells) and Purkinje cells gave a strong signal. Many positive cells were observed in the sparsely populated molecular layer (Fig. 1E and 1F). It appears that not all granule cells were equally labeled: highly positive cell clusters were separated by non- or weakly labeled cells. In all cases, a higher signal was observed using the antisense probe compared with the sense probe (Fig. 1B, 1D and 1G). Experiments on mouse brain suggest a similar distribution of ThTPase mRNA as in rat brain (not shown).

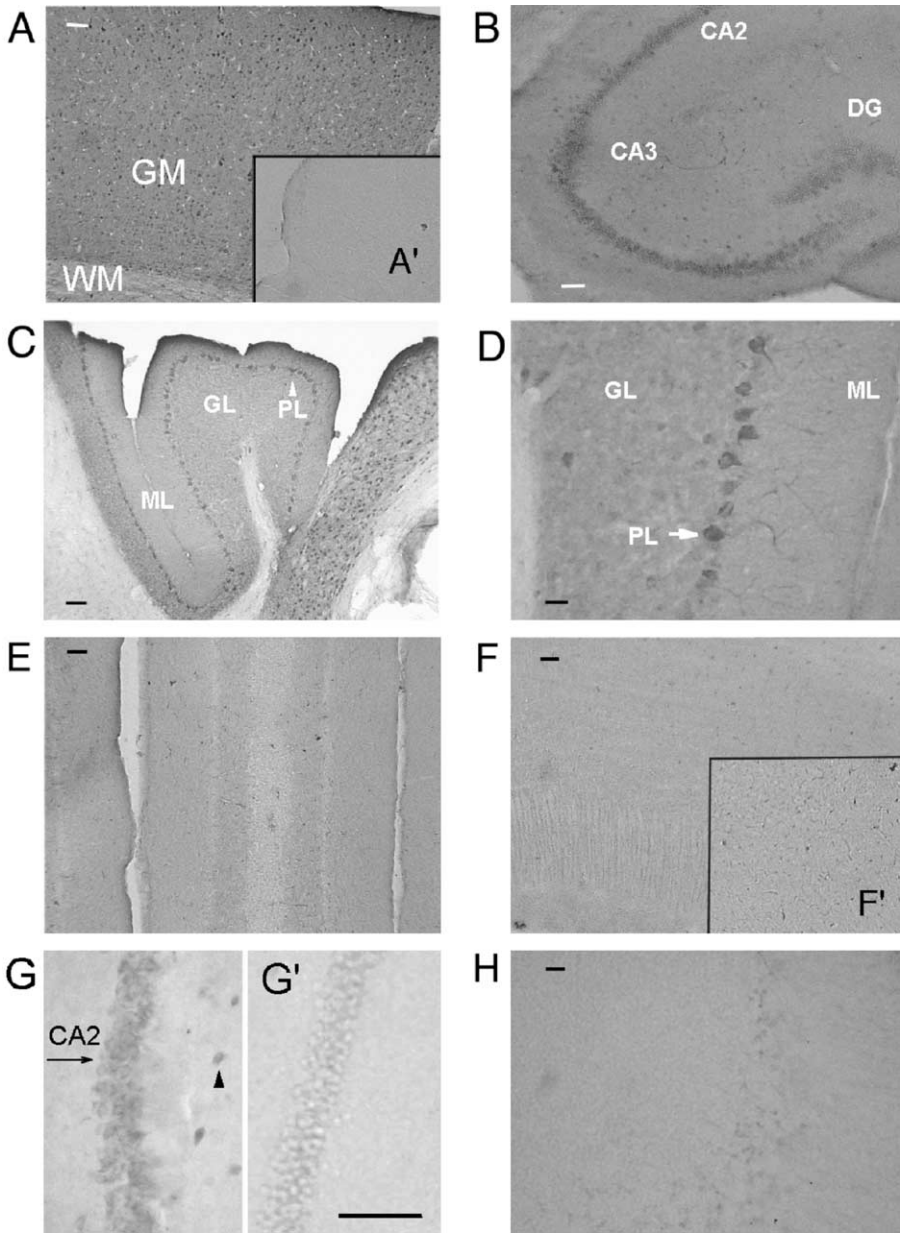

Fig. 2. Distribution of anti-ThTPase immunoreactivity in the mouse brain. Cryostat-made brain sections (30 μm) were processed for ThTPase immunodetection using a chicken polyclonal antibody and a peroxidase-conjugated secondary antibody. The staining was done using 3′,3′-diaminobenzidine as substrate. Specific immunostaining is observed in mouse brain sections from the cortex (A; GM, gray matter; WM, white matter), hippocampus (B; G, an interneuron is indicated by an arrow head) and cerebellum (C; D; ML, molecular layer; GL, granule cell layer; PL, Purkinje cell layer). Light, evenly distributed non-specific staining is present in quail brain sections from cerebellum (E) and encephalon (F). Control sections, without addition of the primary antibody, are shown for mouse (insert A′) and quail (insert F′) cortex. In another series of control experiments the anti-ThTPase antibodies were preabsorbed using purified recombinant human ThTPase, before application on hippocampal (G′) and cerebellar sections (H) of mouse. Scale bars=200 μm (A, A′, B, C, F, F′), 50 μm (D, G, G′, H), 400 μm (E).

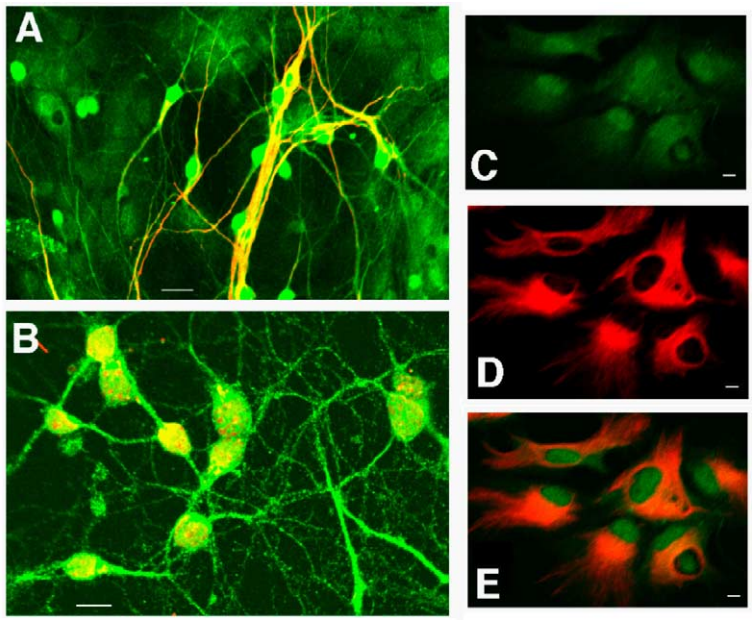

Fig. 3. Anti-ThTPase immunoreactivity distribution in cultures of mouse hippocampal (A, C–E) and cerebellar (B) dissociated cells. Anti-ThTPase staining is in green. In red are represented: anti-MAP2ab staining of hippocampal dendrites (A), ethidium homodimer-1 staining of cell nuclei of cerebellar granule cells (B), and anti-GFAP staining of hippocampal astroglial cells (C, D). Colocalization of anti-ThTPase and anti-GFAP staining was demonstrated by merging the two stainings (E). Scale bars=25 μm (A), 5 μm (B–E).

Immunohistochemical localization of ThTPase in mouse brain

In the mouse cerebral cortex, many ThTPase-immunoreactive cells were observed throughout the gray matter (Fig. 2A), while white matter was poorly stained. No significant staining was observed in the absence of primary antibody (Fig. 2A inset). The hippocampal CA1–CA3 pyramidal cell layers were highly stained, as was the granule cell layer of the dentate gyrus (Fig. 2B and 2G). Some interneurons were also stained in the molecular layer. The cerebellar cortex showed high anti-ThTPase immunoreactivity in Purkinje cells, but only a few cells were stained in the granular layer (Fig. 2C and 2D). No significant labeling was observed in the white matter. The molecular layer was negative, except for the dendritic tree of Purkinje cells.

We have previously shown that the 25-kDa ThTPase is specific for mammalian tissues and that the enzyme does not exist in birds, though birds possess other ThTPase activities (Makarchikov et al., 2003). Therefore, we used quail cerebellum to check the specificity of our antibody. No significant staining was observed in quail cerebellum and forebrain (Fig. 2E and 2F). Furthermore, in mouse cerebellum and hippocampus, the signal disappeared after preabsorption of the antibody with purified recombinant human ThTPase, suggesting a specific interaction of our antibody with endogenous ThTPase (Fig. 2G and 2H).

Intracellular localization of ThTPase in neural cells *in vitro*

The intracellular localization of ThTPase was investigated in hippocampal and cerebellar neural cells in culture. Neurons were identified using an antibody against the neuron-specific microtubule-associated protein MAP2ab. In dissociated hippocampal cell cultures, anti-ThTPase staining was observed in cell bodies as well as in MAP2ab-positive dendrites (Fig. 3A). In cerebellar granule cells, anti-ThTPase staining was also present in cell bodies and dendrites (Fig. 3B). This is in agreement with our immunohistochemical data revealing ThTPase immunoreactivity in both Purkinje cell bodies and dendrites (Fig. 2D). A partial colocalization of ThTPase immunoreactivity and ethidium homomer-1 fluorescence suggests that ThTPase might also be present in the nucleus, in agreement with several studies showing the presence of ThTPase activity in the nuclear fraction after differential centrifugation (Barchi and Braun, 1972; Hashitani and Cooper, 1972). Many MAP2ab-negative cells were observed in dissociated hippocampal cell cultures. Many of these cells are stained with anti-GFAP antibody (Fig. 3C–E), indicating that they are glial cells, mainly astrocytes. In these cells, anti-ThTPase immunoreactivity staining was observed throughout the cytoplasm and the nucleus, but was less intense than in hippocampal neurons (Fig. 3A). No significant contamination with astroglial cells was observed in granule cell cultures.

DISCUSSION

Thiamine pyrophosphokinase (Nosaka et al., 1999), the enzyme catalyzing the synthesis of the cofactor ThDP, and the 25-kDa ThTPase are the only mammalian enzymes of thiamine metabolism characterized so far and no tissular localization study is available for any enzyme involved in thiamine metabolism. ThTP and ThTPases exist in most organisms studied so far, including prokaryotes, but the

25-kDa ThTPase is specific for mammalian tissues. It probably has a role in the control of intracellular ThTP concentration (Makarchikov et al., 2003).

Our previous results suggested that the 25-kDa ThTPase mRNA exists in virtually all mammalian organs (Lakaye et al., 2002), but the tissular localization remained unknown. Here, we compared the results obtained by *in situ* hybridization in the rat and by immunohistochemistry in the mouse. As ThTPase is relatively conserved among mammalian species (Lakaye et al., 2002), chicken antibodies were raised against purified human recombinant ThTPase, which was available in relatively large amounts (Lakaye et al., 2004). After purification, the antibody recognized purified ThTPase on Western blots (Makarchikov et al., 2003). Several control experiments were performed. The signal disappeared after preabsorption of the antibody with recombinant human ThTPase and no specific signal was observed in quail brain, devoid of 25-kDa ThTPase. The results obtained by *in situ* hybridization and immunohistochemistry are in good agreement, suggesting a good specificity of our antibody.

It is well known that thiamine deficiency mainly affects the nervous system. As a specific role of ThTP in nerve cell excitability has been postulated (Cooper and Pincus, 1979), our study was mainly focused on the brain. It is clear, from both our *in situ* hybridization and immunohistochemical studies, that several kinds of nerve cells are highly labeled. Our results suggest a predominantly neuronal expression of ThTPase. The signal was highest in hippocampal neurons and in granule and Purkinje cells of the cerebellar cortex, but other cells, probably interneurons, were also labeled.

In comparison with Purkinje cells, cerebellar granular layer showed a more intense labeling using *in situ* hybridization than immunohistochemistry. This might be related to species differences as *in situ* hybridization was performed on rat brain and immunohistochemistry was in mouse brain. Another possibility would be that ThTPase mRNA is only very inefficiently translated to protein in these cells, due to a regulation at the translational level.

No significant signal was observed in white matter, suggesting that axons and oligodendrocytes contain little ThTPase. This would agree with previous results showing that, after subcellular fractionation of rat brain, the specific activity was low in the myelin fraction (Hashitani and Cooper, 1972). On the other hand, hippocampal astrocytes in culture stained positive for ThTPase immunoreactivity, though less than neurons. It is possible that this is related to a lower degree of differentiation of astrocytes in culture. Indeed, in cerebellar cultures, astrocytes retain many immature features such as expression of vimentin (Pixley and de Vellis, 1984).

Taken together, our results show a predominantly neuronal localization of the specific 25-kDa ThTPase. Previous studies suggested that thiamine diphosphatase activity was also 20-fold higher in the neuronal cell-enriched fraction of rat brain compared with the glial cell-enriched fraction (Laforenza et al., 1988). Furthermore, ThTP is also more abundant in cells of neuronal origin compared with cells of glial origin (Bettendorff et al., 1991, 1993a). This means that ThTP synthesis should be much faster in neurons compared with glial cells.

An earlier study reported the ultrastructural localization of membrane-associated ThTPase activity at pH 6.5 and of the soluble ThTPase activity (presumably the 25-kDa enzyme) at pH 9.2 in mouse liver, kidney and brain sections, using an indirect method of phosphate precipitation (Ogawa et al., 1982). These authors found that alkaline ThTPase activity was mainly associated with synaptic membranes but they did not report any cytosolic activity. Furthermore, they found a very similar distribution for membrane-associated neutral ThTPase and alkaline ThTPase activities. However, under those conditions, a participation of alkaline phosphatases, especially at pH 9.2, cannot be excluded. Moreover, as the tissues were fixed before the enzyme assay, it is difficult to estimate what part of specific activity remained *in situ*.

Immunocytochemical studies using cultured hippocampal neurons, cerebellar granule cells and astroglial cells show that ThTPase immunostaining is rather uniformly distributed throughout the cell body and dendrites, in agreement with a cytosolic localization. In astroglial cells, a nuclear staining was rather obvious. This fits with the biochemical studies showing that, in rat brain, ThTPase activity is soluble and mostly cytosolic (including the cytosolic fraction associated with synaptosomes, Bettendorff et al., 1993b), but it is also present in the mitochondrial and nuclear fractions (Hashitani and Cooper, 1972). Subcellular fractionation of rat brain revealed that ThTP is also found in all fractions (Bettendorff et al., 1994).

Earlier studies had suggested—though not on a very firm basis—that ThTP was implicated in nerve excitation (Itokawa and Cooper, 1969) or in neurotransmitter release (Itokawa et al., 1972; Eder and Dunant, 1980; Yamashita et al., 1993). The present data show a relatively uniform distribution of the specific ThTPase in neuronal perikarya and dendrites, and the enzyme appears essentially cytosolic rather than membrane-associated. Also, ThTPase immunoreactivity does not appear to be associated with a particular neurotransmitter. Indeed, it is well known that cerebellar granule cells and cerebral pyramidal cells release glutamate, while Purkinje cells release GABA.

An important observation is that there is no apparent correlation between ThTPase expression and selective vulnerability of certain brain regions to thiamine deficiency. In Wernicke-Korsakoff syndrome, an alcohol-related thiamine deficiency, neuronal loss occurs predominantly in the thalamus (in particular the mediodorsal nucleus), in mammillary bodies and, in one third of the patients, in the cerebellum (Hazell et al., 1998; Singleton and Martin, 2001). The same holds true in rodent models of thiamine deficiency (Calingasan et al., 1999; Harata and Iwasaki, 1995). In general, thiamine deficiency does not result in an appreciable loss of cortical pyramidal neurons. Thus, a high ThTPase content does not appear to be correlated to a sensitivity of neurons to thiamine deficiency.

In more homogenous tissues such as the liver, ThTPase expression was uniformly observed in practically all

cells (not shown). Thus ThTPase, as well as ThTP, may play a basic role common to most cells, not restricted to nerve cells. In the brain, however, the distribution of the 25-kDa ThTPase appears essentially neuronal, and we have noticed that the steady-state ThTP concentration is also higher in neuronal than in glial cells (Bettendorff et al., 1991, 1993a). Thus the turnover of ThTP may be much higher in neurons suggesting a specific role of the ThTP/ThTPase couple in, for instance, a cellular signaling pathway. An alternative possibility would be a role in the regulation of energy metabolism. Indeed, it is thought that astrocytes transform glucose into lactate, which is captured by neurons and oxidized to pyruvate, that is used aerobically via pyruvate dehydrogenase and the Krebs cycle (Magistretti et al., 1999). As ThTP and ThTPase activity are elevated in other cell types with a high oxidative metabolism such as hepatocytes (Makarchikov et al., 2003), it is tempting to consider that ThTP, as the cofactor ThDP, might be involved in the control of cellular oxidative metabolism.

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