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Immunolocalization of the Proton-Coupled Oligopeptide Transporter PEPT2 in Developing Rat Brain

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Abstract: This study examined the tissue distribution, cellular localization, and developmental expression of the PEPT2 protein in rat brain. Immunoblot and immunocytochemistry analyses were performed with specific rat PEPT1 and PEPT2 antisera developed in our laboratory. Rats were examined from fetus (gestation for 17 days) to adult (day 75). On immunoblot analysis, the PEPT2 protein was detected in cerebral cortex, olfactory bulb, basal ganglia, cerebellum, and hindbrain sections of adult brain, with the strongest signals in cerebral cortex. No PEPT1 protein was found in brain. Expression levels of the PEPT2 protein in cerebral cortex were maximal in the fetus and declined rapidly with advancing age. Adult protein levels were approximately 14% of that observed in fetus. In immunofluorescence experiments, the strongest PEPT2 signals were observed in epithelial cells of the choroid plexus for both adult and neonate brains. The PEPT2 protein was exclusively expressed on the apical membrane (CSF-facing) of choroid plexus epithelia. In double labeling experiments, PEPT2 immunoreactivity in adult brain colocalized with NeuN, a neuronal marker, but not with GFAP, an astrocyte marker. In contrast, in neonatal brain, PEPT2 immunoreactivity colocalized with both GFAP and NeuN. These findings demonstrate that the PEPT2 protein is found throughout the brain. The apical expression of PEPT2 in choroid plexus suggests that it is involved in the export of neuropeptides, peptide fragments, and peptide-like drugs from cerebrospinal fluid. PEPT2 may also play a role in the regulation of neuropeptide concentrations in extracellular fluid, especially during early development.

Keywords: PEPT2; choroid plexus; neurons; astrocytes; brain; localization; development

Introduction

Although the number of amino acids and amines that act as neurotransmitters within the brain is fewer than a dozen,[1] more than 100 biologically active peptides have been identified in the central nervous system.[2] The amino acid and amine neurotransmitters are fast-acting molecules that are rapidly inactivated via presynaptic reuptake mechanisms, while in contrast, neuropeptides appear to be long-acting neuromodulating substances since reuptake systems for peptides are generally lacking. Instead, diffusion and enzymatic degradation are pathways for the termination of neuropeptide activity. Degradative enzymes are found ubiq-

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uitously in brain and include serine proteases, thiol (cysteine) proteases, acid (aspartic acid) proteases, and metalloproteases. Moreover, it has been suggested that the ectoenzyme-mediated metabolism of neuropeptides may be an important regulatory site of peptide-mediated activity.[2]

Peptide biotransformation can have multiple consequences since the metabolic fragments may retain, lose, or exhibit partial activity at the receptor as compared to the parent compound. However, the events following proteolysis are unclear. For example, while peptide fragments may be carried away from the synaptic cleft by diffusion, it is also possible that peptide breakdown products are recycled via uptake mechanisms into neurons and/or glial cells. Functional studies using dipeptide probes have suggested that peptide transporters may be responsible for this uptake mechanism.[3−7] At present, four mammalian proton-coupled oligopeptide transporters (POTs) have been cloned from several species such as rabbit,[8,9] rat,[10−13] human,[14,15] and mouse.[16,17] The low-affinity peptide transporter, PEPT1, is found primarily in the epithelia of intestine and, to a lesser extent, in kidney.[18−20] The high-affinity peptide transporter, PEPT2, is the predominant POT in kidney.[20−22] Together, PEPT1 and PEPT2 function in the assimilation of dietary protein digestion products and the reclamation of peptide-bound amino nitrogen from the glomerular filtrate, respectively.

PEPT2, however, has a wider tissue distribution than PEPT1, and transcripts are found in other tissues, including lung, mammary gland, and brain.[23,24] In particular, PEPT2 mRNA and protein are highly expressed in the choroid plexuses of brain.[24,25] In brain parenchyma, the PEPT2 protein has been reported in crude synaptosomes prepared from adult rat cerebral cortex and cerebellum,[7] and dipeptide uptake has been observed in these preparations.[6,7] Likewise, PEPT2 mRNA and PEPT2-like function were noted in

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neonatal astroglia-rich primary cultures.[3,5] Nevertheless, Berger et al.[24] found PEPT2 mRNA in astrocytes but not neurons of adult rats, while Dieck et al.[5] did not find PEPT2 mRNA in embryonic neuronal cultures. Because of this controversy, further work is required to identify the localization of the PEPT2 protein in different brain regions and cell types, and to determine whether there are age-related differences in protein expression.

Two newer POT members, PHT1[12] and PHT2,[13] have been cloned from brain, and are capable of transporting the amino acid histidine as well as di- and tripeptides. However, the peptide/histidine transporters are not viewed as possible candidates for the removal of peptide fragments from the extracellular space surrounding neurons and/or glial cells. This contention is supported by functional studies in choroid plexus,[25−28] synaptosomes,[6,7] and astroglia-rich primary cultures[3] that have consistently failed to exhibit inhibition of dipeptide uptake by excess L-histidine, or inhibition of L-histidine uptake by excess dipeptide.[7]

In this study, we have examined the tissue distribution, cellular localization, and developmental expression of PEPT2 protein in rat brain. The results are unique in demonstrating, for the first time, age-related differences in the expression of this peptide transporter in cerebral cortex. In particular, definitive evidence is provided for the expression of the PEPT2 protein in neurons (adult and neonate) and astrocytes (neonate but not adult).

Experimental Section

Animals. Sprague-Dawley rats (Charles River Laboratories, Portage, MI) were kept in cages with a 12 h light−dark cycle and given *ad libitum* access to chow and tap water. For immunoblotting experiments, the rats were given a lethal dose of pentobarbital and decapitated. Brains were quickly isolated and placed in ice-cold homogenization buffer. For immunofluorescence experiments, rats were anesthetized with methoxyfluorane and perfused intracardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4), followed by Carnoy's fixative. The brains were immediately removed from the cranium and immersion-fixed overnight at 4 °C in the same fixative, and the brains were dehydrated and embedded in paraffin blocks. Sections (10 μm thick) were cut and placed on Superfrost Plus glass slides.

Pregnant Sprague-Dawley rats were delivered to The University of Michigan Animal Care Facility at day 16 of gestation. Litters were born after gestation for 22 days and were kept with their mothers until they were 21 days old. Animals were routinely weaned at 21 days to a standard laboratory diet (Rodent Diet 5001, PMI Nutrition International, St. Louis, MO). Rats were sacrificed at day 17 of gestation, and at 1−2, 21, and 75 days (adult) following birth. For each developmental age, cerebral cortex samples were pooled from at least four pups (both genders). Data points represent the mean ± standard error of seven independently generated preparations.

All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (NIH Publication 85-23, revised in 1985).

Antisera. Antisera to rat PepT1 and PepT2 were generated against fusion proteins containing glutathione *S*-transferase and rat kidney PEPT1 amino acid residues 390−571 or rat kidney PEPT2 amino acid residues 408−594.[20] These antisera have been shown to react specifically with the appropriate oligopeptide transporter polypeptides and not to cross-react in either immunoblot analysis or immunohistochemical experiments.[20] The mouse antineuronal nuclei (NeuN) monoclonal antibody and the mouse antiglial fibrillary acidic protein (GFAP) monoclonal antibody were obtained from Chemicon International (Temecula, CA).

Immunoblot Analysis. Tissues from different brain regions of adult male rats were prepared as previously described.[29] Samples from three or four animals were pooled, weighed, and transferred into ice-cold RIPA lysis buffer [50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS (pH 7.5)]. Renal brush border membrane vesicles (BBMV) were prepared using a method described previously.[30,31] Lysates were then separated on SDS−PAGE gels as previously described,[20] blotted to stabilized nitrocellulose membranes, blocked with TBS-Tween 20 and 5% milk (TBS-T 5% milk) for 1 h, and then placed in TBS-T 5% milk overnight at 4 °C with either the PEPT1 (at a dilution of 1:1000) or PEPT2 (1:1000) antiserum. After three 10 min washes in TBS-T, the appropriate horseradish peroxidase-linked secondary antibody was then added in TBS-T 5% milk and the mixture incubated at room temperature for 1 h. Membranes were then washed three times (10 min each) in TBS-T. Immunoreactive proteins were visualized by enhanced chemiluminescence (Roche Diagnostics Corp., Indianapolis, IN). For specificity controls, anti-PEPT2 sera were preabsorbed with the respective immunogenic fusion protein (0.9 mg/mL) for 1 h (at room temperature) before the immunoblotting procedure.

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Immunocytochemistry. Indirect immunofluorescence staining was performed as previously described,[20] with minor modifications. Tissue sections from male rats were rehydrated in PBS for 5 min, heated at 100 °C for 5 min in 0.01 M citrate buffer (pH 6.0), and then blocked for 90 min in 10% normal goat serum and 0.1% Triton X-100 (TX100) in TBS. Subsequently, the sections were incubated overnight at 4 °C with rabbit anti-PEPT2 serum (1:150 in blocking buffer). After three washes in PBS and 0.1% TX100, the sections were incubated for 90 min (at room temperature) with a 1:100 dilution of goat anti-rabbit IgG conjugated to FITC (Vector Laboratories, Burlingame, CA), washed twice for 10 min in PBS and 0.1% TX100, washed once for 10 min in TBS alone, and then mounted in an aqueous medium (Vectashield, Vector Laboratories). Fluorescence labeling was assessed microscopically, and photomicrographs were digitally acquired and stored using a SPOT camera and RT software (Diagnostic Instruments, Inc., Sterling Heights, MI).

Some sections were stained simultaneously with a monoclonal antibody (diluted 1:500) directed against the astrocytic marker, GFAP, or with a monoclonal antibody (diluted 1:800) directed against the neuron specific nuclear protein, NeuN. Binding of these mouse monoclonal antibodies was detected with a secondary antibody (diluted 1:400) conjugated to Cy3 (goat anti-mouse IgG, Chemicon).

The following controls were used to assess the specificity of the immunostaining: (1) omission of the primary antibody, (2) blocking competition of the primary antiserum by preincubation with the fusion protein that had been used as an immunogen for the generation of the antiserum, and (3) use of the preimmune serum from the same animal from which the primary antibody was obtained.[20] Further, it is unlikely that PHT1 or PHT2 is being detected in our experiments given their low level of homology with the 187 amino acids of the PEPT2 immunizing fusion protein (i.e., 4.4 or 5.1%, respectively).

Results

Immunoblot analyses were performed on tissue samples from different regions of adult brain using antisera against a fusion protein containing 187 amino acids of the PEPT2 large extracellular loop. The PEPT2 protein was also extracted from renal brush border membrane vesicles (BBMV) for use as a positive control. As shown in Figure 1, PEPT2 was expressed widely throughout the brain, including regions such as the cerebral cortex, olfactory bulb, basal ganglia, cerebellum, and hindbrain. However, the strongest signals were found in the cerebral cortex. In contrast, there was no detection of any band in brain tissues by the PEPT1 antiserum which did, however, detect a specific 90 kDa band in kidney lysates (results not shown), as previously reported.[20]

To characterize the cellular expression of PEPT2 in adult brain, sections of cerebral cortex were examined further for PEPT2 staining. Strong signals were observed in cerebral cortex (Figure 2A), by cells that appear by location and morphology to be neurons, as well as in the choroid plexus and ependymal cells (Figure 2B,C). PEPT2 was specifically

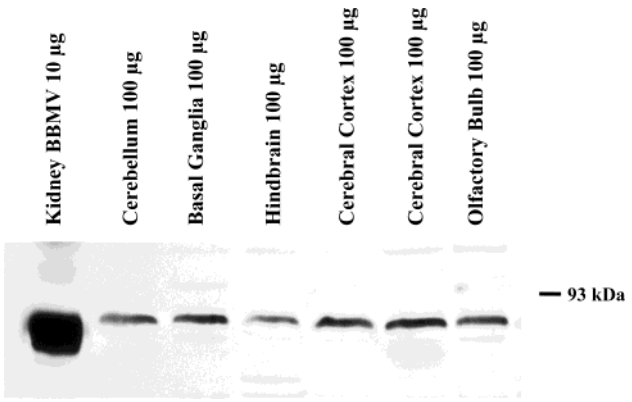

Figure 1. Immunoblot analyses of PEPT2 in membranes prepared from different regions of adult rat brain. Rat kidney brush border membrane vesicles (BBMV) were used as a positive control.

localized to the apical membrane of choroid plexus epithelial cells. In contrast, PEPT2-positive cells with endothelial or astrocytic morphology were absent. This distinction between PEPT2 transporter expression in neurons and its absence in astrocytes was confirmed by double staining experiments. Both PEPT2 (Figure 3A) and NeuN (Figure 3B), a marker for neuronal nuclei, were readily detected in cerebral cortical sections. When immunolocalization for both PEPT2 and NeuN was performed on the same sections in cerebral cortex, an overlapping signal was observed (Figure 3C), confirming the neuronal expression of PEPT2. In contrast, experiments with an antiserum for GFAP, a marker for astrocytes, showed that PEPT2 and GFAP were expressed in different cells (Figure 4). Thus, it appears that in adult rat brain, the PEPT2 protein is expressed in neurons but not in astrocytes. As before, immunolocalization studies did not detect the presence of PEPT1.

Since there is evidence that PEPT2 is present in astrocytic cultures from neonatal rat, the developmental expression of PEPT2 was further examined by immunoblot and immunocytochemistry analyses. As shown in panels A and B of Figure 5, expression levels of the PEPT2 protein were highest in fetal cerebral cortex and then decreased in a monotonic fashion. By day 75 (adult rat), protein levels were only 14% of the maximal expression level observed in embryonic day 17 animals. Detection of the 85 kDa PEPT2 band (as shown in rat kidney BBMV, and cerebral cortex from fetal and neonatal rats) was eliminated by preincubation of the antiserum with the PEPT2 immunizing fusion protein, but not by preincubation of the antiserum with a nonspecific antigen (Figure 5C). Thus, the specificity of the antiserum was confirmed for brain tissue as it had been previously confirmed for detection of PEPT2 in kidney.[20] Similar results were observed in the other immunoblot and immunolocalization experiments.

The cellular expression of PEPT2 in neonatal brain is displayed in Figure 6. Strong signals were observed in cerebral cortex (Figure 6A), as well as in the choroid plexus and some ependymal cells (Figure 6B,C). Further, PEPT2

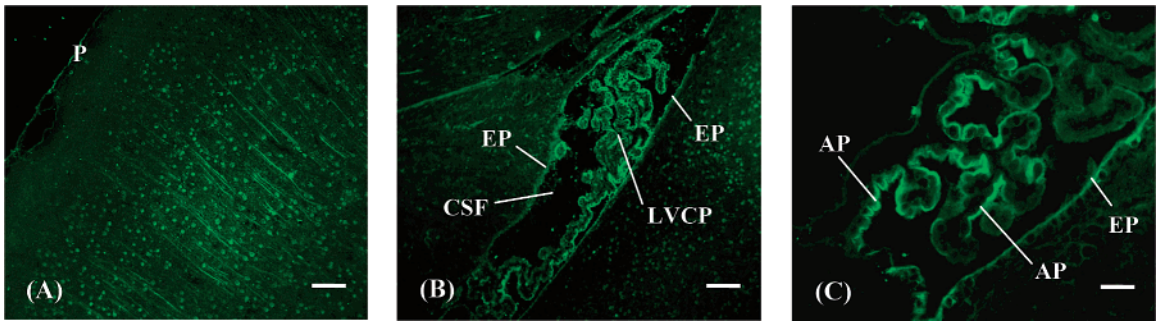

Figure 2. Immunolocalization of PEPT2 expression in the cerebral cortex of adult rat (A). Morphologically, PEPT2 appears to be expressed in neurons of the cerebral cortex (note the axonal profiles). PEPT2 was also present on the apical membrane of choroid plexus epithelial cells and in some ependymal cells (B and C). P denotes the pial surface, CSF the cerebrospinal fluid space, EP the ependyma, LVCP the lateral ventricle choroid plexus, and AP the apical membrane of LVCP. The scale bar is equivalent to 112 (A), 142 (B), and 35 μm (C).

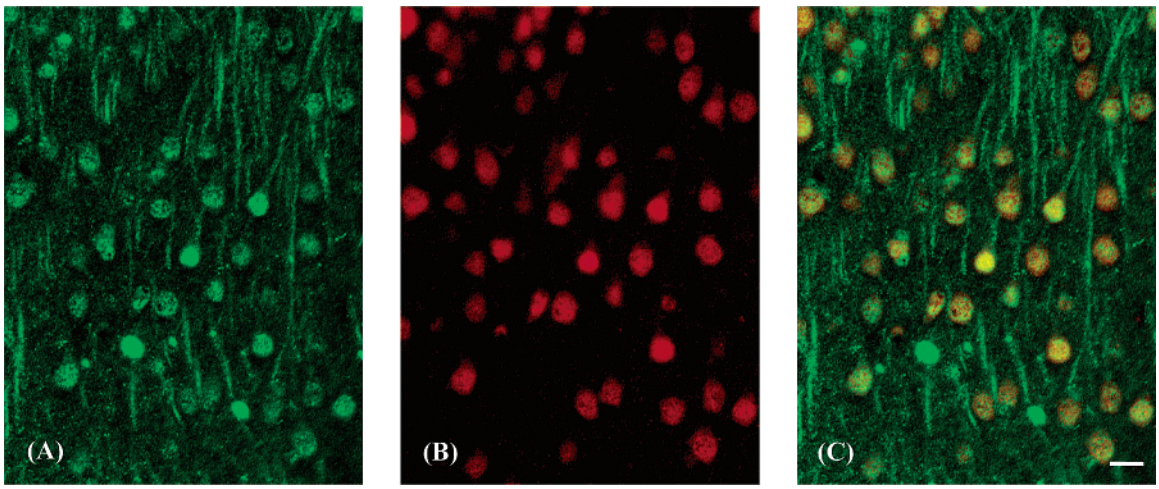

Figure 3. Immunolocalization of PEPT2 expression in cerebral cortical neurons of adult rat by double labeling experiments. PEPT2 is observed in panel A (green signal) and NeuN in panel B (red signal), and the colocalized proteins are observed in panel C (yellow-orange signal). The scale bar is equivalent to 16 μm (C).

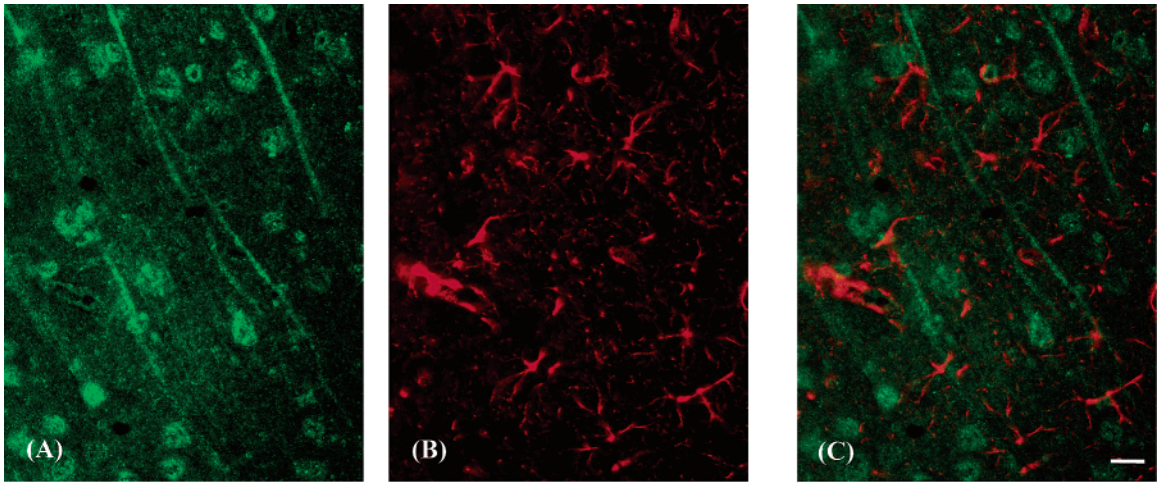

Figure 4. Immunolocalization of PEPT2 expression in cerebral cortex by double labeling experiments indicates that PEPT2 is not expressed in astrocytes of adult rat. PEPT2 is observed in panel A (green signal) and GFAP in panel B (red signal). These two proteins did not colocalize (C). The scale bar is equivalent to 16 μm (C).

is directed to the apical membrane of choroid plexus epithelial cells. Morphologically, PEPT2-positive cells in the brain parenchyma appeared to be both neuronal and astrocytic (Figures 7A and 8A). Colocalization studies with NeuN and GFAP indicated that, indeed, the PEPT2 protein is present in both the neurons (Figure 7) and astrocytes (Figure

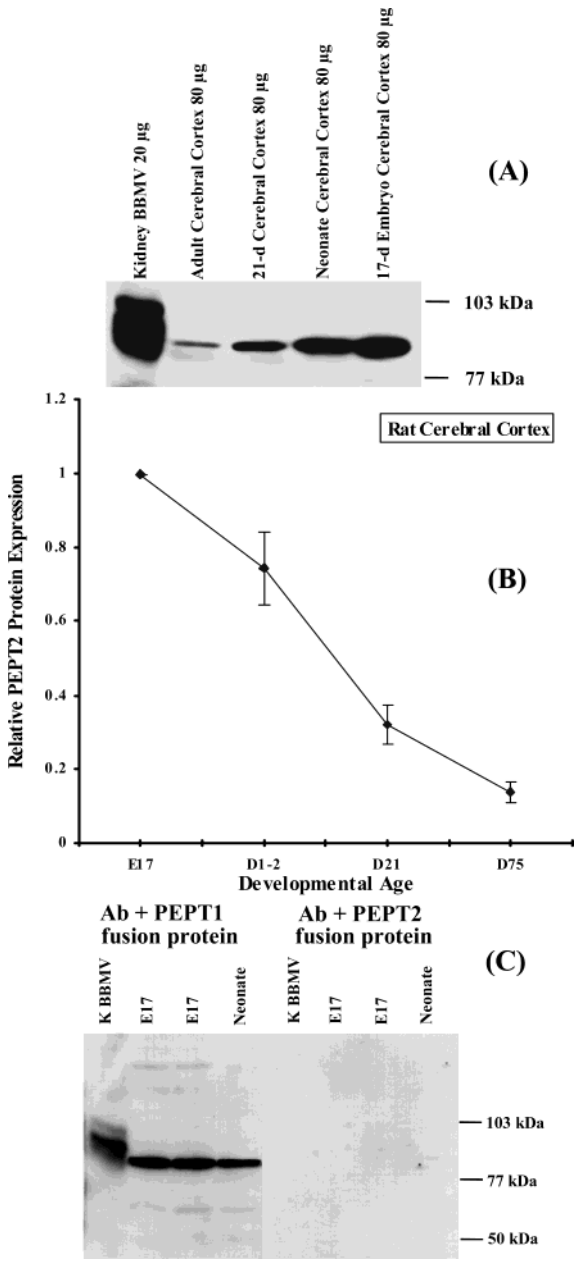

Figure 5. Immunoblot analyses of PEPT2 in membrane proteins (80 μg) prepared from the cerebral cortex of rats at different developmental ages (A). Rat kidney brush border membrane vesicles (BBMV) were used as a positive control. Age-dependent relationship of PEPT2 protein expression in rat cerebral cortex (B). Blots were subjected to scanning densitometry, and protein abundance was expressed relative to that of the maximal observed expression (i.e., 17 day embryo). Data are reported as the means $\pm$ the standard error of seven separate experiments, with equal gender representation in each preparation. Competitive immunoblot analyses of membranes prepared from rat cerebral cortex at day 17 of gestation (E17) and at day 1−2 (neonate) of birth (C). Samples were preincubated with PEPT2 antisera containing either a nonspecific antigen (i.e., PEPT1 fusion protein) or the immunizing antigen (i.e., PEPT2 fusion protein). Rat kidney brush border membrane vesicles (K BBMV) were used as positive controls (10 μg).

8) of neonatal rats. PEPT1 protein expression was sought in neonatal rat tissue as performed previously in adult rat. In all cases, the results were negative.

Discussion

Proton-coupled oligopeptide transporters are responsible for the symport of small peptides across biological membranes via an inwardly directed proton gradient and negative membrane potential. PEPT1 and PEPT2 were the first such transporters that were cloned, and are physiologically important in their ability to absorb protein digestive products arising in the intestine (PEPT1) as well as filtered peptides generated by luminal peptidases in the kidney proximal tubule (PEPT2). These roles are consistent with PEPT1 being abundantly expressed in the intestine (and to a lesser extent in kidney), and PEPT2 being abundantly expressed in the kidney. However, PEPT2 has a broader tissue distribution than PEPT1, and it is also found in the lung, mammary gland, and brain. Moreover, two members of the POT family, PHT1 and PHT2, have been cloned more recently from brain, but their physiological roles have yet to be determined.

The presence of peptide transporters in the brain has generated considerable interest in their physiological role and tissue and cellular distribution in the central nervous system. In the mammalian central nervous system, various amino acids, amines, and small peptides function as neurotransmitters, as neuromodulators, and in the regulation of cellular metabolism, volume, and ion homeostasis. While the mechanisms responsible for turning off the signals produced by amino acids and amines are reasonably well-defined, the mechanism(s) by which neuropeptide signals are attenuated is unclear. One possibility is that the generally long-acting signals are turned off by catabolism of neuropeptides by various aminopeptidases that are present in the central nervous system.[2,32,33] It is also reasonable to suggest that peptide transporters could play an important role in the brain by clearing the peptide fragments that are formed by proteolysis. In doing so, aminopeptidases and peptide transporters, in proximity of each other, would regulate the potentially active (or toxic) neuropeptide metabolites in the extracellular fluid surrounding neurons and/or glial cells. Although functional evidence supports a role for peptide transport within the brain, no previous studies have identified a specific oligopeptide transporter protein in brain tissue or characterized its (sub)cellular localization.

In this study, we report several new findings. First, the PEPT2 protein was found extensively throughout the brain. While it has the strongest signal in cerebral cortex, strong

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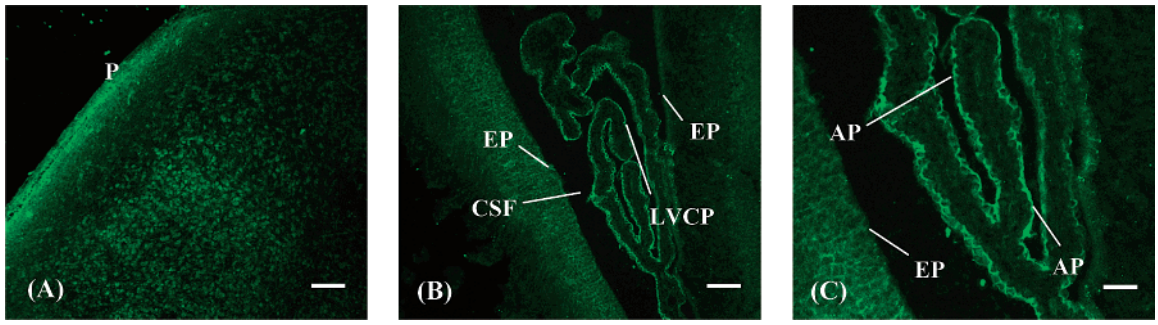

Figure 6. Immunolocalization of PEPT2 expression in the cerebral cortex of neonatal rat (A). PEPT2 was also present on the apical membrane of choroid plexus epithelial cells and in some ependymal cells (B and C). P denotes the pial surface, CSF the cerebrospinal fluid space, EP the ependyma, LVCP the lateral ventricle choroid plexus, and AP the apical membrane of LVCP. The scale bar is equivalent to 112 (A), 108 (B), and 46 μm (C).

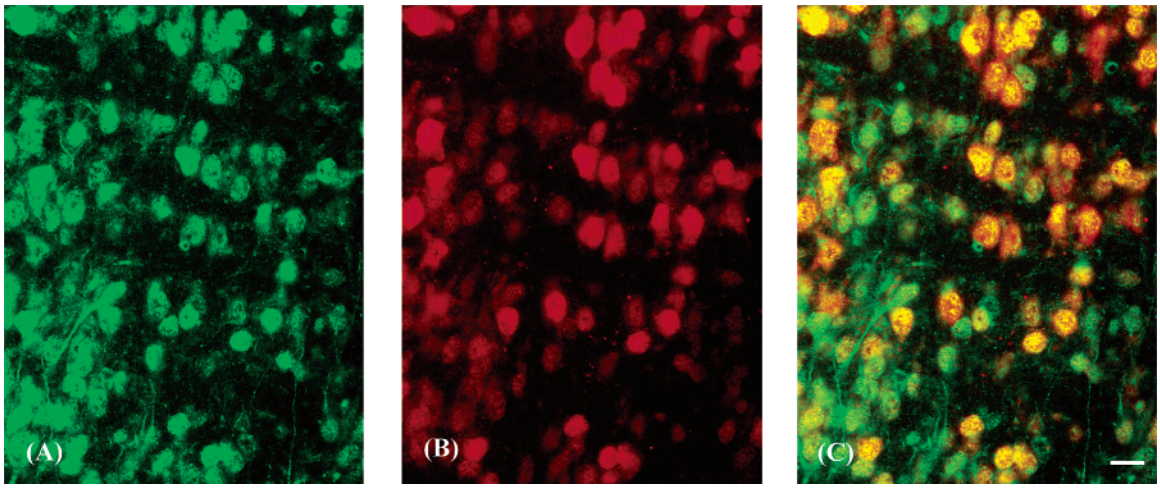

Figure 7. Immunolocalization of PEPT2 expression in cerebral cortical neurons of neonatal rat by double labeling experiments. PEPT2 is observed in panel A (green signal) and NeuN in panel B (red signal), and the colocalized proteins are observed in panel C (yellow-orange signal). The scale bar is equivalent to 16 μm (C).

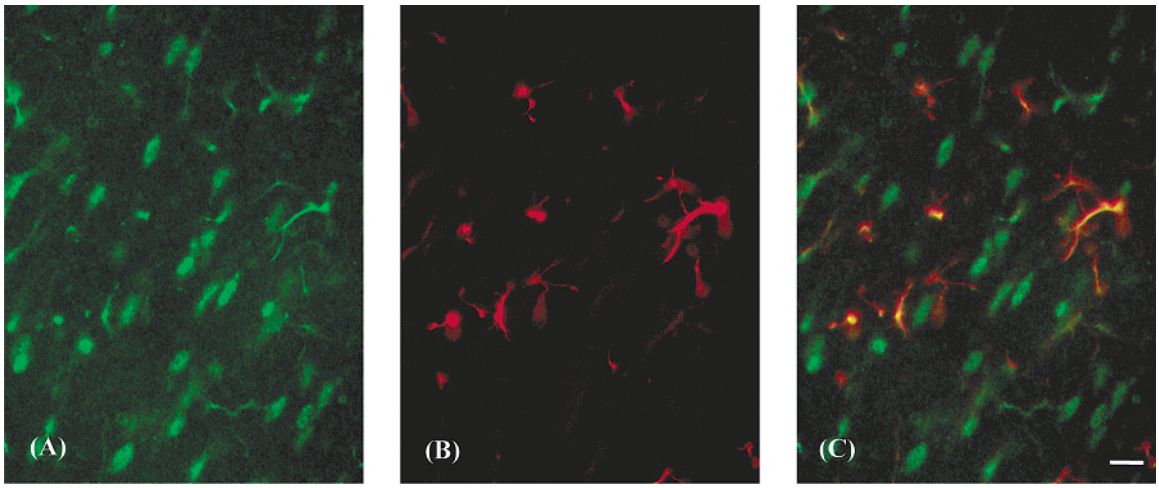

Figure 8. Immunolocalization of PEPT2 expression by double labeling experiments indicates that PEPT2 is expressed in the astrocytes of neonatal rat. PEPT2 is observed in panel A (green signal) and GFAP in panel B (red signal), and the colocalized proteins are observed in panel C (yellow-orange signal). The scale bar is equivalent to 16 μm (C).

signals were also observed in the olfactory bulb, basal ganglia, cerebellum, and hindbrain. Second, PEPT2 was expressed abundantly in epithelial cells of the choroid plexus as well as in ependymal cells. The transporter was expressed exclusively on the apical membrane (i.e., CSF-facing) of choroid plexus epithelia in both adult and neonatal rats, a finding that corroborates our previous studies using choroid plexus whole tissue[27,28] and choroid plexus epithelial cells

in primary culture.[25,34] The preferred apical uptake of GlySar[25] and carnosine[34] in primary cell cultures and results from this study suggest that PEPT2 may function to move peptides from the CSF into the blood. Third, PEPT2 was differentially expressed in cerebral cortex as a function of age, with much greater levels in fetal and neonatal tissue than in adult. Fourth, PEPT2 was expressed in neurons (adult and neonate) as well as in astrocytes (neonate but not adult). It is possible that the age-related decline of PEPT2 in cerebral cortex reflects a loss of astrocytic PEPT2. Fifth, PEPT2 was not found in endothelial cells of the blood−brain barrier, a result that agrees with in situ hybridization studies[24] and the lack of penetration by GlySar in brain microvessel endothelial cells.[35] Finally, PEPT1 was absent from brain.

This study did not examine the presence of PHT1 and/or PHT2 in the central nervous system. While antibodies against the PHT1 transporter are not yet available,[12] antibodies against the PHT2 transporter are thought not to have the required sensitivity for detection of the endogenous protein.[13] In any case, there is still insufficient information to demonstrate that either the peptide/histidine transporter is functionally active in brain.

The neuronal presence of PEPT2 in this study provides molecular validation of earlier studies in which neuropeptides have been shown to be transported in adult rat brain synaptosomes,[6,7,36] in newborn rat olfactory bulb neurons,[37] and in expression systems using PEPT2 cloned from rat brain.[4] In the study by Ueda et al.,[36] the uptake of kyotorphin in whole brain synaptosomes was temperature- and energy-dependent, and the level was significantly decreased by ouabain. Moreover, the K_m of this process was 131 μM, a value indicative of the high-affinity interaction anticipated for PEPT2. In the study by Fujita et al.,[6] the uptake of glycylsarcosine in cerebellar synaptosomes was inhibited competitively by kyotorphin ($K_i = 30$ μM), also suggesting an interaction with the high-affinity transporter PEPT2. These same authors[7] subsequently reported a K_m value of 110 μM for GlySar in cerebral synaptosomes, as well as finding PEPT2 protein in their preparation. Given the dose-dependent inward currents induced by carnosine in olfactory bulb neurons, Kanaki et al.[37] suggested that carnosine is an excitable neuroeffector in the olfactory pathway. PEPT2 might serve as the transporter primarily involved in transporting small neuropeptides (i.e., two or three amino acid residues) since electrophysiological characteristics of PEPT2, cloned from brain, have been reported by Wang et al.[4] for the transport of *N*-acetyl-L-aspartyl-L-glutamate and other charged peptides.

The protein and functional data on neuronal PEPT2 contrast with previous mRNA measurements. Berger and Hediger,[24] using nonisotopic in situ hybridization, failed to demonstrate the presence of PEPT2 mRNA in neurons of adult rat brain, while Dieck et al.,[5] using Northern blot analyses, did not detect PEPT2 transcripts in primary neuron-rich cultures prepared from day 16 rat embryos. Although there are other factors that might account for the discrepancy between the protein/functional and mRNA studies (e.g., the effect of culture conditions and hybridization efficiency), it is possible that there is little turnover of the neuronal PEPT2 protein, and as a result, only low levels of mRNA are required for translation.

In our study, the PEPT2 protein was expressed in astrocytes during the early (i.e., neonates), but not late (i.e., adults), development of rats. The finding of PEPT2 in neonatal astrocytes is consistent with other studies in which PEPT2 functional activity and mRNA expression have been noted in astrocytic preparations from newly born animals. In particular, Dringen et al.[3] reported that PEPT2 mediates the uptake of cysteinylglycine, a glutathione precursor, in astroglia-rich primary cultures prepared from neonatal rats and that RT-PCR indicated the presence of PEPT2 transcripts in these cells. A similar finding was reported by Dieck et al.,[5] in which a fluorescent reporter dipeptide accumulated in astroglia-rich cell cultures prepared from newborn rats by an energy-dependent, saturable process with a K_m value of 28 μM. Northern blot analyses also indicated that PEPT2 mRNA was expressed in glial cell cultures.

The absence of the protein for PEPT2 in adult astrocytes contrasts with the finding of mRNA by Berger and Hediger,[24] using in situ hybridization. This appears to be another example of the presence of mRNA not necessarily being reflected in protein levels. It is interesting to speculate that the presence of PEPT2 mRNA in adult astrocytes may allow the rapid production of protein (under certain conditions) for clearing peptides from the brain extracellular space.

One possible reason for the developmental change in astrocyte PEPT2 protein levels may reside in maturation of the blood−brain barrier. In the rat, there is a marked "tightening" of the blood−brain barrier between the fetus and the adult. This is reflected by an increase in the transendothelial resistance[38] and a reduction in the permeability of low-molecular weight organic compounds[39] with age. Thus, early in development, there may be an influx of di- or tripeptides from blood that is cleared by astrocytes,

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but in the adult, such flux is prevented by a "mature" blood−brain barrier. Interestingly, Groneberg et al.[40] found that glial cells express the PEPT2 protein in the dorsal root ganglia of adult rats. Evidence indicates that some high-molecular weight compounds that are excluded from the brain by the blood−brain barrier can penetrate the dorsal root ganglia.[41,42] Thus, PEPT2 may be necessary in this leakier region of the nervous system.

In conclusion, our findings demonstrate that the PEPT2 protein is found throughout the brain. The apical expression of PEPT2 in choroid plexus suggests that it is involved in the export of neuropeptides, peptide fragments, and peptide-like drugs from cerebrospinal fluid. Its expression in neurons (adult and neonate) and astrocytes (neonate, not adult) further suggests that PEPT2 may play a role in regulating the concentration of neuropeptides in extracellular fluid, especially at birth.

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