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Localization of Peptide Transporter in Nuclei and Lysosomes of the Pancreas

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Summary

Conclusions. These studies show for the first time the localization of a H^+/peptide cotransporter in nuclei of vascular smooth muscle cells and Schwann cells and its localization in lysosomes of the exocrine pancreas. It is likely that the transporter functions to move small peptides from the lysosome to the cytoplasm following intralysosomal protein degradation. The nature of the transporter function in the nucleus remains to be determined, including the possibility that peptide signaling molecules may be transmitted between nucleus and cytoplasm.

Background. PEPT1 transports di- and tripeptides through plasma membranes. Peptides are cotransported with H^+, thus deriving the energy for the active transport process from an electrochemical H+ gradient. The main regions in which PEPT1 has been thought to function are the plasma membranes of the small intestinal epithelial cells for absorption of protein digestion products and in the kidney tubules for recovery of small peptides from the glomerular filtrate.

Methods. Pancreas was removed from rats and quick frozen with liquid nitrogen. Frozen sections were fixed in cold acetone. Sections were incubated with primary antibody against PEPT1, followed by a secondary antibody conjugated with fluorescein, then examined with a fluorescence microscope.

Results. Three major structures were immunopositive with the antibody to PEPT1: the nuclei of smooth muscle cells in the wall of arterioles, the nuclei of Schwann cells in unmyelinated pancreatic nerves, and lysosomes in acinar cells.

Key Words: Peptide transporter; PEPT1; smooth muscle cell nuclei; acinar cell lysosomes; Schwann cell nuclei.

Introduction

PEPT1 is a peptide transporter that is present in the small intestine and kidney of humans and experimental animals *(1)*. It appears in the brush border membranes of enterocytes and has been localized there by fluorescence microscopy *(2)*. Its function in the intestine is to facilitate the absorption of dipeptides and tripeptides resulting from the digestion of protein that has not proceeded to the production of single amino acids *(3)*. It is also capable of transporting peptide-like drugs, such as cephalosporins and penicillins *(4–7)*, and bestatin *(8)*. Its presumed function in the kidney is to recover di- and tripeptides from the lumen of proximal tubules. PEPT1-medi-

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ated transport of peptides is an active process and a transmembrane electrochemical H^+ gradient serves as the driving force *(9,10)*.

Human PEPT1 has been cloned *(7)*. It consists of 708 amino acids and contains 12 putative transmembrane domains. The expression of PEPT1 cDNA by microinjection of cRNA *(5,11)* in *Xenopus laevis* oocytes reveals that, although it transports a variety of di- and tripeptides, it does not transport larger peptides or free amino acids.

Northern blot analysis has shown that mRNA transcripts hybridizing to PEPT1 cDNA are present in the pancreas *(7)*. In the present study, an antibody produced in response to a peptide sequence from PEPT1 was used to study its localization in the pancreas. Surprisingly, it was localized in smooth muscle and Schwann cell nuclei, as well as in lysosomes of acinar cells.

Methods

Antibody to human PEPT1 was raised in rabbits using an 18-amino-acid sequence, RFRHRSKAF PKREHWLDW, corresponding to position 247–264 in the amino acid sequence. A cysteine residue was added to the C-terminus of this peptide for coupling to keyhole limpet hemocyanin before injection into rabbits *(11)*. Polyclonal antibodies were affinity-purified by passing them through a column of Sepharose 4B to which the 18-amino-acid peptide was coupled. This antibody recognized PEPT1 from various sources by immunoblotting, as well as by immunofluorescence in the membrane of *X. laevis* oocytes induced to express PEPT1 by injection of cRNA *(11)*.

Segments of pancreas from four adult male rats were removed for study, each in four different weeks. The specimens were immediately frozen onto stubs using liquid nitrogen. Sections 10 μm thick were cut in a cryostat at –20°C, mounted on slides, and immediately fixed in acetone at the same temperature. The slides were warmed to room temperature and washed through phosphate-buffered saline (PBS), pH 7.4, before beginning incubation with antibodies.

Primary antibody (rabbit antihuman PEPT1), diluted 1:100, was layered on sections for 1 h at room temperature after nonspecific binding was blocked by incubation in 10% normal goat serum in PBS. The secondary antibody was fluorescein-conjugated goat antirabbit IgG (AffiniPure, Jackson ImmunoResearch, West Grove, PA). The secondary antibody, diluted 1:100, was layered onto sections for 1 h after washing through PBS. After final washing, the sections were observed and photographed using a Zeiss fluorescence microscope.

Negative controls to assure against nonspecific binding were prepared by omitting the primary antibody and by preincubating the antibody with the 18-amino-acid peptide originally used for antibody production, but otherwise following the same procedure as above.

In order to identify structures in the sections of pancreas, some sections were stained with hematoxylin and eosin, then observed and photographed using transmitted light with the same microscope used for fluorescence.

Results

In hematoxylin and eosin-stained sections, structures of the normal pancreas were clearly observed. Arterioles, nerves, acinar cells, and islets were easily identifiable. Figure 1 is included for the purpose of allowing comparison of images of arteriole and nerves with subsequent fluorescent images.

The nuclei of smooth muscle cells in the wall of arterioles were immunopositive with the antibody to PEPT1 (Fig. 2). Arterioles with a thicker muscle layer had more positive nuclei than those with a greater proportion of elastic laminae closer to their origin from the aorta. Omission of the primary antibody left only the nonspecific fluorescence of the elastic laminae (Fig. 3).

The nuclei in sections of nerves also were immunopositive with the antibody to PEPT1 (Fig. 4). Because there were no nerve cell bodies in the nerves, the nuclei are identified as Schwann cells. In these nuclei, as in those of smooth muscle cells, there was evidence of localization in the region of the membrane and also some bright punctate fluorescence toward the central area. It is not certain whether the latter represents localization in nucleoli or association with another structure. Omission of the primary antibody resulted in no fluorescence emanating from nerves (not shown).

The nuclei of acinar cells were not immunopositive. However, minute punctate structures corresponding to lysosomes were immunopositive (Fig. 5). Omission of primary antibody resulted in the disap-

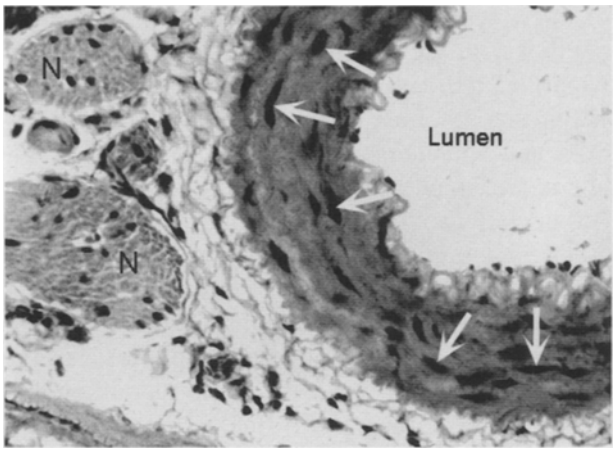

Fig. 1. Frozen section of rat pancreas stained with hematoxylin and eosin. Smooth muscle nuclei in the wall of an arteriole are indicated by arrows. Dark nuclei in sections of nerves (N) are Schwann cell nuclei.

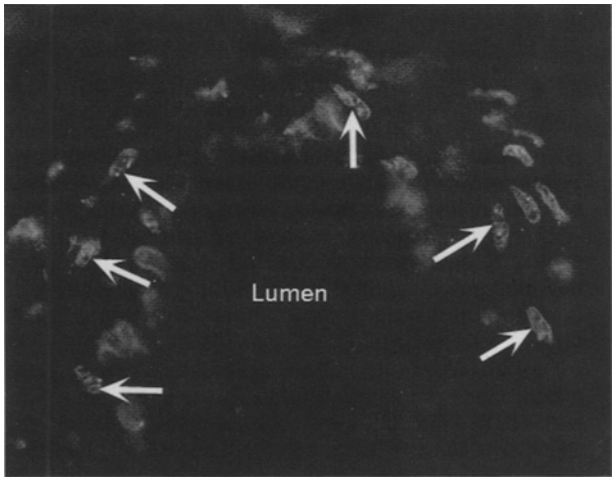

Fig. 2. Fluorescence micrograph of pancreatic arteriole. The nuclei of smooth muscle nuclei (arrows) are immunopositive for PEPT1.

pearance of this immunopositivity, although yellowish autofluorescence of some lysosomes was observable (not shown). Preincubation of antibody with the 18-amino-acid antigen blocked localization in nuclei and lysosomes (not shown).

In the connective tissue through which vessels and nerves ran, occasional cells displayed fluorescent granules. These presumably correspond with the lysosomes of macrophages in these areas.

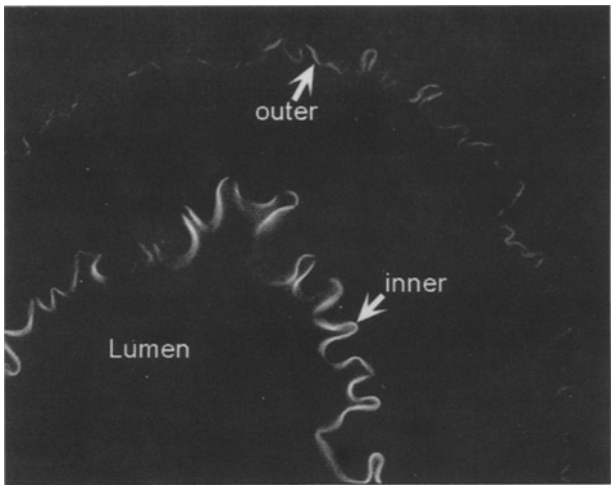

Fig. 3. Negative control for localization in smooth muscle nuclei. The fluorescence that remains after omitting the anti-PEPT1 antibody is the autofluorescence of the inner and outer elastic laminae. Smooth muscle nuclei are negative.

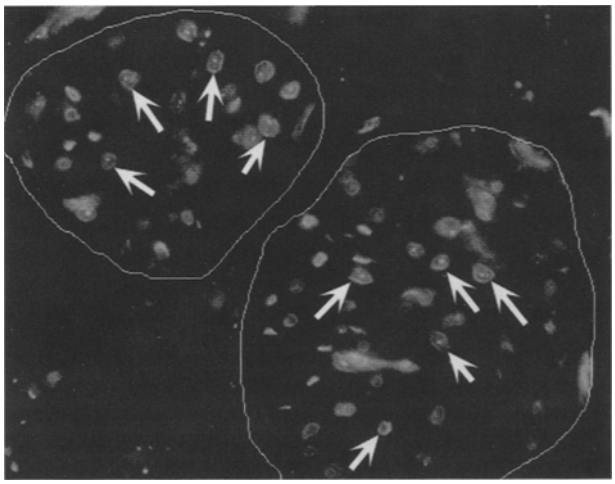

Fig. 4. Sections of two pancreatic nerves. Their perimeters are outlined approximately. The Schwann cell nuclei, indicated by arrows, are immunopositive.

Discussion

This study has provided clear evidence of the localization of a peptide transporter in lysosomes of acinar cells and in nuclei of vascular smooth muscle and Schwann cells.

Several years ago it was speculated that intralysosomal digestion might be incomplete and that

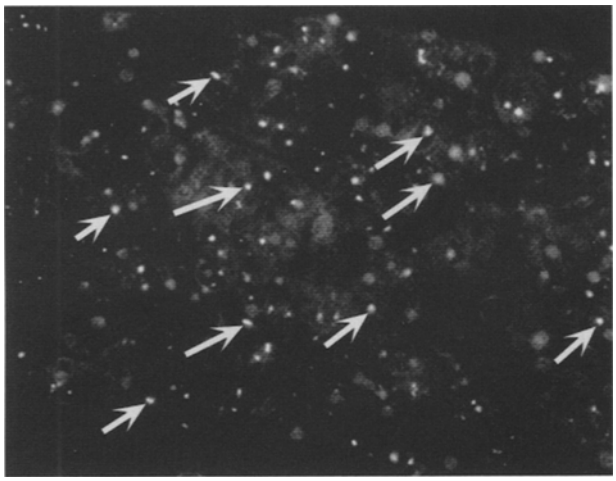

Fig. 5. Section through the exocrine pancreas. Small, punctate immunopositive bodies (arrows) correspond with lysosomes in acinar cells. Those indicated by the arrows are well delimited because they are in focus. Those out of the focal plane appear lighter and more diffuse.

small peptides might be transported through lysosomal membranes from the inside of the lysosome to the cytosol *(12)*. Since then, evidence has accumulated for the selective transport of di- and tripeptides across the lysosomal membrane *(13,14)*. Quite recent evidence has demonstrated the presence of a H^+-coupled peptide transporter in lysosomes from liver *(15)*. It is presumed that in these lysosomes, and in the lysosomes in the pancreas, small peptides resulting from incomplete protein breakdown are cotransferred from the lysosome using the motive force of H^+ transport through the membrane. The demonstration in the present study of a H^+/peptide cotransporter in lysosomes of the pancreas suggests that di- and tripeptide transport from lysosomes may be a common phenomenon.

The observation of localization of a H^+/peptide cotransporter in nuclei is novel. Previous studies have concentrated on the presence of the peptide transporter in the apical cell membrane of enterocytes of the small intestine and absorptive cells of kidney tubules *(1)*. Immunofluorescence studies in the small intestine revealed immunopositive reaction in the brush border *(2)*, consistent with studies using vesicles derived from that source *(16,17)*.

Localization in nuclei is far from universal. Nuclei of exocrine and endocrine cells were negative. Furthermore, nuclei of smooth muscle of the muscularis of intestine, taken at the same time and treated in parallel with the pancreas (not shown here), were negative, indicating a selectivity of localization within smooth muscle nuclei. Similarly, only a few Schwann cell nuclei were positive in sections of sciatic nerve, indicating that localization in Schwann cell nuclei is not universal.

The function of the transporter in nuclei remains to be determined. Suggestions concerning this function can now only be a matter of speculation. It is not known so far what dipeptides or tripeptides can serve as substrates for the nuclear transporter, although it is reasonable to begin with the assumption that a broad range of peptides will serve, as has been shown in other locations. It is not known if transport of protein breakdown products is involved. An intriguing possibility is that this transporter, and perhaps other transporters, could serve to transport small peptides that would act as signaling molecules for events in the nucleus. The pursuit of these possibilities should present exciting new understandings of novel functions, hitherto unrecognized, of the peptide transporters in mammalian cells.

Acknowledgment

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