JOP. J Pancreas (Online) 2009 Sep 4; 10(5):501-506.

ORIGINAL ARTICLE

BACE1 is a Newly Discovered Protein Secreted by the Pancreas which Cleaves Enteropeptidase *In Vitro*

Albrecht Hoffmeister[1], Gunther Dietz[1], Ulrike Zeitschel[2], Joachim Mössner[1], Steffen Rossner[2], Tobias Stahl[3]

[1]Department of Internal Medicine II, [2]Paul Flechsig Institute for Brain Research, and [3]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine; University of Leipzig. Leipzig, Germany

ABSTRACT

Context Activity of beta-site APP-cleaving enzyme1 (BACE1) is required for the generation of beta-amyloid peptides, the principal constituents of plaques in the brains of patients with Alzheimer's disease. Strong BACE1 expression has also been described in pancreatic tissue. **Objective** The aim of the present study was to reveal the cell type-specific expression of BACE1 in the pancreas and to identify a substrate for BACE1 in this organ. **Methods** RT-PCR of microdissected rat pancreatic tissue was carried out in order to analyze BACE1 expression within pancreatic acini. Pancreatic juice was examined by western blot analysis and by an enzymatic activity assay in order to reveal the presence of secreted BACE1. Database analysis suggested enteropeptidase as a putative substrate for BACE1 in pancreatic juice. *In vitro* digestion of enteropeptidase by BACE1 was performed to demonstrate this cleavage. **Results** We demonstrate the expression of BACE1 in the islets of Langerhans and at the apical pole of pancreatic acinar cells. Recombinant BACE1 cleaves enteropeptidase *in vitro*. Furthermore, some results suggested the presence of BACE1 enzymatic activity in pancreatic juice and pancreatic tissue. **Discussion** We hypothesize that enteropeptidase is a BACE1 substrate *in vivo*. If so, BACE1 could protect the pancreas from premature trypsinogen activation due to the occasionally occurring reflux of enteropeptidase.

INTRODUCTION

Pancreatitis is a potentially lethal disease with an incidence of 5-10/100,000 in industrialized nations. In spite of medical progress, particularly in intensive care medicine, acute pancreatitis remains a severe disease with a mortality of 2-10% [1]. The pathophysiology of acute pancreatitis is a multifactorial process which is still only partially understood. The activation of intrapancreatic proteases, and the subsequent autodigestion of pancreatic tissue, has been suggested as a key factor in triggering the inflammatory process in pancreatitis [2]. The elevation of trypsinogen activation peptide in urine during the acute phase of pancreatitis supports this theory [3]. Altered activation of pancreatic proteases has already been shown in patients with hereditary pancreatitis, a minor subgroup of pancreatitis patients. Mutations of the cationic trypsinogen gene associated with this rare disease have been reported to lead to modified stability of pancreatic trypsinogen [4, 5]. For most cases of pancreatitis, however, the cascades leading to inflammation and organ destruction remain largely obscure. Clarification of the first steps in this chain would represent a major advance in the elucidation of the pathological cascades which cause pancreatitis and could potentially offer a more specific treatment option for the early stages of this disease.

Enteropeptidase activation has long been thought to be the first step of the activating cascade of digestive enzymes. This gut enzyme itself is synthesized as an inactive precursor as are many of the pancreatic acinar digestive enzymes [6]. Inactive proenteropeptidase is a single chain protein. Duodenase activates proenteropeptidase by cleavage which results in enteropeptidase heavy and light chains [7]. The light chain contains the catalytic center, which is similar to other trypsin-like serine proteinases [8]. The physiological importance of enteropeptidase is indicated by severe intestinal maldigestion in patients with a congenital deficiency of this enzyme [9, 10, 11]. Enteropeptidase has been described not only at the brush border membrane of the duodenum but also within intestinal mucosal fluid and intestinal contents

Received December 18th, 2008 - Accepted July 20th, 2009

Key words BACE1 protein, human; Enteropeptidase; Pancreas, Exocrine; Pancreatitis

Abbreviations BACE1: beta-site APP-cleaving enzyme1

Correspondence Albrecht Hoffmeister
Department of Internal Medicine II, University of Leipzig, Ph.-Rosenthal-Str. 27, 04103 Leipzig, Germany
Phone: +49-341.971.2251; Fax: +49-341.971.2239
E-mail: albrecht.hoffmeister@medizin.uni-leipzig.de

Document URL http://www.joplink.net/prev/200909/15.html

[12, 13]. The enzyme has a high specificity for the sequence $(Asp)_4$-Lys, a motif found in the amino terminus of trypsinogen, which is highly conserved across various species [14]. Cleavage of trypsinogen at the lysine residue of the recognition site releases the amino terminal trypsinogen activation peptide [15] and results in active trypsin.

Since some studies have shown that duodenobiliary reflux can occur postprandially [16], intrapancreatic activation of trypsinogen by enteropeptidase has been proposed as a potential cause of acute pancreatitis [17]. However, since the pressure in the pancreatic duct is higher than that in the biliary tract, reflux of the duodenal contents into the pancreatic ducts is highly debated and remains controversial.

Beta-site APP-cleaving enzyme1 (BACE1) was recently described by several groups as being up-regulated in the brains of patients with Alzheimer's disease. It is the endoprotease required for the generation of beta-amyloid peptides, the principal constituents of plaques in the brains of Alzheimer's disease patients [18, 19, 20, 21]. BACE1 is a type I transmembrane aspartic protease [18]. However, full-length BACE1 has also been detected in the cerebrospinal fluid of patients with Alzheimer's disease and non-demented subjects [22]. Thus, BACE1 appears to be secreted and might have additional functions unrelated to intracellular amyloidogenesis.

Interestingly, BACE1 mRNA is found at moderate levels in the brain, at low levels in most peripheral tissues but at rather high levels in the pancreas [20, 21, 23, 24, 25]. However, in the pancreas, both the function and organ-specific substrates of BACE1 are unknown. There are only a few reports on the BACE1 protein in the pancreas. Sinha *et al*. [19] measured only very low levels of specific BACE1 activity in the pancreas and proposed a different post-transcriptional regulation of its activity. Moreover, although full-length BACE1 (BACE1-501) is the major transcript in the pancreas [26], this BACE1 isoform was not detected in pancreatic homogenates.

We herein reveal the expression and cellular localization of BACE1 in pancreatic acini. Using western blot analysis, we demonstrate BACE1 protein in pancreatic organ homogenates and in pancreatic juice. However, our results only suggest the presence of BACE1 enzymatic activity in pancreatic juice and pancreatic tissue. Recombinant BACE1 *in vitro* cleaves purified enteropeptidase, indicating a possible role of BACE1 in protecting the pancreas from autodigestion.

MATERIALS AND METHODS

BACE1 Western Blot Analysis

BACE1 was detected by western blot analysis as has recently been described [27]. Briefly, 8 µg protein was loaded on a 8% SDS-PAGE gel, separated by electrophoresis at 150 V for 2 hours and transferred onto a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The membranes were blocked overnight in TBS pH 7.4 containing 1% Tween 20 and 4 % BSA (TBST/BSA) and then incubated with a monoclonal antibody (anti-BACE1; 1:2,000) or a rabbit antiserum (AS-4; 1:1,000) in the same medium at 4°C overnight. After three washes in TBST, the nitrocellulose membranes were incubated with the corresponding peroxidase-conjugated secondary antibodies in TBST/BSA (1:5,000) for 45 minutes, washed again three times in TBST and preincubated in diaminobenzidine (DAB) substrate buffer (50 mM TB; pH 7.6). The presence of the marker enzyme was visualized by incubating nitrocellulose membranes with 4 mg diaminobenzidine and 5 µL H_2O_2 (30%; v/v) per 10 mL substrate buffer.

BACE1 Activity Assay

BACE1 enzymatic activity was quantified in pancreatic homogenates and in pancreatic juice using a commercially available assay (R&D Systems; Wiesbaden, Nordenstedt, Germany) according to the manufacturer's protocol. BACE1 activity in different samples was normalized to protein content and given as units of flourochromated substrate hydrolyzed per µg of protein. In order to inhibit activity of lysosomal cathepsin D, the assay procedure was also performed after the addition of pepstatin to a final concentration of 30 µM.

Immunhistochemical Detection of BACE1 Expression in the Murine Pancreas

Adult mice were sacrificed using an overdose of anesthesia (ketamine/xylazine), and the pancreatic tissue was removed rapidly and fixed by immersion with 4% paraformaldehyde in 0.1 M phosphate-buffered saline for 18 hours. Fixed tissue was paraffin-embedded, and 12 µm-thick sections were cut on a sliding microtome. For immunodetection of BACE1, polyclonal rabbit serum at a dilution of 1:500 was used as has recently been described [27]. After deparaffination, the sections were incubated with proteinase K (5 µg/mL) for 30 minutes at room temperature, followed by destruction of the endogenous peroxidase activity by incubation of the specimens with 0.3% hydrogen peroxide in TBS for 15 min. Unspecific binding sites were blocked by incubation of the slides in TBS containing 5% normal goat serum (blocking solution) just prior to the incubation of the slides with the primary antibody in blocking solution at 4°C overnight. The detection of the primary antibody was carried out by incubation with biotinylated goat-anti-rabbit IgG (1:1,000; DAKO, Glostrup, Denmark). The bound secondary antibody was revealed with an avidin-biotin-peroxidase kit (ABC, Vector Laboratories, Burlingame, CA, U.S.A.) using 3,3'-diaminobenzidine as the chromogen. Control experiments were carried out either by omitting incubation with the BACE1-specific antiserum or by using the rabbit pre-immune serum instead of the antiserum.

Acini Microdissection and RT-PCR for BACE1 and Insulin

Adult rats were sacrificed by inhalation of CO_2. Pancreatic tissue was removed immediately and frozen on dry ice. Cryostat sections (10 µm) were prepared, stained with hematoxylin for 30 seconds, and dried using a graded series of ethanol. Pancreatic acini were sectioned using a laser microdissection microscope (Cell Cut, MMI/IX71, Olympus, Zurich, Switzerland) according to the manufacturer's protocol. The samples were lysed, and RNA was prepared using RNAeasy (Quiagen, Hilden, Germany) supplemented with 4% mercaptoethanol. Two µL of lysate were used for each reverse transcriptase reaction (random primer, Quiagen), and 2 µL of the cDNA obtained underwent PCR, followed by nested PCR. The following primers were used for amplification: BACE1 (-caccatccttcctcagcaat-; -gcagagtggcaacatgaaga-), BACE1 nested (-tacaagttcgccgtctcaca-; -gtgacaaacggaccttccac-) and insulin (-ggggaacgtggtttcttct-; -agtggtggactcagttgcag-), insulin nested (-agaccttggcactggaggtt-; -ttttggtgctcattcaaagg-)

Enteropeptidase Digestion

One microgram of bovine enteropeptidase (Biozyme Laboratories San Diego, CA, U.S.A.) was incubated with 2.9 µg of recombinant BACE1 in assay buffer (R&D Systems, Wiesbaden, Nordenstedt, Germany). At the beginning of the incubation and after 1, 5, and 10 minutes, 1/8 of the reaction mixture was withdrawn, denatured and applied to SDS polyacrylamide gel electrophoresis. Separated proteins were visualized by silver staining of the gels.

ETHICS

No animal experimentation was performed. Animals were only sacrificed according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals (1996)", prepared by the National Academy of Sciences, in order to obtain organs.

RESULTS

BACE1 is Expressed in Pancreatic Acini and Localized at the Apical Cellular Pole of the Acini

There are inconsistent results on BACE1 expression in the pancreas [23, 25, 26]. In one report, BACE mRNA was detected in the pancreas, in particular in beta-cells, but no BACE1 mRNA was found in pancreatic exocrine ductal cells [24]. In order to reveal the cellular source of BACE1 in the pancreas, immunohistochemistry of the tissue sections and RT-PCR of the microdissected tissue were carried out. We observed strong labeling of the islets of Langerhans and only weak labeling of the pancreatic acini (Figure 1a). However, within the stained acini, more intense BACE1 immunoreactivity of the apical cell poles was observed (Figure 1a). To substantiate these results, we separated the acini of the exocrine pancreas from the islets of Langerhans by microdissection and performed RT-PCR using BACE1-specific primers. The presence of BACE1 mRNA was shown in the pancreatic islets as well as in the acini (Figure 1b, top). The quality of the tissue prepared by microdissection was proven by the presence of insulin mRNA within the islets of Langerhans and its absence in the dissected acini (Figure 1b, bottom).

BACE1 Protein is Abundant in Pancreas and Secreted into Pancreatic Juice

To substantiate our findings on the pancreatic expression of BACE1, we also examined the presence of the BACE1 protein in the pancreas by western blot analysis. Using both a monoclonal antibody as well as a polyclonal antiserum, we observed a band in the pancreas corresponding to the molecular weight of recombinant BACE1 (data not shown) and BACE1 in the brain (Figure 2). Thus, we concluded that the BACE1 protein is present in the pancreas.

As mentioned above, in the brain, BACE1 can be secreted into the cerebrospinal fluid. Considering the

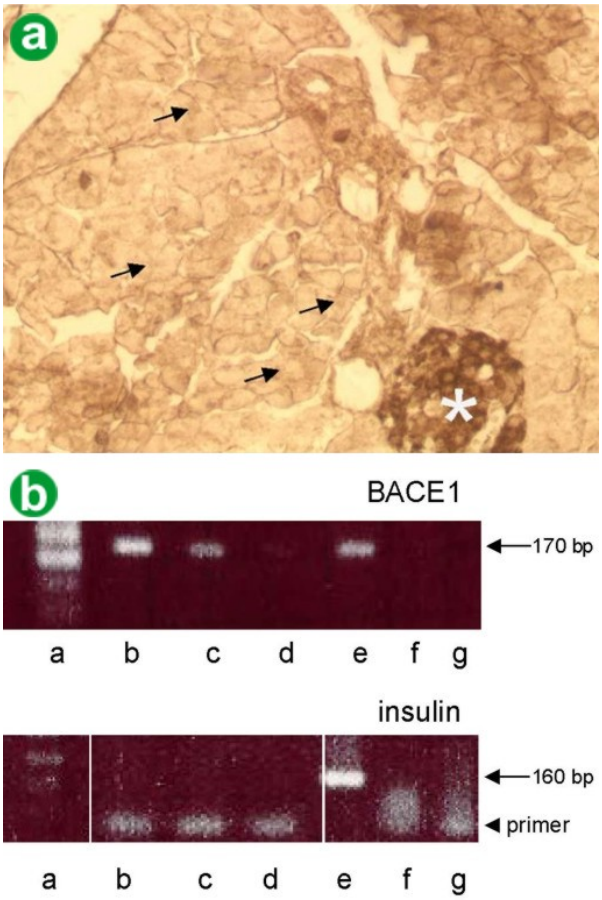

Figure 1. a. Detection of pancreatic BACE1 protein by immunohistochemistry. Note the strong labeling of the islets of Langerhans (asterisk) and the weak staining at the apical pole of the pancreatic acini. **b.** Pancreatic acini and islets of Langerhans were dissected from rat pancreatic slices by laser capture microdissection, and mRNA was prepared and reverse transcribed. BACE1 was detected by nested PCR in the pancreatic acinus-derived cell line AR42J (lane b), pancreatic acini (lane c+d) and islets of Langerhans (lane e). Contamination of microdissected acini by beta-cells was excluded by nested RT-PCR using insulin-specific primers. Lane a: bp standard; lanes f and g: negative controls for PCR.

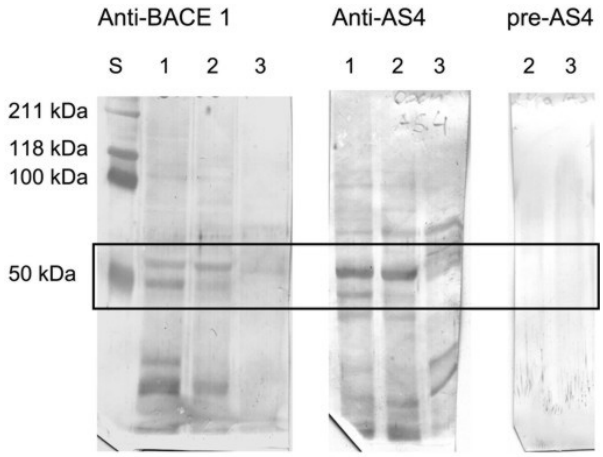

Figure 2. Detection of BACE1 protein by western blot analysis in brain tissue homogenate from BalbC mice (1), and two different pancreatic juices (2+3). A monoclonal antibody (anti-BACE1; S: molecular weight marker) and a rabbit antiserum (AS4) were used for BACE1 detection. Incubation of the same samples with preimmune serum (pre-AS4) did not result in BACE1 immunoreactive bands at the molecular weight of BACE1.

observed apical subcellular localization, we wondered whether BACE1 is secreted into the pancreatic juice as well. To address this question, pancreatic juice was separated by SDS-PAGE electrophoresis, and BACE1 was visualized by western blot analysis. As shown in Figure 2, specific BACE1 immunoreactivity was detected in the pancreatic juice.

BACE1 Activity is found in the pancreas

In the next step, we wanted to determine whether detected BACE1 immunoreactivity in the pancreas and in pancreatic juice derives from intact and enzymatically active BACE1 protein. Therefore, we used a commercial activity assay to specifically detect BACE1 activity. The highest BACE1 activity, ranging from 22 to 53 units/µg of protein, was found in pancreatic tissue homogenates and in homogenates of the exocrine pancreatic cell line AR42J (28 to 38 units/µg protein). Additionally, BACE1 activity was also found in pancreatic juice (6 to 9 units/µg of protein; Figure 3).

We repeated this experiment with pancreatic juice samples in the presence of BACE1 specific inhibitors and pepstatin, a specific cathepsin D inhibitor. In this experiment, BACE1 activity in pancreatic juice was not reduced after the addition of either specific BACE1 or cathepsin D inhibitors (data not shown). The lack of effect of BACE1 inhibitors on enzyme activity may be due to the degradation of BACE1 in our samples because we noticed a reduction in BACE1 enzymatic activity after prolonged storage periods. This was also evident from our western blot analysis (Figure 2). Cleavage of the fluorimetric assay peptide by cathepsin D, which shares a low pH optimum with BACE1, is unlikely because the addition of pepstatin did not alter enzymatic activity.

Enteropeptidase is a Putative Substrate for Pancreatic BACE1

Searching for a physiological substrate of BACE1 in pancreatic juice, we used known sequences cleaved by BACE [28] and screened proteins of pancreatic juice and duodenal mucosa for potential BACE substrates. Within the enteropeptidase light chain, the active part of the enzyme, we found a potential BACE1 cleavage site. Cleavage of the enteropeptidase light chain at this point would result in two fragments of approximately 25 kDa and 10 kDa (Figure 4a). To demonstrate cleavage of the enteropeptidase by BACE1 *in vitro*, we incubated a bovine enteropeptidase light chain with recombinant BACE1. Only one minute after starting the reaction, cleavage products of the expected sizes, 25 kDa and 10 kDa, were detected (Figure 4b), suggesting a specific cleavage. The disappearance of the smaller fragment after five minutes of incubation may have been due to additional cleavage of that fragment.

DISCUSSION

Aspartyl protease BACE1 is a type I transmembrane aspartic protease which has been closely linked to the pathogenesis of Alzheimer's disease [18]. In addition to its expression in the brain, BACE1 is expressed at rather high levels in the pancreas. Different groups have reported the presence of BACE1 mRNA within the pancreas [23, 26, 29], particularly in beta-cells [24]. The same authors report the absence of BACE1 mRNA within the exocrine ductal cells of the pancreas. We report for the first time that BACE1 is expressed in pancreatic acinar cells. Using a microdissectional

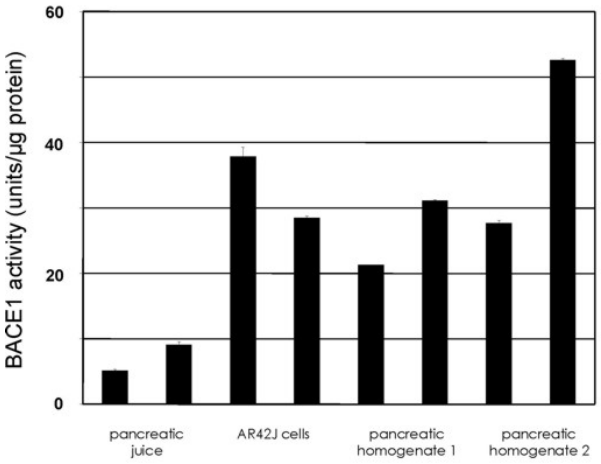

Figure 3. Homogenates from two different mouse pancreata, lysates of the pancreatic acini-derived cell line AR42J and pancreatic juice were screened for BACE1 enzymatic activity using a fluorimetric BACE1 activity assay.Two preparations were produced from all samples and were measured three times each. BACE1 activity was detectable in all samples. Measured activity is shown in units of specific activity per µg protein (mean± SD). Lower amounts detected in pancreatic juice are probably due to proteolysis during sample collection.

approach, we demonstrated the presence of BACE1 mRNA both within pancreatic acini and the islets of Langerhans. The presence of BACE1 mRNA within the islets of Langerhans is consistent with the reports of others who described BACE1 as being expressed in pancreatic beta-cells [24]. However, the same group did not detect BACE1 mRNA within the exocrine part of the pancreas using *in situ* hybridization. These data are not in agreement with our results, but they may be explained by the low concentration of BACE1 mRNA in pancreatic acini and by the superior sensitivity of the nested RT-RCR protocol employed in our study.

We performed western blot analysis to demonstrate the expression of the BACE1 protein in the pancreas. Similar to the distribution of BACE1 mRNA, the BACE1 protein was detected in the islets of Langerhans by immunohistochemical staining whereas pancreatic acini showed only weak labeling. This staining pattern is consistent with BACE1 protein being located at the apical pole of acinar cells where enzyme secretion takes place. The location of BACE1 at this subcellular structure indicates either a role for BACE1 in the secretory process itself or the secretion of BACE1 into the pancreatic juice. To address this question, we examined pancreatic juice by western blot analysis and enzymatic activity assays. We detected BACE1 protein and its fragments in pancreatic juice. We found BACE1 activity in the pancreas and in the pancreatic exocrine cell line AR42J, and less BACE1 activity in pancreatic juice. However, since we could not block the measured activity by the addition of BACE1 inhibitors, there still remains some doubt about the specificity of the activity detected. It is possible that the inhibitor was degraded by another protease within the pancreas or that the fluorimetric substrate of BACE1 activity assay was cleaved in an unspecific manner; however, the detection of specific BACE1 protein in pancreatic juice implies a specific function of BACE1 outside the pancreatic acinar cells. Since BACE1 exhibits specific aspartic protease activity [28], we searched for proteins harboring one of the known BACE1 consensus sequences which represent potential BACE1 substrates. Based on these criteria, the catalytic light chain of enteropeptidase appeared to be a putative substrate of extracellular BACE1. An *in vitro* assay demonstrated that enteropeptidase is indeed cleaved by BACE1 because specific enteropeptidase cleavage products were generated.

Thus, the presence of BACE1 in pancreatic juice might indicate its involvement in a protective mechanism. Duodenopancreatic reflux of the duodenal contents has been implicated in experimental and clinical pancreatitis [30, 31, 32, 33]. Occasional duodenopancreatic reflux could result in trypsinogen activation by enteropeptidase within the pancreas, followed by acute pancreatitis. Since BACE1 activity evolves in an acidic milieu, it would cleave enteropeptidase only after duodenopancreatic reflux. In an alkaline environment, as in the duodenal lumen, BACE1 is less active, and enteropeptidase could stimulate the digestive cascade. Therefore, we propose that pancreatic secretion of BACE1 presents a defense mechanism of the pancreas to protect the organ from premature trypsinogen activation by enteropeptidase.

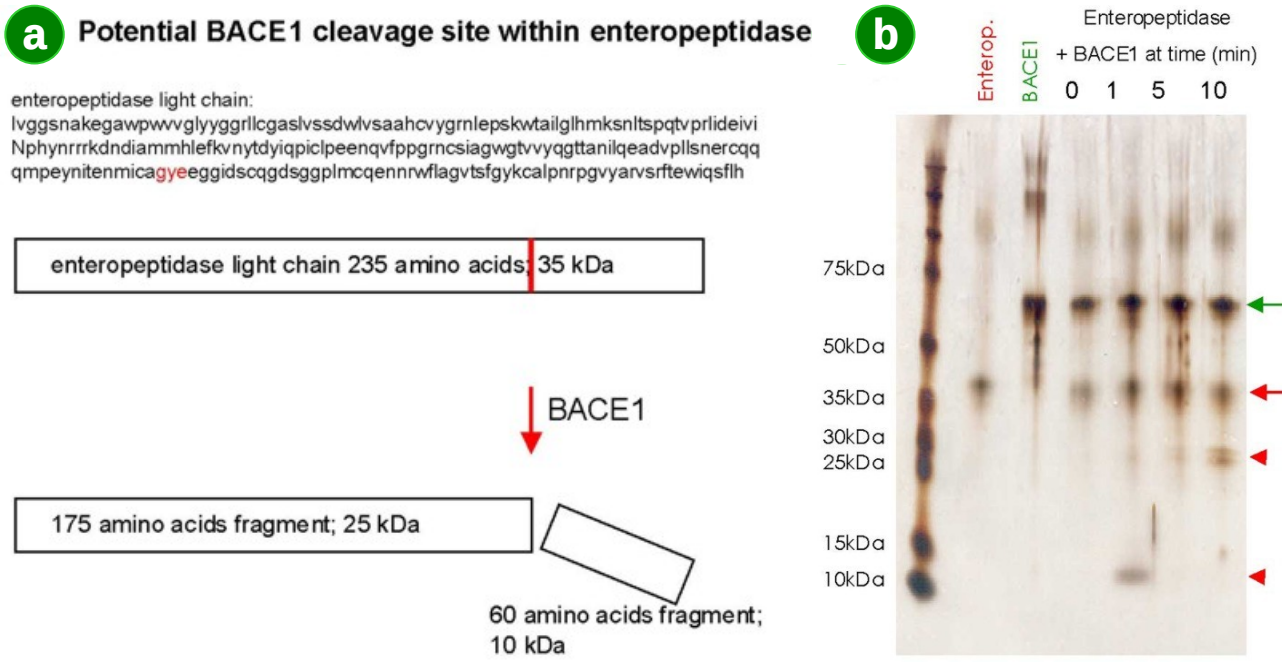

Figure 4. a. Sequence analysis of different pancreatic and duodenal proteins revealed a putative BACE1 cleavage site (red) within the catalytic active light chain of enteropeptidase. Given the molecular weight of 35 kDa of bovine enteropeptidase light chain, cleavage of enteropeptidase at this position would theoretically result in two fragments of 25 kDa and 10 kDa. **b.** Bovine enteropeptidase was incubated with recombinant BACE1. At the indicated times, samples were withdrawn and separated by SDS-polyacrylamide gel electrophoresis. Protein staining revealed the presence of BACE1 (green arrow), enteropeptidase (red arrow), and additional bands of the estimated molecular weight of enteropeptidase cleavage products (red arrowheads).

Acknowledgements We would like to express our gratitude to Prof. Volker Bigl (†), who was the mentor and guiding spirit of A.H., S.R., U.Z. and T.S. for his continuous support, scientific advice and personal patronage. We also thank Susanne Kistner and Renate Jendrek for their technical assistance.

Conflict of interest The authors have no potential conflicts of interest

References

1. Mann DV, Hershman MJ, Hittinger R, Glazer G. Multicentre audit of death from acute pancreatitis. Br J Surg 1994;81:890-893.

2. Bhatia M, Wong FL, Cao Y, Lau HY, Huang J, Puneet P, Chevali L. Pathophysiology of acute pancreatitis. Pancreatology 2005;5:132-144.

3. Neoptolemos JP, Kemppainen EA, Mayer JM, Fitzpatrick JM, Raraty MG, Slavin J, Beger HG, Hietaranta AJ, Puolakkainen PA. Early prediction of severity in acute pancreatitis by urinary trypsinogen activation peptide: A multicentre study. Lancet 2000;355:1955-1960.

4. Teich N, Ockenga J, Hoffmeister A, Manns M, Mossner J, Keim V. Chronic pancreatitis associated with an activation peptide mutation that facilitates trypsin activation. Gastroenterology 2000;119:461-465.

5. Sahin-Toth M. Hereditary pancreatitis-associated mutation asn(21) --> ile stabilizes rat trypsinogen in vitro. J Biol Chem 1999;274:29699-29704.

6. Kitamoto Y, Yuan X, Wu Q, McCourt DW, Sadler JE. Enterokinase, the initiator of intestinal digestion, is a mosaic protease composed of a distinctive assortment of domains. Proc Natl Acad Sci U S A 1994;91:7588-7592.

7. Zamolodchikova TS, Sokolova EA, Lu D, Sadler JE. Activation of recombinant proenteropeptidase by duodenase. FEBS Lett 2000;466:295-299.

8. Kitamoto Y, Veile RA, Donis-Keller H, Sadler JE. Cdna sequence and chromosomal localization of human enterokinase, the proteolytic activator of trypsinogen. Biochemistry 1995;34:4562-4568.

9. Hadorn B, Tarlow MJ, Lloyd JK, Wolff OH. Intestinal enterokinase deficiency. Lancet 1969;1:812-813.

10. Haworth JC, Gourley B, Hadorn B, Sumida C. Malabsorption and growth failure due to intestinal enterokinase deficiency. J Pediatr 1971;78:481-490.

11. Holzinger A, Maier EM, Buck C, Mayerhofer PU, Kappler M, Haworth JC, Moroz SP, Hadorn HB, Sadler JE, Roscher AA. Mutations in the proenteropeptidase gene are the molecular cause of congenital enteropeptidase deficiency. Am J Hum Genet 2002;70:20-25.

12. Fonseca P, Light A. The purification and characterization of bovine enterokinase from membrane fragments in the duodenal mucosal fluid. J Biol Chem 1983;258:14516-14520.

13. Eggermont E, Molla AM, Tytgat G, Rutgeerts L. Distribution of enterokinase activity in the human intestine. Acta Gastroenterol Belg 1971;34:655-662.

14. Bricteux-Gregoire S, Schyns R, Florkin M. Phylogeny of trypsinogen activation peptides. Comp Biochem Physiol B 1972;42:23-39.

15. Davie EW, Neurath H. Identification of a peptide released during autocatalytic activation of trypsinogen. J Biol Chem 1955;212:515-529.

16. Owyang C, Dozois RR, DiMagno EP, Go VL. Relationships between fasting and postprandial pancreaticoduodenal pressures, pancreatic secretion, and duodenal volume flow in the dog. Gastroenterology 1977;73:1046-1049.

17. Grant D. Acute necrotising pancreatitis--a role for enterokinase. Int J Pancreatol 1986;1:167-183.

18. Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C, Christie G. Identification of a novel aspartic protease (asp 2) as beta-secretase. Mol Cell Neurosci 1999;14:419-427.

19. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 1999;402:537-540.

20. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of alzheimer's amyloid precursor protein by the transmembrane aspartic protease bace. Science 1999;286:735-741.

21. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrikson RL, Gurney ME. Membrane-anchored aspartyl protease with alzheimer's disease beta-secretase activity. Nature 1999;402:533-537.

22. Holsinger RM, McLean CA, Collins SJ, Masters CL, Evin G. Increased beta-secretase activity in cerebrospinal fluid of alzheimer's disease subjects. Ann Neurol 2004;55:898-899.

23. Bodendorf U, Fischer F, Bodian D, Multhaup G, Paganetti P. A splice variant of beta-secretase deficient in the amyloidogenic processing of the amyloid precursor protein. J Biol Chem 2001;276:12019-12023.

24. Figueroa DJ, Shi XP, Gardell SJ, Austin CP. Abetapp secretases are co-expressed with abetapp in the pancreatic islets. J Alzheimers Dis 2001;3:393-396.

25. Tanahashi H, Tabira T. Three novel alternatively spliced isoforms of the human beta-site amyloid precursor protein cleaving enzyme (bace) and their effect on amyloid beta-peptide production. Neurosci Lett 2001;307:9-12.

26. Ehehalt R, Michel B, De Pietri Tonelli D, Zacchetti D, Simons K, Keller P. Splice variants of the beta-site app-cleaving enzyme bace1 in human brain and pancreas. Biochem Biophys Res Commun 2002;293:30-37.

27. Hartlage-Rubsamen M, Zeitschel U, Apelt J, Gartner U, Franke H, Stahl T, Gunther A, Schliebs R, Penkowa M, Bigl V, Rossner S. Astrocytic expression of the alzheimer's disease beta-secretase (bace1) is stimulus-dependent. Glia 2003;41:169-179.

28. Gruninger-Leitch F, Schlatter D, Kung E, Nelbock P, Dobeli H. Substrate and inhibitor profile of bace (beta-secretase) and comparison with other mammalian aspartic proteases. J Biol Chem 2002;277:4687-4693.

29. Huse JT, Byant D, Yang Y, Pijak DS, D'Souza I, Lah JJ, Lee VM, Doms RW, Cook DG. Endoproteolysis of beta-secretase (beta-site amyloid precursor protein-cleaving enzyme) within its catalytic domain. A potential mechanism for regulation. J Biol Chem 2003;278:17141-17149.

30. Pfeffer RB, Stasior O, Hinton JW. The clinical picture of the sequential development of acute hemorrhagic pancreatitis in the dog. Surg Forum 1957;8:248-251.

31. Johnson RH, Doppman J. Duodenal reflux and the etiology of pancreatitis. Surgery 1967;62:462-467.

32. McCutcheon AD. Reflux of duodenal contents in the pathogenesis of pancreatitis. Gut 1964;25:260-265.

33. Dreiling DA, Janowitz HD. Inhibitory effect of new anticholinergics on the basal and secretin-stimulated pancreatic secretion in patients with and without pancreatic disease: Therapeutic and theoretic implications. Am J Dig Dis 1960;5:639-654.