Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 319 (2004) 58–65
BBRC
www.elsevier.com/locate/ybbrc

The lysosomal degradation of neuromedin B is dependent on tripeptidyl peptidase-I: evidence for the impairment of neuropeptide degradation in late-infantile neuronal ceroid lipofuscinosis

Sharmila Kopan, Uthayatharsini Sivasubramaniam, and Michael J. Warburton*

Department of Cellular Pathology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

Received 22 April 2004
Available online 12 May 2004

Abstract

Late-infantile neuronal ceroid lipofuscinosis (CLN2), previously known as the late-infantile form of Batten disease, is a lysosomal storage disease which results from mutations in the gene that codes for tripeptidyl peptidase-I (TPP-I). This disease is characterised by progressive neurodegeneration in young children although the molecular mechanisms responsible for neuronal cell death are unclear. TPP-I is an exopeptidase which removes N-terminal tripeptides from small peptides, including several peptide hormones. We report that the degradation of the neuropeptide, neuromedin B, by mouse brain cells is restricted to lysosomes and that the pattern of degradation products is consistent with a predominant role for TPP-I. Neuromedin B is degraded by a similar pathway in a mouse neuronal cell line and also in cultured human fibroblasts. A specific inhibitor of TPP-I is able to abolish neuromedin B degradation in a variety of cell types. Fibroblasts from CLN2 patients, which are deficient in TPP-I activity, are unable to degrade neuromedin B. These observations suggest that TPP-I is the predominant proteolytic enzyme responsible for the intracellular degradation of neuromedin B. The inability of cells from CLN2 patients to degrade neuromedin B and other neuropeptides may contribute to the pathogenesis of the disease.

Keywords: Neuronal ceroid lipofuscinosis; Lysosome; Tripeptidyl peptidase-I; Neuromedin B; Neuropeptide degradation

Mutations in the lysosomal enzyme tripeptidyl peptidase-I result in the inherited neurodegenerative disease, classical late-infantile neuronal ceroid lipofuscinosis [CLN2, the late-infantile form of Batten disease, OMIM (Online Mendelian inheritance In Man) 204500] [1,2]. Tripeptidyl peptidase-I (TPP-I) commonly acts as an exopeptidase that removes tripeptides from the N-terminus of peptides smaller than 5 kDa [3,4]. The identity of the natural substrates for TPP-I remains unclear although the enzyme has been demonstrated to act on a number of biologically active peptides, e.g., angiotensins I and II, glucagon, and cholecystokinin [3–7]. TPP-I also cleaves tripeptides from the N-terminus of mitochondrial ATP synthase subunit c initiating its further degradation by other lysosomal proteases. ATP synthase subunit c is observed to accumulate in several cell types in patients with CLN2 [8,9]. Children with this disease are symptom free until the age of about three. They then suffer from progressive neurodegeneration with associated seizures, increasing spacticity, dementia, and blindness. Death occurs around the age of 10. Autopsy reveals a dramatic loss of neuronal cells and to a lesser extent other brain cell types. Brain cells that remain contain autofluorescent ceroid lipopigment and other proteinaceous material enclosed within lysosomal structures [10].

Although TPP-I has a ubiquitous distribution, the pathological effects of TPP-I mutations are largely manifested in neurons, suggesting that the enzyme may play a critical role in neuronal processes, e.g., the degradation of small neuropeptides brought into the cell by receptor mediated endocytosis. We have previously demonstrated that TPP-I is essential for the degradation

* Corresponding author. Fax: +44-208-725-0064.
E-mail address: mwarburt@sghms.ac.uk (M.J. Warburton).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.04.142

of cholecystokinin-5 and 8 (CCK-5 and CCK-8) by mouse brain lysosomes [7,11]. However, it remains unclear if the requirement for TPP-I activity is specific to the degradation of CCK-5/8 or if it is a general phenomenon. We now investigate the role of TPP-I in the degradation of neuromedin B (NMB), a neuropeptide of the ranatensin family of bombesin-like peptides [12]. NMB has a wide distribution throughout mammalian neural and peripheral tissues and may be involved in regulating a multitude of physiological processes. Immunoreactive-NMB is found in several regions of the brain although the NMB receptor has a much wider distribution [12].

Materials and methods

Materials. Enzyme substrates and inhibitors were purchased from Bachem (St. Helens, England). Peptides were synthesised by Genemed Synthesis (San Francisco, USA).

Preparation of a mouse brain lysosomal fraction. Minced mouse brains were suspended in 0.25 M sucrose/10 mM Tris–HCl, pH 7.4/5 mM EDTA and then homogenised using a nitrogen cavitation bomb operated at 500 psi and an equilibration time of 5 min. The homogenate was centrifuged for 10,000*g* min to remove nuclei and intact cells. The supernatant was centrifuged for 30,000*g* min to remove a high proportion of the mitochondria and plasma membrane vesicles. The supernatant was finally centrifuged for 300,000*g* min to prepare an enriched lysosomal fraction [13]. In some experiments, the supernatant (cytosol) was collected and stored at −85 °C. Enzyme assays indicated that the supernatant contained less than 10% of the total lysosomal activity. Lysosomes were further purified as described previously [7]. The overall enrichment of lysosomes was 15-fold using β-D-galactosidase as a marker.

Digestion of peptides. Neuromedin B (NMB) and related peptides (100 μg) were digested with purified lysosomal fractions (5 μg/ml) at 37 °C in the presence of 100 mM sodium acetate, pH 4.0, 20 mM NaCl, and 5 mM EDTA. Incubation of NMB with purified TPP-I (100 μU) was carried out for 3 h in the standard digestion buffer. Reactions were stopped by the addition of TFA to 0.1% and analysed by reversed phase HPLC using a Dynamax C18-300 column (4.6 × 250 mm) and a 0% for 10 min, 0–37.5% acetonitrile/0.1% TFA gradient for 60 min, and a 37.5–60% acetonitrile/0.1% TFA gradient for 15 min. The flow rate was 0.5 ml/min. The extent of peptide degradation was estimated by measuring the peak areas of neuromedin B and its digestion products. Peaks were identified by ion trap mass spectrometry using a ThermoFinnigan LCQ Deca XP equipped with an electrospray ionisation source and by the elution position of standards.

Enzyme assays. Assays for TPP-I and β-D-galactosidase were performed as described previously [14]. Ala-Ala-Phe-NHMec was used as a synthetic substrate for measuring TPP-I activity. Some assays were carried out in the presence of a specific inhibitor of TPP-I (Ala-Ala-Phe-CH_2Cl). TPP-I was purified from rat spleen [3]. Protein was estimated using the Coomassie Plus Assay Reagent (Pierce).

Tissue culture. A normal human skin fibroblast cell line (MCH65) was obtained from The Montreal Children's Hospital. The CLN2 fibroblasts used were GM09404 (Coriell Cell Repository, Camden, NJ). Mouse neuronal HN9e cells [15] were used in some experiments. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Fibroblasts were used 3–6 passages after receipt (passages 12–15). After washing twice with PBS, cells were harvested by scraping into PBS. The cells were collected by centrifugation (5000*g* min), resuspended in 0.25 M sucrose/10 mM Tris–HCl, pH 7.4/5 mM EDTA, and then homogenised using a nitrogen cavitation bomb operated at 200 psi and an equilibration time of 2 min. The homogenate was centrifuged for 10,000*g* min to remove nuclei and intact cells. The supernatant was finally centrifuged for 300,000*g* min to prepare an enriched lysosomal fraction. The pellet was resuspended in water and frozen at −85 °C.

Results

Time course of NMB degradation by mouse brain lysosomes

NMB (GNLWATGHFM-amide) and the major metabolites that might result from TPP-I activity could readily be separated by reversed phase HPLC and this technique was used to characterise the products of NMB degradation. (Fig. 1). Incubation of NMB with a purified lysosomal fraction isolated from mouse brain at pH 4.0 initially resulted in the appearance of a major degradation product eluting from the reversed phase column at 47 min which is identical

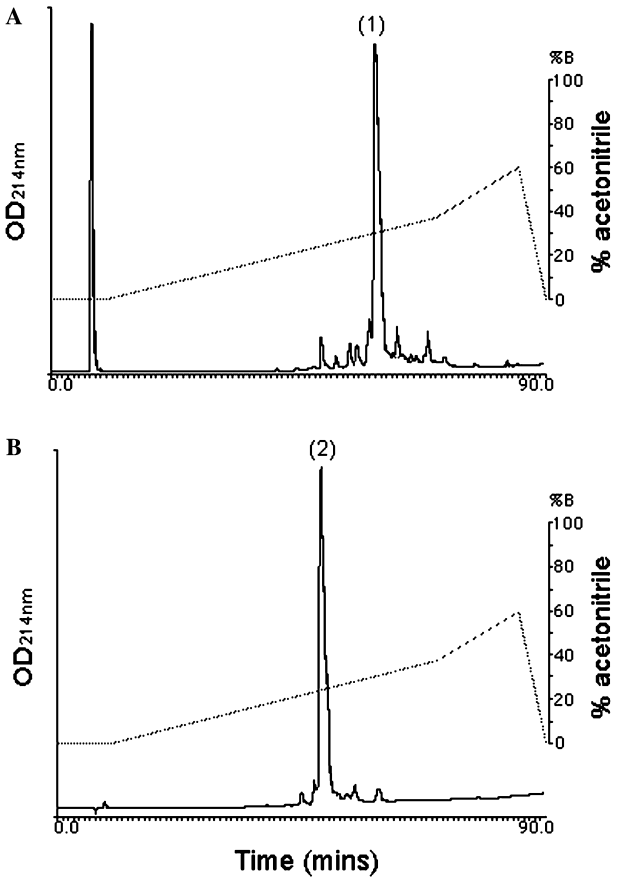

Fig. 1. Elution of (A) NMB and (B) $^{1-3}\Delta$-NMB standards from a C18 reversed phase HPLC column. Standard preparations of NMB and $^{1-3}\Delta$-NMB (75 μg) were chromatographed on a C18 reversed phase HPLC column and the eluant was monitored at 214 nm.

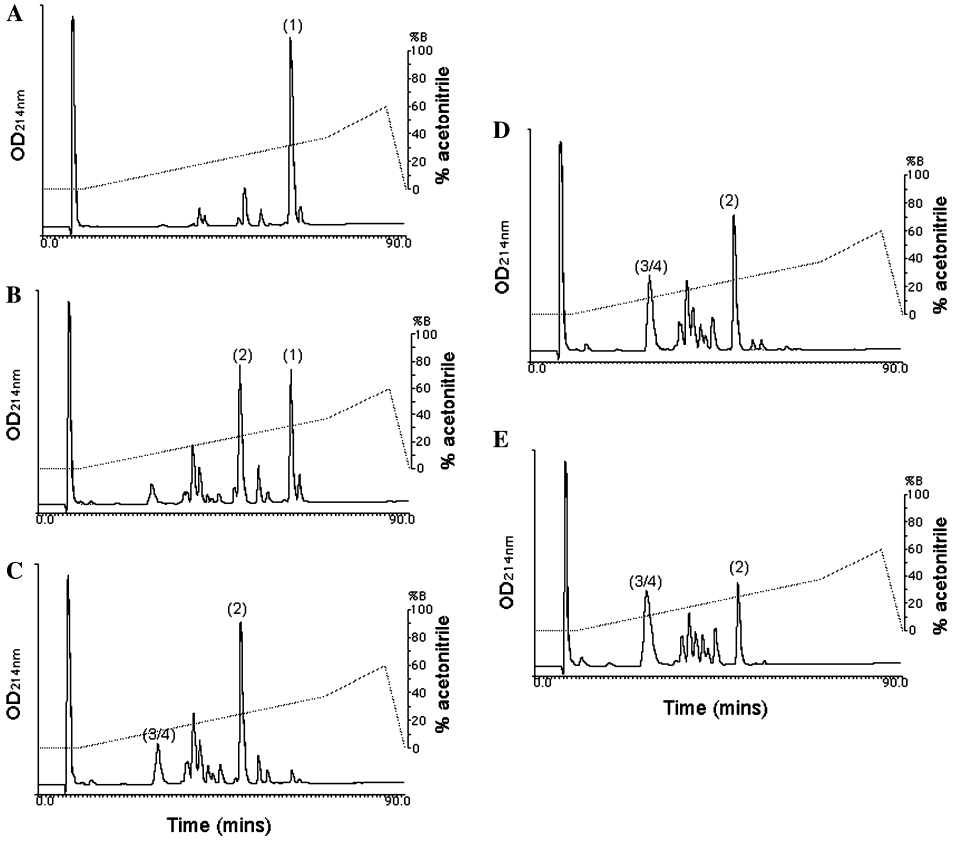

Fig. 2. Time course for the degradation of NMB by mouse brain lysosomes. Purified mouse brain lysosomes were incubated with NMB (100 μg) for 2 h (A), 4 h (B), 8 h (C), 12 h (D) or 20 h (E) and chromatographed on a C18 reversed phase HPLC column. (1) Undegraded NMB, (2) is $^{1-3}\Delta$-NMB, and (3/4) is WAT/GHFM-amide.

to the elution time of $^{1-3}\Delta$-NMB (WATGHFM-amide) (Fig. 2). This peak was confirmed as $^{1-3}\Delta$-NMB by mass spectrometry which indicated a mass of 848.7 ($^{1-3}\Delta$-NMB $M + H^+ = 848.4$). With further incubation, $^{1-3}\Delta$-NMB was degraded to material which eluted as a closely spaced doublet at 27 min and a series of smaller peaks eluting between 31 and 37 min. Standards of WAT and GHFM-amide eluted at 26.8 and 27.8 min, respectively, and mass spectrometry indicated the presence of two peptides with masses of 377.3 and 490.4 (WAT $M + H^+ = 377.2$, GHFM-amide $M + H^+ = 490.2$). The smaller peaks eluting at 31–37 min were not identified.

Distribution of the cellular NMB degrading activity

The pH activity profile for the hydrolysis of NMB had a sharp pH optimum at about 4.0 similar to the pH optimum of TPP-I when assayed with a synthetic substrate (not shown). To determine the distribution of the cellular NMB degrading activity, NMB was incubated, at pH 4 and pH 7, with particulate and supernatant fractions isolated from mouse brain (Fig. 3). Substantial NMB degrading activity was only present in the particulate fraction at pH 4.

Effect of inhibitors on the degradation of NMB by mouse brain lysosomes

Incubation of NMB with mouse brain lysosomes in the presence of Ala-Ala-Phe-CH_2Cl, a specific inhibitor of TPP-I, strongly inhibited the degradation of NMB (Fig. 4). An inhibition of 75% was observed with an inhibitor concentration of 25 μM. Inhibitors of the four main classes of proteases, serine (PMSF), cysteine (E64), metallo (EDTA), and aspartate (pepstatin), had no effect on the degradation of NMB. Inhibitors of aminopeptidases (EDTA, 1,10-phenanthroline, bestatin, and Leu-CH_2Cl) and dipeptidyl peptidases (Gly-Phe-CHN_2 and Lys-Ala-CH_2Cl) also had no effect on the degradation of NMB by mouse brain lysosomes (not shown).

Degradation of NMB by normal CLN2 fibroblasts and neuronal cell lines

Incubation of NMB with lysosomal fractions prepared from normal human fibroblasts (MCH65) resulted in a similar pattern of degradation products to that observed with mouse brain lysosomes (Fig. 5). The TPP-I inhibitor, Ala-Ala-Phe-CH_2Cl, completely abolished the degradation of NMB by these cells. When

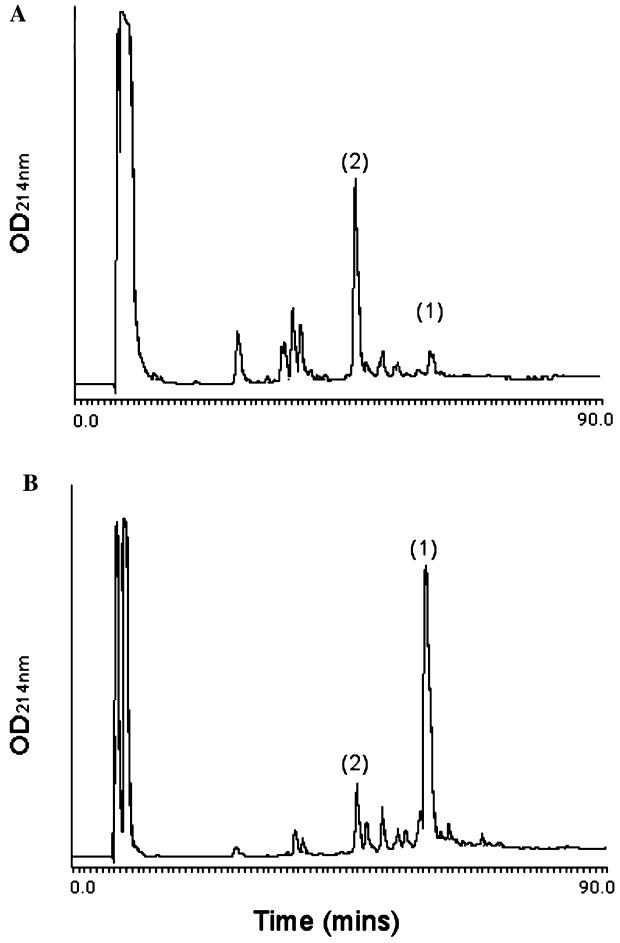

Fig. 3. Effect of inhibition of TPP-I activity on the degradation of NMB by mouse brain lysosomes. NMB (100 μg) was incubated with purified mouse brain lysosomes for 8 h in the absence (A) or presence (B) of Ala-Ala-Phe-CH_2Cl (25 μM) and the degradation products were separated by reversed phase chromatography on a C18 HPLC column. (1) Undegraded NMB and (2) is $^{1-3}\Delta$-NMB.

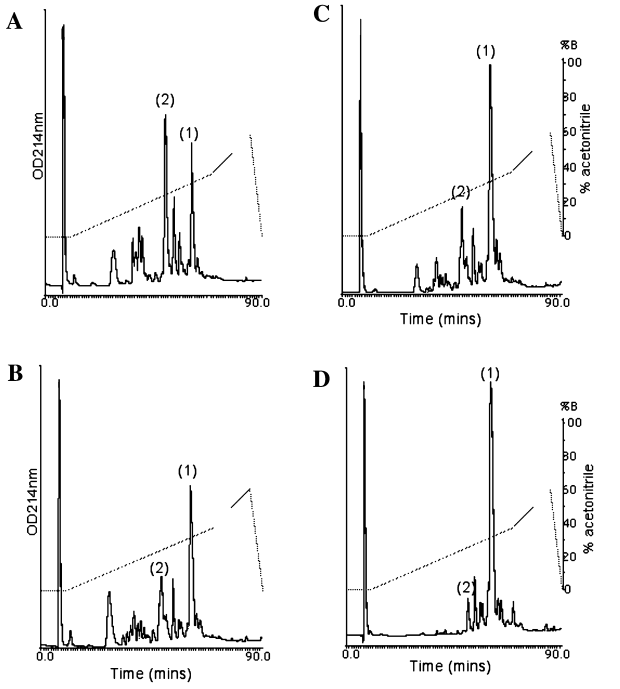

Fig. 4. Comparison of NMB degrading activity at pH 4 and 7 in lysosomal and supernatant fractions derived from mouse brain. NMB (100 μg) was incubated with purified mouse brain lysosomes (A,C) or mouse brain supernatant (B,D) at pH 4 (A,B) or pH 7 (C,D) for 8 h and the degradation products were separated by reversed phase chromatography on a C18 HPLC column. (1) Undegraded NMB and (2) is $^{1-3}\Delta$-NMB.

NMB was incubated with lysosomal fractions prepared from fibroblasts isolated from a patient with CLN2 (GM09404) and which are therefore devoid of TPP-I activity, no degradation of NMB was observed. Incubation of NMB with lysosomal fractions prepared from a mouse neuronal cell line (HN9e) again produced a pattern of degradation products similar to those produced by mouse brain lysosomes (Fig. 6). The major degradation products, $^{1-3}\Delta$-NMB, WAT, and GHFM-amide, were readily identifiable although several unidentified peaks were also observed. Degradation of NMB by HN9e cells was almost completely inhibited by Ala-Ala-Phe-CH_2Cl.

Degradation of $^{1-3}\Delta$-NMB by mouse brain lysosomes and human fibroblast cell lines

Incubation of $^{1-3}\Delta$-NMB (WATGHFM-amide) with purified mouse brain lysosomes produced 3 major degradation products, a doublet eluting at 27 min (WAT and GHFM-amide) and an unidentified peak eluting at 14 min. The production of these degradation products was completely inhibited by Ala-Ala-Phe-CH_2Cl (Fig. 7). A similar pattern of degradation products was observed when $^{1-3}\Delta$-NMB was incubated with lysosomal fractions prepared from normal human fibroblasts (MCH65) with the WAT/GHFM-amide doublet being particularly prominent (Fig. 8). Degradation of $^{1-3}\Delta$-NMB by MCH65 cells was completely inhibited by Ala-Ala-Phe-CH_2Cl and no degradation of $^{1-3}\Delta$-NMB was observed with extracts prepared from CLN2 fibroblasts (GM09404).

Degradation of NMB and $^{1-3}\Delta$-NMB by purified TPP-I

Incubation of NMB with purified TPP-I yielded a major degradation product eluting from the reversed phase column at 47 min ($^{1-3}\Delta$-NMB) and a doublet at 27 min (WAT and GHFM-amide) (Fig. 9A). Incubation of $^{1-3}\Delta$-NMB with purified TPP-I resulted in the production of the 27 min doublet and an unidentified peak at 14 min (Fig. 9B). Both of these degradation profiles are similar to those produced when NMB and $^{1-3}\Delta$-NMB were incubated with the lysosomal fraction purified from mouse brain.

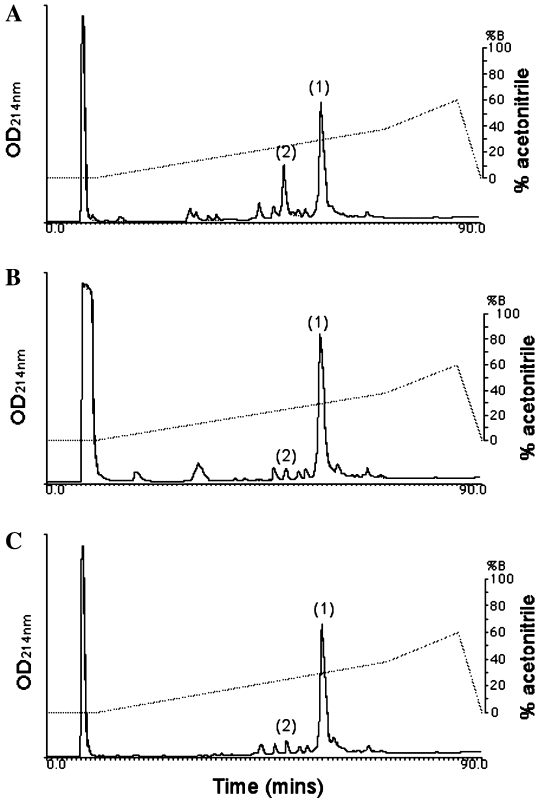

Fig. 5. Degradation of NMB by normal human fibroblasts and fibroblasts derived from CLN2 patients. NMB (100 μg) was incubated with extracts prepared from normal fibroblasts in the absence (A) or presence (B) of Ala-Ala-Phe-CH_2Cl (25 μM) or from CLN2 fibroblasts (C). The degradation products were separated by reversed phase chromatography on a C18 HPLC column. (1) Undegraded NMB and (2) is $^{1-3}\Delta$-NMB.

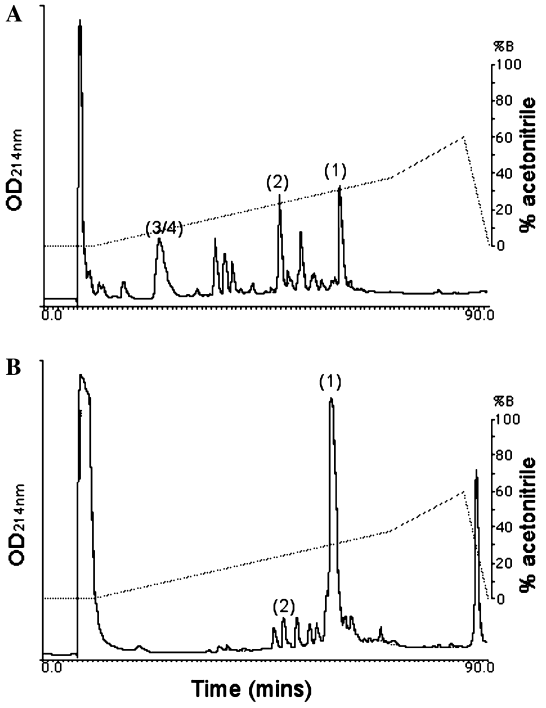

Fig. 6. Degradation of NMB by lysosomal fractions derived from mouse HN9e neuronal cells. An extract prepared from a mouse neuronal cell line, HN9e, was incubated with NMB for 8 h in the absence (A) or presence (B) of Ala-Ala-Phe-CH_2Cl (25 μM) and the degradation products were separated by reversed phase chromatography on a C18 HPLC column. (1) Undegraded NMB, (2) is $^{1-3}\Delta$-NMB, and (3/4) is WAT/GHFM-amide.

Discussion

Interactions between neuropeptides and cells are usually mediated by high affinity cell surface receptors. Binding triggers a cell signalling pathway and the subsequent internalisation of the peptide–receptor complex. These complexes are often initially internalised into early endosomes where the receptor and ligand may dissociate in the acidic environment. Either ligand or receptor or both may be recycled to the cell surface or may be targeted to late endosomes and/or lysosomes for degradation [16]. For example, both transferrin and its receptor are recycled from early endosomes to the cell surface, whereas epidermal growth factor and its receptor progress further into the endosomal/lysosomal system where both are degraded [17,18]. In cases where the receptor alone recycles to the cell surface, it is unclear whether or not degradation of the ligand is necessary for the dissociation which allows the undegraded receptor to return to the cell surface. Internalisation of the ligand–receptor complex has also been reported, in a few cases, to be necessary or to enhance cell signalling [19]. Again, it is unclear if ligand degradation may regulate the duration of the signalling process.

Several lysosomal peptidases and proteases have been implicated in peptide hormone degradation in vitro [20–23]. Experiments with knock-out mice or studies on human genetic diseases further suggest a role for some of these enzymes in the processing of neuropeptides following internalisation into the target cell. However, the susceptibility of peptide hormones to degradation by a specific protease in vitro does not necessarily correlate with the appearance of pathological features when that protease is inactivated in vivo. A deficiency of cathepsin D (in sheep) or TPP-I (in humans) results in severe neurological disease with intralysosomal accumulation of storage material and subsequent death of neurons. However, a deficiency of cathepsins B or L (in knock-out mice) or dipeptidyl peptidase-I (in humans) fails to produce neurological symptoms even though these proteases actively degrade neuropeptides in vitro [1,2,7,10,24–26]. Identification of the enzymes responsible for the intralysosomal degradation of neuropeptides may provide a partial explanation for the pathology of this type of neurodegenerative disease. We have previously shown that TPP-I is required for the degradation of internalised cholecystokinin-related peptides [7,11] and in the present communication we demonstrate, from

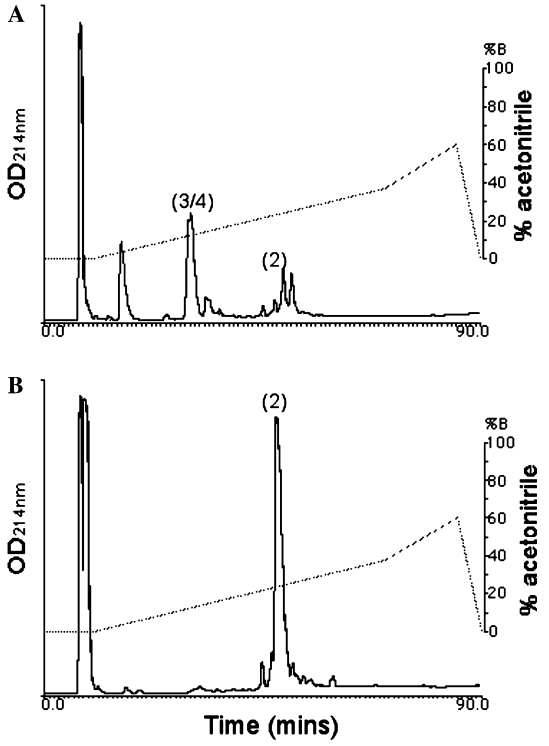

Fig. 7. Effect of inhibition of TPP-I activity on the degradation of $^{1-3}\Delta$-NMB by mouse brain lysosomes. $^{1-3}\Delta$-NMB (100 μg) was incubated with purified mouse brain lysosomes for 8 h in the absence (A) or presence (B) of Ala-Ala-Phe-CH_2Cl (25 μM) and the degradation products separated by reversed phase chromatography on a C18 HPLC column. (2) $^{1-3}\Delta$-NMB and (3/4) is WAT/GHFM-amide.

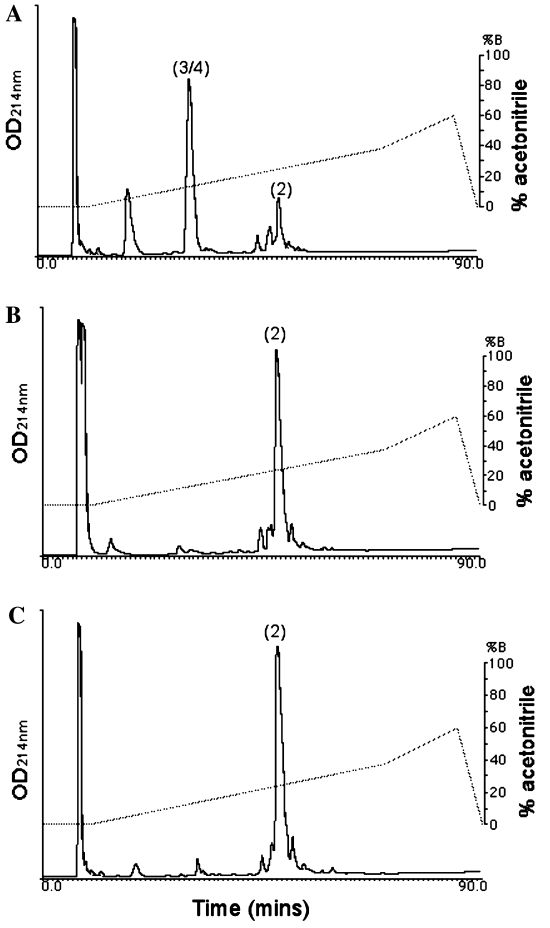

Fig. 8. Degradation of $^{1-3}\Delta$-NMB by normal fibroblasts and fibroblasts derived from CLN2 patients. $^{1-3}\Delta$-NMB (100 μg) was incubated with extracts prepared from normal fibroblasts in the absence (A) or presence (B) of Ala-Ala-Phe-CH_2Cl (25 μM) or from CLN2 fibroblasts (C). The degradation products were separated by reversed phase chromatography on a C18 HPLC column. (2) $^{1-3}\Delta$-NMB and (3/4) is WAT/GHFM-amide.

studies on the degradation of an unrelated neuropeptide, neuromedin B (NMB), that TPP-I may have a more general role in neuropeptide breakdown.

Several results point to an essential role for TPP-I in the degradation on NMB. First, the major degradation product formed when NMB (GNLWATGHFM-amide) was incubated with purified mouse brain lysosomes was $^{1-3}\Delta$-NMB (WATGHFM-amide), NMB from which the N-terminal tripeptide, GNL, had been removed. GNL was not observed amongst the degradation products because it is probably too hydrophilic to bind to the C18 reversed phase column. Inhibitors of aminopeptidases and dipeptidyl peptidases did not prevent the degradation of NMB and it is therefore likely that a tripeptidyl peptidase is responsible for the cleavage of the N-terminal tripeptide. The identity of $^{1-3}\Delta$-NMB was confirmed by the identical elution position of standard $^{1-3}\Delta$-NMB and by determining that the mass of the major degradation product was identical to that of $^{1-3}\Delta$-NMB by mass spectrometry. Second, the production of $^{1-3}\Delta$-NMB from NMB was completely blocked by Ala-Ala-Phe-CH_2Cl, a specific inhibitor of TPP-I under the conditions used. The degradation of NMB was resistant to inhibition by inhibitors of the four major classes of proteolytic enzymes, another property of TPP-I. Third, fibroblasts isolated from CLN2 patients, which have no detectable TPP-I activity, were unable to degrade NMB unlike their normal counterparts which produced a pattern of degradation products very similar to that produced by purified mouse brain lysosomes. The TPP-I inhibitor, Ala-Ala-Phe-CH_2Cl, also blocked the degradation of NMB by normal fibroblasts confirming the essential role for TPP-I in the degradation of NMB in this cell type.

Additional evidence for the role of TPP-I in NMB degradation was obtained by investigating the subsequent breakdown of the initial degradation product, $^{1-3}\Delta$-NMB. Incubation of $^{1-3}\Delta$-NMB (WATGHFM-amide) with purified mouse brain lysosomes resulted in the appearance of the two degradation products, WAT and GHFM-amide, which would be expected from the action of TPP-I. Again, this reaction was blocked by the TPP-I inhibitor, Ala-Ala-Phe-CH_2Cl, but not by inhibitors of aminopeptidases and dipeptidyl peptidases.

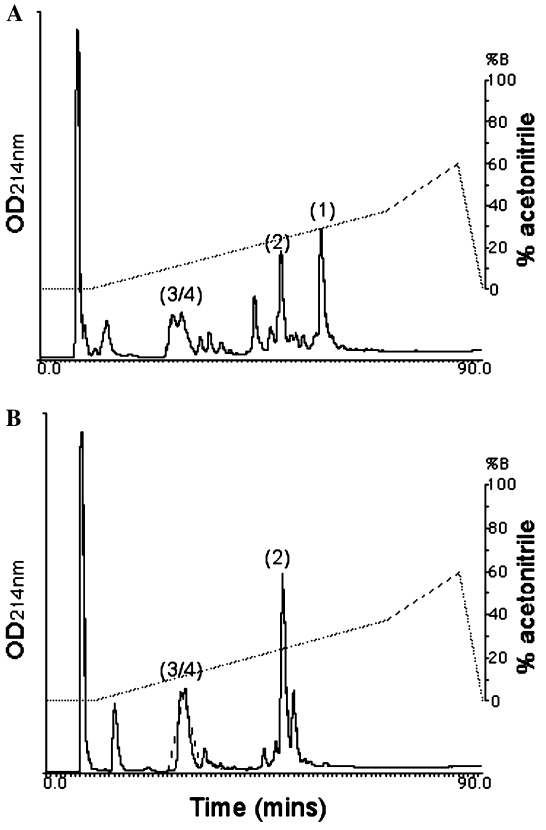

Fig. 9. Degradation of NMB and $^{1-3}\Delta$-NMB by purified TPP-I. NMB (A) or $^{1-3}\Delta$-NMB (B) (100 μg) were incubated with purified TPP-I (5 μg) for 3 h and the degradation products separated by reversed phase chromatography on a C18 HPLC column. (1) Undegraded NMB, (2) $^{1-3}\Delta$-NMB and (3/4) is WAT/GHFM-amide.

CLN2 fibroblasts were also unable to degrade $^{1-3}\Delta$-NMB unlike normal fibroblasts. The rate of degradation of TPP-I by the mouse brain lysosomal fraction was at a maximum at pH 4 which strongly suggests the activity of a lysosomal enzyme. Most of the NMB degrading activity in mouse brain was located in the particulate fraction and was active at pH 4. Little NMB degrading activity was noted in the supernatant fraction at either pH 4 or 7, indicating that the majority of the NMB degrading activity resides in an organelle with an acidic milieu. A mouse neuronal cell line, HN9e, produced a similar pattern of NMB degradation products to those produced by brain and fibroblasts suggesting a common degradation pathway in a variety of cell types. When NMB or $^{1-3}\Delta$-NMB was incubated with purified TPP-I, the pattern of degradation of degradation was very similar to that observed with mouse brain lysosomes, suggesting that the major degradative processes are mediated by TPP-I.

The inherited neurodegenerative disease, classical late-infantile neuronal ceroid lipofuscinosis, results from mutations in the gene that codes for TPP-I [1]. A characteristic of this disease is the progressive accumulation of storage material and lipopigment in the lysosomes of a variety of cell types especially neurons which then undergo apoptosis [10]. Although the loss of TPP-I activity is systemic, cell death is largely restricted to neurons, suggesting that neurons are particularly sensitive to the presence of the storage material or that TPP-I plays a unique and critical role in neuronal survival. In addition, tissues other than brain may possess a backup system for degrading short peptides in the absence of TPP-I. Dipeptidyl peptidase-I, which is expressed in all human and mouse tissues except brain, and which is able to degrade many of the peptides susceptible to TPP-I, may provide a backup system for the degradation of short peptides in the absence of TPP-I activity [7,27]. Consequently, TPP-I may play an exclusive role in the degradation of small peptides in brain. Reduced rates of peptide hormone degradation may interfere with cell signalling and/or receptor recycling with adverse effects on neuronal cell functions or survival. The data presented here provide further evidence for the absolute requirement for TPP-I activity in the degradation of short peptide hormones.

Acknowledgments

We thank Dr. Ted Terelli and Professor Brian Austen for help with mass spectrometry. This work was funded by a grant from The Batten Disease Support and Research Association.

References

[1] D.E. Sleat, R.J. Donnelly, H. Lackland, C.G. Liu, I. Sohar, R.K. Pullarkat, P. Lobel, Association of mutations in a lysosomal protein with classical late infantile neuronal ceroid lipofuscinosis, Science (Washington, DC) 277 (1997) 1802–1805.
[2] D.J. Vines, M.J. Warburton, Classical late infantile neuronal ceroid lipofuscinosis fibroblasts are deficient in lysosomal tripeptidyl peptidase-I, FEBS Lett. 443 (1999) 131–135.
[3] D.J. Vines, M.J. Warburton, Purification and characterisation of a tripeptidyl aminopeptidase-I from rat spleen, Biochim. Biophys. Acta 1384 (1998) 233–242.
[4] F. Bernardini, M.J. Warburton, The substrate range of tripeptidyl peptidase-I, Eur. J. Paediatr. Neurol. A 5 (2001) 69–72.
[5] M.A. Junaid, G. Wu, P.K. Pullarkat, Purification and characterization of bovine brain lysosomal pepstatin-insensitive proteinase, the gene product deficient in the human late-infantile neuronal ceroid lipofuscinosis, J. Neurochem. 74 (2000) 287–294.
[6] M.J. Warburton, F. Bernardini, The specificity of lysosomal tripeptidyl peptidase-I determined by its action on angiotensin-II analogues, FEBS Lett. 500 (2001) 145–148.
[7] F. Bernardini, M.J. Warburton, Lysosomal degradation of cholecystokinin-(29–33)-amide in mouse brain is dependent on tripeptidyl peptidase-I: implications for the degradation and storage of peptides in classical late infantile neuronal ceroid lipofuscinosis, Biochem. J. 366 (2002) 521–529.
[8] K. Kominami, J. Ezaki, D. Muno, K. Ishido, T. Ueno, L.S. Wolfe, Specific storage of subunit c of mitochondrial ATP synthase in lysosomes of neuronal ceroid lipofuscinosis (Batten's disease), J. Biochem. 111 (1992) 278–282.

[9] J. Ezaki, M. Takeda-Ezaki, E. Kominami, Tripeptidyl peptidase-I, the late infantile neuronal ceroid lipofuscinosis gene product, initiates the lysosomal degradation of subunit c of ATP synthase, J. Biochem. 128 (2002) 509–516.
[10] R.M. Boustany, Batten disease or neuronal ceroid lipofuscinosis, in: H.W. Moser (Ed.), Handbook of Clinical Neurology, Neurodystrophies and Neurolipidoses, vol. 22, Elsevier Science, Amsterdam, 1996, pp. 671–700.
[11] M.J. Warburton, F. Bernardini, Tripeptidyl peptidase-I is essential for the degradation of sulphated cholecystokinin-8 (CCK-8S) by mouse brain lysosomes, Neurosci. Lett. 331 (2002) 99–102.
[12] H. Ohki-Hamazaki, Neuromedin B, Prog. Neurobiol. 62 (2000) 297–312.
[13] M.J. Warburton, C.H. Wynn, Characterization of the lysosomal heterogeneity in Chinese hamster fibroblasts, Eur. J. Biochem. 65 (1976) 361–368.
[14] A.E. Page, K. Fuller, T.J. Chambers, M.J. Warburton, Purification and characterization of a tripeptidyl peptidase from human osteoclastomas: evidence for its role in bone resorption, Arch. Biochem. Biophys. 306 (1993) 354–359.
[15] H.J. Lee, D.N. Hammond, T.H. Large, J.D. Roback, J.A. Sim, D.A. Brown, U.H. Otten, B.H. Wainer, Neuronal properties and trophic activities of immortalised hippocampal cells from embryonic and young adult mice, J. Neurosci. 10 (1990) 1779–1787.
[16] N.E. Bishop, Dynamics of endosomal sorting, Int. Rev. Cytol. 232 (2003) 1–57.
[17] A. Dautry-Varsat, A. Ciechanover, H.F. Lodish, pH and the recycling of transferrin during receptor-mediated endocytosis, Proc. Natl. Acad. Sci. USA 80 (1983) 2258–2262.
[18] C.M. Stoscheck, G. Carpenter, Down regulation of epidermal growth factor receptors: direct demonstration of receptor degradation in human fibroblasts, J. Cell Biol. 98 (1984) 1048–1053.
[19] B.P. Ceresa, S.L. Schmid, Regulation of signal transduction by endocytosis, Curr. Opin. Cell Biol. 12 (2000) 204–210.
[20] C.S. Pillay, E. Elliott, C. Dennison, Endolysosomal proteolysis and its regulation, Biochem. J. 363 (2000) 417–429.
[21] F. Authier, J.S. Mort, A.W. Bell, B.I. Posner, J.J.M. Bergeron, Proteolysis of glucagon within hepatic endosomes by membrane-associated cathepsins B and D, J. Biol. Chem. 270 (1995) 15798–15807.
[22] F. Authier, M. Metioui, A.W. Bell, J.S. Mort, Negative regulation of epidermal growth factor signalling by selective proteolytic mechanisms in the endosome mediated by cathepsin B, J. Biol. Chem. 274 (1999) 33723–33731.
[23] F. Authier, M. Metioui, S. Fabrega, M. Kouach, G. Briand, Endosomal proteolysis of internalised insulin at the C-terminal region of the B chain by cathepsin D, J. Biol. Chem. 277 (2002) 9437–9446.
[24] J. Tyynela, I. Sohar, D.E. Sleat, R.M. Gin, R.J. Donelly, M. Baumann, M. Haltia, P. Lobel, A mutation in the ovine cathepsin D gene causes a congenital lysosomal storage disease with profound neurodegeneration, EMBO J. 19 (2000) 2786–2792.
[25] C. Toomes, J. James, A.J. Wood, C.L. Wu, D. McCormick, N. Lench, C. Hewitt, L. Moynihan, E. Roberts, C.G. Woods, A. Markham, M. Wong, R. Widmer, K.A. Ghaffar, M. Pemberton, I.R. Hussein, S.A. Temtamy, R. Davies, A.P. Read, P. Sloan, M.J. Dixon, N.S. Thakker, Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis, Nat. Genet. 23 (1999) 421–424.
[26] T. Reinheckel, J. Deussing, W. Rothand, C. Peters, Towards specific functions of lysosomal cysteine peptidases: phenotypes of mice deficient for cathepsin B or cathepsin L, Biol. Chem. 382 (2001) 735–741.
[27] C.T.N. Pham, R.J. Armstrong, D.B. Zimonjic, N.C. Popescu, D.G. Payan, T.J. Ley, Molecular cloning, chromosomal localization, and expression of murine dipeptidyl peptidase I, J. Biol. Chem. 272 (1997) 10695–10703.