



Dissecting carboxypeptidase E: properties, functions and pathophysiological roles in disease

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Abstract

Since discovery in 1982, carboxypeptidase E (CPE) has been shown to be involved in the biosynthesis of a wide range of neuropeptides and peptide hormones in endocrine tissues, and in the nervous system. This protein is produced from pro-CPE and exists in soluble and membrane forms. Membrane CPE mediates the targeting of prohormones to the regulated secretory pathway, while soluble CPE acts as an exopeptidase and cleaves C-terminal basic residues from peptide intermediates to generate bioactive peptides. CPE also participates in protein internalization, vesicle transport and regulation of signaling pathways. Therefore, in two types of CPE mutant mice, *Cpe^{fat}/Cpe^{fat}* and *Cpe* knockout, loss of normal CPE leads to a lot of disorders, including diabetes, hyperproinsulinemia, low bone mineral density and deficits in learning and memory. In addition, the potential roles of CPE and Δ N-CPE, an N-terminal truncated form, in tumorigenesis and diagnosis were also addressed. Herein, we focus on dissecting the pathophysiological roles of CPE in the endocrine and nervous systems, and related diseases.

Key Words

- ▶ carboxypeptidase E
- ▶ exopeptidase
- ▶ regulated secretory pathway
- ▶ diabetes
- ▶ obesity

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Introduction

Carboxypeptidase E (CPE; EC 3.4.17.10), also known as enkephalin convertase and carboxypeptidase H, is required in the biosynthesis of most peptide hormones and neuropeptides and is localized in secretory granules of endocrine and neuroendocrine cells (1, 2). This enzyme was initially discovered as a carboxypeptidase B-like exopeptidase and isolated from bovine adrenal chromaffin granules, where it was demonstrated to remove the C-terminal basic residues of enkephalin precursors (3). CPE has an optimum pH of 5.5 and exists in both soluble and membrane forms (4). The soluble form, with a molecular weight of 53 kDa, is a processing enzyme that removes C-terminal lysine or arginine residues from neuropeptide intermediates following endopeptidase cleavage to produce biological active hormones and neuropeptides (5). However, the 55 kDa membrane form

acts as a sorting receptor for targeting several prohormones and proneuropeptides to the regulated secretory pathway (RSP) (6, 7, 8).

The importance of CPE is proven in *Cpe^{fat}/Cpe^{fat}* or *Cpe* knockout (KO) mice. *Cpe^{fat}/Cpe^{fat}* mice have a spontaneous point mutation in the coding region of *Cpe* gene, which replaces Ser₂₀₂ with a Pro residue to eliminate the enzymatic activity (9). Although a full-length transcript is produced and the mutant CPE protein is expressed in these mice, this enzyme activity is deficient, unstable and is rapidly degraded in the endoplasmic reticulum before it can be secreted (10, 11). As a result, the inactivation of CPE causes *Cpe^{fat}/Cpe^{fat}* mice (BKS strain, primarily in males) to exhibit a slowly developing obesity at 8–12 weeks of age with mild diabetes, thus this mutation is originally named fat. These mice are hyperglycemic, but sensitive to exogenously



administered insulin. In addition, *Cpe^{fat}/Cpe^{fat}* mice are infertile and develop hyperproinsulinemia with age. Accumulation of hormone and neuropeptide precursors, such as proinsulin, prodynorphin, proneurotensin, promelanin-concentrating hormone, progastrin and pro-opiomelanocortin (POMC), as well as reduced levels of bioactive peptides such as insulin, adrenocorticotrophic hormone (ACTH), neurotensin, cholecystokinin (CCK) and gastrin in *Cpe^{fat}/Cpe^{fat}* mice indicate that the processing of prohormones and proneuropeptides are impaired (6, 9, 11, 12). The reduction of CPE enzymatic activity and the deficiency in CPE sorting function may explain some of these anomalies. In another mutant strain, *Cpe* KO mice, exons 4 and 5 of the *Cpe* gene are deleted, leading to similar endocrinological deficits with *Cpe^{fat}/Cpe^{fat}* mice, such as diabetes, infertility and obesity (13). However, in *Cpe* null model, *Cpe* KO mice are also insulin resistant and exhibit neurological and behavioral abnormalities (13). In addition, *Cpe* KO mice exert reduced muscle strength and coordination, diminished glutamate-transmission-mediated b-wave amplitude (14), a markedly decline in brain-derived neurotrophic factor (BDNF) secretion from cortical neurons (7), low bone mineral density and deficits in learning and memory (15). Therefore, pathophysiological conditions observed in *Cpe^{fat}/Cpe^{fat}* mice and *Cpe* KO mice suggest that CPE plays a variety of roles in both endocrine and nervous systems.

Herein, the properties and functions of CPE in endocrine tissues and in the nervous system were reviewed. We also summarize the recent exciting work on CPE and implications in diseases observed in *Cpe^{fat}/Cpe^{fat}* or *Cpe* KO mice and in human cancers, in hope of deepening the knowledge of CPE and its potential application in translational medicine.

Properties

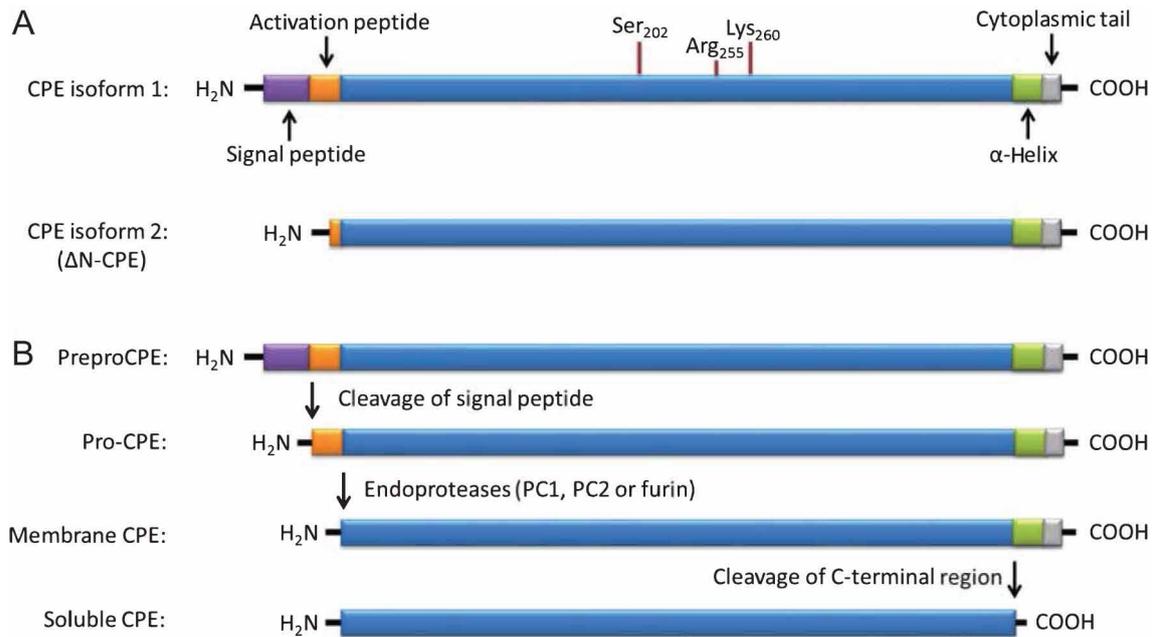
Protein synthesis and structure of CPE

The protein sequence of CPE is highly conserved in bovine, human, mouse and rat, showing more than 90% similarity among these species (9, 16, 17, 18). Moreover, anglerfish CPE shares approximately 80% protein sequence homology with mammalian CPE (19). *Cpe* gene is localized on chromosome 4q32 in human (20), chromosome 8q32.6 in mouse (9) and chromosome 16p13 in rat (17). Although *Cpe* is not from a highly related gene family, human CPE protein sequence shows significant homology with carboxypeptidases M (43% identity

(21)) and carboxypeptidase N (51% identity (22)), which are found in cell membranes and serum, respectively (23). Comparing the three carboxypeptidases with carboxypeptidases A and B, which are previously reported to share 20% and 17% sequence similarity to CPE (16), it reveals that some domains are conserved among these carboxypeptidases, including metal-ion-binding sites and catalytic sites (18). The C-terminus, which has the lowest homology with other enzymes, is responsible for the specialized distribution of CPE in secretory granules.

The isolation and sequence analysis of *Cpe* cDNA clones from bovine (16, 24), rat (17) and human (18) libraries show that the multiple forms of CPE are not from different mRNA species, and southern analysis of genomic DNA indicates a single *Cpe* gene (25), suggesting that the difference between the soluble and membrane forms of CPE is not caused by the translation of different mRNAs but by post-translational modification of a single precursor protein (24). The precursor protein, pro-CPE, contains a 27-amino-acid hydrophobic signal peptide and a short propeptide that ends with five adjacent arginine residues at the N-terminus, compared with the mature soluble and membrane forms of CPE (16). This propeptide is highly conserved among human, rat and mouse pro-CPE (17, 18), indicating that it has an important role, but the exact function of this sequence is unknown. After translation, the pro-CPE is cleaved of the N-terminal signal sequence and passes through the endoplasmic reticulum (ER) and the Golgi apparatus. In secretory granules, after cleavage of propeptide by several endoproteases such as PC1, PC2 and furin (26), CPE bound to membranes and glycosylated, resulting in the membrane CPE (27). In addition, the C-terminal tail was suggested to distinguish the membrane and soluble forms of CPE; thus deletion of a C-terminal region from the membrane CPE can generate the soluble CPE (27) (Fig. 1). However, both forms purified from bovine pituitary gland or produced in rat insulinoma-derived clonal beta-cell lines still contain the intact C-terminus (28, 29).

The C-terminal 25 amino acid region of membrane CPE has an amphiphilic α -helix domain that can be associated with membranes at acidic pH (5.5–6.5) and a cytoplasmic tail of about 6 amino acids (25, 30, 31). The 18 amino acid α -helix domain, containing several pairs of hydrophobic residues separated by hydrophilic residues, interacts with cholesterol-rich lipid rafts, therefore allowing the pH-dependent binding of CPE to secretory granule membranes and facilitating the targeting of peptides to the RSP (31). The CPE cytoplasmic tail has been shown to be involved in the recycling of CPE from

**Figure 1**

CPE is a carboxypeptidase that removes C-terminal basic residues of prohormone. (A) The precursor protein of CPE contains a 27 amino acid hydrophobic signal peptide and a short propeptide that ends with five adjacent arginine residues at the N-terminus. The C-terminal 25 amino acid region has an amphiphilic α -helix domain that can be associated with membranes at acidic pH (5.5–6.5) and a cytoplasmic tail of about 6 amino acids that recycle CPE from the plasma membrane to the TGN. Species-conserved Ser₂₀₂ is required for CPE protein folding, while Arg₂₅₅ and Lys₂₆₀ are necessary for interacting with sorting signal domain of POMC. Δ N-CPE, an N-terminus truncated form of CPE, is expressed in mouse embryos from embryonic day 5.5 to postnatal day 1. Unlike full-length CPE, which is present in both embryonic and adult neurons, Δ N-CPE is expressed only in embryonic neurons and acts in the nucleus of cortical neurons to induce gene transcription. Δ N-CPE exhibits neuroprotection during the embryonic neurodevelopment. (B) CPE exists in membrane and soluble forms. 55 kDa membrane form functions as a sorting receptor for targeting prohormones and proneuropeptides to the regulated secretory pathway. However, the 53 kDa soluble form is a processing enzyme that removes C-terminal lysine or arginine residues from neuropeptide intermediates following endopeptidase cleavage to produce biological active peptide hormones and neuropeptides.

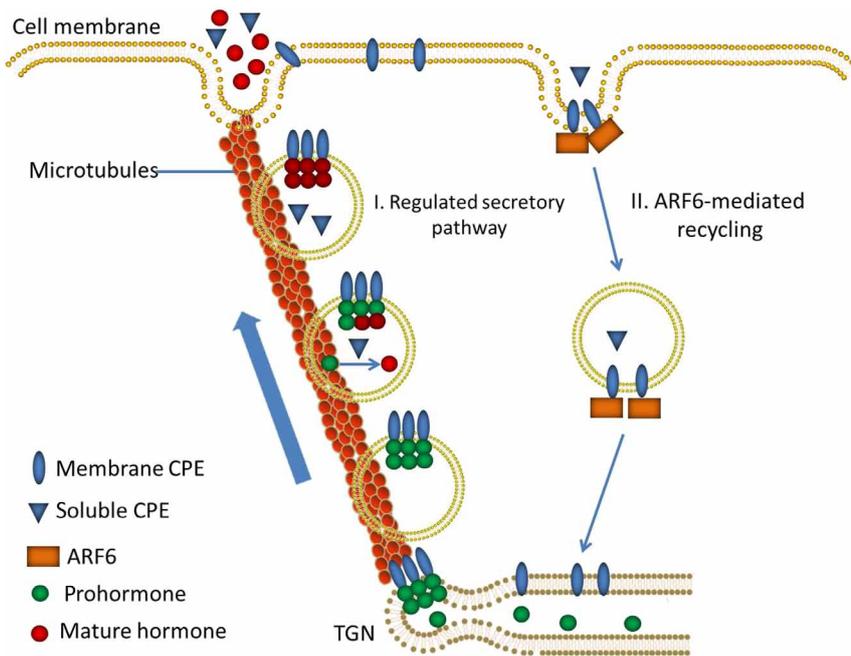
the plasma membrane to the trans-Golgi network (TGN) by interacting with an activated form of the cytoplasmic small GTPase, ADP-ribosylation factor 6 (ARF6) in Neuro2A cells (32). The cytoplasmic tail is also required for anterograde transport of POMC/ACTH vesicles to the secretion site for exocytosis in anterior pituitary cells (33). In addition, the last four residues of the cytoplasmic tail are also necessary for the association and sorting, probably through the interaction with lipids in the outer leaflet of the membrane (34). However, the C-terminal region of CPE is not sufficient for sorting, since the fusion protein N-Tac-CPE25 containing the C-terminal 25 amino acids of CPE was sorted less efficiently compared with full-length CPE (34). Thus, additional regions of CPE may be required for the efficient sorting of CPE. Further studies on the three-dimensional structure of the membrane CPE are required for analyzing its interactions with other molecules and sorting functions.

In targeting of prohormones to the RSP, CPE has been suggested to interact with two acidic residues (Asp₁₀, Glu₁₄) and two hydrophobic residue (Leu₁₁, Leu₁₈) in the

sorting signal domain of POMC at a specific binding site, which contains two basic residues, Arg₂₅₅ and Lys₂₆₀, and is present in a loop of CPE (35). Many proteins, such as proenkephalin, proinsulin and growth hormone (GH) also have similar sorting signal motifs, indicating that these prohormones bind CPE at the same site (36, 37). The interaction between CPE and the sorting signal motif of the prohormones, which is independent of the enzymatic site, leads to the segregation of the prohormones within the TGN and the sorting to the RSP (Fig. 2) (35).

Another important region is between 26 and 33 residues from the C-terminal of membrane CPE. It was found that CPE lacking 33 C-terminal residues was neither active nor secreted from cells (38), while CPE with a deletion of 26 C-terminal residues was active and secreted from cells (27). Thus, the region between 26 and 33 C-terminal residues may be important for the transmembrane orientation of CPE into lipid rafts.

The substitution of a serine in position 202 with a proline was noted to disturb the folding of CPE. Ser₂₀₂ is conserved in different species, such as human, bovine, rat,

**Figure 2**

CPE serves as a regulated secretory pathway sorting receptor of many peptides including proinsulin, proenkephalin, POMC and BDNF. The process of sorting secretory proteins into the regulated secretory pathway is that the protein binds to a sorting receptor at the Golgi network TGN and is then packaged into dense core secretory granules for secretion. In the TGN, the pH decreases along with elevated Ca^{2+} , CPE is activated and some of the CPEs bind to the membranes. Then in mature secretory granules, with an acidic pH of 5–6, CPE is maximally active and a majority of them tightly bind to membranes. Soluble CPE removes the C-terminal basic residues of prohormone for maturation. Finally, when secretory granules fuse with the plasma membrane, the extracellular neutral pH decreases the CPE activity and result in a release of both soluble and membrane CPE as well as hormones. After stimulated secretory granule exocytosis, membrane-bound CPE is recycled from the plasma membrane to the TGN facilitated by a small GTPase ARF6, where CPE is reused.

mouse, fish and *Aplysia* (16, 17, 18, 19), as well as other carboxypeptidases, such as human carboxypeptidase M, carboxypeptidase N and carboxypeptidase D (21, 22, 39), implying its critical role in CPE. The mutation of Ser₂₀₂ to Pro₂₀₂ in *Cpe^{fat}/Cpe^{fat}* mice reduces the activity of CPE and leads to its degradation. Immunofluorescence analysis showed that Ser₂₀₂ CPE was present in secretory vesicles, whereas Pro₂₀₂ CPE was localized in ER and misfolded, and rapidly degraded within the cell and not secreted (10). Ser₂₀₂ is predicted to be localized in a β -sheet, thus it is suggested that the replacement with a proline would perturb CPE structure, leading to inactive CPE (10). More studies on CPE folding are necessary to understand this point mutation.

Regulation of CPE activity

The processing of pro-CPE and the function of CPE are regulated by pH and Ca^{2+} level in the RSP, where pH plays a more important role (40, 41, 42, 43). The disruption of intraluminal balances of pH and Ca^{2+} level can interfere with the proteolytic processing of pro-CPE, possibly by affecting PC1 and PC2 activities (42, 44). CPE is maximally active and most of them tightly bind to membranes at a pH of 5–6 (25). This is consistent with a study showing that the membrane-bound CPE can be extracted by a combination of high salt and detergent at low pH (5–6), or by high pH (>7) without salt or detergents (4, 45). Moreover, the binding of Ca^{2+} destabilizes CPE and

decreases its activity, especially at high pH, whereas low concentration of EGTA stabilizes and activates CPE (9). Thus, within the ER and the Golgi apparatus, where the pH is neutral (46) and the level of Ca^{2+} is low, CPE would be stable due to low Ca^{2+} level, but inactive because of the neutral pH. In the TGN, the pH decreases while the concentration of Ca^{2+} is elevated. CPE would be activated by the acidic pH and some of CPE would be bound to the membranes. Although Ca^{2+} level is high, CPE would not be destabilized due to the low pH. Then in mature secretory granules, with an acidic pH of 5–6 (46, 47, 48) and high concentration of Ca^{2+} , CPE would be stable, active and most of membrane CPE would be associated with the membranes. Finally, when secretory granules fuse with the plasma membrane, the extracellular neutral pH would cause a decrease in CPE activity and result in a release of both soluble and membrane CPE (Fig. 2).

Catecholamines, such as dopamine, norepinephrine, epinephrine and dopamine quinine, can inhibit CPE, with dopamine quinine being the strongest inhibitor. These inhibitors are suggested to affect CPE activity by binding allosteric sites, leading to conformational changes of CPE (49). Reserpine, an agent preventing the entry of catecholamine into granules, is predicted to increase the CPE activity. Indeed, reserpine can increase the processing of the larger precursor peptides, leading to elevated cellular levels of processed peptides (50, 51). It was reported that treatment of chromaffin cells with reserpine caused a 3-fold increase in the CPE activity

within the chromaffin granules (52). However, the level of CPE proteins was not affected. Similarly, long-term treatment with glucocorticoids, which decreases POMC-derived peptide levels, does not change CPE levels (53). These results indicate that CPE levels are not regulated in coordination with the neuropeptide synthesis.

CPE is selectively inhibited by guanidinoethyl-mercaptopropionic acid (GEMSA) (54). Since GEMSA only binds to CPE with a high affinity in tissue sections and homogenates (55), the tritiated GEMSA can be applied to detect the membrane form of CPE by light microscopic autoradiography (56). Additionally, the level of soluble CPE can be measured by determining GEMSA binding with polyethyleneimine-pretreated filters (55).

As a member of the metalloprotease family, CPE requires Zn²⁺ binding to the active site for its catalytic activity (1, 57). Moreover, CPE is activated by Co²⁺ and inhibited by chelating agents (1). Thus, the enzymatic activity of CPE in tissue homogenates can be detected by determining the level of Co²⁺-stimulated activity at pH 5–6.

Tissue distribution of CPE

As a method for detecting CPE, the distribution of GEMSA binding shows that CPE is present in tissues that contain peptide hormones or neurotransmitters, such as brain, pituitary gland, pancreas, heart as well as the adrenal gland (1), and is similar in all of these tissues (58).

High expression of CPE is detected in the central nervous system, including pituitary gland (18, 58, 59). The highest level of CPE mRNA is in the hypothalamus, especially in the median eminence, supraoptic nucleus, paraventricular nucleus and suprachiasmatic nucleus. CPE is also found in axon terminals in the posterior pituitary gland, melanotropes of the intermediate pituitary gland, and select cells of the anterior pituitary gland. Moreover, CPE is detected in the central nucleus of the amygdala and the bed nucleus of the stria terminalis. These results suggest that CPE mRNA localization in the central nervous system corresponds to the distribution of neurotransmitter-synthesis neurons, further indicating the role of CPE in neuropeptide processing (58). In addition, CPE mRNA is localized in reactive glia as well, which is consistent with a study showing that cultured astrocytes are able to synthesize and secrete CPE (60). Since cultured astrocytes can express a number of neuropeptides, such as proenkephalin (60), somatostatin (61) and angiotensinogen (62), this result suggests that

CPE may process neuropeptides in glia. However, high level of CPE mRNA is also shown in some neurons not presently known to express specific neuropeptides (59), implying that in these cells CPE may have functions other than peptide processing, such as transporting neurotransmitter vesicles, or there may be undiscovered peptides processed in these regions.

The fact that CPE is also detected in various peripheral tissues implicates its role in peptide processing. The level of CPE mRNA is relatively high in human adipose tissue as shown by both expressions profiling and Northern blotting (63). CPE has a higher expression in visceral than in subcutaneous adipose tissue of obese subjects, which may explain the elevated release of specific peptides, such as thrombospondin-1, from the visceral tissues compared with the subcutaneous adipocytes (64). However, adipocytes do not contain classical secretory granules, suggesting that adipose tissue may contain unknown (neuro) endocrine-like cells (63). In the rat stomach, CPE is present in antropyloric mucosa. It is reported that gastrin cells and progenitor gastrin-somatostatin cells (G/D) express CPE, but somatostatin cells and most serotonin cells do not contain this enzyme (65), suggesting that CPE may process gastrin in gastrin cells and immature G/D cells. This is consistent with a finding that CPE-deficient obese mice have disturbed gastrin processing (66, 67, 68). In the eyes, CPE mRNA and protein are found in nonpigmented ciliary epithelial cells and aqueous humor by using specific probes. CPE mRNA is detected in many ocular tissues of the human eye, but the level is higher in the ciliary body and retina compared with other ocular tissues (69). Similarly, another study also found a high level of CPE mRNA in rat photoreceptors (70). These results suggest that CPE may confer an endocrine function to these cells. Besides ocular tissues and aqueous humor, other tissues of the human eye, such as lens, may also contain CPE, which, however, has not been reported yet.

In addition to adult tissues, CPE has been reported to be expressed in mammalian embryo (71). *In situ* hybridization histochemistry shows that the expression of CPE mRNA is highly concentrated in the developing nervous system. Some peripheral tissues such as the embryonic heart and cartilage primordia also express CPE mRNA (71). Thus, CPE may be important in the proteolytic maturation of neuropeptides in the nervous system and propeptides in the peripheral tissues. However, it is not clear whether CPE has the same functions in the embryo as it does in adults. In addition, a form of CPE with N-terminus truncated, ΔN-CPE, was found to be transiently expressed

in mouse embryos from embryonic day 5.5 to postnatal day 1 (72). Most of Δ N-CPE expression was present in embryonic brain. However, in contrast to full-length CPE, which is present in both embryonic and adult neurons (71), Δ N-CPE was detected only in embryonic neurons but not in adult mouse brain, suggesting that it may play a role in embryonic neurodevelopment, but the exact function of Δ N-CPE in neurons is not well understood.

CPE acts as an exopeptidase

Soluble CPE is an exopeptidase that has a vital role in the biosynthesis of numerous bioactive peptide (1, 3). Peptide hormones and neuropeptides are initially synthesized as larger inactive precursors that require selective cleavages at specific sites in the TGN and secretory granules before being releasing as bioactive peptides (73). An endopeptidase, such as prohormone convertase PC1/3 or PC2, cleaves either in between or on the C-terminal side of paired basic residues and leaves one or two basic residues at the C-terminus (74, 75, 76). After the action of prohormone convertase, soluble CPE in the secretory granule then removes the remaining basic amino acids (arginine and/or lysine) from peptide intermediates to generate biologically active hormones and neuropeptides (1, 77). For most peptides, CPE is the final step in biosynthesis, whereas some peptides need additional modifications, including C-terminal amidation, N-terminal acetylation and Ser or Thr phosphorylation (78). CPE is specific for removing C-terminal basic residues, with no activity towards nonbasic residues (79). Apart from the requirement for C-terminal basic residues, CPE has a wide range of substrates and can efficiently remove basic residues from all known peptide processing intermediates except for those containing a proline in the penultimate position (80). The action of CPE in peptide processing is confirmed by studies on mice lacking CPE. The deficiency of CPE in *Cpe^{fat}/Cpe^{fat}* mice causes a marked increase in the levels of peptide intermediates, which contain C-terminal basic residues (9, 11, 12). The immediate products of CPE activity were found to decrease substantially in *Cpe^{fat}/Cpe^{fat}* mice. Examples include insulin, BDNF and neurotensin. In the pancreatic islets, the absence of CPE results in the production of C-terminally extended insulin intermediates, which were not detected in the normal mice (9). In the cortical and hippocampal neurons of *Cpe* KO mice, the processing of proBDNF is blocked, leading to a decrease of activity-dependent release of BDNF (7). Similarly, in the brain of *Cpe^{fat}/Cpe^{fat}* mice,

there was a markedly reduced level of neurotensin while the concentration of inactive neurotensin-Lys-Arg was highly elevated (12). These results are consistent with the exopeptidase activity of CPE in the biosynthesis of peptides and suggest that abnormal neuropeptide and peptide hormone processing is a common event in the mice lacking CPE activity.

Although CPE is important in peptide processing, it is surprising that the obese mutation leading to CPE degradation is not lethal. Studies on several types of brain peptide by radioimmunoassay showed that a small amount of correctly processed neuropeptide was produced in *Cpe^{fat}/Cpe^{fat}* mouse brain (11, 12). This finding raises the possibility that another carboxypeptidase may also have CPE-like enzymatic activity, which is later identified as carboxypeptidase D (CPD) and found to be enriched in the TGN (81, 82, 83). Since the subcellular localizations of CPE and CPD are different and the levels of CPD are not sufficient in the brain, it is suggested that CPD just partially compensates for the loss of CPE activity in *Cpe^{fat}/Cpe^{fat}* mouse. Therefore, incomplete processing of the neuropeptide intermediates still lead to the accumulation of peptides containing C-terminal basic residues in *Cpe^{fat}/Cpe^{fat}* mice. The finding of CPD indicates that CPE is the major, but not the only, carboxypeptidase in the processing of neuropeptides. However, in other tissues, such as intestine, CPE shows different activity compared with its action on the brain. For instance, in *Cpe^{fat}/Cpe^{fat}* mouse brain, the concentration of the CPE product, active cholecystokinin (CCK) was markedly reduced, while the level of glycyarginine-extended CCK, which is the CPE substrate, was greatly elevated (84). In contrast, the concentration of active CCK in the intestine was not affected, although there was a smaller increase in glycyarginine-extended CCK level. Similar result is also shown in gastrin processing (67). This suggests that the intestine may have another CPE-independent biosynthetic pathway employing other carboxypeptidases to compensate the absence of intestinal CPE. It is very likely that CPD, which may be involved in the constitutive pathway (85), is largely responsible for peptide processing in the intestine. However, the detailed mechanism of CPD action in the intestine requires further investigation.

CPE also have effects on prohormone convertase activity. Studies on insulin processing show that the level of insulin precursor, proinsulin, is highly increased in *Cpe^{fat}/Cpe^{fat}* mice, indicating that defective CPE affects previous cleavage step (86). It was found that the concentration of PC1 was reduced in pituitaries of

Cpe^{fat}/Cpe^{fat} mice, implying that CPE may influence the activity of this enzyme (87). As the C-terminal tail of PC1 is an inhibitor of this enzyme (88), CPE may remove the C-terminal extension of PC1, thus activating the enzyme. Another explanation is that CPE is required for the processing of proSAAS, an endogenous inhibitor of PC1 (89). Hence, in *Cpe^{fat}/Cpe^{fat}* mice the deficiency of CPE leads to the accumulation of proSAAS, resulting in the inhibition of PC1 activity. The inhibition of PC2 activity in *Cpe^{fat}/Cpe^{fat}* mice may be due to the long-term presence of C-terminal basic residue-containing peptides due to the absence of CPE, since the C-terminal basic residue-containing peptides were reported to inhibit PC2 activity through feedback inhibition (90). Moreover, CPE is able to activate PC2 by blocking the action of 7B2, an endogenous secretory granule inhibitor. 7B2 is released from binding to PC2 by a CPE-mediated removal of C-terminal lysines (91). Thus, loss of CPE increases 7B2 level, which then binds to and inhibits PC2, resulting in the disturbance of peptide processing. More studies are necessary to confirm the action of CPE in regulating the prohormone convertase activity.

CPE acts as a sorting receptor

In neuroendocrine and endocrine cells, neuropeptides and peptide hormones are sorted into the regulated secretory pathway for secretion. However, the mechanism by which peptides are sorted into the regulated secretory pathway is not clear. One proposed mechanism for sorting secretory proteins into the regulated secretory pathway is that the protein binds to a sorting receptor at the TGN and is then packaged into dense core secretory granules for secretion (6).

In addition to its exopeptidase activity, CPE is suggested as a sorting receptor for directing prohormones from the TGN to the regulated secretory granules (Fig. 2) (6). Several experiments have led to the conclusion that CPE is a regulated secretory pathway sorting receptor of many peptides including POMC, proinsulin, proenkephalin and BDNF. Studies using pituitary cells from *Cpe^{fat}/Cpe^{fat}* mice showed that ACTH, POMC and GH, which are directed to the regulated pathway in normal mice, were missorted to the constitutive pathway, showing no response to stimulation by secretagogues (6, 36). Additionally, deletion of CPE by using RNA interference resulted in loss of regulated secretion of proinsulin from INS-1 cells (92). Similarly, immunocytochemical studies using CPE antisense RNA to inhibit CPE expression in

Neuro-2a cells showed that proinsulin and proenkephalin were released constitutively while these prohormones were secreted in a regulated manner in wild-type cells (37). BDNF was also constitutively secreted from neurons and pituitary cells of *Cpe* KO mice rather being released by the regulated secretory pathway in normal mice (7). These results suggested that CPE is essential for sorting peptides to regulated secretory pathway. Following the sorting action of CPE, inactive proforms of peptide hormones and neuropeptides in the regulated secretory granules are processed to active forms and secreted into the extracellular space. Thus, missorting of the prohormones or proneuropeptides to the constitutive pathway leads to incomplete peptide processing and secretion of unprocessed or partially processed precursors in an unregulated manner, resulting in endocrine and nervous system disorders observed in *Cpe^{fat}/Cpe^{fat}* mice and the *Cpe* KO mice.

However, experiments by other groups showed conflicting results. Regulated secretion of proinsulin was observed in pancreatic islets and β -cell lines from *Cpe^{fat}/Cpe^{fat}* mice with the addition of secretagogues, while proinsulin was constitutively released in elevated manner as well (93, 94). Similarly, ACTH was found to be targeted to the regulated secretory pathway in *Cpe* siRNA-treated AtT-20 cells, where the expression of CPE was suppressed (95). Moreover, pituitary cells from *Cpe^{fat}/Cpe^{fat}* mice released gonadotropins LH and FSH in response to GnRH (96). Taken together, these results challenge the notion that CPE is a sorting receptor for the regulated secretory pathway.

There are a few possible explanations for these observations. First, CPE may be not a generic sorting receptor for all prohormones. Some prohormones may be directed to secretory granules by other sorting mechanisms independent of CPE. In both wild-type and CPE-depleted Neuro-2A cells, chromogranin A was sorted to the regulated secretory pathway (37), implying the presence of other sorting receptors. Indeed, secretogranin III (SgIII) was found to be responsible for the sorting of chromogranin A (CgA) in the pituitary and pancreatic endocrine cells (97). Furthermore, another study reported that pituitaries from *Cpe^{fat}/Cpe^{fat}* mice secreted ACTH in response to secretagogues, with the enhanced expression of SgIII and CgA (98). Since the constitutive secretion from the *Cpe^{fat}/Cpe^{fat}* pituitary was still increased, it suggests that these proteins may partly compensate for the sorting function of CPE (98, 99). Secondly, the role of CPE as a sorting receptor may vary with the cell types. In pancreatic

β -cells, CPE may cooperate with other sorting receptors for the transport of prohormones, thus the absence of CPE have little effect on the regulated secretion of proinsulin. However, in Neuro-2A cells, where CPE may play a more important role, CPE depletion leads to the constitutive secretion of proinsulin. This may be related to the distribution of CPE and other sorting receptors, thus it is necessary to measure and compare levels of CPE and other sorting receptors, such as SgIII, in various tissues. More studies on the sorting action of CPE in different tissues and for different prohormones are required. Thirdly, in *Cpe^{fat}/Cpe^{fat}* mice mutant CPE may not be fully degraded in the ER, and some of them may escape degradation. The mutant CPE protein was differentially degraded in various tissues. This protein was detected in pancreas and brains of *Cpe^{fat}/Cpe^{fat}* mice, although it was not found in pituitary (9, 11). In addition, the finding that mutant CPE could efficiently bind proinsulin suggests that it may act as a sorting receptor (35). Thus, in the pancreatic tissues, some mutant CPE could escape degradation, bind proinsulins and target proinsulins to the regulated secretory pathway. Whether mutant CPE functions as a sorting receptor with the same efficiency with normal CPE is unclear. Moreover, further investigation is necessary for determining the role of mutant CPE in sorting other prohormones. Fourthly, it is likely that the RNA interference method downregulated the CPE expression in *Cpe* siRNA-treated cells, but did not totally eliminate the protein. The remaining CPE, although in a small amount, is still capable of sorting prohormones to the secretory granules.

The exact mechanism by which CPE targets prohormones to the regulated secretory pathway has been studied for years. CPE is able to bind specific sorting motifs in prohormones, which is suggested to be critical in the sorting of prohormones. Cross-linking experiments show that the N-terminal peptide of POMC, which contains a 13-residue amphipathic loop, interacts with CPE in the secretory granules (6). Moreover, molecular modeling studies show that similar sorting motifs are also present in proinsulin and proenkephalin, but not in CgA. This is consistent with the finding that CPE is a sorting receptor for POMC, proinsulin and proenkephalin, but not for CgA (6, 37). Specific sorting motifs in these prohormones contain two acidic residues and two aliphatic hydrophobic residues exposed on the surface of a disulfide loop structure (100). The binding site on CPE for interacting with the sorting signal of prohormones, containing two basic residues, Arg₂₅₅ and Lys₂₆₀, is independent of the catalytic domain in soluble

CPE (101), indicating that the binding of prohormones is not related to its enzymatic activity. The pH range of binding the sorting signals to CPE is between 5.5 and 6.5, which is consistent with the pH of the TGN (102). The C-terminal region of CPE was shown to be associated with cholesterol-glycolipid rafts at the TGN, thus CPE is anchored to the membrane for binding prohormones (31). Before interaction with CPE, POMC and proinsulin have been demonstrated to undergo aggregation independent of pH (103). In addition, the acidic pH and the level of Ca²⁺ in the TGN also stimulate the aggregation of CPE (43), which may provide a scaffold for the binding and package the aggregating prohormones in the TGN. Therefore, the possible mechanism of CPE sorting may start with the aggregations of prohormones, which occurs before or at the TGN. CPE self-aggregation and binding to the lipid rafts at the TGN provide binding position for prohormone aggregates, which then bind CPE via specific sorting signals, followed by budding and granule formation.

CPE facilitates endocytic internalization

After stimulated secretory granule exocytosis, CPE is rapidly recycled from the plasma membrane to the TGN, where CPE is reused as a sorting receptor or exopeptidase, through early endosomes and the endocytic recycling compartment (32). A yeast two-hybrid screen and *in vitro* binding assay show that the six residues in the cytoplasmic tail of CPE interact specifically with a small GTPase ADP-ribosylation factor 6 (ARF6). Mutations in the six residues block the interaction with ARF6 and inhibit the recycling of CPE to the TGN, suggesting that CPE recycling is mediated by the binding of ARF6 (32).

Moreover, ARF6-mediated recycling of CPE is considered to facilitate internalization of other proteins. Yeast two-hybrid screening and immunoprecipitation demonstrated the interaction between CPE and eosinophil cationic protein (ECP). Additionally, the expression of mutant CPES473A, E474A in GH3 cells blocked the internalization of ECP (104), suggesting that CPE is involved in the internalization of ECP to neuroendocrine cells. Since S471A and E472A point mutations on the cytoplasmic tail of CPE inhibit the binding of ARF6 (32), the recycling of CPE from the plasma membrane to the TGN was blocked. Therefore, the internalization of ECP may be related to the recycling of CPE in neuroendocrine cells. However, the mechanism underlying the role of CPE in ECP internalization is not clearly known.

CPE mediates vesicle transport

After targeting prohormones to regulated secretory granules, CPE is found to mediate the bidirectional transport of granules to maintain vesicle homeostasis and secretion. The BDNF vesicle in hippocampal neurons, for example, is transported anterogradely to neurite terminals for activity-dependent secretion (105). If the vesicles are not trapped in the nerve terminal for release, they are retrogradely transported back to the cell body for degradation or reuse (106). In a study, the cytoplasmic tail of CPE was shown to play a significant role in anchoring BDNF vesicles to the microtubule-based transport system (107). Overexpression of the CPE cytoplasmic tail leads to a significant decrease in the level of BDNF localized in neurites of hippocampal neuron, and a reduction in the velocity and distance of movement of CPE or BDNF vesicles, indicating the critical role of the CPE cytoplasmic tail in driving transport of BDNF vesicle (107). Pull-down assays and competition assays show that the CPE tail directly interacts with dynactin, which then recruits kinesins for anterograde transport or dynein for retrograde transport. Therefore, cytoplasmic tail of CPE recruits dynactin and kinesin-2/kinesin-3 to facilitate the anterograde movement of BDNF vesicles to the secretion sites, while the interaction between the cytoplasmic tail and dynactin/dynein mediates the retrograde transport of BDNF vesicles (107). Similarly, in mouse AtT-20 cells, the CPE cytoplasmic tail was also shown to mediate the anterograde transport of ACTH vesicles to the release site (108). Overexpression of the CPE cytoplasmic tail disrupted the K⁺-stimulated secretion, but not the constitutive secretion of ACTH, suggesting that the cytoplasmic tail is specific for the transport of regulated secretory pathway granules. The CPE tail also mediates ACTH anterograde transport by binding dynactin along with kinesin-2 and kinesin-3 and the retrograde transport by interacting with dynactin and dynein (33). This function of CPE may be generally used by the peptide hormone and neuropeptide vesicles for moving to the secretion site.

Additionally, CPE is also involved in the transport of synaptic vesicles. *Cpe* KO mice showed impaired glutamate neurotransmission (14) and the lack of CPE ortholog, *egl-21*, in *Caenorhabditis elegans* resulted in the impaired release of acetylcholine at the neuromuscular junction (109), suggesting that CPE is necessary for neurotransmitter secretion. Moreover, electron microscopy revealed that the localization of synaptic vesicles in the pre-active zone was greatly reduced in the hypothalamic neurons of *Cpe* KO mice, along with

the impaired secretion of glutamate (110). This result indicates that CPE may affect neurotransmitter release by transporting synaptic vesicles, since the localization of synaptic vesicles in the pre-active zone is critical for regulating the release of neurotransmitters. Indeed, it has been found that the CPE cytoplasmic tail also mediates the localization of glutamatergic and acetylcholine synaptic vesicles to the pre-active zone of hypothalamic neurons and PC12 cells, respectively, by interacting with γ -adducin and actin, thereby facilitating neurotransmitter secretion (110). However, it is unclear whether other proteins, such as synapsin, are also required for the localization of synaptic vesicles by the CPE cytoplasmic tail.

CPE regulates signaling pathways

Recently, CPE has been shown to function extracellularly, independent of its enzymatic activity, through signaling pathways. It was reported that *Cpe* KO mice exhibit neuronal degeneration in the hippocampal region after emotional and physical stress at weaning, while wild-type mice show good survival of the hippocampal neurons (111). Furthermore, the hippocampal neurons derived from *Cpe* KO mice were rescued by the addition of CPE, indicating a role of extracellular CPE in neuroprotection (112). In addition, the level of Bcl-2 was elevated and both ERK and AKT were phosphorylated in the CPE-treated hippocampal neurons. The activation of pro-apoptotic executioner caspase-3 induced by hydrogen peroxide was also inhibited by the CPE treatment (112). Therefore, CPE is able to upregulate the expression of anti-apoptotic protein Bcl-2 and inhibit caspase-3 activation by activating ERK and AKT signaling pathways, suggesting that it may function as a trophic factor to provide neuroprotection against the oxidative-stress-induced cell death (112). For this reason, CPE is also named as neurotrophic factor- α 1 (NF- α 1). However, the detailed mechanisms of the CPE neuroprotection, such as how CPE activates the ERK and AKT signaling pathways, still need further investigation. It is possible that CPE may bind a specific receptor to initiate ERK and AKT signaling (Fig. 3).

Moreover, an N-terminal truncated isoform of CPE, Δ N-CPE, was suggested to exhibit neuroprotection during embryonic neurodevelopment (72). The overexpression of Δ N-CPE into rat embryonic cortical and hippocampal neurons protected them against glutamate- and H₂O₂-induced cell death and increased the expression of FGF2. Since FGF2 can activate ERK and AKT signaling pathways, it results in the elevation of Bcl-2 level and inhibition

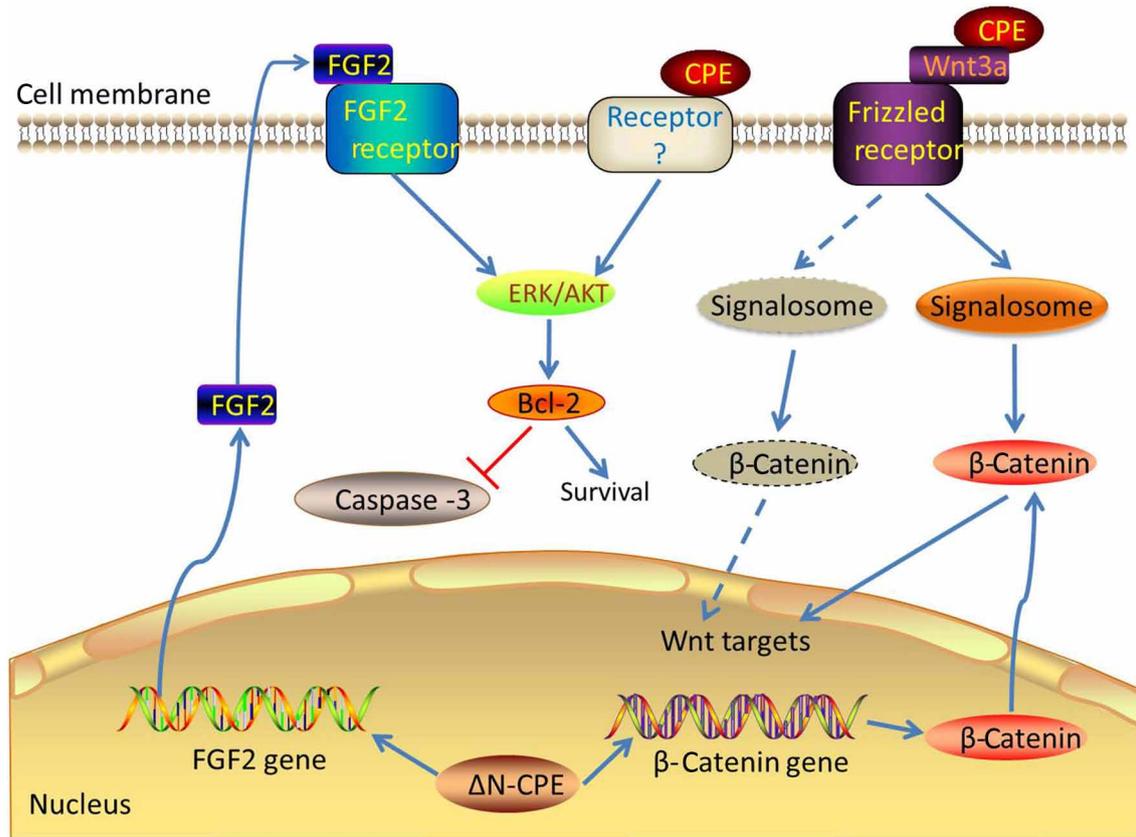


Figure 3

Both full-length CPE and potential Δ N-CPE regulates signaling pathways. CPE upregulates the expression of anti-apoptotic protein Bcl-2 and inhibits caspase-3 activation. This may be realized through binding of CPE to a specific receptor to initiate the ERK and AKT signaling. CPE also interacts with the Wnt3a and the frizzled receptor to form a complex, thereby disrupting disheveled-induced signalosomes of the Wnt signaling and leading to the degradation of β -catenin. However, Δ N-CPE was suggested to act in the nucleus of cortical neurons to induce gene transcription, increase the expression of β -catenin and FGF2, and activate the ERK/AKT signaling or Wnt pathways.

of caspase-3 activity, thereby inducing cell survival (72). Thus, both full-length CPE and Δ N-CPE prevent the neuronal cell death by activating ERK and AKT signaling pathways. However, unlike full-length CPE, which functions extracellularly to activate a signaling pathway, Δ N-CPE acts in the nucleus of cortical neurons to induce gene transcription.

In addition, CPE and Δ N-CPE are identified as regulators of Wnt signaling pathway. Δ N-CPE was suggested to enhance the expression of β -catenin, a key effector protein of the canonical Wnt pathway, and Wnt target gene, whereas full-length CPE decreases the protein level of β -catenin and Wnt signaling (113). Thus, CPE and Δ N-CPE may be the opposite regulators of Wnt signaling. Moreover, full-length CPE was found to interact with Wnt3a ligand and the frizzled receptor to form a complex, thereby leading to the disruption of disheveled-induced signalosomes, which play a critical role in Wnt signaling (113). More studies are required to elucidate how Δ N-CPE

upregulates the Wnt signal and how CPE disrupts the signalosomes (Fig. 3).

Pathophysiological roles of CPE

Diabetes

Since both *Cpe^{fat}/Cpe^{fat}* mice and *Cpe* KO mice exhibit diabetes, loss of CPE activity is strongly related to the development of diabetes in these mice. CPE is an essential enzyme for the processing and maturation of insulin. *Cpe^{fat}/Cpe^{fat}* mice exhibit high levels of proinsulin and incompletely processed intermediates in the pancreatic islets and the circulation (9), producing the phenotype of hyperproinsulinemia and leading to diabetes as a result of deficient proinsulin processing (Table 1).

The apoptosis and dysfunction of β -cells also play significant roles in diabetes pathogenesis. Reducing CPE

Table 1 CPE involves in diseases including diabetes, obesity, infertility, bone metabolism, gastrointestinal deficits, neurological diseases and cancer.

Diseases	Mouse models	Pathogenesis	Related neuropeptides and hormones	References
Diabetes	<i>Cpe^{fat/fat}</i> , <i>Cpe</i> KO	Hyperproinsulinemia, β -cell stress	Insulin, IAPP	Naggert <i>et al.</i> 1995, Marzban <i>et al.</i> 2005
Obesity	<i>Cpe^{fat/fat}</i> , <i>Cpe</i> KO	Defects in energy balance regulation, satiety and nutrient partitioning	CCK, neurotensin, GLP-1, TRH, MCH, enkephalin, α -MSH, heptapeptide, β -endorphin 1–31	Lacourse <i>et al.</i> 1998, Rovere <i>et al.</i> 1996, Tang <i>et al.</i> 1996, Nillni <i>et al.</i> 2000, Fricker 2007, Plum <i>et al.</i> 2009
Infertility	<i>Cpe^{fat/fat}</i> , <i>Cpe</i> KO	Loss of sexual behavior	?	Srinivasan <i>et al.</i> 2004
Low bone mineral density (BMD)	<i>Cpe</i> KO	Increased bone resorption	CART, α -MSH, NPY	Cawley <i>et al.</i> 2010
Inflammatory bowel disease	<i>Cpe^{fat/fat}</i>	Increased cytokine expression, decreased intestinal barrier function	NPY	Bar <i>et al.</i> 2014
Cancer	–	CPE induces cell survival and proliferation; Δ N-CPE promotes tumor growth and metastasis	–	Horing <i>et al.</i> 2012, Lee <i>et al.</i> 2011
Gastrointestinal deficits	<i>Cpe^{fat/fat}</i>	Reduced meal-induced gastrin secretion	Gastrin	Lacourse <i>et al.</i> 1997
Neurodegenerative disease	<i>Cpe</i> KO	Absent neuroprotection	BDNF, SST	Lou <i>et al.</i> 2005, Billova <i>et al.</i> 2007

protein levels by palmitate and shRNA in β -cells increases ER stress and cell death, whereas overexpression of CPE protects β -cells from palmitate-induced ER stress and apoptosis (114). This suggests that CPE is able to inhibit β -cell apoptosis by altering ER stress, thus CPE deficiency contributes to β -cell death in diabetes. Moreover, the addition of palmitate was found to increase proinsulin concentration, indicating that the accumulation of unprocessed proinsulin, possibly because of the loss of CPE, may cause ER stress in β -cell. Also, β -cells were found to be clearly granulated in *Cpe^{fat}/Cpe^{fat}* mice but the secretory granules were less dense, suggesting that these granules store proinsulin instead of fully processed insulin (86). However, it is not clear how proinsulin leads to ER stress.

In addition, CPE is critical for the maturation of islet amyloid polypeptide (IAPP), a peptide hormone secreted from the β -cell (115). In islets from *Cpe^{fat}/Cpe^{fat}* mice, the unprocessed forms of IAPP were elevated approximately 86% while levels of bioactive amidated IAPP was reduced approximately 75%, suggesting that the processing of IAPP was impaired in the absence of CPE (115). It has been found that PC2, which is controlled by CPE, is responsible for the N-terminal processing of proIAPP, whereas CPE removes the C-terminal dibasic residues from proIAPP (115). Therefore, the absence of CPE results in a reduction in processing of proIAPP at both N-terminus and C-terminus. As the aggregation of proIAPP contributes to

the formation of islet amyloid deposits, which are toxic to β -cell (116), incomplete processing of IAPP, due to the loss of CPE, is likely to induce β -cell apoptosis.

Taken together, the absence of functional CPE leads to deficient processing of insulin and IAPP, resulting in hyperproinsulinemia and β -cell stress, which is the feature of type 2 diabetes mellitus.

Obesity

One study reported that 11–14 weeks old *Cpe^{fat}/Cpe^{fat}* mice are more obese and hyperphagic than wild-type littermates (117). Even when *Cpe^{fat}/Cpe^{fat}* mice are given access to exercise wheel or limited amount of food, they still gain more weight than wild-type mice (118). These results suggest that obesity observed in *Cpe^{fat}/Cpe^{fat}* mice is due to overeating and alterations in nutrient partitioning. The molecular mechanisms for obesity development in *Cpe^{fat}/Cpe^{fat}* mice are not yet well understood, but defects in processing of a variety of peptide hormones in brain and endocrine tissues are found to be associated with the obese phenotype in these mice.

CCK, which has multiple functions in the gastrointestinal tract and central nervous system, is able to inhibit food intake (119, 120), although the exact mechanism underlying the regulation of feeding by CCK is not clear. Maturation of CCK requires CPE to remove the C-terminal arginine residues from proCCK.

Thus, the deficiency of CPE may lead to impaired CCK processing, which, due to the satiety function of CCK, possibly further contributes to the obese phenotype of *Cpe^{fat}/Cpe^{fat}* mice. This is confirmed by the finding that after a meal, CCK concentrations were decreased both in the brain and plasma of *Cpe^{fat}/Cpe^{fat}* mice, while levels of arginine-extended form increased, indicating a decline in satiety function of CCK (84). Moreover, the synergistic interaction of CCK with leptin and their effects in neuron may explain why CCK deficiency causes profound obesity in *Cpe^{fat}/Cpe^{fat}* mice (121, 122).

Levels of active neurotensin were also found to be reduced greatly in the brain and hypothalamus of *Cpe^{fat}/Cpe^{fat}* mice, whereas the C-terminal extended neurotensin were elevated (12). Since hypothalamic neurotensin has anorexigenic effects (123), defects in neurotensin processing may lead to the obese phenotype of *Cpe^{fat}/Cpe^{fat}* mice.

Glucagon-like peptide-1 (GLP-1) is a peptide hormone that plays a role in downregulating blood glucose in the intestine and is involved in the regulation of satiety when expressed in the brain (124, 125). CPE is in charge of the C-terminal removal of arginine residue from GLP-1 precursors. This is supported by the observation of a great reduction of mature GLP-1 in *Cpe^{fat}/Cpe^{fat}* mice. Thus, the lack of mature GLP-1 may partly explain the obesity phenotype in these mice.

Thyrotropin releasing hormone (TRH) is important for the regulation of metabolism by controlling hypothalamic–pituitary–thyroid axis. TRH in pituitary stimulates the biosynthesis and secretion of thyroid-stimulating hormone (TSH), which induces the thyroid hormone biosynthesis and release in the thyroid. The processing of proTRH also needs CPE for removing basic residues from the C-terminus. In *Cpe^{fat}/Cpe^{fat}* mice, the conversion of proTRH to mature TRH is attenuated due to CPE mutation. Moreover, *Cpe^{fat}/Cpe^{fat}* mice cannot maintain their body temperatures when exposed to cold, suggesting impaired hypothalamic–pituitary–thyroid axis, which can affect weight gain and appetite (126). Hence, TRH processing deficits owing to CPE deficiency may lead to obesity by disrupting hypothalamic–pituitary–thyroid axis. In addition, α -melanocyte-stimulating hormone (α -MSH) maturation also required CPE activity in POMC neurons. FoxO1 ablation in POMC neurons increases the CPE expression and subsequent α -MSH, resulting in food intake in mice (127).

In contrast to the peptides that decrease body weight, levels of some peptides that increase body weight

are normal or even elevated in the hypothalamus of *Cpe^{fat}/Cpe^{fat}* mice. For example, melanin concentrating hormone (MCH), enkephalin heptapeptide and β -endorphin 1–31, which are present on the C-terminus of their precursors, are higher in *Cpe^{fat}/Cpe^{fat}* mice than in wild-type mice, suggesting that their biosynthesis does not require CPE cleavage and is increased in *Cpe^{fat}/Cpe^{fat}* mice (118). However, it is not clear why these peptides are elevated and how enhancement of their biosynthesis relates to CPE deficiency. As a result, the overall effect of CPE in the *Cpe^{fat}/Cpe^{fat}* mouse brain is toward increasing body weight due to the decreased levels of body-weight-reducing peptides and the increased levels of body-weight-elevating peptides.

Hence, obesity development in *Cpe^{fat}/Cpe^{fat}* mice is attributed to changes in the biosynthesis of a number of hypothalamic, pituitary and gastrointestinal peptide hormones involved in the regulation of energy balance, satiety and nutrient partitioning.

Disorders in bone metabolism

Cpe KO mice have been reported to have low bone mineral density (BMD) with high levels of C-telopeptide of type I collagen (CTX-1) and osteocalcin, which indicate an increase of bone turnover (15). BMD is regulated by a balance between two sequential cellular events, which are bone formation by osteoblasts and bone resorption by osteoclasts. The expression level of receptor activator for NF- κ B ligand (RANKL), which is the osteoclast differentiation factor in osteoblast progenitor cells (128), was highly elevated in the femur of *Cpe* KO mice compared to wild-type littermates, suggesting that osteoclastic activity was increased in *Cpe* KO mice. This is consistent with a finding showing that *Cpe* KO mice have 60% more osteoclasts in the femur than wild-type mice (15). Moreover, the hypothalamic expression of mature cocaine- and amphetamine-regulated transcript (CART), which inhibits bone resorption by blocking RANKL expression (129), was not detected in *Cpe* KO mice, although total levels of CART-IR were increased compared with those in wild-type mice. This would lead to increased bone resorption (15). However, α -MSH and neuropeptide Y (NPY), which play roles in bone resorption through unknown mechanisms (130, 131), were severely diminished in the hypothalamus of *Cpe* KO mice, leading to increased bone formation (15). Since *Cpe* KO mice exhibit decreased BMD, it suggests that CART plays a more significant role in the hypothalamus than

α -MSH and NPY. Therefore, RANKL in the femur and CART in the hypothalamus contribute to the bone phenotype of *Cpe* KO mice. It is necessary to investigate whether peptides in other tissues of *Cpe* KO mice are also related to the low BMD and how CPE affects the expression of these peptides.

However, in another study, CPE was found to directly enhance RANKL-induced osteoclast differentiation (132). High level of CPE was reported in mature osteoclasts, suggesting its role in osteoclastogenesis. Moreover, c-Fos and NFATc1, key transcription factors of osteoclastogenic genes, were increased by overexpression of CPE, and the deficiency of CPE attenuates osteoclast formation in response to RANKL (132). These results indicate that CPE may regulate RANKL-induced osteoclast differentiation by inducing the expression of c-Fos and NFATc1. Nevertheless, this regulation may not be major in osteoclast differentiation. In *Cpe* KO mice, the increased formation of osteoclast suggests that the great enhancement of RANKL is likely to counteract the influence of CPE deficiency in osteoclastogenesis, leading to decreased BMD.

Infertility

CPE may also play a role in the reproductive system, as *Cpe^{fat}/Cpe^{fat}* mice have been shown to be infertile. Mating studies revealed that the pregnancy rate is as low as 5% with homozygous *Cpe^{fat}/Cpe^{fat}* pairings, while the pairing between 50-day-old *Cpe^{fat}/Cpe^{fat}* males and heterozygous females has an increased rate of 43% (96). However, the rate rapidly declines with the increase of age before obesity is evident. Moreover, the evaluation of gonadal status of *Cpe^{fat}/Cpe^{fat}* males showed that the morphology and function of testis were abnormal, which may be caused by the incomplete processing of peptides due to the lack of CPE. Infertility in *Cpe^{fat}/Cpe^{fat}* mice is suggested to be due to a deficit in gonadotropin-releasing hormone (GnRH) processing (96). The level of GnRH was substantially decreased in the hypothalamus while the concentrations of pro-GnRH and C-terminal extended intermediates were highly elevated. Comparable findings have been reported in *Cpe* KO mice (13). Since GnRH plays a major role in regulating mammal reproduction, the abnormal processing of GnRH, possibly due to the lack of CPE, may contribute to infertility of *Cpe^{fat}/Cpe^{fat}* mice (133). However, it was found that baseline levels of gonadotropin were similar in wild-type, heterozygous and homozygous mice, suggesting that the small amount of

fully processed GnRH detected in hypothalamus, which may be attributed to the presence of CPD, is sufficient to maintain gonadotrope functions (96). Thus, the disturbance of GnRH processing is not the main reason of infertility in *Cpe^{fat}/Cpe^{fat}* mice. In addition, studies on 90-day-old *Cpe^{fat}/Cpe^{fat}* males showed that a loss of sexual behavior may play a major role in reproductive deficits. Since CPE is essential for the processing of many neuropeptides and peptide hormones, it is likely that alterations in the maturation of other peptides may be responsible for the infertility of *Cpe^{fat}/Cpe^{fat}* mice.

Gastrointestinal deficits

Enteroendocrine cells (EEC) maintain the intestinal barrier by producing neuropeptides, thus EEC dysfunction is likely to cause decreased intestinal barrier function, resulting in inflammatory bowel disease (IBD) (134). CPE has been found in EEC of the colon and ileum and is linked to the level of NPY and peptide YY produced by EEC, suggestive of its role in EEC function, intestinal homeostasis and barrier function (135). In addition, deficiency of CPE increased susceptibility of *Cpe^{fat}/Cpe^{fat}* mice to dextran sulfate sodium (DSS)-induced chronic colitis as well as the expression of specific cytokines, such as mucosal interleukin 6 (IL-6) and chemokine (C-X-C motif) ligand 1 (KC), under baseline conditions (135). As co-administration with NPY reduces IL-6 and KC expression, it suggests that CPE plays an anti-inflammatory role in the intestines and regulates cytokine production by influencing the intestinal levels of neuropeptides. Moreover, a chemotaxis experiment showed that bone-marrow-derived macrophages exhibited increased migration toward the supernatants obtained from *Cpe^{fat}/Cpe^{fat}* mice, indicating that CPE further controls the migration of primary immune cells (135). Therefore, CPE deficiency reduces neuropeptide processing and increases cytokine expression, leading to the migration of primary immune cells to intestinal mucosa, which then causes the proinflammatory phenotype observed in *Cpe^{fat}/Cpe^{fat}* mice upon DSS stimulation. Further studies on the molecular mechanisms of intestinal CPE effects are needed.

In the stomach, CPE is required for the cleavage of C-terminal extended gastrin followed by amidation to produce bioactive gastrin (136). In *Cpe^{fat}/Cpe^{fat}* mice, the concentration of amidated bioactive gastrin was found to be slightly reduced compared to that in wild-type mice, while the progastrin processing intermediates, such as glycylarginine-extended gastrin and glycine-extended

gastrin, accumulated excessively with increased gastrin mRNA (67). These results show that the deficiency of CPE disturbs progastrin processing, but the synthesis of gastrin is reinforced to maintain a normal level of bioactive gastrin (67). One of the possible mechanisms is that the transcriptional machinery or the proliferation of gastrin-producing cells (G-cells) is enhanced, thus leading to elevated gastrin mRNA levels (137). Alternatively, somatostatin, which is present in D-cells and inhibits the gastrin biosynthesis (138), may be reduced to abolish the inhibitory mechanisms. More studies are needed to clarify which mechanism plays a major role.

Although gastrin levels were not significantly decreased in *Cpe^{fat}/Cpe^{fat}* mice, meal-induced gastrin secretion was much lower than that in wild-type mice (67). Since gastric acid secretion is stimulated by gastrin (66), the reduction of gastrin may be accompanied by a decreased acid secretion, suggesting that CPE mutation may affect the stomach function by attenuating gastrin secretion. This is in agreement with studies showing that both basal gastric acid secretion and challenged gastrin secretion in response to inhibition of gastric acid secretion were decreased in *Cpe^{fat}/Cpe^{fat}* mice (66, 68). Therefore, CPE is important for the stomach's secretory activity and defective CPE processing leads to reduced stomach function, although the exact mechanism of CPE in challenged gastrin secretion is unknown.

Neurological diseases

CPE plays multiple roles in the central nervous system, such as maintenance of normal cognitive function, proper neuronal structure and neuron survival. Thus, mice lacking CPE activity will exhibit various neurological and behavior abnormalities.

The absence of CPE leads to degeneration of hippocampal neurons and memory deficits. Studies using the water maze, object preference and social transmission of food preference tests showed that adult *Cpe* KO mice have defects in memory consolidation. Moreover, neuronal degeneration occurred in the hippocampal CA3 region that normally contains a high concentration of CPE, indicating that CPE is essential for the hippocampal neuron survival (139). Indeed, another study showed that after transient global ischemia levels of CPE expression are substantially elevated in the CA3 area, resulting in the survival of those neurons, whereas in CA1 region there is only a transient increase of CPE expression, making neurons more vulnerable to ischemia (140).

Additionally, overexpression of CPE protects hippocampal neurons against H₂O₂-induced oxidative stress cell death (139). These results are suggestive of a role of CPE in neuroprotection. Immunohistochemistry and calbindin staining revealed gliosis in the CA3 region as well as the early termination of the mossy fiber before reaching the CA1 region in *Cpe* KO mice, indicating the perturbation of hippocampus cytoarchitecture that has a significant effect on function. However, antiepileptic agent like carbamazepine was suggested to prevent hippocampal neurodegradation in *Cpe* KO mice (141). Thus, CPE is responsible for maintaining levels of hippocampal neurons in the brain and the proper structure and functions of the hippocampus.

In addition, CPE is required for proper dendritic patterning, which is important for appropriate synaptogenesis and formation of neuronal network. *Cpe* KO mice exhibited more dendritic intersections and more branch points, reflecting more dendritic complexity compared to wild-type mice (142). Moreover, with the increase of age, dendritic pruning did not occur in the *Cpe* KO neuron, and the number of non-functional D-type spines, an indicator of spine degeneration, significantly increased in cortical neurons of *Cpe* KO mice. Since dendrite development is related to the establishment of proper neuronal connectivity and non-functional D-type spines affect synaptogenesis, these results suggest that CPE plays a critical role in maintaining neuronal functions by regulating appropriate arborization and spine morphology (142). Thus, dendritic architectural defects due to CPE deficiency can partly explain some neurological deficits in *Cpe* KO mice.

Although the neuroprotective function of CPE through ERK, AKT and Bcl-2 expression pathway is well established, several other pathways may also contribute to the neuroprotective activity of CPE (72). It is possible that some neuropeptides, processed and sorted to the secretory granules by CPE, are associated with neuron survival. One example is BDNF which is targeted to the RSP by CPE and involved in promoting neuronal survival. Disturbance of BDNF secretion is observed in *Cpe* KO mice, and thus possibly results in degeneration of neurons in the hippocampal CA3 region (7, 143). Additionally, immunohistochemistry identified the colocalization of CPE and somatostatin (SST) in the cortex and striatum, suggesting that CPE may be involved in processing and sorting of SST in these regions (144). Loss of SST has been shown in some neurodegenerative diseases. Hence, SST may be also required for neuron survival (144).

More studies are needed to clarify the mechanism by which CPE affects the processing or sorting of SST. Besides its processing and sorting functions, CPE may also associate with signaling molecules for the maintenance of normal brain function. The long isoform of nitric oxide synthase 1 adaptor protein (NOS1AP-L), which regulates dendrite outgrowth and branching, is shown to interact with CPE, suggesting that CPE may control proper dendrite patterning through NOS1AP-L (145). However, it is not clear how this interaction affects dendrite morphology and whether additional signaling proteins are also involved.

CPE is recently found to associate with the development of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease and Huntington's disease (146, 147). In normal human cortex, CPE is primarily present in dendrites and perikarya, while in cortices of AD patients and an AD mice model, high accumulation of CPE is found in dystrophic neurites surrounding amyloid beta (146). As a result, the processing and sorting of neuropeptides in the cortex may be altered due to the aberrant accumulation of CPE. Indeed, it has been shown that vesicles containing neuropeptides, such as BDNF, also accumulate in dystrophic neuritis (148). On the other hand, the accumulated CPE may be a self-defense mechanism to protect neurons against the amyloid beta toxicity, thus delaying the onset and progression of AD (112). Further investigation is required for analyzing the exact action of CPE in AD and other neurodegenerative diseases.

Cancer

CPE was reported to promote cell survival in pheochromocytoma (PC12) and hepatocellular carcinoma (MHCC97H) under nutrient starvation and hypoxic conditions, but does not induce cell proliferation (149). In MHCC97H cells, CPE increases the expression of pro-survival/anti-apoptotic proteins, such as BCL-2, through ERK signaling pathway. Thus, CPE stimulates cell survival by activating ERK signaling pathway. Additionally, CPE negatively regulates the Wnt signaling and reduces the expression of β -catenin, which plays a major role in cancer cell proliferation (113, 150), suggesting that CPE may inhibit cell proliferation by blocking the Wnt signaling. However, another study in gliomas shows that overexpression of CPE enhances cell proliferation, indicating its pro-proliferative role in tumor (151). Additionally, hypoxia and glucose starvation reduce

the CPE expression. Therefore, in nutrient starvation and hypoxic conditions, the level of CPE may be too low to induce PC12 and MHCC97H cell proliferation but sufficient to protect cells from metabolic stress. The mechanism underlying how CPE regulates cell proliferation needs further investigation. It is possible that CPE may regulate another pathway to counteract the effect of β -catenin, thus the net effect of CPE is toward enhancing cell proliferation. CPE also has been found to reduce migration and invasion in fibrosarcoma (HT1080) cells (149), and CPE overexpression is able to delay and decrease glioma cell motility (151), indicating its anti-metastatic effects. More evidence is provided by studies on neuroendocrine tumors, small-cell lung cancers and large-cell neuroendocrine carcinomas showing that high levels of CPE expression predict good prognosis (152). Mechanisms by which CPE inhibits migration and invasion are not fully understood. One of the possible mechanisms is related to the Wnt signaling pathway, as some components of this pathway regulate cell invasion (153).

Increased expression of Δ N-CPE has been reported in hepatocellular carcinoma (HCC) cell lines, where it is localized in the nucleus and interacts with histone deacetylase 1/2 to stimulate the transcription of neural precursor cell expressed developmentally downregulated protein 9 (NEDD9) (154). Since NEDD9 promotes cell proliferation and invasion *in vitro* (155, 156), it is possible that Δ N-CPE can induce tumor growth and metastasis through NEDD9 expression. Δ N-CPE may also enhance cell proliferation by increasing β -catenin, as it is a positive regulator of the Wnt signaling pathway (113). Another possible mechanism of Δ N-CPE-induced cancer growth and invasion is related to the activation of NF- κ B expression, which is involved in the development and progression of many cancers (157, 158). The overexpression of CPE was detected in pancreatic cancer with high levels of NF- κ B, while the downregulation of CPE reduced the NF- κ B expression and inhibited cell proliferation and invasion, suggesting that CPE regulates pancreatic cancer through NF- κ B (159). However, expression levels of Δ N-CPE and CPE were not separated in this study; possibly it is Δ N-CPE, not full-length CPE, that was involved in pancreatic cancer growth and invasion. Moreover, Δ N-CPE was reported to be associated with the recurrence and metastasis of lung adenocarcinoma (160). However, more evidence is needed to confirm the expression of Δ N-CPE among different cancers as well as its role in cancer growth, invasion, metastasis and prognosis.

Conclusions and perspectives

CPE is well established for the biosynthesis of a variety of peptide hormones and neuropeptides in the endocrine tissue and nervous system. Recently, additional roles of CPE have been identified in protein internalization, vesicle transport, regulation of signaling pathway and neuroprotection. Due to the significant role of CPE, it is not surprising that loss of CPE activity in *Cpe^{fat}/Cpe^{fat}* mice and *Cpe* KO mice lead to disorders in a number of physiological processes, such as feeding and body weight regulation, reproduction, memory, glucose homeostasis and bone metabolism. Consistently, in clinical trial a human with a null mutation has been identified and reported to be obese and have type 2 diabetes, hypogonadotrophic hypogonadism and intellectual disability (161). Thus, *Cpe^{fat}/Cpe^{fat}* mice and *Cpe* KO mice can serve as useful research models to understand the biological functions and clinical relevance of CPE. Moreover, Δ N-CPE, a splice form of CPE, was recently suggested to promote tumorigenesis and metastasis. Thus, it potentially acts as a prognostic/diagnostic biomarker for cancers, including HCC and colon cancer, and may be a novel target for the treatment of these cancers. Further studies especially in clinical trials are required to investigate the role of CPE in translational medicine, which will be beneficial to human being.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author's contribution statement

L R F has written, edited and submitted this manuscript. Q X Y has edited and approved this manuscript. J L and W H T have written this manuscript.

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