Carboxypeptidase E Activity Is Deficient in Mice with the fat Mutation

EFFECT ON PEPTIDE PROCESSING

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Carboxypeptidase E (CPE) is involved in the biosynthesis of many peptide hormones and neurotransmitters. Mice with the fat mutation have previously been found to have a point mutation in the cpe gene, and to have greatly reduced levels of CPE-like enzyme activity in the pituitary and pancreatic islets (Naggert, J. K., Fricker, L. D., Varlamov, O., Nishina, P. M., Rouille, Y., Steiner, D. F., Carroll, R. J., Paigen, B. J., and Leiter, E. H. (1995) Nat. Genet. 10, 135–142). In the present report, we examined CPE-like activity and peptide processing in several tissues of C57BLKS/LtJ-Cpefat/Cpefat mutant (Cpefat/Cpefat) mice. Whereas CPE-like activity is detected in homogenates of Cpefat/Cpefat mouse tissues, the majority of this activity is not due to CPE based on the sensitivity to p-chloromercuriphenyl sulfonate. In addition, the Cpefat/Cpefat activity does not bind to a substrate affinity column under conditions that bind CPE. Furthermore, the enzyme activity and immunoreactive properties of the enzyme purified from Cpefat/Cpefat brain are distinct from those of CPE. Taken together, these data suggest that CPE is completely inactive in the Cpefat/Cpefat mice, and that all of the CPE-like activity is due to other carboxypeptidases such as carboxypeptidase D. Levels of Leu-enkephalin in Cpefat/Cpefat mouse brain are approximately 5-fold lower than those in control brain. Treatment of the Cpefat/Cpefat brain extract with carboxypeptidase B restores the level of Leu-enkephalin to the level in control brain. Interestingly, the large molecular weight enkephalin-containing peptides are elevated 2–3-fold in Cpefat/Cpefat mouse brain. These data indicate that CPE plays an important role in the processing of peptide hormones in various tissues, but that other carboxypeptidases also contribute to peptide processing. Furthermore, the increase in levels of high molecular weight enkephalin peptides in the Cpefat/Cpefat mouse suggests that CPE is required for efficient peptide processing by the endopeptidases.

Peptide hormones and neurotransmitters are usually produced as larger pro-peptides, requiring a series of enzymes to generate the bioactive peptide (1–5). Most of these cleavages occur at specific basic residue sites, and enzymes that initially cleave the precursor have been identified (4–8). Following this initial cleavage, a carboxypeptidase is then usually required to remove the C-terminal basic residues from the peptide to produce the bioactive moiety (9, 10). For many years, a single carboxypeptidase was thought to be involved with the processing of most secreted peptides (9, 10). This enzyme is alternatively known as carboxypeptidase E (CPE),1 carboxypeptidase H, and enkephalin convertase, and has been designated EC 3.4.17.10 (11). CPE was initially discovered associated with the production of enkephalin in the adrenal medulla (12), and has been found in all neuroendocrine tissues (9, 10, 13, 14). However, the view that CPE is the only intracellular peptide-processing carboxypeptidase has been challenged by the finding that mice with the fat mutation are still capable of producing insulin, albeit at lower levels (15).

The fat mutation has been mapped to the CPE locus on chromosome 8, and a point mutation has been found in the coding region (15). This point mutation converts Ser202 into a Pro residue. When this mutation is created in the homologous rat CPE and the protein expressed in S9 cells using the baculovirus system, the enzyme is inactive and is not secreted into the medium (15). Comparison of the enzyme activity measured between mutant and wild type CPE in the baculovirus system showed that the mutation resulted in less than 0.1% of the activity of the wild type CPE. However, the levels of CPE-like activity in Cpefat/Cpefat mouse pituitary and pancreatic islets were found to be 5–10% of the levels in tissues from control mice (15). Furthermore, the C-terminal processing of insulin is not completely eliminated in the Cpefat/Cpefat mice (15), suggesting that an active carboxypeptidase is present in the insulin secretory pathway of the Cpefat/Cpefat mouse.

A newly reported enzyme, carboxypeptidase D (CPD), may be involved in the processing of secretory pathway peptides and partially compensate for the defective CPE in Cpefat/Cpefat mice (16). CPD is present in bovine pituitary and adrenal (16) and in many rat tissues.7 In contrast, CPE is present mainly in neuroendocrine tissues, with high levels in pituitary and pancreatic islets, lower levels in other neuroendocrine tissues, and undetectable levels in liver (14, 17). The major difference between the two enzymes is the size: CPE is approximately 50–56 kDa, whereas CPD is 180 kDa in bovine pituitary (16) and

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1 The abbreviations used are: CPE, carboxypeptidase E; CPD, carboxypeptidase D; CPB, carboxypeptidase B; dansyl, 5-methylamino-naphthalene-1-sulfonyl; GEMSA, guanidinoethylmercaptosuccinic acid; PCMPs, p-chloromercuriphenyl sulfonate.

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100–180 kDa in various rat tissues. CPD is not recognized by antisera raised against the N- or C-terminal regions of CPE (16).

The major purpose of the present study was to investigate whether CPE activity is present in the Cpefat/Cpefat mouse, or whether all of the detected activity is due to other enzymes (such as CPD). A related goal was to examine whether peptide processing was affected in Cpefat/Cpefat mouse tissues. For this, Leu-enkephalin (enkephalin) was chosen as a representative peptide that requires endopeptidase and carboxypeptidase cleavages. The finding that the level of enkephalin with a correctly processed C terminus is greatly reduced in the Cpefat/Cpefat mouse brain indicates that CPE is physiologically important for the processing of this peptide. However, CPE is not essential since some correctly processed enkephalin is detected in the Cpefat/Cpefat mouse brain. Furthermore, the finding that the peptide precursors are greatly elevated in the Cpefat/Cpefat mouse tissues suggests that CPE is required for an efficient endopeptidase processing reaction.

MATERIALS AND METHODS

Animals—Mice were bred at The Jackson Laboratory as described previously (15). In each litter, the Cpefat/Cpefat animals were identified by genetic markers and by the development of obesity. Non-obese litter-mates were controls in the various experiments. A 1:1 mixture of testis and heart (Table I) and mouse CPE. The C-terminally directed antiserum was raised against peptides corresponding to regions of CPE that are not conserved in other carboxypeptidases. The N-terminally directed antiserum was raised against a 15-residue peptide corresponding to the N terminus of the bovine form of bovine CPE (21). This antiserum reacts with rat and mouse CPE. The C-terminally directed antiserum was raised against the 9-residue peptide KMMSETLNF corresponding to the C terminus of the full-length mouse CPE. The peptide was coupled to keyhole limpet hemocyanin using the glutaraldehyde procedure, as described (22). Antisera were used at final dilutions of 1:1000. The enzyme immunoassay method (Amersham) as described by the manufacturer to detect the primary antiserum.

For the study examining the levels of CPD immunoreactive protein in control and Cpefat/Cpefat mouse tissues, the tissues were extracted directly with 1 mM NaCl and 1% Triton X-100 and purified on 0.5 ml of affinity resin, as described above. Brain extracts were pooled from four control mice and from four Cpefat/Cpefat mice, and approximately 2 mg of protein used for the affinity column purification. Testis and duodenum extracts were pooled from two control mice and two Cpefat/Cpefat mice, and approximately 10 mg of protein used for the affinity purification. Heart extracts from two control mice and two Cpefat/Cpefat mice were pooled, and approximately 30 mg used for the affinity purification. The affinity columns were eluted first with 1.8 ml of Tris-Cl, pH 8.0, containing 100 mM NaCl and 0.01% Triton X-100 and then with 1.8 ml of 5 mM Arg in the same buffer. One-hundred ml of the affinity column elute was analyzed on a Western blot, as described above, using an antiserum raised against rat brain CPD.

Analysis of Enkephalin and Prodynorphin—Brains were homogenized in 10 volumes of boiling acetic acid (0.1 M) and incubated for 30 min in a boiling water bath. Following centrifugation at 10,000 × g for 30 min, the supernatants were lyophilized and stored at −70 °C until analysis. An aliquot (30% of the total) of extract from two brains was rehydrated with 500 ml of 0.15 M acetic acid containing 0.1% Triton X-100 and 0.15 M NaCl and centrifuged again at 10,000 × g for 10 min. The supernatant was applied to a Sephadex G-50 column (1 × 57 cm) pre-equilibrated with 0.15 M acetic acid containing 0.15 M NaCl and 0.1% Triton X-100. The column flow rate was 9 ml/h, and 3.5-min fractions were collected. Fractions were lyophilized and reconstituted in 200 ml of 0.15 M sodium phosphate buffer. One aliquot (60 μl) of each fraction was treated with 5 μg/ml tosylphenylalanlychloromethyl ketone-treated trypsin (Sigma) for 16 h followed by treatment with 5 μg/ml carboxypeptidase B (Sigma) for 12 h. The samples were then analyzed by boiling for 2 min. Another aliquot of 60 μl was treated only with CPB for 120 min. A third aliquot (untreated) of 60 μl was subjected to the same incubation and boiling conditions as above except that enzymes were omitted.

Immunoreactive Leu-enkephalin was measured in the untreated and enzyme-treated fractions by radioimmunoassay as described previously (23). The antisera used to measure Leu-enkephalin in the radioimmunoassay does not recognize peptides with C- or N-terminal extensions of Leu-enkephalin (24). To measure prodynorphin (pro-enkephalin B), aliquots of the gel filtration fractions eluting at 30 kDa (18 ml) were pooled, dried in a vacuum centrifuge, reconstituted in gel loading buffer, and subjected to electrophoresis and Western blot analysis as described above. The 13 S antiserum that recognizes the midportion of the mature form of bovine CPE (21) was used to detect CPE activity in the Cpefat/Cpefat mouse, and the C-terminally directed antiserum was used to detect CPE activity in the Cpefat/Cpefat mouse.

RESULTS

When assayed with dansyl-Phe-Ala-Arg at pH 5, many tissues contain a Co2+-activated GEMSA-inhibited carboxypeptidase activity (Table I). This assay will detect both CPE and CPD (16, 18), and could also detect the neutral pH optimal carboxypeptidase N and carboxypeptidase M (19). High levels of carboxypeptidase activity are detected in the pancreatic islets and pituitary of the control mice, moderate levels are detected in the brain, adrenal, and duodenum, and low levels are detected in the testis and heart. Table I compares the Cpefat/Cpefat mouse, the level of carboxypeptidase activity is only 6–7% of the control level in pancreatic islets and pituitary (Table I). In brain, adrenal, and testis, the levels of carboxypeptidase...
activity in the Cpefat/Cpefat mice are approximately 50–57% of the levels in control tissue. In heart and duodenum, the levels of carboxypeptidase activity in Cpefat/Cpefat mice are not statistically different from the levels in control mice (Table I).

The finding that carboxypeptidase activity is not uniformly lower in the Cpefat/Cpefat mouse tissues, compared to the corresponding control mouse tissues, suggests that either the point mutation affects the enzyme differently in the various tissues, or that other enzymes are responsible for the carboxypeptidase activity detected at pH 5.0. To directly investigate these possibilities, brain extracts were fractionated on a substrate affinity column. The majority of the carboxypeptidase activity in control brain elutes from the column when the pH is raised from 5.5 to 8 (elute 1, Table II); this condition has been previously shown to elute CPE from the affinity column (21). A smaller amount of enzyme activity is retained on the column in the high pH buffer, and subsequently elutes when 5 mM Arg is included in the buffer (elute 2, Table II); this condition has been previously shown to elute CPD from the affinity column (16). In contrast to the results with control mouse brain, the Cpefat/Cpefat brain extracts contains very little carboxypeptidase activity that elutes with the high pH treatment (Table II). However, the amount of carboxypeptidase that elutes with the Arg is similar in Cpefat/Cpefat and control brain extracts (Table II).

The enzymatic properties of the carboxypeptidase in the elute 1 fraction from control brain (Table III) are similar to those previously reported for CPE (16, 20). The activity is greatly stimulated by CoCl₂, and inhibited by HgCl₂, p-chloromercuri-mercuricbenzenesulfonate (PCMS), GEMSA, aminopropylmercuric captosuccinic acid, and 1,10-phenanthroline (Table III). In addition, the enzyme is virtually inactive at pH 7.4 (Table III). The carboxypeptidase activity present in the second affinity column elute from control brain has properties similar to those of CPD (16). This activity is stimulated by CoCl₂ to a smaller extent than CPE, and is less sensitive to inhibition by HgCl₂ and PCMS (Table III). In contrast, GEMSA and the peptide Hipp-Arg are better inhibitors of the elute 2 carboxypeptidase activity than the elute 1 fraction (Table III). The properties of the carboxypeptidase activity in the second elute of the Cpefat/Cpefat mouse brain are similar to those of the corresponding fraction of control mouse brain (Table III), and to CPD (16). In contrast, the properties of the activity in the first elute from Cpefat/Cpefat brain are distinct from those of the control brain (Table III) or from CPE (16, 20).

Western blot analyses of the material in the first and second elute fractions were performed to determine if any CPE protein was present in the Cpefat/Cpefat mouse extracts. Antisera to either the N- or C-terminal regions of CPE recognize proteins of 50–56 kDa in the first elute fraction of control mouse brains (Fig. 1). The size of these proteins correspond to the range previously found for CPE (20, 21). Immunoreactive CPE is not detected in any of the other fractions (Fig. 1).

Western blot analysis of affinity purified material from several tissues showed results similar to those found with brain (Fig. 2). Immunoreactive CPE was present in the affinity column eluates only from control mouse tissues and not from Cpefat/Cpefat mouse tissues. In the control tissues, the amount of immunoreactive CPE is highest in the brain (Fig. 2). Levels of immunoreactive CPE are lower in the testis, very low in heart, and not detectable in the duodenum.

To investigate whether CPD is up-regulated in the Cpefat/Cpefat mice to compensate for the absence of CPE activity, mouse tissues from 2–4 animals were extracted, purified on the affinity resin, and analyzed on Western blots using the antiserum raised against purified rat brain CPD (Fig. 3). No CPD is detected in the elute 1 fraction (not shown), consistent with previous studies (16). The elute 2 fractions of brain, testis, and heart show a major band of 180 kDa. Minor bands of 120 and 100 kDa are detected in brain and testis, and of 125 and 100 kDa in heart. Duodenum shows a major band of approximately 125 kDa. In all tissues, the relative amount of CPD immunoreactive protein is comparable between the control and Cpefat/Cpefat mouse tissues. This result suggests that CPD protein is not up-regulated to compensate for the defect in CPE activity in the Cpefat/Cpefat mouse. However, it is possible that the recovery of CPD during the purification process was not identical for the two samples. To investigate whether the CPD-like activity in whole brain extracts is altered in Cpefat/Cpefat mice, the sensitivity to PCMS was examined. In this experiment, only the Co²⁺ stimulated activity was measured, and the assay was performed at pH 5.5. Control brain extract shows a biphasic response to PCMS, with approximately 60–70% of the activity inhibited by 10–100 μM PCMS (Fig. 4). In contrast, only 20–30% of the activity in Cpefat/Cpefat mouse brain is inhibited by these low concentrations of PCMS (Fig. 4). Whereas the activity measured in the absence of PCMS is approximately 3-fold higher in the control brain compared to the Cpefat/Cpefat brain, there is no difference in carboxypeptidase activity measured in the presence of 30 μM PCMS (Fig. 4). This finding shows that only carboxypeptidase activities that are sensitive.

### Table I

| Tissue                  | Carboxypeptidase activity<sup>a</sup> | Ratio fat:control |
|-------------------------|--------------------------------------|-------------------|
|                         | Control ± S.E. (n)<sup>b</sup>         |                   |
|                         | fat/fat ± S.E. (n)<sup>b</sup>         |                   |
|                         | (nanomoles/min/mg)                     |                   |
| Pancreatic islets       | 294 ± 94 (4/2)                        | 20.5 ± 6.1* (4/3) |
| Pituitary               | 126 ± 18 (5/5)                        | 8.0 ± 1.4* (6/5)  |
| Brain                   | 7.0 ± 6.6 (6/4)                       | 3.5 ± 0.2* (7/5)  |
| Adrenal                 | 12.4 ± 0.9 (2/5)                      | 6.8 ± 0.7* (3/5)  |
| Testis                  | 1.68 ± 0.12 (4/-)                     | 0.95 ± 0.04* (4/-) |
| Heart                   | 0.28 ± 0.02 (4/0)                     | 0.21 ± 0.03 (4/0) |
| Duodenum                | 14.1 ± 1.5 (4/0)                      | 12.1 ± 1.9 (4/0)  |
|                         |                                       | 0.85              |

<sup>a</sup> Carboxypeptidase activity (±S.E. of the mean) is defined as the enzyme activity measured at pH 5.0 that is stimulated by 1 mM CoCl₂ and inhibited by 1 μM GEMSA (18).

<sup>b</sup> Number of animals (males/females).

<sup>c</sup> Statistically significant difference with control value (p < 0.01) using Student’s t-test.

### Table II

| Tissue                  | Carboxypeptidase activity following purification on a p-aminobenzoyl-Arg Sepharose affinity column |
|-------------------------|---------------------------------------------------------------------------------------------------|
|                         | Units (±S.E. of the mean) as nanomol/min of carboxypeptidase activity after purification, per mg of protein in tissue before purification. n = 3 (1 male pool, 2 female pools). |
|                         | Elute 1 | Elute 2 |
|                         | Control | Fat/fat | Control | Fat/fat |
| Brain                   | 2.77 ± 0.58 | 0.11 ± 0.06<sup>a</sup> | 0.47 ± 0.07 | 0.43 ± 0.05 |

<sup>a</sup> p < 0.01, control versus fat, using Student’s t test.
TABLE III

| Compound                      | Concentration | pH  | Relative activity (% no inhibitor control) |
|-------------------------------|---------------|-----|-------------------------------------------|
|                               |               |     | Control Fat Control Fat                   |
| No addition                   |               | 5.5 | 100 100 100 100                           |
| CoCl₂                         | 1 mM          | 5.5 | 770 400 199 197                           |
| HgCl₂                         | 1 μM          | 5.5 | 3 51 75 74                               |
| PCMPS                         | 1 μM          | 5.5 | 10 65 96 89                              |
| GEMSA                         | 1 μM          | 5.5 | 23 9 8 7                                |
| Hipp-Arg                      | 0.5 mM        | 5.5 | 110 43 61 58                            |
| Aminopropylmercaptosuccinic acid | 10 μM        | 5.5 | 52 47 48 47                             |
| 1,10-Phenanthroline           | 1 mM          | 5.5 | 1 8 ND ND                               |
| No addition                   |               | 7.4 | <1 6 ND ND                              |

*ND, not determined.

FIG. 2. Western blot analysis of CPF in control and Cpefat/Cpefat mouse tissues after purification on a substrate affinity resin. Tissues from two to four animals were pooled, purified on the affinity resin, and analyzed on a Western blot using an antiserum raised against purified rat brain CPF. Different amounts of protein were used for the affinity column procedure: brain (2 mg), testis (10 mg), heart (30 mg), and duodenum (10 mg). Approximately 5% of the affinity column eluate was used for the Western blot. The positions and molecular weights (in kDa) of prestained protein standards (Bio-Rad) are indicated.

FIG. 4. Effect of PCMPS on carboxypeptidase activity in control and Cpefat/Cpefat mouse brain. Homogenates were treated with the indicated concentration of PCMPS for 15 min at 4°C and then substrate was added and carboxypeptidase activity determined (at pH 5.5) as described under “Materials and Methods.”

with 0.1 M acetic acid, and fractionated on gel filtration columns. The peak of enkephalin immunoreactivity in control brain extracts is detected in the low molecular mass fraction, consistent with a molecular mass of 555 Da (Fig. 5A). The antiserum used for this analysis does not react to a significant amount with enkephalin precursor peptides. To measure the amount of enkephalin with a C-terminally extended basic residue (i.e. Lys and/or Arg), the fractions were treated with CPB after separation on the gel filtration column. This treatment did not substantially alter the amount of enkephalin immunoreactivity (Fig. 5A). To measure the total amount of enkepha-
Molecular weight calibration standards: a, bovine serum albumin (Mr = 68,000); b, ovalbumin (Mr = 45,000); c, cytochrome c (Mr = 12,400); d, aprotinin (Mr = 6,500); e, Leu-enkephalin (Mr = 580). These standards were separated on a precast 12% polyacrylamide gel and stained prior to Western blot analysis.

To directly compare the level of prodynorphin in the control and Cpefat/Cpefat mouse brains, gel filtration fractions representing 25–30-kDa sized proteins were analyzed on a Western blot using an antiserum directed against the midportion of prodynorphin. The amount of immunoreactive prodynorphin is approximately 3-fold higher in the Cpefat/Cpefat mouse brain extracts compared to control brain extracts (Fig. 6).

**DISCUSSION**

The major finding of the present study is that there is no active CPE in the Cpefat/Cpefat mouse. This conclusion is based on the absence of immunoreactive CPE in the material purified from the Cpefat/Cpefat mouse tissues using a substrate affinity column. This material does show a small amount of carboxypeptidase activity in the affinity column fractions corresponding to CPE, but the enzymatic properties are not identical to those of CPE. Thus, it is likely that the Ser202 to Pro mutation of CPE found in the Cpefat/Cpefat mice causes the enzyme to be completely inactive. This result supports the previous finding that mutation of Ser202 of rat CPE into a Pro results in an inactive protein when expressed in either insect cells using the baculovirus system, or expressed in mouse AtT-20 cells (26). The previous report that CPE activity was not completely eliminated, but was reduced by 90–95% in the Cpefat/Cpefat mouse pituitary and pancreatic islets (15), did not take into account the possibility that other carboxypeptidases were responsible for the low levels of CPE-like activity in these tissues.

Until the recent discovery of CPD, CPE was the only known metallocarboxypeptidase with an acidic pH optimum and so this was the primary criteria previously used to distinguish CPE from carboxypeptidases M, N, B, and A (18). In addition to CPD, other members of the metallocarboxypeptidase gene family have recently been discovered, including a protein designated AEBP1 (27), and a novel cDNA isolated by homology cloning and tentatively designated CPZ.4 Nothing is presently known regarding the enzymatic properties or the subcellular distribution of CPZ protein. AEBP1 was identified as a tran-

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scription repressor present in the nucleus and based on the lack of an N-terminal signal peptide, this protein is not expected to enter the secretory pathway where peptide processing occurs (27). Based on the immunoreactivity (Fig. 3), as well as previous studies on CPD (16), it is likely that CPD accounts the CPE-like activity in the second elute fraction of the affinity column. However, a small amount of CPE-like activity is detected in the high pH eluate from the affinity columns of the Cpefat/Cpefat mouse brain, and since this activity does not appear to be CPE based on the immunoreactivity and enzyme properties, it is possible that this activity is a novel carboxypeptidase. Still, this activity represents a minor amount of CPE-like activity compared to either the levels of CPE in control mouse tissues, or to the amount of CPD in the control or Cpefat/Cpefat tissues.

The absence of active CPE in the Cpefat/Cpefat mice causes a large decrease in the levels of the fully processed peptides that were examined in the present study. As expected, the levels of the peptide precursors which contain C-terminal basic amino acids are markedly elevated in the Cpefat/Cpefat mouse tissues examined. In the brain extracts of Cpefat/Cpefat mice, CPD treatment produces a large increase in immunoreactive enkephalin (Fig. 5B) indicating a large amount of C-terminally extended peptide. In contrast, there is very little of the enkephalin precursor with C-terminal basic residues in control mouse brain since treatment with CPD fails to produce a measurable increase in enkephalin immunoreactivity (Fig. 5A). These results are similar to those found previously for proinsulin and proneurotensin in the Cpefat/Cpefat mouse (15, 28). In the islets, the C-terminally extended processing intermediate form of insulin was undetectable in the control mice, but was abundant in the Cpefat/Cpefat mouse (15). In the brain, the majority of the neurotensin was found to be present as neurotensin-Lys-Arg (28). Taken together, these results indicate that the deficiency of CPE in the Cpefat/Cpefat mice leads to a dramatic accumulation of peptides with C-terminal basic residues, and a decrease in the levels of correctly processed peptides.

The finding that some C-terminal processing of the peptides occurs in the Cpefat/Cpefat mice implies that an alternative pathway exists. It is possible that CPD contributes to the processing of peptides. Previously, CPD has been found in vesicles of the bovine pituitary (16), and 5–10% of the total cellular pool of the duck homologue (gp180) has been detected on the surface of hepatocytes (29), indicating transit through a secretory pathway. The lack of up-regulation of CPD protein in Cpefat/Cpefat mouse tissues (Fig. 3) implies that this enzyme is not induced to compensate for the absence of CPE activity. In addition to CPD, it is possible that other carboxypeptidases are present in the secretory pathway and contribute to peptide processing. Alternatively, some of the endopeptidases may initially cleave the peptide precursors to the N-terminal side of the basic residues, thereby generating the C-terminally processed peptide without the need for a carboxypeptidase. Although the well studied prohormone convertases 1 and 2 do not appear to cleave to the N-terminal side of the basic residues within the cleavage site (30, 31), other enzyme activities have been reported which can perform this cleavage (32, 33).

The increase in prodynorphin and other high molecular weight enkephalin-containing peptides in the Cpefat/Cpefat mouse brain is not predicted from the known function of CPE. The absence of CPE activity was expected to cause only the accumulation of peptides with C-terminal basic residues attached. The high molecular weight enkephalin-containing pep-

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