Altered processing of procholecystokinin in carboxypeptidase E-deficient *fat* mice: differential synthesis in neurons and endocrine cells

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Abstract **The *fat* mouse strain exhibits a late-onset obesity syndrome associated with a mutation in the gene encoding carboxypeptidase E (CPE). CPE plays a central role in the biosynthesis of many regulatory peptides. Therefore, we examined the processing of procholecystokinin (proCCK) in the brain (neurons) and small intestine (endocrine cells) of *fat/fat* mice. In the brain, bioactive CCK was markedly reduced (7.9 ± 1.0 pmol/g in *fat/fat* mice vs. 82.5 ± 11.2 pmol/g in controls), but the concentration of the CPE substrate, glycyl-arginine-extended CCK, was elevated 105-fold. In contrast, the concentration of bioactive CCK in intestinal endocrine cells was unaffected. Endocrine cell processing was, nevertheless, altered with a 33-fold increase in glycyl-arginine-extended CCK. Interestingly, although total proCCK products were normal in the brain they were elevated 3-fold in the intestine, indicating that biosynthesis is upregulated in endocrine cells but not neurons to compensate for the processing defect. These results demonstrate that the CPE mutation differentially affects CCK processing in these two cell types. Intestinal CCK synthesis more closely resembles progastrin processing, suggesting the presence of an endocrine-specific biosynthetic regulatory mechanism not present in neurons.**

Key words: Cholecystokinin; Prohormone processing; Carboxypeptidase E; Obesity; *fat* mouse

1. Introduction

The CPE^{fat} mouse strain (*fat*) exhibits a slowly developing adult-onset obesity with mild diabetes due to a genetic mutation ($Ser^{202}Pro$) in the peptide processing enzyme carboxypeptidase E (CPE), which abolishes enzyme activity [1,2]. CPE is expressed in a variety of neurons and endocrine cells, thus, the posttranslational processing of many peptide hormones and neuropeptides could be altered in *fat/fat* mice. Regulatory peptides are synthesized as large propeptides that undergo a series of modifications in the trans-Golgi network and secretory vesicles before being released as active peptides. These modifications include endo- and exoproteolytic cleavages, sulfation, phosphorylation, and amidation [3–7].

CPE cleaves C-terminal basic residues during protein processing; thus, peptides requiring removal of C-terminal basic residues for bioactivity may be deficient in the *fat/fat* mouse. Accordingly, *fat/fat* mice do not process proinsulin, prodynorphin, proneurotensin, promelanin-concentrating hormone and pro-opiomelanocortin properly as demonstrated by an accumulation of hormone precursors and a marked decline in the concentration of bioactive peptides [2,8–10]. Moreover, the processing of progastrin is also affected [11,12]. However, in contrast to the other regulatory peptides examined, we have shown that gastrin biosynthesis is upregulated to maintain normal production of bioactive peptide [11]. It has become increasingly difficult to predict the complete range of peptides affected in the *fat/fat* mouse particularly since the discovery of another carboxypeptidase, carboxypeptidase D, which may compensate for CPE in some tissues [1,9,13].

Cholecystokinin (CCK) is a neuroendocrine peptide synthesized in intestinal endocrine cells (I cells) and many neurons of the peripheral and central nervous systems. It is closely related to gastrin. CCK regulates a variety of physiological functions in both the gastrointestinal tract and central nervous system. Specifically, both peripheral and central administration of CCK dose-dependently inhibits food intake [14–16]. ProCCK processing involves endoproteolytic cleavage by prohormone convertases yielding a C-terminally glycyl-arginine extended CCK, which by carboxypeptidase removal of the arginyl residues exposes a glycine-extended CCK for amidation (Fig. 1). Thus, the potential for CCK processing deficiencies or attenuated postprandial CCK release is of particular interest considering the proposed satiety function of CCK and the obese phenotype of *fat/fat* mice.

Using the *fat* mouse as an in vivo model, we have now tested the hypothesis that CPE is required for the maturation of proCCK. Moreover, we examined how alterations in processing might affect CCK biosynthesis differentially in neurons and intestinal endocrine cells to ameliorate the effects of reduced maturation. Finally, we measured plasma concentrations of bioactive CCK to determine if *fat/fat* mice respond adequately to a meal.

2. Materials and methods

2.1. Mice and genotyping

Heterozygous *fat/+* mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed on a 12-h light-dark cycle and fed Purina 5008 chow ad lib. Homozygous *fat/fat* and wild-type (+/+) mice were generated by intercrossing heterozygotes and genotyping the offspring. Genomic DNA was prepared from tail biopsies [17]. The *fat* and wild-type CPE alleles were detected using an allele specific polymerase chain reaction (PCR) assay as previously described [11].

2.2. Tissue isolation and extraction

Adult mice 2–6 months old were anesthetized, and the brain and small intestine (excluding duodenum) were rapidly dissected and frozen in liquid nitrogen. The tissue was washed gently in phosphate buffered saline, on ice, before freezing. Tissue extracts for radioimmunoassay (RIA) were prepared as previously described [18]. Briefly, frozen tissues were boiled in water (1 ml/mg) for 20 min, homogenized

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Abbreviations: CCK, cholecystokinin; CPE, carboxypeptidase E; RIA, radioimmunoassay

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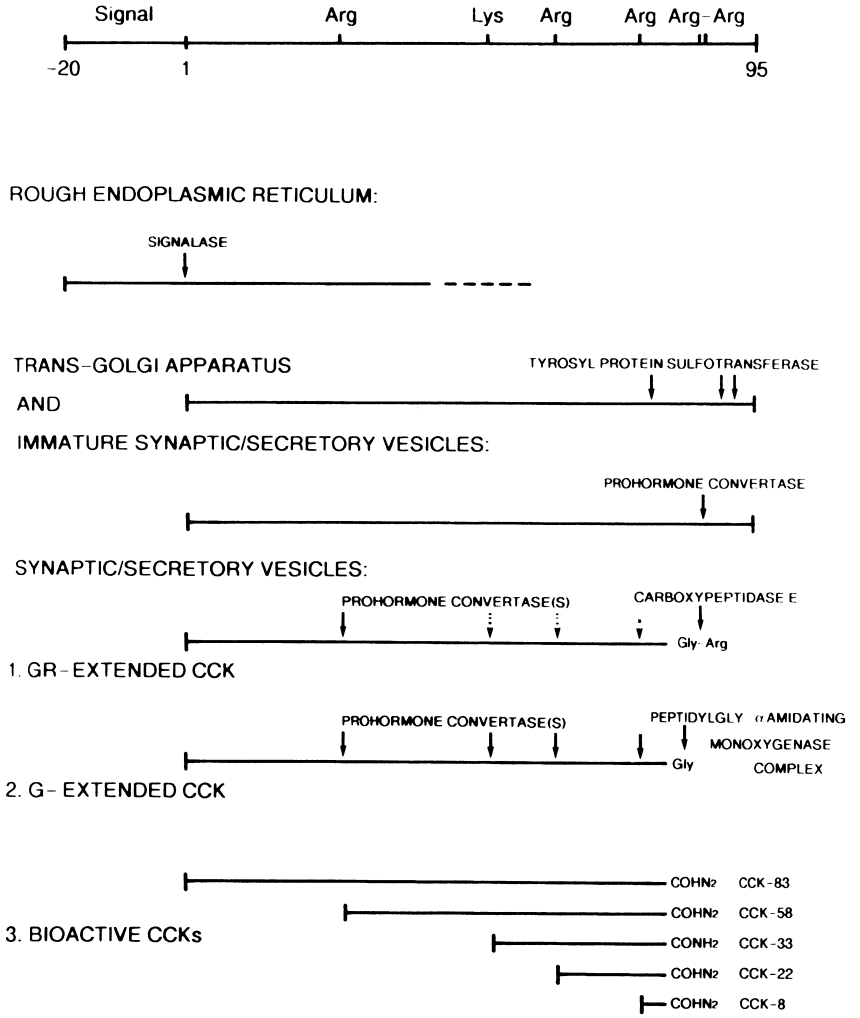

Fig. 1. Diagrammatic presentation of the co- and posttranslational modification of preprocholecystokinin (proCCK). Activation of the CCK amidation site occurs via a series of carboxy-terminal cleavages and modifications. Endoproteolytic cleavage by prohormone convertases produces the carboxypeptidase E substrate (R_1-Phe-Gly-Arg). Carboxypeptidase E then acts in secretory granules to remove the C-terminal arginine residue yielding glycine-extended CCK (R_1-Phe-Gly). Carboxyamidation of glycine-extended CCK by a-amidating mono-oxygenase results in the production of bioactive CCK (R_1-Phe-NH_2). Concomitant N-terminal cleavage by prohormone convertases produces pro- and bioactive CCK of varying sizes (e.g. CCK-83, CCK-33, CCK-22, etc.). A library of sequence-specific antibodies was used in combination with in vitro protease treatments to measure bioactive CCK and various precursor peptides, as described in Section 2.

(polytron) and centrifuged for 30 min at 10 000 rpm. The supernatants were withdrawn and the pellets re-extracted in 0.5 M CH_3COOH (1 ml/mg), rehomogenized, incubated at room temperature for 30 min and centrifuged. The water and acid supernatants were stored at −20°C until RIA analysis.

2.3. Chromatography

One or two ml of extracts were applied to Sephadex G-50 superfine columns (10×1000 mm), which were eluted at 4°C with 0.02 M barbital buffer, pH 8.4, containing 0.1% bovine serum albumin. Fractions of 1.0 ml were collected at a rate of 4.0 ml/h. The columns were calibrated with [^{125}I]albumin (void volume), human CCK-33, -22, -8 (sulfated) and with ^{22}NaCl (total volume). The elutions were monitored with sequence-specific radioimmunoassays as described below.

2.4. Radioimmunoassay

A library of sequence specific antibodies against proCCK was used to measure the different forms of bioactive CCK and processing intermediates (Fig. 1). The sum of carboxyamidated and *O*-sulfated CCKs (CCK-8, -22, -33, -39, -58, and -83) were measured using the CCK-specific antiserum no. 92128, with [^{125}I]CCK-8 as tracer and CCK-8 as standard [19]. Antibody no. 92128 binds all carboxyamidated and *O*-sulfated forms of CCK (i.e. bioactive) with equimolar affinity irrespective of size and sulfation. Crossreactivity with homologous gastrin peptides is negligible [19]. Glycine-extended intermediates of CCK were measured using antiserum no. 3208 with [^{125}I]glycine-extended CCK-8 as tracer and glycine-extended CCK-8 as standard [20]. Glycine-arginine-extended intermediates were measured using antiserum no. 3208 following enzymatic pretreatment with carboxypeptidase B. To measure all precursor forms of CCK, samples were pretreated with trypsin and carboxypeptidase B followed by RIA with antiserum no. 3208, as described previously [20]. Carboxypeptidase B mimics the effect of CPE, while trypsin mimics the effects of prohormone convertases. To confirm that our measurements accounted for only CCK peptides, control measurements of gastrin peptides were performed with a gastrin-specific RIA using the antibody no. 2604 [21].

2.5. RNA analysis

Tissues for RNA analysis were dissected from four *fat/fat* mice and four wild-type mice following an overnight fast. Total RNA was extracted from frozen tissue by a guanidine thiocyanate homogenization-CsCl centrifugation method [22]. For Northern blot analysis, total brain and small intestine RNA samples (10 μg) were electro-

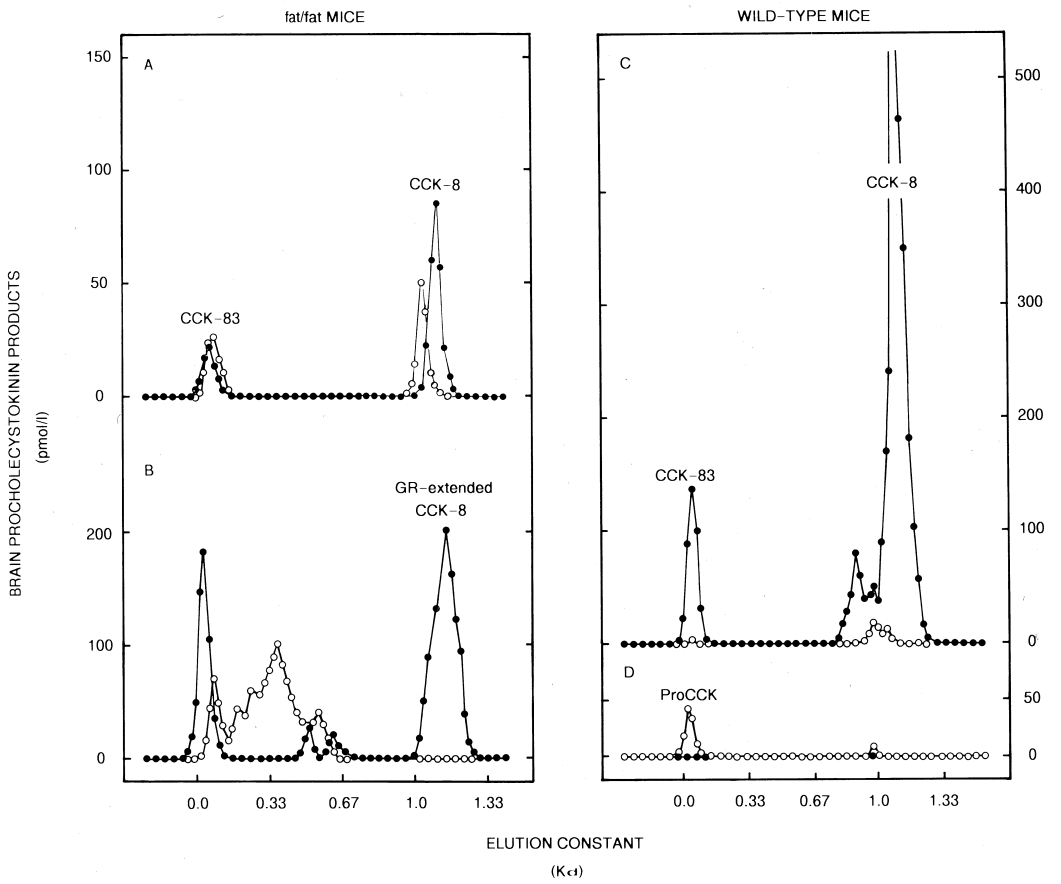

Fig. 2. Gel chromatography of brain extracts from CPE-mutant *fat/fat* mice (left) and wild-type control mice (right). The upper panels (A and C) show the elution of carboxyamidated and *O*-sulfated CCK as measured using Ab. no. 92128 (●–●), and glycine-extended CCK (measured using Ab. no. 3208, ○–○). The lower panels (B and D) show the elution of glycyl-arginine-extended CCKs (measured using Ab. no. 3208 after carboxypeptidase B treatment, ●–●) and further C-terminally extended gastrins (measured using Ab. no. 3208 after trypsin and carboxypeptidase B treatment, ○–○). The peaks are identified relative to the elution position of the CCK peptides used for calibration.

phoresed in agarose gels containing 2.2 M formaldehyde and transferred to Zeta-Probe nylon membrane (Bio-Rad). Probes were ^{32}P-labeled and hybridized to filters as previously described [23]. Final wash concentrations were 0.5× SSC (0.075 M NaCl, 7.5 mM trisodium citrate, pH 7.0) and 0.1% SDS at 60°C. Imaging and quantitation were performed on a GS-250 Molecular Imager (Bio-Rad). The CCK probe was a 0.8-kb *Bgl*II fragment containing exon 3 isolated from a genomic clone described by Vitale et al. [24]. After hybridization, filters were stripped and rehybridized with a mouse ribosomal protein L32 (rpL32) probe [25] to control for RNA loading.

2.6. Plasma hormone measurements

To examine changes in plasma CCK concentrations in response to feeding, mice were fasted for 24 h prior to blood collection by retro-orbital bleeding. A second group of mice was fasted and then refed for 40 min prior to bleeding. Blood was collected into tubes containing 10 mM EDTA. Plasma concentrations of bioactive CCK (carboxyamidated and *O*-sulfated) were measured by RIA using antibody no. 92128 as previously described [19].

2.7. Statistics

Statistical analysis was carried out using an unpaired *t*-test with the SYSTAT software. All values are expressed as means ± S.E.M.

3. Results

3.1. Brain proCCK processing

The concentrations of sulfated carboxyamidated CCK in the brain were 10-fold lower in *fat/fat* mice compared to wild-type controls (Table 1). To determine the effect of the

Table 1
ProCCK products in *fat/fat* and wild-type mice (pmol/g tissue (wet weight); mean ± S.E.M.)

CCK peptides	Brain		Small intestine	
	fat/fat	Wild-type	*fat/fat*	Wild-type
Carboxyamidated	7.9 ± 1.0	82.5 ± 11.2	18.3 ± 2.1	18.3 ± 4.4
Glycine-extended	7.4 ± 0.5	11.0 ± 1.2	11.4 ± 1.7	3.3 ± 0.3
Glycyl-arginine-extended	69.0 ± 9.8	0.66 ± 0.7	29.3 ± 2.3	0.9 ± 0.5
Further C-terminally extended	34.5 ± 3.6	19.5 ± 1.8	13.2 ± 3.1	4.1 ± 0.9
Total proCCK product	118.8	113.7	72.2	26.6

$N = 4$ mice/group.

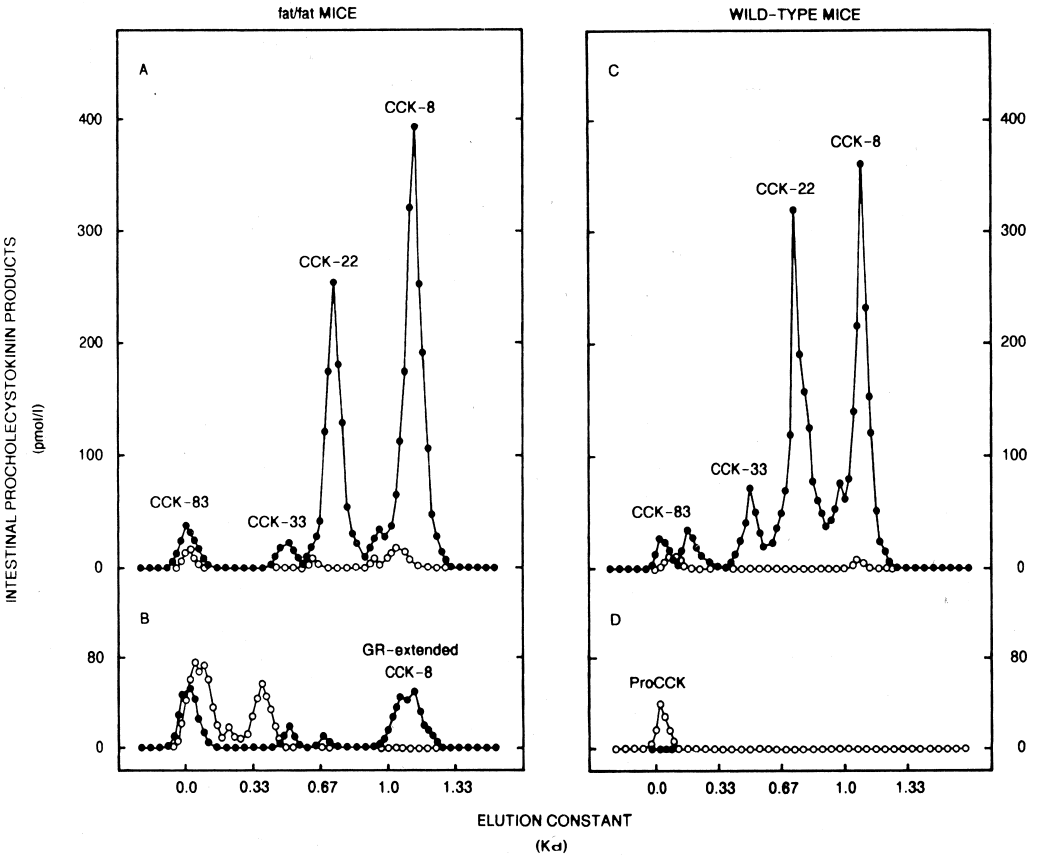

Fig. 3. Gel chromatography of small intestinal extracts from the CPE-mutant *fat/fat* mice (left) and wild-type control mice (right). The upper panels (A and C) show the elutions of carboxyamidated and *O*-sulfated CCK (measured using Ab. no. 92128, ●–●) and glycine-extended CCKs (measured using Ab. no. 3208, ○–○). The lower panels (B and D) show the elution of glycyl-arginine-extended CCKs (measured using Ab. no. 3208 after carboxypeptidase B treatment, ●–●) and further C-terminally extended CCKs (measured using Ab. no. 3208 after trypsin and carboxypeptidase B treatment, ○–○). The peaks are identified relative to the elution position of the CCK peptides used for calibration.

CPE mutation on further proCCK processing in cerebral neurons, we measured the concentrations of processing intermediates (Fig. 1). The concentration of glycyl-arginine-extended CCK, the CPE substrate, was elevated 105-fold in *fat/fat* mice (69.0 ± 9.8 vs. 0.66 ± 0.7 pmol/g), whereas the concentration of glycine-extended CCK decreased slightly (7.4 ± 0.5 vs. 11.0 ± 1.2 pmol/g). The concentrations of further C-terminally extended products almost doubled in *fat/fat* mice (Table 1). Summing up all forms of CCK (bioactive and precursor peptides = total proCCK products), *fat/fat* mouse brains contained an equivalent amount of total proCCK products compared to wild-type controls (Table 1).

Gel chromatography revealed additional differences in the endoproteolytic processing of proCCK in *fat/fat* mice (Fig. 2). Glycine-extended and further C-terminally extended CCKs were less efficiently cleaved at N-terminal processing sites in *fat/fat* mice, leading to a smaller fraction of short molecular forms of CCK. This molecular pattern suggests that CPE activity influences endoproteolytic prohormone convertase cleavage (Table 1, Figs. 1 and 2).

3.2. Intestinal proCCK processing

Unlike the brain, the concentration of bioactive CCK in the gut was identical in *fat/fat* mice and wild-type controls (Table 1). Upon further analysis of processing intermediates we found that the concentration of glycyl-arginine-extended CCK, the CPE substrate, was elevated 33-fold in *fat/fat* mice (29.3 ± 2.3 vs. 0.9 ± 0.5 pmol/g). Similarly, the concentrations of glycine-extended CCK and further C-terminally extended CCK increased approximately 3-fold in intestinal extracts from *fat/fat* mice compared to wild-type controls (Table 1). Summing up the total proCCK products, the small intestine from *fat/fat* mice contained nearly three times more total proCCK products compared to wild-type controls (Table 1).

Gel chromatography revealed similar patterns of carboxyamidated CCK in *fat/fat* compared to controls, with only minor differences occurring in the N-terminal proteolytic processing of proCCK in *fat/fat* mice (Fig. 3). Glycyl-arginine-extended CCK was present in chromatographically detectable forms only in *fat/fat* mice, where they constituted a mixture of long and short forms. The molecular pattern suggests that CPE influences endoproteolytic prohormone convertase cleavage only marginally in the small intestine in contrast to the greater accumulation of longer proCCK forms observed in the brain (Figs. 2 and 3).

3.3. Plasma CCK

Fasting plasma concentrations of bioactive CCK in *fat/fat* mice were not significantly altered compared to wild-type controls (Fig. 4). To examine whether *fat/fat* mice exhibit the normal postprandial rise in CCK, we also measured CCK concentrations in plasma after refeeding. In wild-type mice

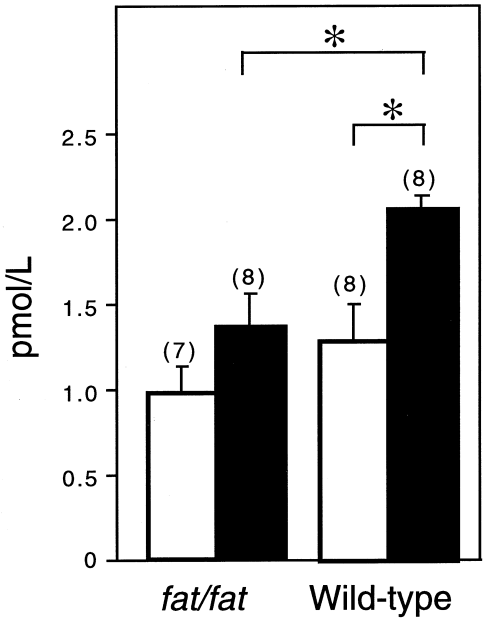

Fig. 4. Plasma concentrations of bioactive CCK in fasted and refed mice. Fasted mice were food deprived for 24 h, and refed mice were fasted then refed for 40 min before plasma collection. Open bars represent fasted mice and solid bars represent refed mice. The mean values and S.E.M. are expressed as pmol per liter plasma. The number of mice is shown for each group (n). *$P < 0.01$.

CCK concentrations significantly increased 40 min after refeeding. However, this response was attenuated in *fat/fat* mice. Postprandial concentrations of CCK in *fat/fat* mice did not rise significantly and were 34% lower than control mice ($P < 0.01$).

3.4. CCK mRNA levels

CCK mRNA levels measured by Northern blot analysis were unchanged in both the brain and the small intestine of *fat/fat* mice (Fig. 5). This indicates that the 3-fold increase in intestinal proCCK products is not a result of increased gene expression.

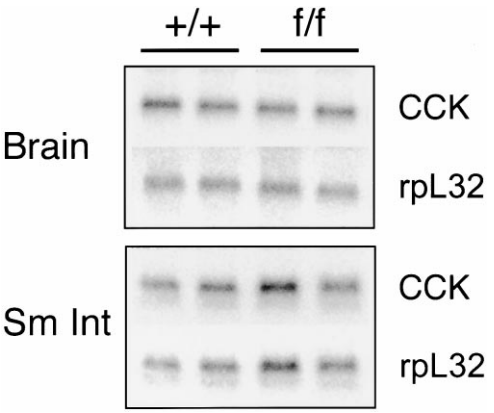

Fig. 5. CCK mRNA abundance in *fat/fat* mice. Displayed are the results from Northern blot analysis of brain and small intestinal RNA hybridized with a CCK probe from two wild-type (+/+) and two *fat/fat* (*f/f*) mice. The top half of each panel shows hybridization with the CCK probe, while the bottom half of each panel shows the same filters after stripping and rehybridizing with the rpL32 probe to control for loading. Phosphoimager analysis was used to quantitate CCK mRNA abundance in four wild-type and four *fat/fat* mice as described in Section 2.

4. Discussion

This study displays remarkable differences in the way carboxypeptidase E deficiency affects the biosynthesis of CCK in its two major sites of expression, the brain (representing neuronal synthesis) and the small intestine (representing endocrine synthesis).

In cerebral neurons, the synthesis of bioactive CCK is grossly reduced, while the concentration of the total translated proCCK product is maintained at a strictly normal level (Table 1). A particularly striking change is the 105-fold increase in the concentration of the CPE substrate, glycyl-arginine extended CCK (Table 1). In contrast, endocrine CCK synthesis is affected in an entirely different way. The tissue concentration of bioactive CCK is maintained at normal levels, while the concentrations of processing intermediates are markedly increased. Not only is the concentration of glycyl-arginine extended CCK (the CPE substrate) elevated 33-fold, but the levels of glycine- and further C-terminally extended CCK are also increased (Table 1). Hence, the intestinal total proCCK products combined are nearly 3-fold higher in the intestines of *fat/fat* mice compared to controls.

The gross reduction in cerebral CCK synthesis resembles the previously described deficits of insulin, neurotensin, melanin-concentrating hormone, dynorphin and ACTH in CPE-deficient mice [2,8–10]. All are characterized by a large reduction in bioactive peptide and the accumulation of arginine-extended precursor forms. In contrast, CCK synthesis in intestinal endocrine cells (I-cells) more closely resembles gastrin synthesis in gastric endocrine cells (G-cells). Bioactive gastrin and intestinal CCK are both produced in normal amounts and show elevated prohormone biosynthesis [11]. The precise mechanism of increased biosynthesis may be different for gastrin and CCK since elevated gastrin biosynthesis correlates with a 2-fold increase in gastrin mRNA [11,12], but intestinal CCK mRNA levels do not change in *fat/fat* mice compared to controls (Fig. 5). As suggested for gastrin [11], the pattern of intestinal CCK synthesis in CPE-deficient mice suggests that endocrine cells in the gastrointestinal tract have another biosynthetic pathway independent of CPE activity. The increased concentrations of glycine-extended processing intermediates in G- and I-cells further supports this hypothesis (Table 1 and [11]). This alternative pathway likely utilizes other carboxypeptidases to compensate for the absence of intestinal CPE [1,9,13].

During the preparation of this paper, Cain et al. [26] reported the results of a similar study on proCCK processing in *fat/fat* mice. Their conclusions generally correspond to those presented here, however, their results deviate in several important respects. First, they measured CCK in duodenal extracts and observed a significant decrease in bioactive CCK and no change in arginine-extended CCK, while we observed no decrease in bioactive CCK and elevated arginine-extended CCK in intestinal extracts. Their report did not take into account crossreactivity with gastrin precursors in the duodenum. Since gastrin is also synthesized in the duodenum, and since there is a substantial increase in gastrin precursor concentrations [11], the analysis of duodenal CCK processing is more difficult to interpret. Second, Cain et al. [26] did not

measure the full complement of CCK precursors, which is central to the argument for the existence of alternate biosynthetic pathways in neurons and endocrine cells. Our results also allow for a more direct comparison with the previous report on the endocrine cell processing of gastrin and lead us to suggest that endocrine I- and G-cells may similarly regulate prohormone biosynthesis. In addition, we have measured CCK mRNA and plasma concentrations which further adds to our understanding of possible regulatory mechanisms of hormone biosynthesis as well as possible physiological effects resulting from altered circulating hormone concentrations (see below). Finally, it should be pointed out that our CCK measurements in the brain are 10-fold higher than those reported by Cain et al. [26], most likely due to different extraction procedures.

Although we have shown that the concentration of bioactive CCK in intestinal tissue extracts was normal, *fat/fat* mice did not show the normal meal-stimulated rise in circulating CCK. This observation is consistent with the debated suggestion [8] that CPE also functions as a sorting receptor for packaging hormones into the regulated secretory pathway. The decrease in postprandial hormone levels nevertheless indicates that the regulated secretory pathway is dysfunctional. The lower postprandial CCK concentrations in plasma, as well as the marked reduction in neural CCK concentrations, suggest that CCK function is diminished in this mutant. This is of particular interest considering the potential satiety function of CCK.

The present study does not show whether the CCK deficiency contributes to the obese phenotype of the *fat* mouse, however, others have demonstrated that both exogenous and endogenous CCK induce satiety in rodents [14–16,27]. In addition, exogenous administration of CCK produces a synergistic interaction with leptin, heightening leptin's satiety effects [28,29]. Barrachina et al. and Matson et al. [28,29] have also demonstrated that devazepide, a CCK antagonist, blocks leptin-induced inhibition of food intake. Therefore, reduced CCK levels in the brain, as well as a reduction in circulating CCK following a meal, may well contribute to the obese phenotype in *fat/fat* mice.

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References

[1] Xin, X., Varlamov, O., Day, R., Dong, W., Bridgett, M.M., Leiter, E.H. and Fricker, L.D. (1997) DNA Cell Biol. 16, 897–909.
[2] 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.
[3] Smeekens, S.P., Chan, S.J. and Steiner, D.F. (1992) Prog. Brain Res. 92, 235–246.
[4] Seidah, N.G., Day, R., Marcinkiewicz, M. and Chretien, M. (1993) Ann. NY Acad. Sci. 680, 135–146.
[5] Steiner, D.F., Smeekens, S.P., Ohagi, S. and Chan, S.J. (1992) J. Biol. Chem. 267, 23435–23438.
[6] Fricker, L.D. and Snyder, S.H. (1982) Proc. Natl. Acad. Sci. USA 79, 3886–3890.
[7] Fricker, L.D. (1991) in: Peptide Biosynthesis and Processing (Fricker, L.D., Ed.) pp. 199–228, CRC Press, Boca Raton, FL.
[8] Cool, D.R., Normant, E., Shen, F.S., Chen, H.C., Pannell, L., Zhang, Y. and Loh, Y.P. (1997) Cell 88, 73–83.
[9] Fricker, L.D., Berman, Y.L., Leiter, E.H. and Devi, L.A. (1996) J. Biol. Chem. 271, 30619–30624.
[10] Rovere, C., Viale, A., Nahon, J.L. and Kitabgi, P. (1996) Endocrinology 137, 2954–2958.
[11] Lacourse, K.A., Friis-Hansen, L., Rehfeld, J.F. and Samuelson, L.C. (1997) FEBS Lett. 416, 45–50.
[12] Udupi, V., Gomez, P., Song, L., Varlamov, O., Reed, J.T., Leiter, E.H., Fricker, L.D. and Greeley, G.H. (1997) Endocrinology 138, 1959–1963.
[13] Song, S. and Fricker, L.D. (1995) J. Biol. Chem. 270, 25007–25013.
[14] Moran, T.H., Ameglio, P.J., Schwartz, G.J. and McHugh, P.R. (1992) Am. J. Physiol. 262, R46–R50.
[15] Silver, A.J., Flood, J.F., Song, A.M. and Morley, J.E. (1989) Am. J. Physiol. 256, R646–R652.
[16] Smith, G.P. and Gibbs, J. (1994) Ann. NY Acad. Sci. 713, 237–241.
[17] Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) Nucleic Acids Res. 16, 1215.
[18] Rehfeld, J.F., Bardram, L. and Hilsted, L. (1992) Gastroenterology 103, 424–430.
[19] Rehfeld, J.F. (1998) Clin. Chem. 44, 991–1001.
[20] Hilsted, L. and Rehfeld, J.F. (1986) Anal. Biochem. 152, 119–126.
[21] Rehfeld, J.F., Stadil, F. and Rubin, B. (1972) Scand. J. Clin. Lab. Invest. 30, 221–232.
[22] Samuelson, L.C., Keller, R.R., Darlington, G.H. and Meisler, M.H. (1988) Mol. Cell. Biol. 8, 3857–3863.
[23] Samuelson, L.C., Isakoff, M.S. and Lacourse, K.A. (1995) Mamm. Genome 6, 242–246.
[24] Vitale, M., Vashishitha, A., Linzer, E., Powell, D.J. and Friedman, J.M. (1990) Nucleic Acids Res. 19, 169–177.
[25] Dudov, K.P. and Perry, R.P. (1984) Cell 37, 457–468.
[26] Cain, B.M., Wang, W. and Beinfeld, M.C. (1997) Endocrinology 138, 4034–4037.
[27] Weller, A., Smith, G.P. and Gibbs, J. (1990) Science 24, 1589–1591.
[28] Matson, C.A., Waiter, M.F., Kuijper, J.L. and Weigle, D.S. (1997) Peptides 18, 1275–1278.
[29] Barrachina, M.D., Martinez, V., Wang, L., Wei, J.Y. and Tache, Y. (1997) Proc. Natl. Acad. Sci. USA 94, 10455–10460.