Pancreatic function in CCK-deficient mice: adaptation to dietary protein does not require CCK

KAREN A. LACOURSE, LISA J. SWANBERG, PATRICK J. GILLESPIE, JENS F. REHFELD, THOMAS L. SAUNDERS, AND LINDA C. SAMUELSON. Pancreatic function in CCK-deficient mice: adaptation to dietary protein does not require CCK. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1302–G1309, 1999.—A CCK-deficient mouse mutant generated by gene targeting in embryonic stem cells was analyzed to determine the importance of CCK for growth and function of the exocrine pancreas and for pancreatic adaptation to dietary changes. RIAs confirmed the absence of CCK in mutant mice and demonstrated that tissue concentrations of the related peptide gastrin were normal. CCK-deficient mice are viable and fertile and exhibit normal body weight. Pancreas weight and cellular morphology appeared normal, although pancreatic amylase content was elevated in CCK-deficient mice. We found that a high-protein diet increased pancreatic weight, protein, DNA, and chymotrypsinogen content similarly in CCK-deficient and wild-type mice. This result demonstrates that CCK is not required for protein-induced pancreatic hypertrophy and increased proteolytic enzyme content. This is a novel finding, since CCK has been considered the primary mediator of dietary protein-induced changes in the pancreas. Altered somatostatin concentrations in brain and duodenum of CCK-deficient mice suggest that other regulatory pathways are modified to compensate for the CCK deficiency.

MATERIALS AND METHODS

Generation of CCK-deficient mice. CCK-deficient mice were generated by gene targeting in the R1 ES cell line (28). The targeting vector contained 7.5 kb of mouse CCK genomic DNA, including 6.5 kb in the 5′ arm and 1.0 kb in the 3′ arm (BssH II-Sal I) (Fig. 1). The bacterial lacZ reporter gene from plasmid pCH110 (Pharmacia) and a neomycin phosphotransferase selection cassette controlled by the phosphoglycerate kinase-1 promoter from plasmid pPN1 (45) were inserted into exon 2 at the site of a 168-bp deletion of CCK sequence. The deletion completely removed the NH2 terminus of CCK, including the signal sequence. The lacZ reporter was positioned at the CCK translational start site. R1 cell culture and gene targeting were performed as described previously (9). Correctly targeted cell lines were identified after screening by PCR and confirmed by long-range PCR and Southern blot analysis.

Some experimental conditions (29, 31, 44, 47). These studies suggest that CCK plays an important role in pancreatic growth and maintenance. However, there are studies that fail to demonstrate growth inhibition following the administration of CCK antagonists in rodents (12, 30, 47, 51).

Increases in dietary protein also stimulate pancreatic hypertrophy and lead to increases in the expression of proteolytic enzymes (12, 13). These changes in the pancreas are believed to result from elevated CCK concentrations in plasma, since dietary protein stimulates the release of endogenous CCK (14, 23). Accordingly, Green and colleagues (12, 27) have demonstrated that CCK antagonists inhibit the induction and maintenance of pancreatic hypertrophy produced by dietary protein.

It is clear from these studies that CCK stimulates pancreatic growth. However, whether CCK is required for normal growth and development of the pancreas is less clear, since the effectiveness of CCK receptor antagonists in vivo appears variable. To address the physiological requirement for CCK in whole animals, we have established a CCK-deficient mouse model by gene targeting in mouse embryonic stem (ES) cells. We have examined the overall histology of the gastrointestinal tract, as well as the ability of the pancreas to adapt to changes in dietary protein in this model. Because CCK may function as a satiety factor, we also tracked normal body weight gain in the CCK-deficient mice.
analysis as described in Lay et al. (22). Six targeted cell lines were microinjected into C57BL/6j blastocysts, with three ES clones yielding chimeric mice that transmitted the targeted mutation through the germ line. Germ line chimeric males were mated to 129/SvJ females, and heterozygous progeny were intercrossed to generate homozygous CCK-deficient and wild-type mice. Mice used in this study were generated by homozygous matings. Mouse genotypes were determined by PCR analysis of tail DNA preparations with primers specific for the wild-type (CS1 and CS2) and mutant (CS1 and LZ) alleles as follows: CS1, 5'-CTGGTTAGAAGAGATGAGCTAGAGATC-3'; CS2, 5'-TAGGACTGCACCCCAGCAGACAGAAC-3'; LZ, 5'-TGTAGATGGGCGCATCGTAACCGTGCATCT-3'.

All mice were subjected to a 12:12-h light-dark cycle and fed Purina 5001 Chow ad libitum (basal diet; Table 1), except where otherwise indicated. Growth curves were established by weighing mice once per week from the time of weaning (3 wk) to 18 wk of age. Histology. Gastrointestinal tissues were dissected from adult mice, rinsed in ice-cold PBS, and fixed in 4% paraformaldehyde in PBS (4–6 h). The fixed tissues were embedded in paraffin, sectioned (3 µm), and stained with hematoxylin and eosin.

Table 1. Formulation of specialized diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein, %</th>
<th>Carbohydrate, %</th>
<th>Fat, %</th>
<th>kcal/g</th>
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<tr>
<td>Pro</td>
<td>78.4</td>
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<td>6.6</td>
<td>3.57</td>
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<tr>
<td>CHO</td>
<td>14.6</td>
<td>79.2</td>
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<tr>
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<td>15.0</td>
<td>15.0</td>
<td>70.0</td>
<td>5.95</td>
</tr>
<tr>
<td>Basal</td>
<td>28.4</td>
<td>59.4</td>
<td>12.3</td>
<td>3.30</td>
</tr>
</tbody>
</table>

Values represent the percent contribution of protein, carbohydrate, and fat to the total energy. Specialized diet formulas mixed by Purina (Pro, CHO, and Fat) were based on previous studies (4, 11, 39, 43) and contained carbohydrates in the form of sucrose, protein in the form of vitamin-free casein, and fat in the form of lard and corn oil. Pro, high protein; CHO, high carbohydrate; Fat, high fat; basal, Purina 5001 chow.

Pancreatic adaptation to specialized diets. Six- to seven-week-old male mice were divided into three groups and fed either high-protein, high-carbohydrate, or high-fat diets for 15 days (see Table 1 for diet formulations). Both wild-type and CCK-deficient mice were fed ad libitum for the duration of the study, and food intake was monitored. After 2 wk, mice were weighed and killed. The pancreas was dissected, weighed, and frozen in liquid nitrogen.

For measurement of protein, DNA, and enzyme content, each pancreas was homogenized (Polytron) for 1 min in 4 ml of homogenate buffer (5 mM MgCl₂, 0.1% Triton X-100) and sonicated for 30 s. Protein measurements were made using the Bio-Rad protein assay kit (Bradford method) using BSA as standard. Aliquots for DNA analysis contained 2.5 M NaCl, and DNA was measured fluorometrically using the TKO 100 minfluorometer (Hoefer Scientific) with Hoechst 33258 dye and calf thymus DNA as standard.

Pancreatic enzyme assays. Amylase activity was assayed in pancreatic homogenates using a method modified from previous studies (3, 19). Reactions containing 50 µl of diluted pancreatic homogenate and 50 µl of 1% starch solution in amylase buffer (20 mM NaPO₄, pH 6.9, 6 mM NaCl, 1 mg/ml BSA) were incubated at 30°C for 5 min. At the end of the incubation, 100 µl of stop solution were added (1:1 mixture of 2% dinitrosalicylate in 0.4 M NaOH and 60% sodium potassium tartrate in 0.4 M NaOH), and the samples were placed in a boiling water bath for 5 min. Reactions were cooled to room temperature for 10 min, 1 ml H₂O was added, and absorbance was read at 540 nm. All samples and standards...
were assayed in duplicate. Samples for the standard curve contained 0–0.3 µmol maltose in amylase buffer. One unit of amylase activity liberates 1 µmol maltose/min at 30°C (3, 19).

Chymotrypsinogen content was measured in duplicate in pancreatic homogenates using a protocol adapted from Erlanger et al. (8). Diluted pancreatic homogenate (100 µl) was activated with 10 µl of trypsin (0.1 mg/ml amylase buffer) and placed on ice for 1 h. One milliliter of substrate buffer (2% glutaryl-l-phenylalanine p-nitroanilide in 50 mM Tris-HCl, 20 mM CaCl₂, 2H₂O, 3 M NaCl, pH 7.6) was added to the trypsin-activated samples and incubated at 37°C for 30 min. Reactions were stopped with 1 ml of 20% acetic acid, and the absorbance was read at 410 nm. Chymotrypsinogen content in the pancreatic homogenates was adjusted to chymotrypsin weight by comparison to a standard curve containing 0–100 µg chymotrypsin (Sigma type 1-S) in homogenate buffer.

RNase protection analysis. Total RNA was extracted from frozen tissue by a guanidine thiocyanate homogenization-CsCl centrifugation method (38). The CCK-A receptor riboprobe was generated by in vitro transcription of a 310-bp EcoRI-PstI fragment from the 3' untranslated region of the mouse gene (21) using the riboprobe system (Promega), [³²P]UTP, and T3 polymerase. RNase protection was performed with the RPA II ribonuclease protection assay kit (Ambion) as previously described (21). Pancreatic RNA (50 µg) was hybridized overnight at 45°C with 5 × 10⁶ cpm of spin-column-purified riboprobe. After hybridization, the samples were RNase digested for 1 h at 37°C. To test probe specificity, digests were ethanol precipitated in the presence of 10 µg of yeast tRNA as a carrier and resolved by electrophoresis on 8% denaturing polyacrylamide gels (not shown). To quantitate transcript abundance, RNase-digested samples were precipitated on ice for 1 h in 10% TCA and 50 µg salmon sperm DNA. Precipitates were collected on Whatman GF/C filters and washed five times with 10% TCA and five times with 95% ethanol. Filters were air dried and immersed in scintillation fluid for counting. Fifty micrograms of yeast tRNA and mouse liver RNA were used as negative controls. All samples were assayed in triplicate.

Intracellular Ca²⁺ signaling. Pancreatic acinar cells were isolated from two to three free-fed mice by collagenase digestion as previously described (5). Acini were suspended in HR incubation buffer (10 mM HEPES, pH 7.4, 127 mM NaCl, 4.7 mM KCl, 0.6 mM MgCl₂, 1.3 mM CaCl₂, 0.6 mM Na₂HPO₄, 2.0 mg/ml glucose, MEM amino acid supplement, 2 mM L-glutamine, 1% BSA, and 0.01% soybean trypsin inhibitor), preincubated for 1 h with 1 µM fura 2-AM, and then washed with HR buffer. Fura 2-loaded acinar cell clusters were transferred to a closed chamber, mounted on the stage of a Zeiss Axiovert-inverted microscope, and continuously perfused with HR buffer alone or HR buffer containing secretin (10 µM), CCK (10 pM), or carbachol (10 µM). Intracellular Ca²⁺ measurement by fura 2 utilized an Attoflour dual-excitation wavelength digital imaging system (Rockville, MD) (15, 24, 49).

Statistics. Statistical analysis was carried out using SYSTAT software. An unpaired t-test was used for two-group comparisons, and ANOVA followed by Tukey's post hoc test was used for multiple-group comparisons. All values are expressed as means ± SE.

RESULTS

Generation of CCK-deficient mice. A mouse strain with a CCK gene mutation was generated by gene targeting in mouse ES cells. The targeting event disrupted the protein coding region by insertion of the bacterial lacZ reporter gene at the translational start site (Fig. 1). Heterozygous matings resulted in the expected Mendelian ratios of homozygous CCK-deficient mutants, heterozygotes, and wild-type mice (1:2:1). The homozygous CCK-deficient mice produced from these matings were viable and fertile and developed without gross abnormalities. Measurement of body weight in males and females showed that mutant mice grow at a normal rate compared with wild-type controls (Fig. 1B). RIA of tissue extracts confirmed the absence of bioactive CCK in the brain and duodenum (Table 2), demonstrating that the targeted gene rearrangement produced a null mutant mouse.

Pancreatic growth was examined, since CCK is thought to function as a trophic factor in this tissue. However, there was no difference in pancreatic weight between CCK-deficient (9.22 ± 0.72 mg/g body wt) and wild-type (9.02 ± 0.67 mg/g body wt) mice (n = 8–9 per group). Analysis of pancreatic histology in hematoxylin- and eosin-stained paraffin sections showed no differences between mutant mice and wild-type mice (Fig. 1C). Similarly, the histologies of the stomach, small intestine, colon, and liver from CCK-deficient mice were indistinguishable compared with those from controls (data not shown). CCK-deficient mice were still viable at 1 yr of age and revealed no gross anatomic changes.

Pancreatic enzyme content. Although overall pancreatic growth and morphology appeared normal in CCK-deficient mice, digestive enzyme content differed. Analysis of pancreatic enzymes in CCK-deficient mice fed the basal diet revealed 60% more pancreatic amylase compared with wild-type mice (Table 3). Chymotrypsinogen content was unaffected, suggesting that the high pancreatic amylase content in CCK-deficient mice is most

<table>
<thead>
<tr>
<th>Brain</th>
<th>Wild-type</th>
<th>CCK-KO</th>
<th>Wild-type</th>
<th>CCK-KO</th>
<th>Wild-type</th>
<th>CCK-KO</th>
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<tr>
<td>CCK</td>
<td>99.7 ± 8.5</td>
<td>&lt;0.1*</td>
<td>76.3 ± 8.2</td>
<td>&lt;0.1*</td>
<td>318.7 ± 20.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Gastrin</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>36.7 ± 3.6</td>
<td>30.6 ± 6.5</td>
<td>286.2 ± 30.4</td>
<td></td>
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<tr>
<td>Somatostatin</td>
<td>150.8 ± 6.2</td>
<td>177.1 ± 9.9*</td>
<td>60.0 ± 3.7</td>
<td>45.8 ± 6.0*</td>
<td>660.7 ± 52.3</td>
<td>528.4 ± 49.4</td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>6.7 ± 0.5</td>
<td>6.3 ± 0.3</td>
<td>5.9 ± 0.5</td>
<td>6.7 ± 0.7</td>
<td>15.0 ± 2.3</td>
<td>12.3 ± 1.1</td>
</tr>
</tbody>
</table>

Values for peptide concentrations [in pmol/g tissue (wet wt)] are means ± SE (n = 8–10 mice per group; n = 4 for stomach CCK measurements and brain gastrin). Peptide concentrations were determined by RIA as described in MATERIALS AND METHODS. Statistical comparisons were made between wild-type and CCK-deficient (CCK-KO) mice (⁎ P < 0.05).
likely due to elevated amylase synthesis and not a general inhibition of pancreatic enzyme secretion.

Pancreatic adaptation to dietary protein. CCK-deficient and wild-type mice were fed diets high in protein, carbohydrate, or fat for 2 wk to determine if CCK is required for pancreatic adaptation to diet. Mice fed the high-protein diet maintained normal body weight compared with mice fed the standard chow (basal) (Fig. 2A). CCK was previously thought to be necessary for dietary protein-induced pancreatic growth; however, CCK-deficient mice still exhibited increased pancreatic weight when fed the high-protein diet compared with mice fed the other three diets (basal, high carbohydrate, and high fat) (Fig. 2B). The basal diet contains a moderate amount of protein. Accordingly, the pancreatic weights of mice maintained on the basal diet were intermediate between the high-protein and low-protein (the high-carbohydrate and high-fat) diets. There were no differences in the pancreatic weights of mutant and wild-type mice fed similar diets.

To determine if the increase in pancreatic weight observed in mice fed the high-protein diet was due to hypertrophy or hyperplasia, total pancreatic protein and DNA were measured. This analysis focused on the groups fed the specialized diets (high protein, carbohydrate, and fat), since these diets are formulated from the same base components and thus are more directly comparable to each other than to the basal diet (Table 1). Total pancreatic protein increased significantly in both CCK-deficient and wild-type mice fed the high-protein diet (Fig. 3A), whereas DNA content was not significantly altered in any group (Fig. 3B). The protein-to-DNA ratio increased similarly in both wild-type and CCK-deficient mice fed the high-protein diet, demonstrating that CCK is not required for protein-induced pancreatic hypertrophy (Fig. 3C).

Amylase and chymotrypsinogen contents were measured to determine if pancreatic enzymes also changed with diet. Chymotrypsinogen content increased two- to threefold in both mutant and wild-type mice fed the high-protein diet, whereas amylase content did not change (Table 3). These changes in enzyme content were consistent with the increase in total pancreatic weight. Chymotrypsinogen content was increased in both wild-type and CCK-deficient mice fed the high-protein diet (Fig. 4A), whereas amylase content was not altered in any group (Fig. 4B). These results demonstrate that CCK is not required for dietary protein-induced stimulation of pancreatic enzyme synthesis. The basal diet contains a moderate amount of protein. Accordingly, the pancreatic weights of mice maintained on the basal diet were intermediate between the high-protein and low-protein (the high-carbohydrate and high-fat) diets. There were no differences in the pancreatic weights of mutant and wild-type mice fed similar diets.

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threefold in wild-type mice and CCK-deficient mice fed the high-protein diet compared with mice fed the low-protein diets (i.e., high carbohydrate and high fat) (Fig. 4A). Amylase enzyme activity was not significantly altered with respect to diet. Although the pancreas from CCK-deficient mice fed the specialized diets displayed a tendency toward higher amylase content, these differences were not significant (Fig. 4B). Thus the ratio of proteolytic to nonproteolytic enzymes (i.e.: chymotrypsinogen to amylase) increased similarly in both CCK-deficient and wild-type mice (Fig. 4C).

CCK receptor expression. Because the CCK-A-type receptor mediates CCK regulation of the exocrine pancreas in the mouse, we measured CCK-A receptor mRNA levels to determine if hormone deficiency alters receptor gene expression. CCK-A receptor transcripts were quantitated by RNase protection assay using a riboprobe derived from the 3′ untranslated region of the mouse CCK-A receptor gene. The concentration of CCK-A receptor mRNA was normal in CCK-deficient mice compared with wild-type controls (Fig. 5), suggesting that agonist binding does not regulate receptor gene expression. In addition, pancreatic acinar cells from CCK-deficient mice responded to physiological doses of CCK (10 pM) to produce normal oscillatory changes in intracellular Ca²⁺, even though the acinar cells were naive to the CCK hormone (Fig. 6). Secretin did not alter intracellular Ca²⁺ concentrations in acinar cells from CCK-deficient mice, a result consistent with wild-type mice, since the secretin receptor is normally coupled to the cAMP signaling pathway.

Other gastrointestinal peptides. We examined other gastrointestinal peptides to determine if their concentrations changed as a consequence of the CCK deficit. Gastrin levels in the brain, stomach, and duodenum from CCK-deficient mice were normal compared with wild-type controls, supporting the conclusion that gastrin is not upregulated to compensate for the loss of CCK (Table 2). However, somatostatin concentrations were significantly different in the CCK mutant. In the duodenum, CCK-deficient mice have 25% less somatostatin. In contrast, somatostatin concentrations increased 18% in the brain of CCK-deficient mice (Table 2). In the stomach, somatostatin concentrations were not significantly different in CCK-deficient mice compared with wild-type controls. Chromogranin A, a marker of neuroendocrine cells, remained unchanged, indicating that CCK deficiency does not grossly alter neuroendocrine cell numbers in the brain or the duodenum.

DISCUSSION

A CCK-deficient mouse model was successfully generated by gene targeting in ES cells. The RIA data presented here confirm that this new mutant mouse

![Figure 4](https://example.com/fig4.png)

**Figure 4.** Pancreatic enzyme content in response to a high-protein diet. A: feeding a high-protein diet increased pancreatic chymotrypsinogen content compared with mice fed low-protein diets (high carbohydrate and high fat). Responses were similar in both CCK-deficient and wild-type mice. Chymotrypsinogen is measured as chymotrypsin weight (mg) as described in MATERIALS AND METHODS. B: pancreatic amylase activity is not significantly altered by the consumption of a high-protein diet in either wild-type or CCK-deficient mice. Measurement of amylase activity in enzymatic units is described in MATERIALS AND METHODS. C: ratio of proteolytic to nonproteolytic enzymes (chymotrypsin to amylase) increased with a high-protein diet in comparison to the high-carbohydrate diet. Solid bars, Pro; open bars, CHO; hatched bars, Fat (*P < 0.05; n = 8–9 per group).

![Figure 5](https://example.com/fig5.png)

**Figure 5.** CCK-A receptor expression in the pancreas of CCK-deficient mice. CCK-A receptor transcript levels were unchanged in CCK-deficient mice compared with wild-type controls. mRNA levels were quantitated in 50 µg total RNA by RNase protection assay followed by TCA precipitation, as described in MATERIALS AND METHODS. Liver RNA and yeast tRNA were used as negative controls (n = 6 per group). CPM, counts/min.
strain does not produce CCK. CCK-deficient mice are viable and grow at a normal rate. Aging mice (1 yr and older) continued to thrive, with no obviously abnormal pathologies. The body weight, rate of weight gain (Fig. 1B), and total daily food intake (data not shown) of CCK-deficient mice do not differ from wild-type mice, suggesting that CCK is not essential for maintaining normal energy balance (intake vs. expenditure).

Analysis of pancreatic enzyme content in CCK-deficient mice fed the basal diet revealed an increase in amylase enzyme activity (Table 3). Because chymotrypsinogen content remained unaltered in the mutant, we concluded that the increase in amylase is not due to a general reduction in enzyme secretion but a specific increase in amylase synthesis. Previous studies have demonstrated an inhibitory effect of CCK on amylase synthesis (43). This effect has not always been apparent in mice (29, 31). Yet our results indicate that CCK does exert some inhibition on amylase synthesis in the mouse pancreas, resulting in elevated amylase content when CCK is chronically absent. In contrast to CCK, insulin acts as a potent stimulator of pancreatic amylase content in rats and mice (6, 20). Thus we propose that lack of CCK results in an imbalance of inhibitory and stimulatory signals, resulting in elevated amylase synthesis.

Previous studies have produced conflicting reports regarding the role of CCK in normal development and growth of the pancreas; however, the CCK-deficient mouse clearly demonstrates that CCK is not a required growth factor for the pancreas. Moreover, our analysis showed that CCK is not necessary for pancreatic adaptation to a high-protein diet. This result was unexpected considering the wealth of literature demonstrating a primary role for CCK in protein-induced pancreatic hypertrophy. Both short- and long-term studies in the mouse demonstrated that the CCK-A receptor antagonist CR-1409 blocks pancreatic hypertrophy in response to feeding the trypsin inhibitor camostat (29, 30). Moreover, short-term (10 days) administration of CCK-A receptor antagonists alone produced pancreatic atrophy (29, 31). However, long-term CR-1409 administration did not induce atrophy, suggesting that over time other pathways may compensate for the loss of CCK (30). Our analysis of the CCK-deficient mouse supports the conclusion that compensatory mechanisms may arise when CCK function is blocked long term. The CCK-deficient mouse model will be useful for identifying alternative regulatory peptides or neural mechanisms that can regulate the pancreas.

Although gastrin is closely related to CCK and they both recognize the CCK-B receptor, it is unlikely that gastrin compensates for CCK deficiency. Our data show no change in gastrin tissue concentrations or sites of expression. Likewise, RNase protection analysis indicated that CCK-B receptor expression does not replace CCK-A receptor in the pancreas of CCK-deficient mice (data not shown). Similarly, our laboratory has generated a double mouse mutant by crossing the CCK-deficient mouse described here to the gastrin-deficient mouse described by Fris-Hansen et al. (9). The double mutant exhibits no change in pancreatic morphology (data not shown), supporting the hypothesis that gastrin is not compensating for the lack of CCK.

Interestingly, somatostatin concentrations in CCK-deficient mice were significantly lower in the duodenum, whereas brain concentrations were increased. Previous studies have demonstrated that CCK stimulates somatostatin secretion and synthesis (7, 23, 50). In addition, somatostatin also decreases CCK release from the intestine (17, 37, 40). Thus CCK and somatostatin may participate in a feedback loop affecting pancreatic function. In the brain, somatostatin may act as an inhibitor of feeding. Because CCK has been shown to inhibit short-term food intake, it is intriguing to propose that somatostatin may be upregulated in the brain to somehow compensate for the loss of CCK. Some of the obesity mouse mutants show similar patterns of decreased somatostatin expression in the gastrointestinal tract and increases in the central nervous system (32). Because somatostatin expression occurs in many tissues and affects a variety of systems, it may play a modulatory role and help in compensating for physiological imbalance, such as in the case of the CCK-deficient mouse.

In addition to providing a useful model to study alternative regulatory pathways compensating for the lack of CCK stimulation of the pancreas, the CCK-deficient mouse generated in this study will be useful for investigating CCK functions in other gastrointestinal...
nal processes, such as gallbladder contraction, gastric emptying, and intestinal motility. In addition, analysis of feeding behavior in this mutant can test the importance of CCK in regulating short-term satiety responses and long-term food intake. Both endogenous and exogenous CCK can inhibit short-term food intake, and recent studies suggest that CCK may contribute to leptin action (1, 2, 25). Further analysis of CCK-deficient mice will help elucidate CCK’s contribution to the complex mechanisms regulating food intake and body weight.

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