Carboxypeptidase E (CPE) Deficiency in Mice with the Fat Mutation Have Reduced Stomach Function (44344)

PABLO GOMEZ, LANCE HALLBERG, AND GEORGE H. GREELEY, JR.[1]
Department of Surgery, The University of Texas Medical Branch, Galveston, Texas 77555-0725

Abstract. **An obese mouse model (Cpe^{fat}/Cpe^{fat}) that has hyperproinsulinemia and late onset obesity has been described. Cpe^{fat}/Cpe^{fat} mice have a missense mutation in carboxypeptidase E (CPE), a processing enzyme essential for production of biologically active endocrine and neuroendocrine peptides. We have reported previously that CPE activity was absent in the antrum of the stomach and that processing of progastrin to the amidated biologically active form of gastrin is reduced. Since gastrin is a major secretagogue for gastric acid secretion, the purpose of the present experiments was to examine gastric acid secretion in Cpe^{fat}/Cpe^{fat} mice. In addition, secretion of amidated gastrin in response to inhibition of acid secretion was tested in Cpe^{fat}/Cpe^{fat}. Both gastric acid and challenged gastrin secretion are reduced in Cpe^{fat}/Cpe^{fat} mice. We conclude that stomach CPE activity is essential for gastric secretory activity and for challenged gastrin release.** [P.S.E.B.M. 1999, Vol 220]

Endocrine and neuroendocrine peptides are cleaved endoproteolytically by prohormone convertases with subsequent excision of basic residues at the carboxyl terminal by carboxypeptides (1). An obese mouse model (Cpe^{fat}/Cpe^{fat}) that has hyperproinsulinemia and late-onset obesity has been described (2). Cpe^{fat}/Cpe^{fat} mice have a missense mutation in carboxypeptidase E (CPE) with marginal pancreatic CPE-like activity. We reported earlier that processing of the stomach hormone, progastrin, to C-terminally-amidated gastrin is reduced significantly in Cpe^{fat}/Cpe^{fat} mice (3). Basal stomach levels of amidated gastrin were reduced to approximately one-third those levels measured in control mice. Because gastrin is a major secretagogue for gastric acid secretion (4), the purpose of this study was to examine the influence of reduced stomach gastrin on basal gastric acid secretion in Cpe^{fat}/Cpe^{fat} mice. In addition, the serum and antral gastrin response to inhibition of gastric acid secretion in Cpe^{fat}/Cpe^{fat} mice was examined.

Materials and Methods

Mice-Cpe^{fat}/Cpe^{fat} and control mice ($Cpe^{fat}/+$, +/+) were generated in the research animal facilities of E. Leiter at the Jackson Laboratory (Bar Harbor, ME).

Experiment 1. The purpose of this experiment was to examine basal gastric acid secretion in fasted Cpe^{fat}/Cpe^{fat} mice. Adult Cpe^{fat}/Cpe^{fat} and control mice (~5 months old) were fasted for 24 hr with access to water. Under ether anesthesia, a laparotomy was done, the stomach exposed, and the esophagus and pylorus ligated; the stomach was removed, and 0.5 ml of saline were injected into the stomach lumen. The stomach fluid contents were collected, clarified, and titrated to determine the basal gastric acid secretory outputs.

Experiment 2. The purpose of this experiment was to measure the serum gastrin response and the increase in antral gastrin concentration to pharmacologic inhibition of gastric acid secretion by famotidine treatment. Famotidine inhibits gastric acid secretion by blocking the stomach H_2 receptor (5). Cpe^{fat}/Cpe^{fat} and control mice were treated with famotidine IP (80 mg/kg BW/2 times/day) for 7 days. *Ad libitum*-fed mice were sacrificed, and serum was collected to determine serum gastrin levels. The stomach antrum was excised, homogenized in distilled water (1:10),

[1] To whom requests for reprints should be addressed at Department of Surgery, The University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas 77555-0725. E-mail: ggreeley@utmb.edu
This work was supported by grants from the National Institutes of Health (RO1 DK15241 and PO1 DK35608).

Received July 24, 1998. [P.S.E.B.M. 1999, Vol 220]
Accepted September 14, 1998.

0037-9727/99/2201-0052$10.50/0

Table I. Basal Gastric Acid Secretion in Cpe^{fat}/Cpe^{fat} Mice and Serum and Antral Gastrin Levels in Famotidine-Treated Cpe^{fat}/Cpe^{fat} Mice

Group	Gastric acid secretion	Serum gastrin[a] response (pg/ml)	Antral gastrin[a] concentration (μg/g tissue)
Control	1.9 ± 0.4 (5)	308 ± 49 (10)	19 ± 2 (11)
Cpe^{fat}/Cpe^{fat}	0.69 ± 0.28[b] (4)	107 ± 17[b] (10)	12 ± 2[b] (9)

[a] Acid secretion was inhibited by IP administration of famotidine.
[b] $P < 0.05$ vs control mice.
() Indicates the number of mice/group.

boiled for 20 min, and clarified by centrifugation. Antral supernatants were assayed for gastrin levels.

Measurement of Fasting Gastric Acid Secretion. The acid contents of the stomach were quantitated by titrating with 0.001 *M* NaOH to pH7 using an automatic titrator (Radiometer, Copenhagen, Denmark). Gastric acid output values are expressed as μEq.

Gastrin Radioimmunoassay (RIA). A double antibody RIA procedure was used to measure serum and antral gastrin levels. This RIA uses an antiserum (5135, provided by the Center for Ulcer Research and Education, University of California-Los Angeles, Digestive Diseases Center Antibody Core) that recognizes only C-terminally amidated gastrin and cholecystokinin (CCK) and does not cross-react with C-terminally extended forms of either peptide (6). The sensitivity and ID_{50} (50% inhibition of bound [^{125}I] gastrin) for amidated gastrin are 6 and 50 pg/tube, respectively. The antiserum was used at an initial dilution of 1:50,000.

Statistical Analysis. Results are the mean ± SE. Data were analyzed by the Student's *t* test. Differences with $P < 0.05$ were considered significant.

Results

In Cpe^{fat}/Cpe^{fat} mice, basal gastric acid outputs were significantly decreased ($P < 0.05$) when compared to basal gastric acid secretion of control mice (Table I). In addition, the serum gastrin response and the increase in antral gastrin levels in response to inhibition of acid secretion by famotidine were reduced significantly in Cpe^{fat}/Cpe^{fat} mice when compared to control mice.

Discussion

The results of the present study indicate that stomach secretory function is reduced in Cpe^{fat}/Cpe^{fat} mice. Basal gastric acid secretion, the serum gastrin response, and the increase in antral gastrin peptide expression in response to pharmacologic inhibition of gastric acid secretion are reduced in Cpe^{fat}/Cpe^{fat} mice when compared to control mice. Previously, we showed the absence of CPE expression, a processing enzyme involved in the production of biologically active gastrin (6, 7), in the stomach of Cpe^{fat}/Cpe^{fat} mice (3). Our previous report showed that, despite the absence of stomach CPE activity, the formation of amidated gastrin was significant, suggesting that another CP in the antrum of the stomach can process progastrin to gastrin in CPE-deficient mice. The important point of the present paper is that the findings indicate that stomach CPE activity is essential for adequate production of C-terminally amidated gastrin, when gastrin secretion is challenged by inhibition of acid secretion. Gastrin secretion from the stomach is linked tightly to acid secretion such that acute inhibition of acid secretion increases gastrin expression and secretion (4, 8). Interestingly, another report indicated that the serum gastrin response to ingestion of food was reduced significantly in Cpe^{fat}/Cpe^{fat} mice (9). Serum gastrin levels after a meal were 2.4-fold lower in Cpe^{fat}/Cpe^{fat} mice when compared to control mice. The findings, when considered together, indicate that stomach CPE is essential for production of amidated gastrin, when gastrin secretion is challenged.

Our results support the idea that gastric acid secretion receives a major stimulatory input from gastrin. In Cpe^{fat}/Cpe^{fat} mice, the reduced basal gastric acid secretion is apparently due to a reduced stimulatory input of amidated gastrin. In conclusion, CPE activity is essential for normal stomach secretory function.

1. Fricker LD. Peptide processing exopeptidases: Amino- and carboxypeptidases involved with peptide biosynthesis. In: Fricker LD, Ed. Peptide Biosynthesis and Processing. Boca Raton: CRC Press, pp 199–229, 1991.
2. Naggert JK, Fricker LD, Varlamov O, Nishina PM, Rouille Y, Steiner DF, Carroll RJ, Paigen BJ, Leiter EH. Hyperproinsulinaemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nat Genet **10:**135–142, 1995.
3. Udupi V, Gomez P, Song L, Varlamov O, Reed JT, Leiter EH, Fricker LD, Greeley GH Jr. Effect of carboxypeptidase E deficiency on progastrin processing and gastrin messenger ribonucleic acid expression in mice with the fat mutation. Endocrinology **138:**1959–1963, 1997.
4. Lloyd KCK, Walsh JH. Regulation of acid secretion *in vivo*. In: Walsh JH, Ed. Gastrin. New York: Raven Press, pp 221–242, 1993.
5. Decktor DL, Pendleton RG, Kellner AT, Davis MA. Acute effects of ranitidine, famotidine and omeprazole on plasma gastrin in the rat. J Pharmacol Exp Ther **249:**1–5, 1989.
6. Kochman ML, DelValle J, Dickinson CJ, Boland CR. Post-translational processing of gastrin in neoplastic colonic human tissues. Biochem Biophys Res Commun **189:**1165–1169, 1992.
7. Dickinson CJ. Relationship of gastrin processing to colon cancer. Gastroenterology **109:**1384–1388, 1995.
8. Brand SJ, Stone D. Reciprocal regulation of antral gastrin and somatostatin gene expression by omeprazole-induced achlorhydria. J Clin Invest **82:**1059–1066, 1988.
9. Lacourse KA, Friis-Hansen L, Rehfeld JF, Samuelson LC. Disturbed progastrin processing in carboxypeptidase E-deficient *fat* mice. FEBS Lett **416:**45–50, 1997.