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PRELIMINARY REPORT

# Dose-Responses for the Slowing of Gastric Emptying in a Rodent Model by Glucagon-Like Peptide (7-36)$NH_2$, Amylin, Cholecystokinin, and Other Possible Regulators of Nutrient Uptake

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**Several peptides have been proposed as regulators of nutrient release from the stomach and subsequent uptake from the gut. Using a phenol red gavage method, we compared the potencies of subcutaneously preinjected amylin, glucagon-like peptide-1 (7-36)amide (GLP-1), cholecystokinin octapeptide (CCK-8), gastric inhibitory peptide (GIP), glucagon, and pancreatic peptide on slowing the release of an acaloric gel from rat stomach. The latter three peptides did not fully inhibit gastric emptying at subcutaneous doses up to 100 μg. Amylin, GLP-1, and CCK-8 fully inhibited gastric emptying, with $ED_{50}$s of 0.42 ± 0.07, 6.1 ± 0.12, and 8.5 ± 0.20 nmol/kg ± SE of log, respectively.**


CONTROL OF NUTRIENT release from the stomach is becoming recognized as an important component of overall fuel homeostasis. In human volunteers, over a range of carbohydrate concentrations in a liquid meal, energy release from the stomach was remarkably constant at approximately 2 kcal/min,[1] equivalent to about 500 mg glucose/min. This release rate is about the same as the rate of glucose disposal that insulin-sensitive individuals can attain at peak plasma insulin concentrations (1 nmol/L).[2] Thus, the rate at which carbohydrate is released from the stomach and absorbed is normally matched to the rate at which it can be metabolized.

Several feedback loops may control nutrient efflux from the stomach. These loops need to include elements that sense the nutrient load that has passed the stomach and effector elements that inhibit gastric emptying. Exploring their potential roles as regulators of gastric nutrient efflux, we have measured the gastric inhibitory effects of six peptides secreted in response to meals that have at some time been considered possible moderators of nutrient absorption: the pancreatic β-cell peptide, amylin,[3] glucagon-like peptide-1(7-36) amide (GLP-1),[4] cholecystokinin (CCK),[5] gastric inhibitory peptide (GIP),[6] pancreatic glucagon,[7] and pancreatic polypeptide.[8]

Gastric emptying was assessed by an established method[9] that measured loss from the stomach at 20 minutes after gavage of an acaloric cellulose gel dyed with phenol red. A dose-inhibition curve was constructed for each of six peptides to allow comparison of the potency of these peptides in controlling gastric emptying.

## MATERIALS AND METHODS

### *Animals*

Male Harlan-Sprague-Dawley rats were housed at 22.7 ± 0.8°C on a 12-hour light/dark cycle (experiments were performed during the light cycle) and fed and watered ad libitum (Diet LM-485; Teklad, Madison, WI). To ensure that stomachs were empty before gavage, animals were deprived of food for approximately 20 hours before experiments.

### *Measurement of Gastric Emptying*

Gastric emptying was measured using a modification[9] of the original method of Scarpignato et al.[10] Briefly, conscious rats received by gavage 1.5 mL of an acaloric gel containing 1.5% methyl cellulose (M-0262; Sigma Chemical, St Louis, MO) and 0.05% phenol red indicator. Compared with the flow of water through a constriction, the methyl cellulose gel had a treacle-like relative viscosity of approximately 6.7. Twenty minutes after gavage, rats were anesthetized using 5% halothane and the stomach was exposed and clamped before being opened into a fixed

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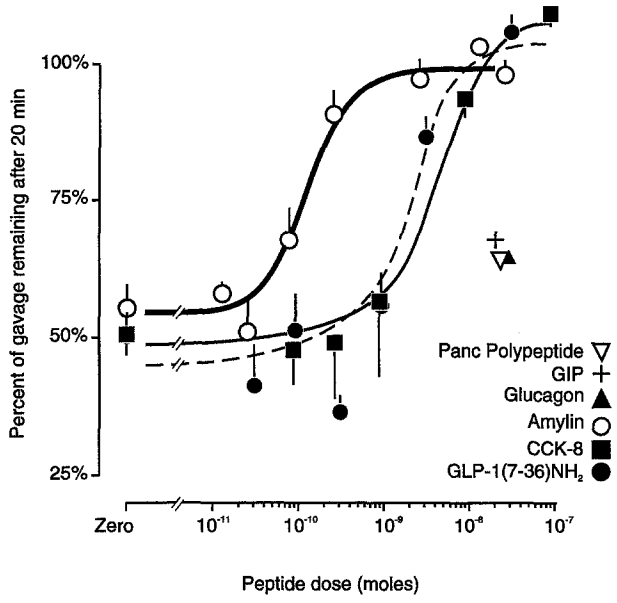


**Fig 1. Dose-response for preinjected amylin, GLP-1, and CCK-8 on gastric emptying in conscious rats. For clarity, only responses to the highest doses of glucagon, GIP, and pancreatic polypeptide are shown. Symbols are the mean ± SEM.**

volume of 0.1 mol/L NaOH. Stomach dye content was derived from absorbance of this alkaline solution at a wavelength of 560 nm. A small fraction of gavaged dye (11% ± 4%) could not be recovered from within the gastrointestinal tract, with part of it appearing to be irreversibly bound to the mucosal surface. To compensate for this loss of soluble dye, stomach contents remaining after 20 minutes were expressed as a fraction of gastric contents recovered from control rats killed immediately after gavage in the same experiment: % gastric contents remaining = (absorbance at 20 minutes)/(absorbance at 0 minutes) × 100. The coefficient of variation of the denominator (absorbance at $t$ = 0 minutes) was 9.8%.

### *Treatments*

Five minutes before gavage, rats were injected subcutaneously with 0.1 mL saline vehicle alone or 0.1 mL containing a dose of peptide that ranged between 0.1 and 100 μg. Peptides injected were as follows: rat amylin, n = 39 (Bachem, Torrance, CA); GLP-1, n = 87, and glucagon, n = 36 (American Peptide, Sunnyvale, CA); and CCK octapeptide (CCK-8), n = 51, GIP, n = 54, and pancreatic polypeptide, n = 50 (Peninsular Laboratories, Belmont, CA).

### *Numerical Methods*

Prism (GraphPad Software, San Diego, CA) was used for dose-response analyses. Comparisons with control responses used Dunnett's multiple comparisons test and $P$ less than .05 as the level of statistical significance (Instat; GraphPad Software).

## RESULTS AND DISCUSSION

Amylin, GLP-1, and CCK-8 were able to completely inhibit gastric emptying at subcutaneous doses of approximately 1, 10 to 100, and 10 μg, respectively (Fig 1). Glucagon did not significantly inhibit gastric emptying at any dose up to 100 μg. GIP and pancreatic polypeptide produced only partial inhibition of gastric emptying at 100-μg-doses.

Amylin, GLP-1, and CCK-8 each inhibited gastric emptying in a dose-dependent manner. $ED_{50}$s were, respectively, 0.42 ± 0.07, 6.1 ± 0.12, and 8.5 ± 0.20 nmol/kg ± SE of log. That is, amylin was 15- and 20-fold more potent than GLP-1 and CCK-8, while GLP-1 and CCK-8 were similar in potency for this response.

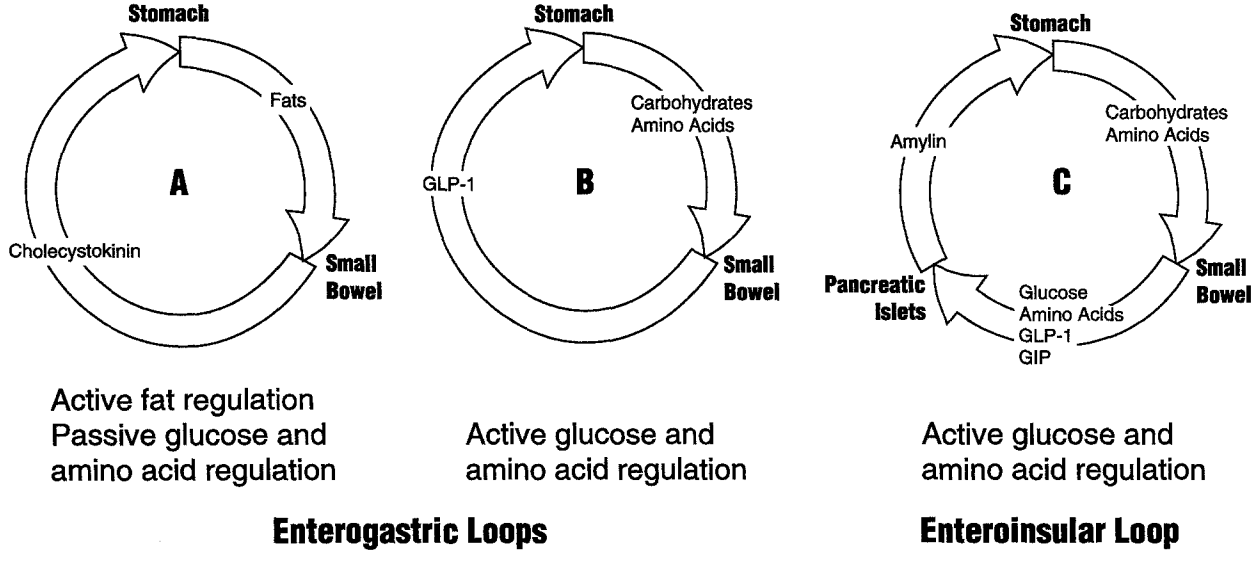


**Fig 2. Postulated feedback loops controlling rate of nutrient release from the stomach. (A) CCK-mediated loop actively controls fat release from the stomach. It does not include carbohydrate-responsive elements, but passively controls carbohydrate release by carbohydrate usually being a component of fat-containing meals. (B) A direct enterogastric GLP-1 loop that can operate in the absence of pancreatic β cells. (C) An enteroinsular loop where amylin, secreted in response to nutrients (glucose, amino acids, etc.) and incretins (GLP-1 and GIP), potently inhibits nutrient release from the stomach.**

In the fasted-to-fed transition in humans, plasma amylin concentrations are reported to increase from approximately 5 to approximately 20 pmol/L,[11] GLP-1 from 10 to 25 pmol/L,[12] and CCK from 1 to 7 pmol/L.[5] That is, amylin and GLP-1 are present at similar concentrations in vivo, several times higher than circulating CCK concentrations. Thus, if plasma levels following a given molar subcutaneous dose are similar for these peptides, one may predict that an amylin-mediated loop would exert much more feedback control over nutrient release from the stomach than would GLP-1 or CCK, at least in the rat.

Loops that could mediate the observed tight control of nutrient release from the stomach are displayed in Fig 2. They include loops where GLP-1 and CCK directly inhibit gastric emptying. Also shown is an enteroinsular loop, where glucose, other secretagogues, and incretins (GLP-1 and GIP) increase secretion of amylin, the most potent inhibitor of gastric emptying of which we are aware.

The gastric inhibitory effect of GLP-1 presumably includes an effect mediated via its enhancement of amylin secretion in the presence of elevated glucose (the incretin effect).[13] To assess only the effects of exogenous hormones and to eliminate gastric actions of endogenous hormones secreted in response to nutrients, an acaloric gel was used in the present study. Under these conditions, GLP-1 enhancement of amylin secretion may have been minimal. Via its stimulation of amylin secretion, GLP-1 may have more of a role in controlling nutrient release when glucose and other secretagogues are elevated. Studies evaluating gastric responses in the presence of more physiological (and potentially synergistic) mixtures of hormones and their secretagogues are warranted.

CCK, secreted by duodenal and jejunal I cells in response to fat, is considered part of a loop that actively regulates gastric release of fat. Because carbohydrate is usually a component of fat-containing meals, this "fat" loop may incidentally control carbohydrate absorption.

Several feedback loops may simultaneously participate in the physiologic regulation of efflux from the stomach and subsequent absorption of carbohydrate and other nutrients. Of these loops, the amylin-mediated enteroinsular loop, based on dose-response data presented here, appears particularly influential.

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