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Truncated and Full-Length Glucagon-Like Peptide-1 (GLP-1) Differentially Stimulate Intestinal Somatostatin Release

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Glucagon-like peptide-1$^{7\text{-}36NH2}$ (GLP-1$^{7\text{-}36NH2}$) is a potent stimulator of insulin secretion, as well as of somatostatin-14 (SS-14) release from the pancreatic and gastric D-cells. To investigate the possible effects of this peptide on release of intestinal somatostatin (SS-28 and SS-14), rat intestinal cultures were treated with 10^{-12}–$10^{-6}M$ GLP-1$^{7\text{-}36NH2}$, as well as with the structurally related peptides, GLP-1$^{1\text{-}36NH2}$ and GLP-2. Both forms of GLP-1 stimulated dose-dependent increases in intestinal somatostatin; secretion reached 643 ± 126% of controls ($p < 0.001$) after treatment with $10^{-6}M$ GLP-1$^{7\text{-}36NH2}$, and 398 ± 76% of controls ($p < 0.001$) after $10^{-6}M$ GLP-1$^{1\text{-}36NH2}$. Thus, GLP-1$^{7\text{-}36NH2}$ was more effective than GLP-1$^{1\text{-}36NH2}$ in stimulating secretion of intestinal somatostatin-like immunoreactivity (SLI) ($p < 0.05$). GLP-2 did not affect intestinal somatostatin release. Gel permeation analysis demonstrated that $10^{-6}M$ GLP-1$^{7\text{-}36NH2}$ stimulated SS-28 by 2.9 ± 0.4-fold and SS-14 by 9.1 ± 3.7-fold, whereas GLP-1$^{1\text{-}36NH2}$ exerted equivalent effects (2.8 ± 0.9-fold) on both forms of somatostatin. These findings define a novel biological role for GLP-1$^{7\text{-}36NH2}$ in the regulation of intestinal somatostatin secretion, and demonstrate that GLP-1$^{1\text{-}36NH2}$ exerts unique biological activities in this system.

Key Words: Glucagon-like peptide-1 (GLP-1); somatostatin; intestine.

Introduction

The intestinal proglucagon-derived peptide, glucagon-like peptide-1 (GLP-1) is a potent stimulator of glucose-dependent insulin secretion from the pancreatic B-cell *(1–4)*. This effect is specific for the truncated form of GLP-1 (GLP-1$^{7\text{-}36NH2}$), since the full-length peptide, GLP-1$^{1\text{-}36NH2}$, does not affect insulin release *(5,6)*. GLP-1$^{7\text{-}36NH2}$ is, therefore, a highly attractive candidate for therapeutic use in the treatment of type II diabetes. In addition to its insulinotropic effects, GLP-1$^{7\text{-}36NH2}$ is also characterized by several biological activities that are complementary in the regulation of glycemia, including inhibition of pancreatic glucagon secretion *(7)* and gastric function *(8–10)*, as well as stimulation of pancreatic *(11)*, and gastric *(9,12)* somatostatin release. Consistent with these diverse functions, GLP-1 receptors have been localized in the endocrine pancreas *(13)*, as well as in gastric parietal cells *(14)*. GLP-1 receptor mRNA transcripts have also been detected in high levels throughout the small intestine and colon *(15)*; however, a function has yet to be ascribed to GLP-1 in the regulation of intestinal function. Because the intestine is a major source of circulating somatostatin *(16,17)*, the authors have examined the possible role of GLP-1 as a determinant of intestinal somatostatin secretion. For these studies, they have utilized an in vitro rat intestinal culture model that has been validated for studies on secretion of both of the major forms of intestinal somatostatin, somatostatin-28 (SS-28), and -14 (SS-14) *(18,19)*.

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Materials and Methods

The methodologies for preparation and treatment of fetal rat intestinal cultures (FRIC) have been described in detail previously *(18,19)*. All animal protocols were approved by the University of Toronto Animal Care Committee. In brief, intestinal cells from a litter of term fetal Wistar rats were enzymatically dispersed using 4 mg/mL collagenase (Sigma Blend Type H, Sigma, St. Louis, MO), 5 mg/mL hyaluronidase, and 0.5 mg/mL DNase-I (Sigma), and were placed into monolayer culture overnight at a cell density of 0.6 fetal rat intestines per 60 mm dish. Each preparation was used in an independent experiment to make $n = 1$, and each experiment was repeated three to seven times, as indicated.

On the day of the experiment, the cells were washed and incubated for 2 h with synthetic test peptides obtained from Bachem California (Torrance, CA) or Penninsula Laboratories (Belmont, CA). Test peptides were >98% pure with no detectable contamination by other peptides. Groups of two dishes were used for all secretion experiments, except those for molecular weight analysis in which groups of 10 dishes were used. Peptides contained in the media and cells

were then collected separately using established reversed-phase adsorption techniques, that permit >95% recovery of intact SS-28 and SS-14 *(18)*. In brief, cells were homogenized in 1*N* HCl containing 5% HCOOH, 1% trifluoroacetic acid, and 1% NaCl, and peptides were collected by passage through a cartridge of C18 silica (C18 SepPak, Waters Associates, Milford, MA).

Separation of SS-28 and SS-14 in media and cell extracts was carried out by gel permeation analysis, using a 9 × 1000 mm Sephadex G-50 superfine column as described previously *(18–20)*. Aliquots from reversed-phase extracts or gel columns were then analyzed by RIA for total somatostatin-like immunoreactivity (SLI), using an antiserum that recognizes SS-28 and SS-14 equally, and synthetic SS-14 as the standard *(20)*. The detection limit of the assay is 0.3 fmol/tube, with an IC_{50} of 9.5 fmol/tube. The intra- and interassay variations are 4.3 and 7.5%, respectively.

All data is expressed as the mean ± SEM. The total cell content of SLI was not changed by any of the experimental treatments; thus, secretion is calculated as the percent of the total cell content that was released into the medium during the incubation period. Total SLI in analyses of gel columns was determined as the sum of the SLI in each fraction under the peak. Statistical differences were determined by analysis of variance using a general linear model with n – 1 custom hypotheses tests on a SAS program for IBM computers (Statistical Analysis Systems, Cary NC).

Results

To determine the effects of GLP-1 on intestinal somatostatin secretion, FRIC cultures were incubated with increasing concentrations of either GLP-$1^{7\text{-}36NH2}$ or GLP-$1^{1\text{-}36NH2}$ (Fig. 1). GLP-$1^{7\text{-}36NH2}$ stimulated release of total SLI at concentrations as low as $10^{-10}M$, reaching 643 ± 126% of controls at $10^{-6}M$ ($p < 0.001$). This degree of stimulation was not different from that of dbcAMP, which reached 546 ± 157% of controls ($p < 0.001$) in paired experiments. Unexpectedly, GLP-$1^{1\text{-}36NH2}$ also stimulated SLI release, but was less effective than GLP-$1^{7\text{-}36NH2}$, reaching a value of 398 ± 76% of controls ($p < 0.001$). The addition of protease inhibitors to the culture media did not alter the response to GLP-$1^{1\text{-}36NH2}$ (data not shown), suggesting that degradation to GLP-$1^{7\text{-}36NH2}$ could not account for the biological activity of GLP-$1^{1\text{-}36NH2}$. Furthermore, SLI release stimulated by GLP-$1^{7\text{-}36NH2}$ was greater than that induced by GLP-$1^{1\text{-}36NH2}$ at all effective doses ($p < 0.05$ at 10^{-10}, 10^{-8}, and $10^{-6}M$). In contrast to the actions of GLP-1 on SLI release, the structurally related peptide, glucagon-like peptide-2 (GLP-2) had no effect on FRIC cultures (Fig. 1).

As total SLI in FRIC cultures is comprised of SS-28 and SS-14, with only small amounts of putative prosomatostatin *(18,19)*, gel permeation chromatography was used to determine which of these peptides was released in response to GLP-1 treatment (Fig. 2). In control media and cells, the

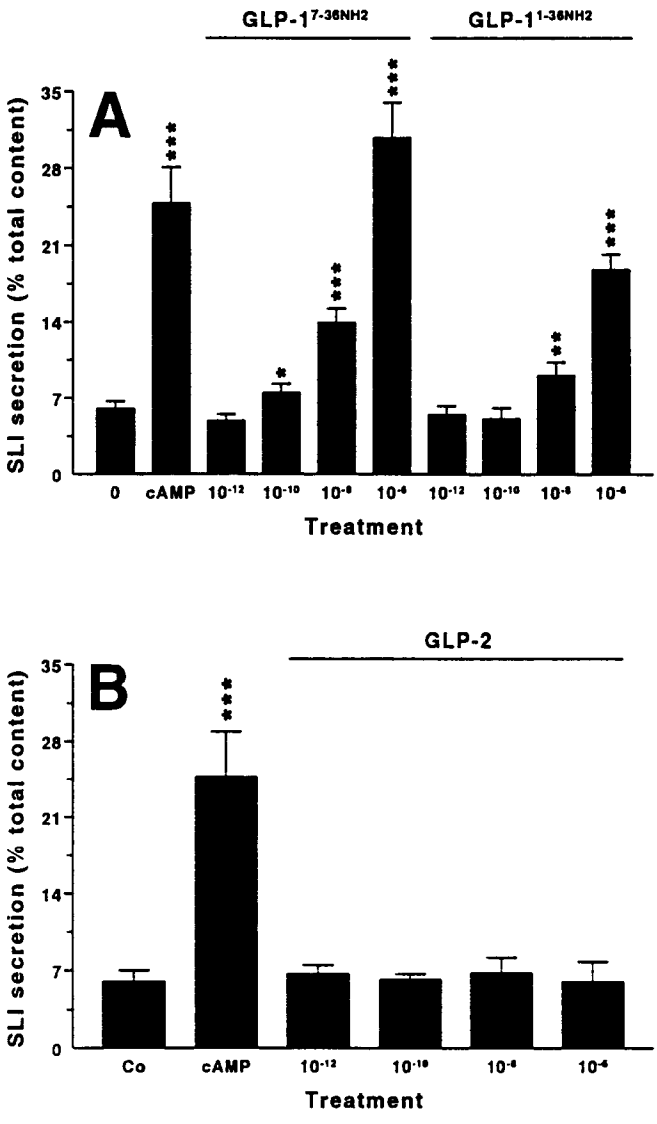

Fig. 1. Secretion of total SLI by FRIC cultures in response to 2 h of treatment with control medium (Co), or 5 m*M* dbcAMP (cAMP), or 10^{-12}–$10^{-6}M$ GLP-$1^{7\text{-}36NH2}$, GLP-$1^{1\text{-}36NH2}$ or GLP-2 (n = 6–7). Peptides in media and cells were collected separately by reversed-phase adsorption techniques and analyzed by radioimmunoassay. Secretion of SLI into the media is expressed as a percentage of the total culture content of SLI (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

predominant SLI peptides were SS-28 (48 ± 3 and 47 ± 3% of total SLI, respectively) and SS-14 (33 ± 2 and 49 ± 5% of total SLI, respectively), as reported previously *(18,19)*. Whereas treatment with GLP-$1^{7\text{-}36NH2}$ augmented secretion of SS-28, from 20.8 ± 6.7 fmol/10 dish in controls to 55.3 ± 12.8 fmol/10 dish (2.9 ± 0.4-fold; $p < 0.05$), there was a preferential stimulation of SS-14 release, from 14.0 ± 4.4 fmol/10 dish in controls to 96.6 ± 4.2 fmol/10 dish (9.1 ± 3.7- fold; $p < 0.001$). Thus, the ratio of SS-14 to SS-28 in the media was increased from 0.7 ± 0.1 in controls to 2.1 ± 0.7 in GLP-$1^{7\text{-}36NH2}$-treated cells ($p < 0.01$). The distribution of peptides in the cells of GLP-$1^{7\text{-}36NH2}$-treated cultures was not altered, however, with SS-28 and SS-14 constituting 51 ± 3 and 46 ± 3% of the total SLI, respec-

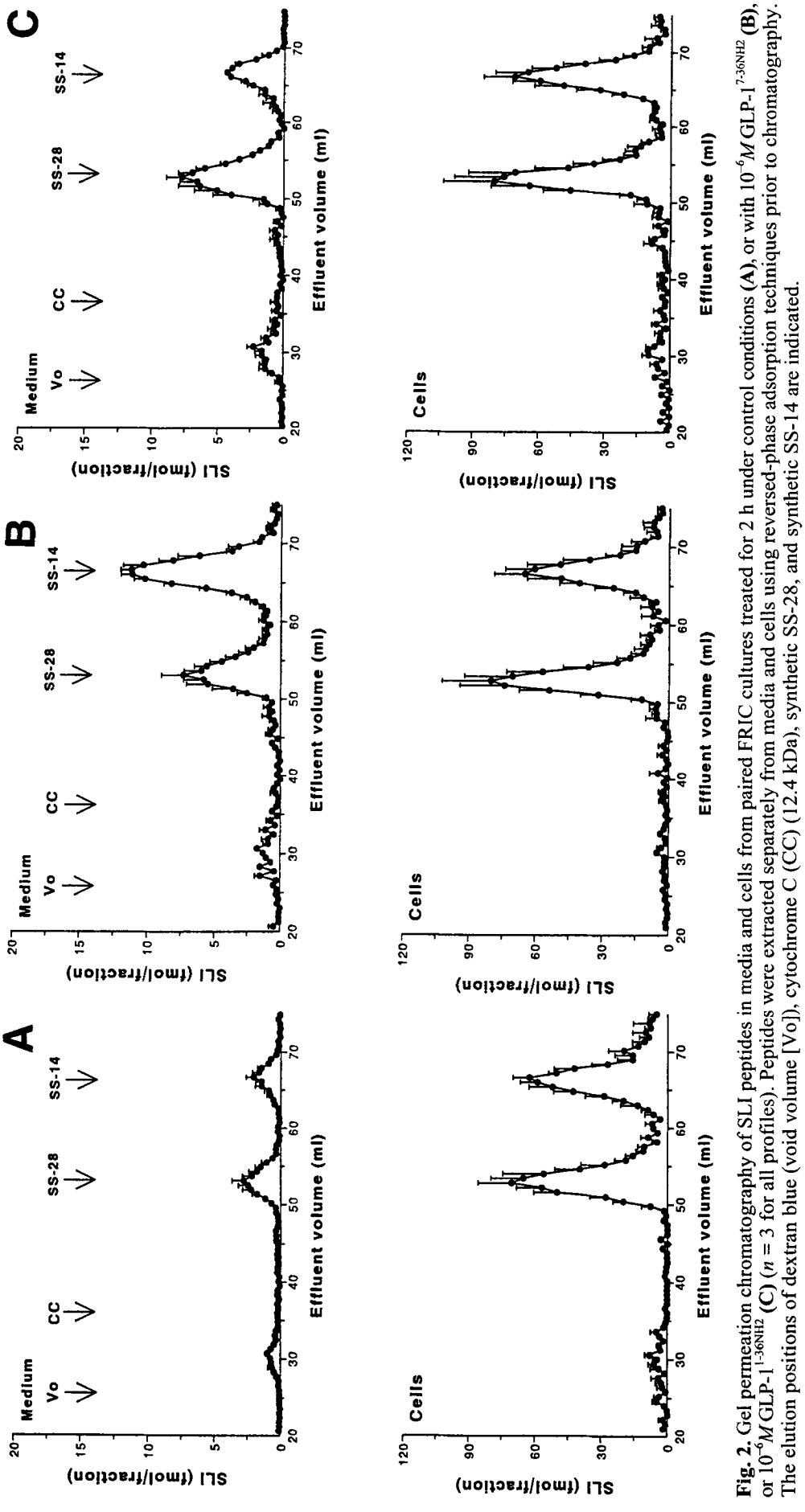

Fig. 2. Gel permeation chromatography of SLI peptides in media and cells from paired FRIC cultures treated for 2 h under control conditions (**A**), or with 10^{-6} *M* GLP-$1^{7\text{-}36NH2}$ (**B**), or 10^{-6} *M* GLP-$1^{1\text{-}36NH2}$ (**C**) ($n = 3$ for all profiles). Peptides were extracted separately from media and cells using reversed-phase adsorption techniques prior to chromatography. The elution positions of dextran blue (void volume [Vo]), cytochrome C (CC) (12.4 kDa), synthetic SS-28, and synthetic SS-14 are indicated.

tively. In contrast to the effects of GLP-1$^{7\text{-}36NH2}$, treatment of FRIC cultures with GLP-1$^{1\text{-}36NH2}$ caused a similar increase in release of SS-28 (from 20.8 ± 6.7 fmol/10 dish to 61.4 ± 11.7 fmol/10 dish, or 3.4 ± 0.7-fold) and SS-14 (from 14.0 ± 4.4 fmol/10 dish to 31.6 ± 4.6 fmol/10 dish, or 2.8 ± 0.9-fold); the proportions of these peptides remaining constant in media (56 ± 3 and 30 ± 4% of total SLI, respectively) and cells (49 ± 5 and 44 ± 3% of total SLI, respectively).

Discussion

GLP-1$^{7\text{-}36NH2}$ is released from the distal intestine in response to nutrient ingestion *(2,3)*, whereupon a diverse series of biological activities serve to coordinate subsequent glycemic responses *(7–12)*. The results of the present study delineate a novel role for this intestinal peptide as a stimulator of somatostatin secretion from the rat intestinal D-cell. The effectiveness of this stimulation was quite remarkable, equalling that of the pharmacologic agent, dbcAMP, and greatly exceeding that of all other known SLI secretagogues in this model system, including calcitonin gene-related peptide-1 (CGRP), gastrin-releasing peptide, secretin, and sodium oleate *(19,21)*. As GLP-1$^{7\text{-}36NH2}$ was also effective at near-physiologic concentrations in this system ($10^{-10}M$), these studies suggest that secretion of GLP-1$^{7\text{-}36NH2}$ in vivo is associated with concomitant release of intestinal somatostatin. Interestingly, it was previously demonstrated that both SS-28 and SS-14 inhibit the release of the intestinal proglucagon-derived peptides, a family of peptides that are synthesized from the same prohormone as GLP-1, and that are secreted in parallel with GLP-1 from the intestinal L-cell *(22–24)*. Furthermore, SS-28 is up to 100 times more effective than SS-14 in regulating L-cell secretion *(22)*. Thus, it is hypothesized that secretion of GLP-1 and SS-28 by the intestine may be regulated coordinately in a classical endocrine feedback loop.

Analysis of the molecular forms of somatostatin that were released in response to $10^{-6}M$ GLP-1$^{7\text{-}36NH2}$ indicated that, although both forms were stimulated, release of SS-14 was augmented to a greater extent than that of SS-28. This occurred in the absence of any changes in the cellular distribution of these peptides, suggestive of an effect at the level of the regulated secretory pathway only. Although not established for the intestinal D-cell, GLP-1 receptors in the endocrine pancreas *(13,25)*, as well as in gastric parietal cells *(26)*, are linked to activation of the adenylyl cyclase pathway. However, it was previously demonstrated that activation of the protein kinase A pathway with dbcAMP in FRIC cultures leads to identical increments in SS-28 and SS-14 *(18)*. In contrast, CGRP, which also exerts its effects through cAMP *(27)*, stimulates release of SS-14 only, from the same intestinal cultures. It was, therefore, proposed that the intestine contains two distinct populations of somatostatin-producing D-cells, both of which are responsive to cAMP, but only one of which produces SS-14 in response to CGRP *(19)*. The present findings support this hypothesis and extend it to include differential distribution of the receptor for GLP-1$^{7\text{-}36NH2}$ on the intestinal SS-14-producing D-cell. This would provide a mechanism whereby release of SS-14 may be dissociated from that of SS-28 in response to circulating secretagogues. These findings are also consistent with the known distribution of SS-14 and SS-28 in the intestinal mucosa, whereby SS-14 predominates in the proximal gut, and SS-28 is more distally distributed *(16,17)*. Physiologically, therefore, distal release of GLP-1$^{7\text{-}36NH2}$ in response to nutrient ingestion may act at the level of the proximal gut, to release SS-14, and at the level of the distal intestine to stimulate SS-28 secretion. The ultimate targets of these different forms of somatostatin are not known, but may include the parietal cell *(28)* and/or gastric motor neurons *(29)*, as well as the GLP-1-producing L-cell itself *(22)*.

Unexpectedly, the full-length form of GLP-1, GLP-1 $^{1\text{-}36NH2}$, was also found to stimulate intestinal somatostatin release. Interestingly, although stimulation of SS-14 secretion was reduced as compared to the truncated peptide, GLP-1$^{1\text{-}36NH2}$ was as effective as GLP-1$^{7\text{-}36NH2}$ in releasing SS-28. Furthermore, these findings are in marked contrast to the total lack of biological activity that has been reported for GLP-1$^{1\text{-}36NH2}$ on the pancreatic B-cell *(5,6)*. GLP-1 $^{1\text{-}36NH2}$ does inhibit gastric acid secretion in vivo in humans, however, albeit with a potency of <5% that of the truncated peptide *(10)*. GLP-1$^{1\text{-}36NH2}$ has also been reported to stimulate parietal cAMP production, although again, it is much less potent than GLP-1$^{7\text{-}36NH2}$ *(26)*. This reduced bioactivity could potentially arise as a consequence of limited interaction of GLP-1$^{1\text{-}36NH2}$ with the GLP-1 receptor, which binds the truncated peptide with a 100-fold higher Kd than that for the full-length peptide *(30)*. However, our finding of differential effects of the two forms of GLP-1 on SS-14 release indicates that GLP-1$^{1\text{-}36NH2}$ can exert distinct biological effects from those of GLP-1$^{7\text{-}36NH2}$, and implicate GLP-1$^{1\text{-}36NH2}$ as a biologically active peptide. Whether GLP-1$^{1\text{-}36NH2}$ exerts its effects through a receptor or receptor isoform that is distinct from that for GLP-1$^{7\text{-}36NH2}$, or is consequent upon differing affinities of the two peptides for the same receptor *(31,32)* requires further study, although to date, only a single GLP-1$^{7\text{-}36NH2}$ receptor band has detected by polymerase chain reaction (PCR) in mouse intestine *(15)*.

In summary, the authors have demonstrated a novel biological activity of GLP-1$^{7\text{-}36NH2}$ as a stimulator of intestinal somatostatin secretion in the rat. Physiologically, such an activity likely augments the role of GLP-1$^{7\text{-}36NH2}$ as a stimulator of insulin secretion, whereby intestinal transit and subsequent nutrient absorption is delayed. Their finding of a unique role for GLP-1$^{1\text{-}36NH2}$ in regulating intestinal somatostatin is suggestive of the existence of an alternate receptor or receptor isoform for this peptide.

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