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Functional receptors for the insulinotropic hormone glucagon-like peptide-I(7–37) on a somatostatin secreting cell line

Hans C. Fehmann and Joel F. Habener

Laboratory of Molecular Endocrinology, Massachusetts General Hospital and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02114, USA

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Glucagon-like peptide-I (7–37) [(GLP-I(7–37)] is an intestinal peptide hormone that has potent insulinotropic activities in vivo in response to oral nutrients, in the isolated perfused pancreas, and in vitro in cultured B cells. GLP-I(7–37) receptor binding and GLP-I(7–37)-induced cAMP generation and hormone secretion was studied using cell lines producing insulin/B cell (βTC-1), glucagon/A cell (INR1G9) and somatostatin/D cell (RIN 1027-B2). [^{125}I]GLP-I(7–37) bound specifically to both B and D cells but not to A cells. GLP-I(7–37) induced cAMP-formation in B and D cells with a maximum response at 10 nmol/l (B cells) or at 100 nmol/l (D cells). Insulin secretion from perifused B cells was stimulated by GLP-I(7–37) (maximum at 10 nmol/l) and 10 nmol/l GLP-I(7–37) released somatostatin from perifused D cells. GLP-I(7–37) did not influence cAMP or glucagon secretion from A cells. These data indicate that pancreatic B and D cells, but not the A cells are influenced directly by GLP-I(7–37) via binding to specific receptors. Our findings support a model of physiologic regulation of insulin secretion whereby GLP-I(7–37) released from the intestine in response to oral nutrients potently stimulates insulin secretion via an endocrine mechanism that in turn may be dampened by a feed-back suppression by the release of somatostatin. In addition, suppression of the secretion of glucagon, a hormone whose actions are counter-regulatory to those of insulin, may occur by paracrine mechanisms involving GLP-I(7–37)-mediated stimulation of both insulin and somatostatin secretion.

Glucagon-like peptide-I(7–37); Receptor; Somatostatin-secreting cell; Pancreatic islet

1. INTRODUCTION

The proglucagon gene is expressed in the A cells of the endocrine pancreas (Islets of Langerhans) and the neuroendocrine L cells of the intestine. The translated prohormone contains glucagon and 3 additional peptides: glicentin-related peptide and the glucagon-like peptides-I and -II [1,2]. The intestinal processing of proglucagon results in 3 different forms of glucagon-like peptide-I: GLP-I(1–37), a biologically inactive precursor, and two N-terminally truncated peptides, GLP-I(7–36) amide and GLP-I(7–37), both equally potent in stimulating the pancreatic B cell to generate cAMP and to release insulin [3–5].

GLP-I(7–37) is secreted postprandially from the intestinal L cells into the circulation [6] whereby it stimulates insulin secretion at picomolar concentrations in vivo [6] and in vitro [4,7] via interactions with specific receptors on pancreatic B cells in a glucose-dependent manner [4,8,9]. Thus GLP-I(7–37) is an incretin hormone that may have an important role in glucose homeostasis. Up to now it has not been established whether or not GLP-I(7–37) influences directly hormone secretions from not only the B cells, but also the glucagon (A cells) and somatostatin (D cells) contained within the islets of Langerhans. A and D cells represent only a few percent of the endocrine cells of the pancreatic islets and it is difficult to obtain experimentally suitable amounts of pure glucagon- and somatostatin-producing cells. Therefore, we studied GLP-I(7–37) receptor-binding, GLP-I(7–37) induced cAMP generation and hormone secretion using insulin-(βTC-1), glucagon-(INR1G9) and somatostatin-(RIN1027-B2) producing cell lines.

Correspondence address: H.C. Fehmann, Massachusetts General Hospital, Laboratory of Molecular Endocrinology, 50 Blossom Street, Boston MA 02114, USA

2. MATERIALS AND METHODS

2.1. *Peptides*

Synthetic GLP-I(7–37) was from Cal-Bio (Mountain View, CA) and [^{125}I]GLP-I(7–37) was prepared as described [3]. Synthetic porcine GIP and glucagon were from Bachem (Philadelphia, PA).

2.2. *Cell culture*

βTC-1 cells [10], RIN 1027-B2 cells [11] and INR1G9 cells [12] were grown in RPMI medium supplemented with 10% fetal bovine serum, 5% horse serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a 95% O_2 and 5% CO_2 humidified atmosphere.

2.3. *Receptor binding studies*

After gentle trypsinization cells were washed twice with Krebs–Ringer-buffer (KRB) (pH 7.4) supplemented with 10 mM Hepes and 1% human serum albumin (Sigma) and were incubated in this medium for 30 min at 37°C. Unlabeled GLP-I(7–37) (50 μl, final concentration 10 pmol/l to 1 μmol/l) and labeled GLP-I(7–37) tracer (50 μl, 20 000 cpm) were added to 200 μl cell suspension with a 5 min time interval. Incubations were carried out for 30 min at 37°C and terminated by centrifugation (1 min, 10 000 rpm). The supernatant was

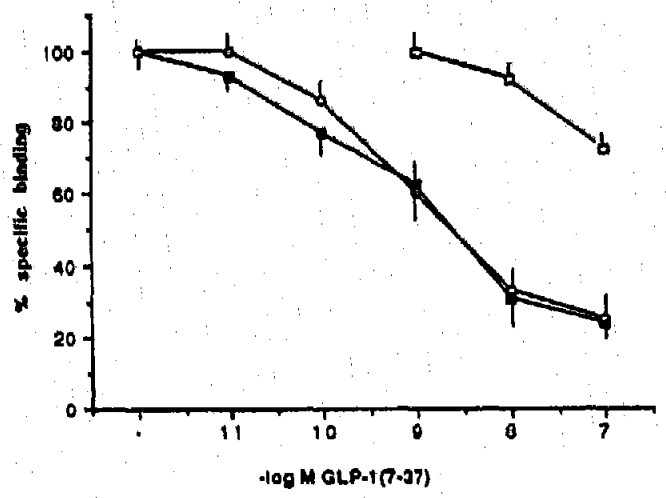

Fig. 1. Displacement of binding of [^{125}I]GLP-1(7-37) by GLP-1(7-37) from βTC-1 cells (O—O, $n = 4$) and RIN 1027-B2 cells (■—■, $n = 4$), and by glucagon from βTC-2 cells (□—□, $n = 4$).

carefully aspirated and the radioactivity bound to the pellet was determined using a gamma counter. Specific binding was defined as total binding minus binding in the presence of 1 μmol/l GLP-1(7-37) and expressed as the percent of binding in the absence of GLP-1(7-37).

2.4. *Static incubation experiments (cAMP release)*

Two days before the experiment approximately 5×10^5 cells were transferred to each well of 24 well plates. At the beginning of the experiment cells were washed twice with KRB buffer and incubated in this medium (1 ml) for 60 min (37°C). Then the buffer was changed to a buffer (300 μl) containing the test substances as indicated for 30 min. The entire supernatant was lyophilized and reconstituted in assay buffer for cAMP determination.

2.5. *Perifusion experiments (hormone release)*

The day before the experiments ca 2×10^6 cells were planted into 35 mm diameter culture dishes. Cells were washed twice with KRB buffer supplemented with 0.1% human serum albumin (pH 7.4, 37°C) and incubated in this medium for one h (37°C). Three inflow needles and one outflow needle were inserted in the roof of the culture dish prepared with the respective holes (angle 90° each). The culture dishes were placed on a temperature-regulated plate (37°C) and each inflow needle was connected with one syringe containing KRB buffer plus the respective test substances. A Harvard Apparatus pump 22 (Harvard Apparatus, South Natick, MA) generated a constant flow of 0.2 ml/min. The outflow needle was connected to a peristaltic pump that was adjusted to 0.6 ml/min. After a basal period (10 min) media were changed to buffer containing the test substances as indicated. One-minute fractions were collected and each fraction was assayed for the respective hormone concentration. Hormone secretion data are expressed as percent of the average secretion values during the last 5 min of the unstimulated period (Figs 2 and 3). Integrated secretion values, area under the curve (AUC), obtained were calculated by addition of the percentage values during the first (1-10 min) and the second (11-20 min) phase (Tables II and III).

2.6. *Radioimmunoassays*

Insulin was determined by radioimmunoassay using an antiserum raised in rabbits against pork insulin (Sigma) and pork insulin (Sigma) as standard [13]. Glucagon was determined with a radioimmunoassay using a rabbit antiserum and synthetic glucagon as standard [14]. Somatostatin was measured by radioimmunoassay with a rabbit anti-somatostatin antiserum (Immunonuclear) and synthetic somatostatin as standard (Immunonuclear, [13]). Cyclic AMP was determined using a rabbit anti-cAMP antiserum (Chemicon, El Segundo, CA), cAMP (Sigma) as standard [15], and samples were acetylated with 20 μl triethylamine/acetic anhydride 1:3 (vol/vol). In all 4 systems bound and free ligand were separated with dextran-coated charcoal. Inter- and intra-assay variance for all assay systems were within 12%.

2.7. *Statistics*

Data are given as mean ± standard error of (n) experiments. When indicated Student's t-test was used for statistical analysis. Statistical significance was set at the 5% level.

3. RESULTS

3.1. *Binding experiments*

GLP-1(7-37) inhibited specific binding of [^{125}I]GLP-1(7-37) to βTC-1 cells and RIN 1027-B2 cells in a concentration-dependent manner (Fig. 1). Non-specific binding (bound radioactivity in the presence of 1 μmol/l GLP-1(7-37)) was 19.8% (βTC-1 cells) and 18.6% (RIN 1027-B2 cells). The K_d (50% inhibition) was 3.3 nmol/l (βTC-1 cells) and 3.5 nmol/l (RIN-1027-B2 cells). Glucagon, but not GIP inhibited binding of [^{125}I]GLP-1(7-37) to βTC-1 cells only weakly: 100 nmol/l, 32% inhibition; 10 nmol/l, 11% inhibition (Fig. 1). There was no detectable binding of [^{125}I]GLP-1(7-37) to INR1G9 cells.

Table I

Effect of GLP-1(7-37) on cAMP secretion from βTC-1, INR1G9 and RIN 1027-B2 cells. Four to six independent experiments were performed in triplicate for each condition

Glucose (mmol/l)	GLP-1(7-37) (-log M)	cAMP secretion (fmol/well/30 min)		
		βTC-1	INR1 G9	RIN 1027-B2
0	0	38 ± 2	1674 ± 155	576 ± 158
5	0	39 ± 18	2055 ± 117	778 ± 289
5	11	38 ± 18		576 ± 142
5	10	63 ± 30		421 ± 147
5	9	140 ± 45*		1223 ± 485
5	8	330 ± 136*	1524 ± 69	1827 ± 353*
5	7	129 ± 42*		2624 ± 376*
5	6	53 ± 18#		1754 ± 593*
0	8	41 ± 4		926 ± 123

*significant difference vs control experiments (5 mmol/l glucose)
#significant difference vs experiments with 10 nmol/l GLP-1(7-37)

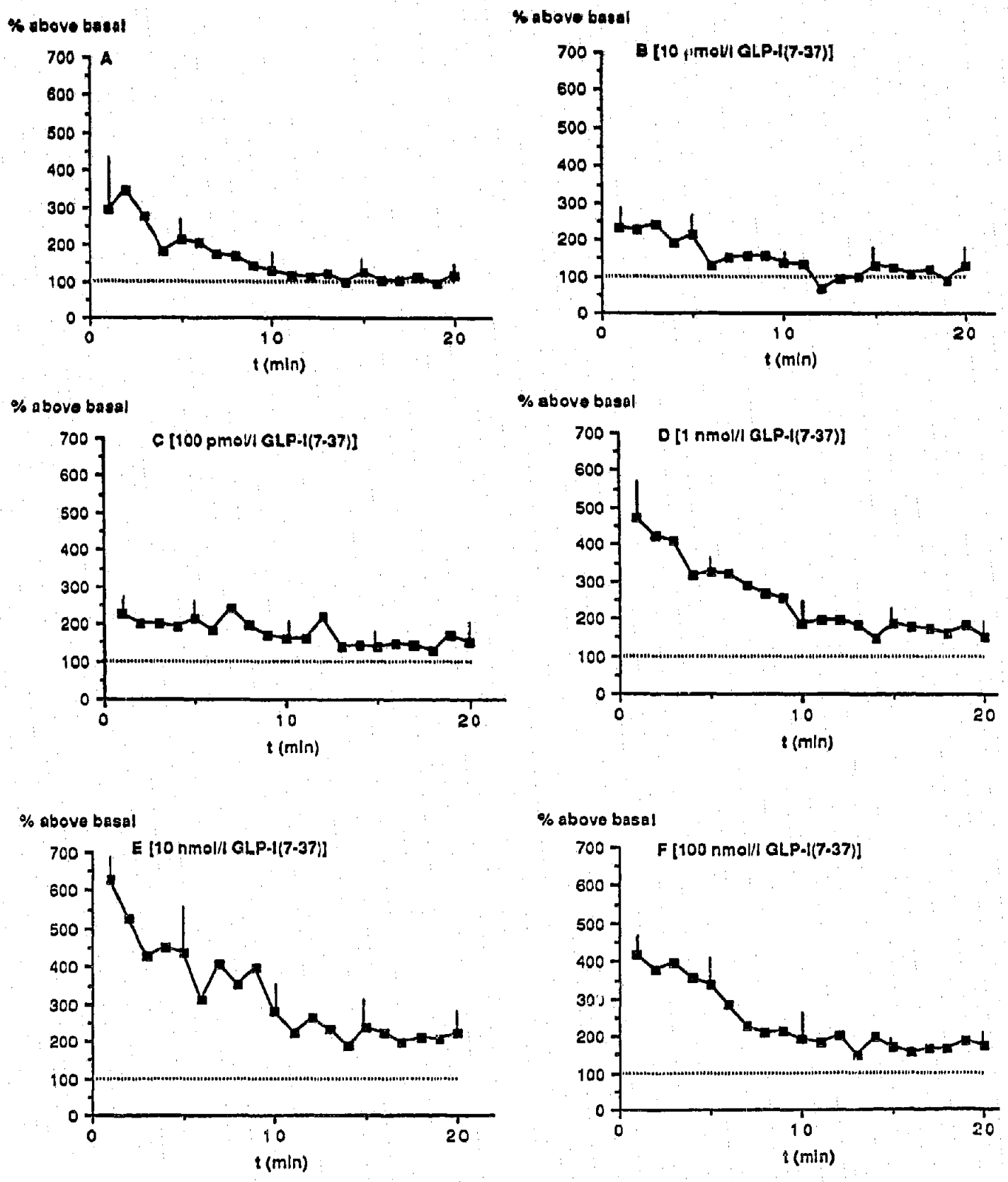

Fig. 2. Effect of GLP-I(7-37) on glucose-stimulated (5 mmol/l) insulin release from perifused βTC-1 cells. (A) 5 mmol/l glucose ($n=6$), (B) 5 mmol/l glucose + 10 pmol/l GLP-I(7-37) ($n=6$), (C) 5 mmol/l glucose + 100 pmol/l GLP-I(7-37) ($n=6$), (D) 5 mmol/l glucose + 1 nmol/l GLP-I(7-37) ($n=6$), (E) 5 mmol/l glucose + 10 nmol/l GLP-I(7-37) [6], (F) 5 mmol/l glucose + 100 nmol/l GLP-I(7-37) ($n=3$).

Table II

Integrated insulin secretory responses to glucose and GLP-1(7-37) from perifused βTC-1-cells during the first (1-10 min) and the second phase (11-20 min)

Glucose (mmol/l)	GLP-1(7-37) (-log M)	First phase (AUC/1-10 min)	Second phase (AUC/11-20 min)
0	0	1092 ± 95	772 ± 80
5	0	2330 ± 226*	1089 ± 125
10	0	2781 ± 222*	1359 ± 58
20	0	4299 ± 364*	1356 ± 144
5	11	2074 ± 387	1289 ± 194
5	10	2035 ± 85	1542 ± 44
5	9	3299 ± 671*	1757 ± 267
5	8	4950 ± 1057*	2204 ± 518
5	7	3068 ± 333	1751 ± 54
0	8	1302 ± 191	855 ± 88

#Significant difference vs control experiments (buffer alone)
*Significant difference vs control experiments (3 mmol/l glucose)
AUC = area under the curve, the integrated measurement of insulin secretion.

3.2. *cAMP secretion*

Incubation of βTC-1, INR1G9 and RIN 1027-B2 cells with buffer supplemented with 5 mmol/l glucose had no effect on secreted cAMP levels compared to experiments with buffer alone [Table I]. GLP-I(7-37) induced cAMP secretion from βTC-1 cells in a concentration-dependent manner with a maximum at 10 nmol/l as reported previously [16] and from RIN 1027-B2 cells with a maximum at 100 nmol/l. GLP-I(7-37) had no influence on cAMP secretion from INR1G9 cells. The biphasic response of cAMP formation with an attenuated response at higher concentrations of GLP-1(7-37) was shown in earlier studies of the effect of GLP-1(7-37) on a rat B cell line (RIN 1046-38 cells, [17]). The diminished response at higher concentrations of ligand is due to a rapid homologous desensitization of the GLP-1(7-37) receptor (H.C. Fehmann and J.F. Habener, unpublished observations). In the absence of glucose GLP-1(7-37) had no effect on cAMP secretion from βTC-1 and RIN 1027-B2 cells.

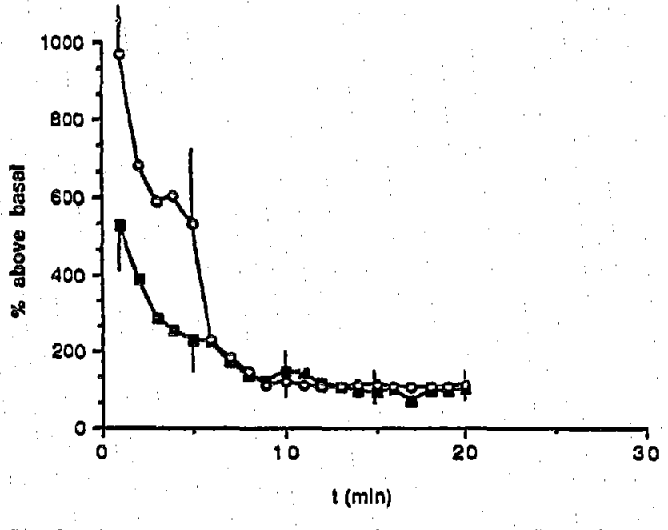

Fig. 3. Effect of 10 nmol/l GLP-1(7-37) (○—○; $n = 6$) on glucose-induced (5 mmol/l) somatostatin release from perifused RIN 1027-B2 cells. Control experiments (5 mmol/l glucose (■—■), $n = 6$).

3.3. *Hormone secretion*

βTC-1 cells secreted insulin in response to glucose in a concentration-dependent manner as described from static incubation experiments with this cell line [18]. The secretory pattern was monophasic as known from studies with another glucose-responsive cell line, HIT-T-15 [19] and insulin secretion values returned to basal levels after 10-12 min in the continuous presence of glucose (Fig. 2). Addition of GLP-I(7-37) enhanced insulin secretion concentration-dependently during the first phase (1-10 min) with a maximum at 10 nmol/l and induced a small second phase of insulin release (Fig. 2, Table II). A similar phenomenon has been observed in studies with HIT cells and IBMX [19]. The insulinotropic action of GLP-I(7-37) was glucose-dependent (Table II).

Perifusion of RIN 1027-B2 cells with KRB buffer supplemented with 5 mmol/l glucose induced somatostatin release for 12 min. Addition of 10 nmol/l GLP-I(7-37) enhanced the somatostatin response during the first 5 min in a glucose-dependent manner (Fig. 3; Table III). 10 mmol/l glucose plus 10 mmol/l arginine stimulated glucagon release from INR1G9 cells with a monophasic pattern for 15 min. Additon of 10 nmol/l GLP-I(7-37) had no effect on glucagon secretion (AUC; first phase: controls: 2975 ± 199, GLP-I(7-37): 2574 ± 219; second phase: controls: 1477 ± 226, GLP-I(7-37): 1771 ± 189).

4. DISCUSSION

The insulinotropic action of GLP-I(7-37) in vivo and in vitro is well established and GLP-I(7-37) is believed to represent an important mediator of the entero-insulinar axis and glucose homeostasis. Using the

Table III

Integrated somatostatin secretory responses to glucose and GLP-I(7-37) from perifused RIN 1027-B2-cells during the first (1-10) and the second phase (11-20 min)

Glucose (mmol/l)	GLP-I(7-37) (-log M)	First phase (AUC/1-10min)	Second phase (AUC/11-20 min)
0	0	937 ± 125	1157 ± 161
0	8	598 ± 63	741 ± 179
5	0	1578 ± 390	956 ± 105
5	8	3332 ± 282*	1089 ± 105

*Significant difference vs control experiments (5 mmol/l glucose)
AUC = area under the curve, the integrated measurement of somatostatin secretion.

glucose-responsive cell line βTC-1 we demonstrate the presence of a specific receptor for GLP-I(7-37) on these cells (K_d 3.5 nmol/l) as was reported previously in studies with the pancreatic B cell lines RINm5F [8] and RIN 5AH [9]. The different cell lines used might account for the slightly different K_ds (RINm5F cells: 204 pmol/l [8], RIN 5AH: 3.3 nmol/l [9], βTC-1 cells: 3.5 nmol/l). The failure of GIP to compete with binding of radiolabeled GLP-I(7-37) suggests the presence of distinct receptors for GLP-I(7-37) and GIP on pancreatic B cells. In addition, our data demonstrate that GLP-I(7-37) stimulates concentration-dependently cAMP and insulin secretion from βTC-1 cells as reported previously in studies with this and other B cell lines [8,6,17].

Previous studies with rat islet cell monolayer cultures [20] and the perfused rat pancreas [21] suggested a somatostatin-releasing action of GLP-I(7-37). Our data provide evidence for a direct role of GLP-I(7-37) in the regulation of pancreatic somatostatin release: RIN 1027-B2 cells possess specific receptors for GLP-I(7-37) with nearly identical binding characteristics compared with insulin-producing cell lines βTC-1, RIN 5AH [9] and RIN m5F [8]. These data suggest that the same receptor is expressed on insulin- and somatostatin-producing cell lines. In addition, GLP-I(7-37) induces cAMP and somatostatin secretion from RIN 1027-B2 cells. These observations extend those of earlier reports that glucose is a stimulator of pancreatic somatostatin secretion [22] and that cAMP is involved in the stimulus-secretion coupling of the pancreatic D cell [23].

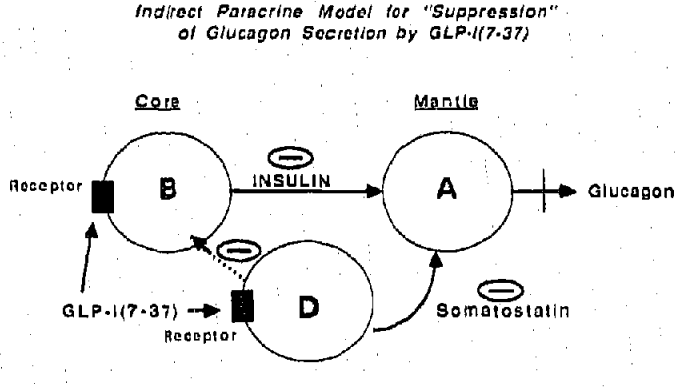

Fig. 4. Model for postulated negative feedback suppression of glucagon secretion by the actions of GLP-I(7-37) to stimulate secretion of both insulin and somatostatin via specific receptors in B and D cells. Because of the portal blood flow from the core to the mantle of the pancreatic islet, it is envisioned that the known suppressive effects of insulin and somatostatin on glucagon secretion are mediated by paracrine mechanisms. Somatostatin may in theory feed back negatively to dampen the GLP-I(7-37)-induced insulin secretion, perhaps by an endocrine pathway.

Some controversy exists whether or not GLP-I(7-37) influences pancreatic A cell secretion. Studies with the isolated perfused rat pancreas [4] and rat pancreatic islet cell monolayer cultures [20] revealed no effect of GLP-I(7-37) on pancreatic glucagon release, whereas other reports using the isolated pig [24] and rat pancrease [5,25] and isolated rat islets [26] described, compared to the insulinotropic action, a weak glucagonostatic action of GLP-I(7-37) that requires 10 to 100-fold higher GLP-I(7-37) levels than the insulinotropic effect. Our data now indicate that INR1G9 (glucagon-producing) cells do not possess binding sites for GLP-I(7-37), and presumably binding sites are absent on pancreatic A cells. It is well established that at least in the rat pancreas a directed blood flow from the B cells to the A cells and then to the D cells exists and in this vascular compartment insulin represents a physiological inhibitor of glucagon secretion [27]. There is also strong evidence that somatostatin suppresses A cells by paracrine mechanisms [28]. Thus, the glucagonostatic action of GLP-I(7-37) in the perfused pancreas can be explained by the stimulation of both insulin and somatostatin release. This concept is supported by the recent finding that GLP-I(7-36) amide does not suppress glucagon secretion in perfused pancreata from streptozotocin-treated rats which had a 50% reduced insulin release during stimulation with GLP-I(7-36)amide or GIP compared to non-diabetic controls [29]. In addition, such paracrine mechanisms seem to be involved in the reported inhibitory action of GLP-I(7-37) on glucagon gene transcription in isolated rat islets [26] and the hamster glucagonoma cell line INR1G9 [30].

Taken together our data support the concept of GLP-I(7-37) as an important intestinal factor in the regulation of hormone secretion from the endocrine pancreas.

In addition to the stimulation of insulin release from B cells GLP-I(7–37) can also stimulate somatostatin release by specific receptors present on D cells but is without direct effect on the A cells (Fig. 4). The demonstration of the existence of functional GLP-I(7–37) receptors on D cells raises the possibility that with respect to GLP-I(7–37) the entero-islet axis may involve more than just the augmentation of insulin secretion. It seems reasonable to postulate that the effect of GLP-I(7–37) to stimulate somatostatin secretion is designed to dampen the stimulation of insulin secretion invoked by GLP-I(7–37). In addition, the combined secretion of both insulin and somatostatin augmented by GLP-I(7–37) may act to suppress by paracrine mechanisms the secretion of glucagon, a known potent hormone that is counter-regulatory to the metabolic actions of insulin.

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