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Synthesis and secretion of glucagon-like peptide-1 by fetal rat intestinal cells in culture

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Secretion of the intestinal proglucagon-derived peptides (PGDPs) including the incretin glucagon-like peptide-1 (GLP-1) is regulated, at least in part, by the duodenal hormone glucose-dependent insulinotropic peptide (GIP) through a protein kinase (PK) A-dependent pathway. It has been demonstrated that the activation of PKA increases the synthesis of some intestinal PGDPs, particularly the glucagon-like immunoreactive (GLI) peptides glicentin and oxyntomodulin. However, the effects of GIP on GLI and GLP-1 synthesis are not known. Fetal rat intestinal cells in culture were therefore treated for up to 24 h with 5 mM dbcAMP or 10^{-6} M GIP and the changes in glicentin, oxyntomodulin, GLP-1^{x-37} and GLP-$1^{x-36NH2}$ secretion and synthesis were examined by RIA and HPLC. Both dbcAMP and GIP increased the acute (2 h; to 224 ± 21 and 256 ± 20% of controls, respectively, $P<0.001$) and chronic (24 h; to 230 ± 22 and 130 ± 6% of controls, respectively, $P<0.001$) secretion of intestinal PGDPs. In contrast, the total culture content of PGDPs was increased only after 24 h of incubation (to 156 ± 15 and 125 ± 7% of controls for dbcAMP and GIP, respectively, $P<0.01$). HPLC analysis confirmed that the intestinal cultures produced the GLI peptides glicentin and oxyntomodulin, as well as the biologically active forms of GLP-1, GLP-$1^{7-37}$and GLP-$1^{7-36NH2}$. The relative proportion of these peptides was not altered by treatment with dbcAMP or GIP. Thus, in addition to its effects on GLP-1 release from the rat intestine, GIP appears to be an important regulator of the synthesis of this insulinotropic peptide.

Keywords: Proglucagon; intestine; processing; glucagon-like peptide-1; protein kinase A; glucose-dependent insulinotropic peptide

Introduction

Glucagon-like peptide-1 (GLP-1) is an intestinal hormone that acts as a potent incretin, stimulating glucose-dependent insulin secretion (Kreymann *et al.*, 1987; Mojsov *et al.*, 1987; Gutniak *et al.*, 1992; Nathan *et al.*, 1992). GLP-1 is synthesized by tissue-specific post-translational processing of the C-terminal end of proglucagon in the intestinal L cell, and occurs in a variety of molecular forms, including the biologically active incretins (GLP-1^{7-37} and GLP-$1^{7-36NH2}$), and the inactive full-length precursors (GLP-1^{1-37} and GLP-$1^{1-36NH2}$) (Mojsov *et al.*, 1986; Orskov *et al.*, 1986; Mojsov *et al.*, 1990). The function played by the amidation of GLP-1 is not clear, as this modification does not appear to affect biologic activity (Suzuki *et al.*, 1989; Weir *et al.*, 1989).

The secretion of GLP-1 *in vivo* is modulated by ingested nutrients (Kreymann *et al.*, 1987; Roberge & Brubaker, 1991; Elliott *et al.*, 1993; Roberge & Brubaker, 1993; Andreasen *et al.*, 1994), hormones and neuropeptides (Roberge & Brubaker, 1993; Brubaker *et al.*, 1994). To examine the regulation of proglucagon-derived peptide (PGDP) synthesis and secretion by the intestinal L cell, we have previously established cultures of fetal rat intestinal cells (FRIC). We have used this model to demonstrate that synthesis of the N-terminally derived PGDPs, glicentin and oxyntomodulin, is stimulated through a protein kinase (PK) A-dependent pathway (Brubaker & Vranic, 1987; Brubaker, 1988; Drucker & Brubaker, 1989), likely through stimulation of proglucagon gene transcription (Drucker *et al.*, 1994). Secretion of the N-terminal PGDPs in FRIC cultures is also stimulated by a PKA-dependent pathway (Brubaker & Vranic, 1987; Brubaker, 1988; Drucker & Brubaker, 1989, 1994), as well as by several intestinal peptides known to act through PKA, including glucose-dependent insulinotropic peptide (GIP) (Brubaker, 1991). The stimulatory effects of GIP on secretion of the N-terminal PGDPs have also been demonstrated *in vivo* in the rat, in which GIP functions as a key regulator of acute intestinal PGDP release after nutrient ingestion (Roberge & Brubaker, 1991, 1993). Together, these findings suggest that GIP may stimulate both the synthesis and secretion of the C-terminal PGDP, GLP-1, by the intestinal L cell. We have therefore utilized the FRIC culture model to examine the effects of GIP and its signal transduction pathway (PKA) on the synthesis and secretion of GLP-1.

Results

Control values for secretion and total culture content of GLI, GLP-1^{x-37} and GLP-$1^{x-36NH2}$ after 2 and 24 h of incubation are shown in Table 1. No significant differences between the levels of GLI and GLP-1 could be detected in either the medium or the whole culture dish.

Treatment of FRIC cultures for 2 h with 5 mM dbcAMP (Figure 1) stimulated secretion of all of the PGDPs (GLI, GLP-1^{x-37} and GLP-$1^{x-36NH2}$) to 224 ± 21% of paired control values (combined data; $P<0.001$). Similarly, treatment with 10^{-6} M GIP also increased PGDP secretion to 256 ± 20% of controls ($P<0.001$). No significant increments in the GLI or total GLP-1 content of the cultures were detected during the 2 h incubation period. Treatment of the cells with dbcAMP or GIP for 24 h also increased secretion of the PGDPs, to 230 ± 22 and 130 ± 6% of control values, respectively ($P<0.001$). In contrast to the 2 h data, however, after 24 h of incubation, a significant stimulation of the total culture content of GLI and GLP-1^{x-37} (to a combined value of 156 ± 15 and 125 ± 7% of controls, for dbcAMP and GIP treatment, respectively ($P<0.01$)) was also observed. Unexpectedly, total GLP-$1^{x-36NH2}$ levels were not stimulated by

Table 1 Absolute values (in pg/dish) of GLI, GLP-1^{x-37} and GLP-$1^{x-36NH2}$ in medium or total FRIC cultures, after treatment for 2 or 24 h under control conditions ($n = 4–11$)

	2 h		*24 h*	
	Medium	*Culture*	*Medium*	*Culture*
GLI	98 ± 22	1673 ± 404	605 ± 62	3501 ± 483
GLP-1^{x-37}	38 ± 9	300 ± 77	168 ± 20	576 ± 93
GLP-$1^{x-36NH2}$	40 ± 9	959 ± 438	147 ± 29	648 ± 117

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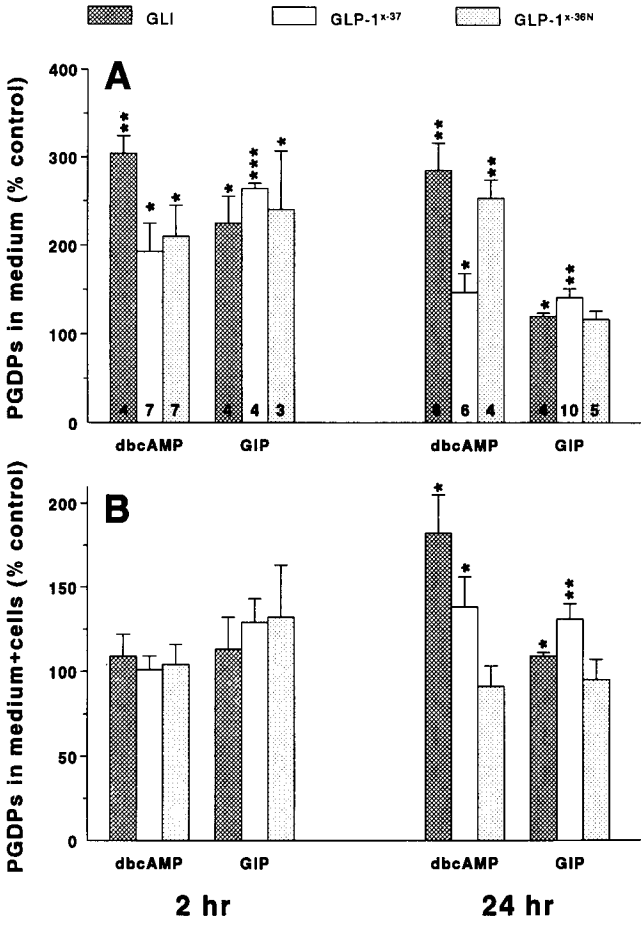

Figure 1 (**A**) secretion and (**B**) total content of PGDPs in FRIC cultures. FRIC cultures were treated for 2 or 24 h under control conditions or with 5 mM dbcAMP or 10^{-6} M GIP. Cells and cell media were extracted and assayed for GLI (closed) and immunoreactive GLP-1^{x-37} (open) and GLP-$1^{x-36NH2}$ (shaded) ($n = 3-10$). Secretion was determined as the media content of immunoreactive peptide after the incubation period, while synthesis was determined as the total (media plus cell) content of immunoreactive peptide at the end of the incubation period. All data are expressed as a percent of paired control values, which are shown in Table 1. *, $P<0.05$, **, $P<0.01$, ***$P<0.001$ *vs* controls

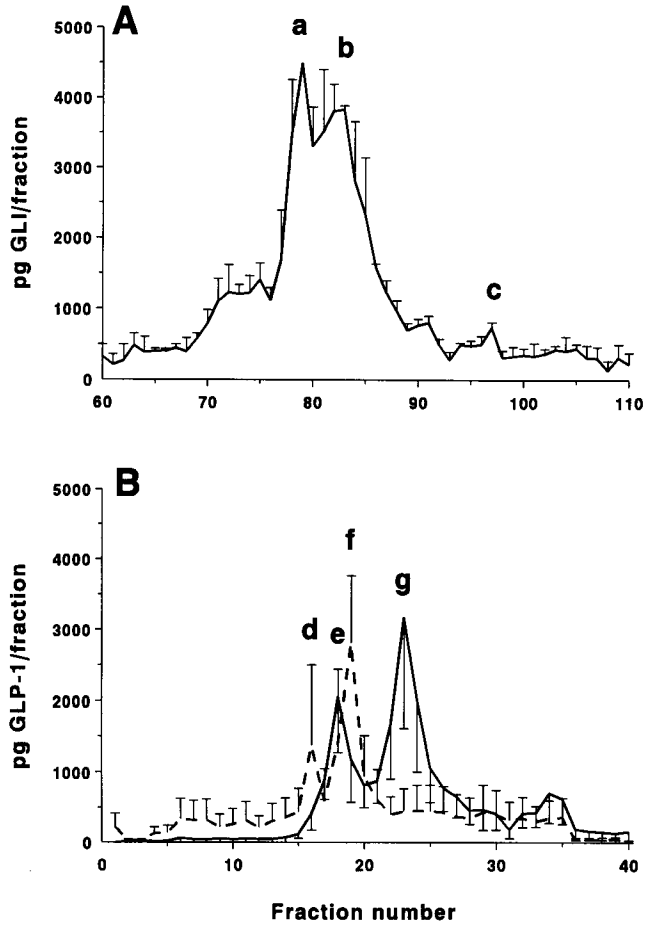

Figure 2 HPLC analysis of PGDPs contained in FRIC cells after treatment for 24 h under control conditions. Peptides were separated by gradient elution on a reversed-phase C18 column. (**A**) GLI, and (**B**) GLP-$1^{x-36NH2}$: solid line, GLP-1^{x-37}: dashed line ($n = 3-4$). The peaks are identified as: (a) glicentin, (b) oxyntomodulin, (c) glucagon, (d) GLP-1^{1-37}, (e) GLP-$1^{1-36NH2}$, (f) GLP-1^{7-37} and (g) GLP-$1^{7-36NH2}$

24 h of treatment with dbcAMP or GIP, remaining at 91 ± 12 and $95 \pm 12\%$ of controls, respectively.

To determine the molecular distribution of the PGDPs synthesized by FRIC cultures, cell extracts were analysed by HPLC after 24 h of incubation under control conditions (Figure 2) or with 5 mM dbcAMP (Figure 3) or 10^{-6} M GIP (Figure 4). Regardless of the treatment, FRIC cells contained a predominance of glicentin and oxyntomodulin, rather than glucagon (46 ± 4, 48 ± 4 and $5 \pm 1\%$ of total GLI, respectively; $n = 9$). Radioimmunoassay for the presence of immunoreactive glucagon in fractions 85–100 confirmed the absence of significant amounts of pancreatic-type glucagon (fraction 97) in the cells (data not shown). Similar analyses of FRIC cell extracts for GLP-1 demonstrated that most of the peptide was present in truncated, biologically active forms, and not in full-length inactive forms (GLP-1^{7-37} *vs* GLP-1^{1-37}: 70 ± 5 *vs* $30 \pm 5\%$ of total GLP-1^{x-37}; and GLP-$1^{7-36NH2}$ *vs* GLP-$1^{1-36NH2}$: $59 \pm 4\%$ *vs* $41 \pm 4\%$ of total GLP-$1^{x-36NH2}$). No marked differences could be detected between the profiles obtained for control cells *vs* dbcAMP- or GIP-treated cells.

Analysis of the HPLC profiles for GLP-1 demonstrated equivalent amounts of amidated and non-amidated forms of GLP-1. These findings agreed with the similar amounts of these peptides found in the whole culture after 24 h of incubation (ratio of amidated to non-admidated = 1.1; Table 1), but differed from the 2 h extracts in which amidated forms of GLP-1 predominated (ratio = 3.1). These findings suggested that the amidating capacity of the cultures was reduced during prolonged incubation periods. As ascorbate depletion is known to reduce the capacity of cultured cells to amidate α-melanocyte stimulating hormone (Eipper *et al.*, 1983a,b; Glembotski, 1986), FRIC cultures were incubated in the absence or presence of 50 μM ascorbate for 24 h ($n = 7$). At the end of the incubation period, the ratio of amidated to non-amidated GLP-1 was increased 1.4-fold in the presence of ascorbate, from 1.6 ± 0.7 in control cells to 2.5 ± 0.7 in ascorbate-treated cells ($P<0.01$).

Discussion

The intestinal proglucagon-derived peptide GLP-1 is an important regulator of insulin secretion in response to nutrient ingestion (Elliott *et al.*, 1993; Kreymann *et al.*, 1987; Andreasen *et al.*, 1994). Although previous experiments have demonstrated that the PKA pathway stimulates both synthesis and secretion of the N-terminal PGDPs, glicentin and oxyntomodulin, in FRIC cultures (Brubaker, 1988; Drucker & Brubaker, 1989), no examination of the effects of PKA on GLP-1 synthesis and secretion has been reported to date. The results of the present study confirm that activation of the PKA pathway with dbcAMP increases both the content and secretion of glicentin and oxyntomodulin, and extend these findings by demonstrating that the increments in both the

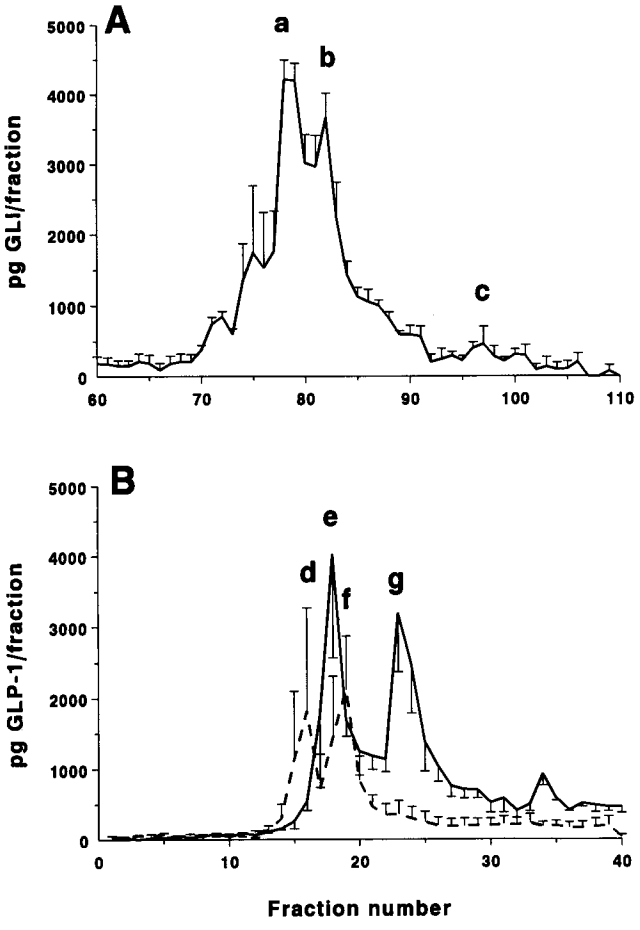

Figure 3 HPLC analysis of PGDPs contained in FRIC cells after treatment for 24 h with 5 mM dbcAMP. Peptides were separated by gradient elution on a reversed-phase C18 column. (**A**) GLI and (**B**) GLP-$1^{x\text{-}36NH2}$: solid line, GLP-$1^{x\text{-}37}$: dashed line ($n = 3$–4). The peaks are identified as: (a) glicentin, (b) oxyntomodulin, (c) glucagon, (d) GLP-$1^{1\text{-}37}$, (e) GLP-$1^{1\text{-}36NH2}$, (f) GLP-$1^{7\text{-}37}$ and (g) GLP-$1^{7\text{-}36NH2}$

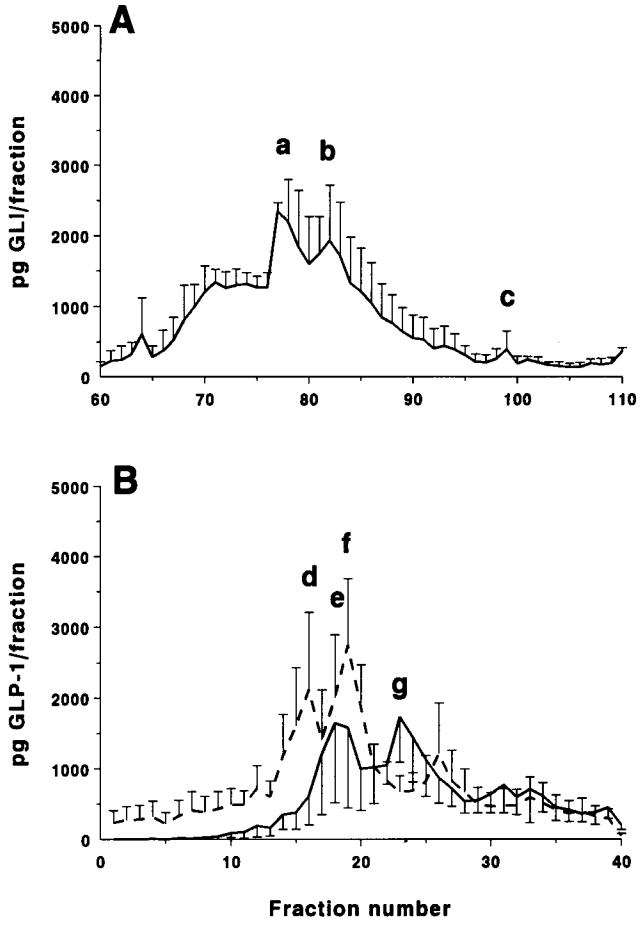

Figure 4 HPLC analysis of PGDPs contained in FRIC cells after treatment for 24 h with 10^{-6} M GIP. Peptides were separated by gradient elution on a reversed-phase C18 column. (**A**) GLI and (**B**) GLP-$1^{x\text{-}36NH2}$: solid line, GLP-$1^{x\text{-}37}$: dashed line ($n = 3$–4). The peaks are identified as: (a) glicentin, (b) oxyntomodulin, (c) glucagon, (d) GLP-$1^{1\text{-}37}$, (e) GLP-$1^{1\text{-}36NH2}$, (f) GLP-$1^{7\text{-}37}$ and (g) GLP-$1^{7\text{-}36NH2}$

total content and secretion of GLP-1 parallel those of the N-terminal PGDPs in FRIC cultures. These increases in total culture content of PGDPs presumably reflect increased synthesis of the peptides, as intestinal proglucagon gene transcription is increased by activation of the PKA pathway (Drucker & Brubaker, 1989; Drucker *et al.*, 1994), likely through the cyclic AMP-response element located in the 5′-flanking region of the gene (Philippe *et al.*, 1988). Since proglucagon encodes for equivalent amounts of glicentin/oxyntomodulin and GLP-1, it would be expected that the levels of these different peptides should be not only similar, but also should change concomitantly in response to various stimuli.

GIP is a key regulator of N-terminal PGDP secretion in response to nutrient fluxes into the duodenum of the rat (Brubaker, 1991; Roberge & Brubaker, 1991, 1993). Recent studies using the isolated rat intestine have confirmed that GIP infusion stimulates GLP-1 release from the L cell (Herrmann *et al.*, 1993; Plaisancie *et al.*, 1994). Cloning of the GIP receptor has demonstrated direct coupling of GIP to cAMP biosynthesis (Usdin *et al.*, 1993). Consistent with such a mechanism of action and with our findings that the PKA pathway increases GLI and GLP-1 production in FRIC cultures, GIP treatment was found to increase the synthesis and secretion of all of the PGDPs by the intestinal L cell. These findings therefore implicate GIP not only in the regulation of GLP-1 secretion from the intestine, but also in the replenishment of this incretin within the L cell upon prolonged stimulation. As GIP itself is also insulinotropic (Pederson *et al.*, 1975; Sarson *et al.*, 1982), these findings suggest that GIP and GLP-1 act together in an integrated fashion to regulate the enteroinsular axis.

As expected from previous findings (Mojsov *et al.*, 1986; Brubaker & Vranic 1987; Brubaker *et al.*, 1989), the profile of N-terminal PGDPs in FRIC cultures was found to be identical to that of the adult rat intestinal L cell. Unexpectedly, however, the GLP-1 profiles differ slightly between the two rat models. While both the adult L cell *in vivo* and the fetal L cell *in vitro* truncate a predominance of their GLP-1 [80 *vs* 60–70%, respectively [(Mojsov *et al.*, 1990) and P.L.B.: unpublished data)], the degree of amidation of GLP-1 appeared to become limiting after 24 h of incubation under the *in vitro* conditions utilized [>90% amidation for the adult L cell *vs* 50–60% in 24 h FRIC cultures (P.L.B.: unpublished data)]. Consistent with these findings, both the synthesis and secretion of GLI and GLP-$1^{x\text{-}37}$ were increased by dbcAMP and GIP after 24 h of incubation, whereas only the release of GLP-$1^{x\text{-}36NH2}$ was affected. These findings suggest that release of preformed amidated GLP-1, but not new synthesis, may be occuring in this time frame. Amidation of many regulatory peptides is carried out by peptidyl α-amidating monooxygenase (PAM) (Eipper *et al.*, 1983b, 1992; Ouafik *et al.*, 1992), a bifunctional enzyme that has been localized in a number of neuroendocrine cells, including the intestinal L cell (Sakata *et al.*, 1986; Martinez *et al.*, 1993). Studies of α-melanocyte-stimulating hormone amidation in primary rat intermediate pituitary cultures have demonstrated that concentrations of one of the cofactors for

PAM, ascorbate, become limiting with time leading to a fall in the amidation rate (Eipper *et al.*, 1983b). Replenishment of ascorbate restores PAM activity in these cells (Eipper *et al.*, 1983a; Glembotski, 1986). Similarly, we have found that replenishment of ascorbate in FRIC cultures increases the ratio of amidated to non-amidated GLP-1. These findings therefore provide further evidence for a role of PAM in the amidation of GLP-1 in the intestinal L cell.

In summary, the results of the present study demonstrate that both the synthesis and secretion of GLP-1 by the rat intestinal L cell are increased by GIP, likely through a PKA-dependent pathway. These findings lend further support to the concept that GIP has a key role in regulating the intestinal L cell response to nutrient ingestion.

Materials and methods

Monolayer cultures of fetal rat intestinal cells (FRIC) were prepared as described in detail previously (Brubaker & Vranic, 1987; Brubaker, 1988; Drucker & Brubaker, 1989). In brief, term fetal rat intestines were dispersed enzymatically with 4 mg/ml collagenase (SigmaBlend Type H), 5 mg/ml hyaluronidase (Type I-S) and 0.5 mg/ml DNase-I (Sigma Chemical Co., St Louis MO). The cells were placed into 60 × 15 mm dishes at a density of 0.6 fetal rat intestines per dish in 2 ml of Dulbecco's Minimal Essential Medium (DMEM) containing 5% (v/v) fetal bovine serum, 4.5 g/l glucose and 50 IU/ml penicillin-50 μg/ml streptomycin, and were allowed to recover for 24 h before use.

Cells were washed twice with Hank's Balanced Salt Solution and then incubated with test agents for 2 or 24 h in DMEM containing 0.5% (v/v) fetal bovine serum, 1 g/l glucose, 20 μU/ml insulin and 50 IU/ml penicillin-50 μg/ml streptomycin. DbcAMP was obtained from Sigma Chemical Co., and synthetic human GIP from Bachem California (Torrance CA). After the incubation period, media were centrifuged to remove any floating cells and then made to 0.1% (v/v) trifluoroacetic acid (TFA). Cells were scraped and homogenized in 2 ml of 1 N HCl containing 5% (v/v) formic acid, 1% (v/v) TFA and 1% (w/v) NaCl, as described previously (Brubaker & Vranic, 1987; Brubaker, 1988; Drucker & Brubaker, 1989). Media and cell extracts were then passed separately through a C18 cartridge (SepPak, Waters Associates, Milford, MA) and the adsorbed peptides were eluted with 80% (v/v) isopropanol containing 0.1% (v/v) TFA. We have previously demonstrated that greater than 88% of intact PGDPs are recovered using this methodology (Brubaker & Vranic, 1987; Brubaker, 1988). Eluates were stored at −20°C prior to analysis by HPLC and/or RIA.

HPLC was carried out using a Waters Associates C18 μBondapak column on a Waters Liquid Chromatography System. N-terminal PGDPs were separated using a 45 min linear gradient of 25–62.5% (v/v) Solvent B followed by a 10 min purge with 99% (v/v) Solvent B [Solvent A: 1% (v/v) TFA made to pH 2.5 with diethylamine; Solvent B: 80% (v/v) acetonitrile], as previously described (Brubaker *et al.*, 1992; Drucker *et al.*, 1992; Drucker *et al.*, 1994; Ehrlich *et al.*, 1994). Iodinated glucagon (<300 c.p.m.) was added to all samples as an internal control. The elution positions of glucagon and oxyntomodulin were determined by comparison with those of synthetic standards, and that of glicentin by comparison with the results obtained by others using the same system (Kervran *et al.*, 1987). Different forms of GLP-1 were separated using a 30 min linear gradient of 45–68% (v/v) Solvent B, followed by a 10 min purge with 99% (v/v) Solvent B [Solvent A: 0.1% (v/v) phosphoric acid and 0.3% (v/v) triethylamine, made to pH 7.0 with NaOH; Solvent B: 60% (v/v) acetonitrile and 40% (v/v) Solvent A], as previously described (Brubaker *et al.*, 1992; Drucker *et al.*, 1992, 1994; Ehrlich *et al.*, 1994). Immediately prior to and following each set of samples, the elution positions of synthetic GLP-1 standard peptides (GLP-$1^{1\text{-}37}$, GLP-$1^{1\text{-}36NH2}$, GLP-$1^{7\text{-}37}$ and GLP-$1^{7\text{-}36NH2}$) were determined. As the peptide levels were low in media samples, HPLC analysis was only performed on cell extracts.

RIA for N-terminal PGDPs was carried out using 2 antisera: antiserum K4023 (Biospacific, Emeryville CA), which recognizes the mid-sequence of glucagon (e.g. glicentin, oxyntomodulin, glucagon and proglucagon) and thus detects all glucagon-like immunoreactive (GLI) peptides; and antiserum 04A (Dr R. Unger, Dallas TX), which cross-reacts with the free C-terminal end of glucagon, and thus detects immunoreactive glucagon (Brubaker & Vranic, 1987; Brubaker, 1988; Brubaker *et al.*, 1992; Drucker *et al.*, 1992, 1994; Ehrlich *et al.*, 1994). RIA for the free C-terminal end of GLP-1 was carried out using: antiserum b5 (a gift from Dr S. Mojsov, New York NY), which recognizes GLP-$1^{1\text{-}37}$ and GLP-$1^{7\text{-}37}$; and antiserum GLP-$1^{7\text{-}36NH2}$ (Affiniti Research, Nottingham UK), which cross-reacts with both GLP-$1^{1\text{-}36NH2}$ and GLP-$1^{7\text{-}36NH2}$ (Brubaker *et al.*, 1992; Drucker *et al.*, 1992, 1994; Ehrlich *et al.*, 1994).

Statistical significance was determined by ANOVA on a SAS (Statistical Analysis System, SAS Institute, Cary NC) program for IBM computers.

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References

Andreasen, J.J., Orskov, C. & Holst, J.J. (1994). *Digestion*, **55**, 221–228.

Brubaker, P.L. & Vranic, M. (1987). *Endocrinology*, **120**, 1976–1985.

Brubaker, P.L. (1988). *Endocrinology*, **123**, 220–226.

Brubaker, P.L., So, D.C.Y. & Drucker, D.J. (1989). *Endocrinology*, **124**, 3003–3009.

Brubaker, P.L. (1991). *Endocrinology*, **128**, 3175–3182.

Brubaker, P.L., Lee, Y.C. & Drucker, D.J. (1992). *J. Biol. Chem.*, **267**, 20728–20733.

Brubaker, P.L., Dhanvantari, S., Gronau, K.A., Huang, T.H.J., Jin, T., Roberge, J.N., Rocca, A.S., Seidah, N.G., Tucker, J.D., Young, T.A. & Drucker, D.J. (1994). *Intern Diabetes Fed.*, 08B1SY1304: (Abstract).

Drucker, D.J. & Brubaker, P.L. (1989). *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3953–3957.

Drucker, D.J., Lee, Y.C., Asa, S.L. & Brubaker, P.L. (1992). *Mol. Endocrinol.*, **6**: 2175–2184.

Drucker, D.J., Jin, T., Asa, S.L., Young, T.A. & Brubaker, P.L. (1994). *Mol. Endocrinol.*, **8**, 1646–1655.

Ehrlich, P., Tucker, D., Asa, S.L., Brubaker, P.L. & Drucker, D.J. (1994). *Am. J. Physiol.*, **267**, E662–E671.

Eipper, B.A., Glembotski, C.C. & Mains, R.E. (1983a). *J. Biol. Chem.*, **258**, 7292–7298.

Eipper, B.A., Mains, R.E. & Glembotski, C.C. (1983b). *Proc. Natl. Acad. Sci, USA*, **80**, 5144–5148.

Eipper, B.A., Green, C.B.-R., Campbell, T.A., Stoffers, D.A., Keutmann, H.T., Mains, R.E. & Ouafik, L'H. (1992). *J. Biol. Chem.*, **267**, 4008–4015.

Elliott, R.M., Morgan, L.M., Tredger, J.A., Deacon, S., Wright, J. & Marks, V. (1993). *J. Endocrinol.*, **138**, 159–166.

Glembotski, C.C. (1986). *Endocrinology*, **118**, 1461–1468.

Gutniak, M., Orskov, C., Holst, J.J., Ahrén, B. & Efendic, S. (1992). *N. Engl. J. Med.*, **326**: 1316–1322.

Herrmann, C., Voge, A. & Goke, B. (1993). *Digestion*, **54,** 367 (Abstract).
Kervran, A., Blache, P. & Bataille, D. (1987). *Endocrinology*, **121,** 704–713.
Kreymann, B., Ghatei, M.A., Williams, G. & Bloom, S.R. (1987). *Lancet* Dec 5; 1300–1304.
Martinez, A., Burrell, M.A., Kuijk, M., Montuenga, L.M., Treston, A., Cuttitta, F. & Polak, J.M. (1993). *J. Histochem. Cytochem.*, **41,** 1617–1622.
Mojsov, S., Heinrich, G., Wilson, I.B., Ravazzola, M., Orci, L. & Habener, J.F. (1986). *J. Biol. Chem.*, **261,** 11880–11889.
Mojsov, S., Weir, G.C. & Habener, J.F. (1987). *J. Clin. Invest.*, **79,** 616–619.
Mojsov, S., Kopczynski, M.G. & Habener, J.F. (1990). *J. Biol. Chem.*, **265,** 8001–8008.
Nathan, D.M., Schreiber, E., Fogel, H., Mojsov, S. & Habener, J.F. (1992). *Diabetes Care*, **15,** 270–276.
Orskov, C., Holst, J.J., Knuhtsen, S., Baldissera, F.G.A., Poulsen, S.S. & Nielsen, O.V. (1986). *Endocrinology*, **119,** 1467–1475.
Orskov, C., Holst, J.J., Seier Poulsen, S. & Kirkegaard, P. (1987). *Diabetologia*, **30,** 874–881.
Ouafik, L'H., Stoffers, D.A., Campbell, T.A., Johnson, R.C., Bloomquist, B.T., Mains, R.E. & Eipper, B.A. (1992). *Mol. Endocrinol.*, **6,** 1571–1584.
Pederson, R.A., Schubert, H.E. & Brown, J.C. (1975). *Diabetes*, **24,** 1050–1056.
Philippe, J., Drucker, D.J., Knepel, W., Jepeal, L., Misulovin, Z. & Habener, J.F. (1988). *Mol. Cell. Biol.*, **8,** 4877–4888.
Plaisancie, P., Bernard, C., Chayvialle, J.-A. & Cuber, J.-C. (1994). *Endocrinology*, **135,** 2398–2403.
Roberge, J.N. & Brubaker, P.L. (1991). *Endocrinology*, **128,** 3169–3174.
Roberge, J.N. & Brubaker, P.L. (1993). *Endocrinology*, **133,** 233–240.
Sakata, J., Mizuno, K. & Matsuo, H. (1986). *Biochem. Biophys. Res. Commun.*, **140,** 230–236.
Sarson, D.L., Wood, S.M., Holder, D. & Bloom, S.R. (1982). *Diabetologia*, **22,** 33–36.
Suzuki, S., Kawai, K., Ohashi, S., Mukai, H. & Yamashita, K. (1989). *Endocrinology*, **125,** 3109–3114.
Usdin, T.B., Mezey, E., Button, D.C., Brownstein, M.J. & Bonner, T.I. (1993). *Endocrinology*, **133,** 2861–2870.
Weir, G.C., Mojsov, S., Hendrick, G.K. & Habener, J.F. (1989). *Diabetes*, **38,** 338–342.