

Short Communication

Structure-Activity Relationships of Glucose-Dependent Insulinotropic Polypeptide (GIP)

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Six GIP_{1–30NH₂} analogs were synthesized with modifications (de-protonation, N-methylation, reversed chirality, and substitution) at positions 1, 3, and 4 of the N-terminus, and additionally, a cyclized GIP derivative was synthesized. The relationship between altered structure to biological activity was assessed by measuring receptor binding affinity and ability to stimulate adenylyl cyclase in CHO-K1 cells transfected with the wild-type GIP receptor (wtGIPR). These structure-activity relationship studies demonstrate the importance of the GIP N-terminus and highlight structural constraints that can be introduced in GIP analogs. These analogs may be useful starting points for design of peptides with enhanced *in vivo* bioactivity.

Key words: Chinese hamster ovary cells/Cyclic AMP/Enteroinsular axis/Gastric inhibitory polypeptide/Incretin/Radioligand binding competition.

Hormonal components of the enteroinsular axis that are released from the intestine in response to nutrient ingestion, and potentiate insulin secretion in the presence of elevated glucose, are classified as incretins. Gastric Inhibitory Polypeptide/Glucose-dependent Insulinotropic polypeptide (GIP) and the N-terminally truncated (t) forms of glucagon-like peptide-1 (tGLP-1), GLP-1_(7–37) and GLP-1_{(7–36)NH₂}, are considered to be the two most important incretins in humans and other species (Pederson, 1994; Kieffer and Habener, 1999). Incretins also increase insulin gene transcription and translation, as well as stimulate expression of hexose transporters and hexokinase

in the β -cell (Fehmann and Göke, 1995; Fehmann *et al.*, 1995; Wang *et al.*, 1996). While it is well established that incretins improve glucose tolerance *via* enhancement of insulin secretion, it is now apparent that insulin-independent pathways, such as suppression of glucagon release, enhancement of peripheral glucose uptake, and inhibition of gastric emptying also contribute to the antidiabetogenic action of these hormones (Pederson, 1994; Fehmann *et al.*, 1995; Kieffer and Habener, 1999; Holst and Ørskov, 2001).

To date, there has been a paucity of reports on structure-activity relationships of GIP. These studies have been limited primarily to fragment analysis (Hinke *et al.*, 2001 and references therein). Recently, we developed enzyme-resistant analogs of GIP by altering the amino-terminus (Kühn-Wache *et al.*, 2000), one of which was deemed suitable for *in vivo* analysis. Independently, we (Hinke *et al.*, 2002) and others (O'Harte *et al.*, 2000) have been able to show antidiabetic action of analogues of GIP with improved plasma stability using different approaches. Incorporation of D-Ala² during synthesis (Hinke *et al.*, 2002) or post-synthesis chemical glycation of Tyr¹ (O'Harte *et al.*, 2000) both resulted in superactive GIP analogs *in vivo*, likely due to resistance to the incretin inactivating enzyme, dipeptidyl peptidase (DP) IV. However, many peptides that showed resistance to degradation *in vitro* were unable to bind or activate the GIP receptor at comparable potency to the native hormone (Kühn-Wache *et al.*, 2000). The N-terminus of GIP appears to be particularly important for receptor activation, given that GIP_{6–30NH₂} is a potent receptor antagonist with similar binding affinity to native hormone (Gelling *et al.*, 1997), whereas GIP_{1–30NH₂} is equipotent to full-length GIP_{1–42} (Hinke *et al.*, 2001). Hence, detailed structure-function studies of GIP's amino-terminus are required to develop potent analogs for *in vivo* studies.

In the current study, we designed a series of synthetic peptides based on the human sequence of GIP_{1–30NH₂}, with single modifications to the amino-terminus: D-Tyr or desamino(Ppa)-Tyr at position one, L-Pro, D-Glu or L-(N-Me)Glu at position three, or D-Ala at position four. An additional cyclic peptide (cyclo[Lys¹⁶,Asp²¹]_{GIP_{1–30NH₂}) was also tested to establish the feasibility of introducing structural constraints while retaining receptor binding and activation. GIP and analogs were tested on CHO-K1 cells transfected with the rat pancreatic islet GIP receptor, wild-type (wt) GIPR cells, a well-characterized *in vitro* model. These GIP analogs may be useful starting points}

for the development of more potent metabolically stable insulinotropic peptides for use *in vivo*, and alone, serve to increase our understanding of structure-activity relationships of GIP.

Basal cyclic AMP levels in wtGIPR cells were 2.82 ± 0.16 fmol/1000 cells, and maximal GIP_{1-30NH2}-stimulated cAMP production reached 360 ± 39 fmol/1000

cells. Half-maximal cAMP production (EC_{50}) occurred at a concentration of 230 ± 39 pM of GIP_{1-30NH2} (Figure 1) and, in binding competition experiments, 50% of bound [¹²⁵I]-GIP was displaced by 3.75 ± 0.55 nM GIP (IC_{50} ; Figure 2). These values are equivalent to those measured using full-length GIP₁₋₄₂ (Hinke *et al.*, 2001), and are characteristic of wtGIPR cells.

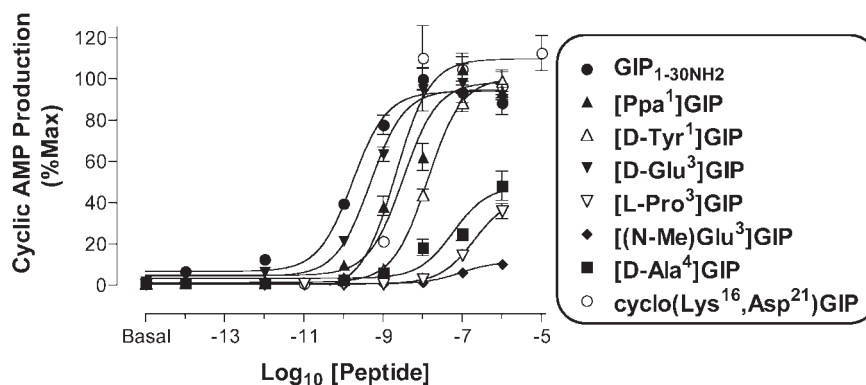


Fig. 1 Cyclic AMP Production in CHO-K1 Cells Stably Overexpressing the wtGIP Receptor (wtGIPR Cells) Stimulated by GIP_{1-30NH2} and Analogs.

Peptides were synthesized at Probiobdrug AG (Halle, Germany) or the peptide research facility at the Tulane School of Medicine (New Orleans, USA), using established Fmoc/solid phase methodology (Kühn-Wache *et al.*, 2000; Mellor *et al.*, 2000) on either a Milligen/Biosearch 9050 or a Rainin Symphony automated peptide synthesizer. Preparative HPLC (LiChrograph HPLC system; Merck-Hitachi) using CH₃CN/H₂O gradients with 0.1% trifluoroacetic acid purified peptides to >95%; analytical HPLC and mass spectrometry (MALDI-TOF) was used to confirm purity and identity of peptides (cyclo-GIP was additionally subjected to trypsin mapping to confirm correct cross-bridge formation) as previously described (Kühn-Wache *et al.*, 2000; Mellor *et al.*, 2000). Stimulation of cyclic AMP production in wtGIPR cells was performed exactly as previously described (Hinke *et al.*, 2001, 2002). Cells were seeded at 50 000 cells/well in 24-well plates (Falcon), and grown for two days (reaching a density of approximately $3-5 \times 10^5$ cells/well). Cells were washed twice with, and pre-incubated for one hour at 37 °C in 15 mM HEPES-buffered (pH 7.4) DMEM/F12 (Gibco-BRL) with 0.1% BSA (Sigma). Stimulation occurred for 30 min at 37 °C in the same buffer additionally supplemented with 0.5 mM IBMX (Research Biochemicals Int.) and the peptide concentrations shown. Cells were lysed in ice-cold 70% ethanol, dried, and cAMP content measured using an RIA kit (Bio-medical Technologies Inc.). Data are presented as mean \pm SEM of ≥ 3 individual experiments. Non-linear regression analysis was accomplished with the GraphPad Prism software package.

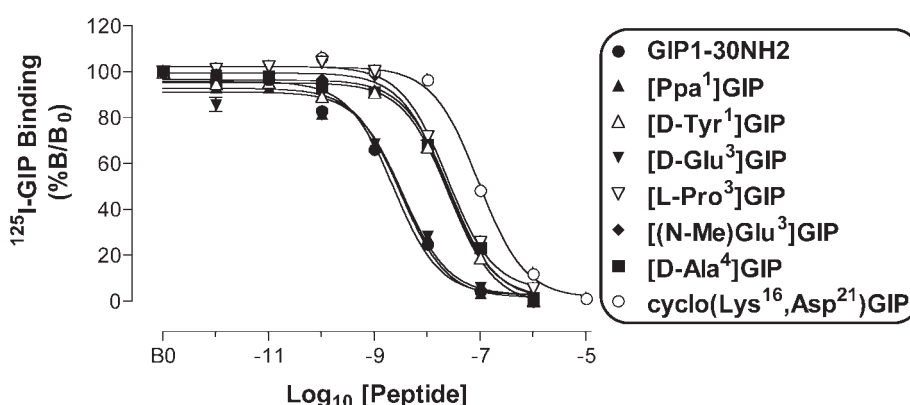


Fig. 2 Competitive Displacement of [¹²⁵I]-GIP Binding on wtGIPR cells by GIP_{1-30NH2} and Analogs.

Binding studies using HPLC-purified [¹²⁵I]-GIP, were performed essentially as described previously (Hinke *et al.*, 2001, 2002). Wild-type GIPR Cells ($3-5 \times 10^5$ /well) were washed twice at 4 °C in HEPES-buffered (pH 7.4) DMEM/F12 (Gibco-BRL), 0.1% BSA (Sigma), and 1% Trasylol (Bayer). They were incubated for 12–16 h at 4 °C with [¹²⁵I]-GIP (50 000 cpm) in the presence or absence of unlabeled GIP_{1-30NH2} or analog. Following incubation, cells were washed twice with ice cold buffer, solubilized with 0.1 M NaOH (1 ml), and transferred to culture tubes for counting of cell-associated radioactivity. Nonspecific binding was defined as that measured in the presence of 1 μ M GIP_{1-30NH2}, and specific binding expressed as percent of binding in the absence of competitor (%B/B₀). Data are presented as mean \pm SEM of ≥ 3 individual experiments. Note that error bars are present, but too small to be observed in most cases.

In general, the amino-terminus of GIP was fairly tolerant to amino acid substitution or modification, when examining binding affinity only. Desamidation of Tyr¹ by introduction of a 3-phenylpropionate (Ppa) moiety or altering the chirality of Glu³ produced peptides with no change in receptor affinity relative to native hormone. Changing the stereochemistry of amino acids 1 or 4 from levorotatory (L) to dextrorotatory (D), substitution of a proline residue or N-methylating glutamate in position 3 resulted in analogs with 6- to 8-fold reduced affinities (Figure 2, Table 1). However, when examining bioactivity at the GIP receptor, modification of the N-terminus can have dramatic effects. While binding affinity was unchanged for [Ppa¹]GIP or [D-Glu³]GIP, modest reductions (2- to 4-fold) in potency were observed, although maximal cAMP production was still attained (Figure 1, Table 1). However, for [D-Tyr¹]GIP, [L-Pro³]GIP, [L-(N-Me)Glu³]GIP and [D-Ala⁴]GIP, despite having similar reductions in receptor binding, potency was greatly reduced. [D-Tyr¹]GIP was able to stimulate maximal cyclic AMP, but showed an EC₅₀ value nearly 60 times greater than native hormone; [L-Pro³]GIP, [L-(N-Me)Glu³]GIP and [D-Ala⁴]GIP were unable to stimulate maximal cyclic AMP so EC₅₀ values could not be calculated for these analogues (Figure 1, Table 1).

The confirmation that the ubiquitous aminopeptidase, dipeptidyl peptidase IV, was the primary enzyme responsible for inactivation of incretins *in vitro* and *in vivo* (Mentlein *et al.*, 1993; Kieffer *et al.*, 1995; Pauly *et al.*, 1996; Pederson *et al.*, 1998; Pauly *et al.*, 1999), has resulted in the development of degradation resistant incretins for use in type II diabetes. Extensive study has only been performed on analogs of GLP-1 (Holst, 1999), likely because GIP was initially considered to lack bioactivity in type II diabetics (Holst *et al.*, 1997; Lynn *et al.*, 2001). However we have challenged the notion that this reduced activity translates into a lack of therapeutic potential for GIP (Hinke *et al.*, 2002), as bolus injections of GIP and DP IV-resistant GIP analogs display enhanced *in vivo* bioactivity in type II diabetic animal models (O'Harte

et al., 2000; Hinke *et al.*, 2002). The substrate specificity of DP IV requires a protonated α -amino group (P₂), that amino acid residues in the second (P₁) and third (P_{1'}) positions be L-isomers and the linkage between them (*i.e.* the scissile bond) be in the *trans* conformation; thus DP IV preferentially cleaves peptides with proline or alanine at position 2, provided amino acid 3 is not proline (Fischer *et al.*, 1983). We have previously examined the effect GIP position 2 substitution with respect to enzyme resistance, receptor binding and activation (Kühn-Wache *et al.*, 2000). The current work extends the structure-activity relationships by looking at modification of positions 1, 3 and 4 of GIP, and the effect of lactam cyclization.

The relation between DP IV resistance by amino acid substitution in GIP and retention of biological activity is currently unclear. This is important for determining which analogs warrant continuation of studies *in vivo*. Of previously published analogs to be tested for DP IV resistance, [D-Ala²]GIP was selected for *in vivo* studies, as it was completely resistant against DP IV and possessed minimal changes in receptor binding or activation parameters (Kühn-Wache *et al.*, 2000; Hinke *et al.*, 2002). Substitution of L-amino acids (Gly, Ser or Val) in position 2 of GIP similarly resulted in peptides with minor changes at the GIP receptor, but only moderately increased resistance to DP IV proteolysis (Kühn-Wache *et al.*, 2000). In the current report, [L-Pro³]GIP and [L-(N-Me)Glu³]GIP were the only peptides that were completely resistant to purified pork kidney DP IV *in vitro*, [D-Tyr¹]GIP, [Ppa¹]GIP, and [D-Glu³]GIP were moderately DP IV resistant, and [D-Ala⁴]GIP and cyclo[Lys¹⁶,Asp²¹]GIP were degraded at similar rates to native GIP by purified enzyme (Kühn-Wache *et al.*, 2000; K. Kühn-Wache and F. Rosche, unpublished data). Unfortunately, despite being completely resistant to DP IV (Kühn-Wache *et al.*, 2000), [L-Pro³]GIP and [L-(N-Me)Glu³]GIP displayed poor potency at the GIP receptor (Figures 1 and 2, Table 1). Similar findings, complete DP IV resistance but dramatically reduced bioactivity, were also shown for Tyr¹-Ala²Ψ(CH₂NH)-GIP_{3-30NH₂} (Kühn-Wache *et al.*, 2000). The remaining moderately re-

Table 1 Summary of Competitive Binding (IC₅₀) and Cyclic AMP Responses (EC₅₀ and % of Maximal GIP-Induced cAMP Production) of GIP Analogs.

Analog	IC ₅₀ (nM)	EC ₅₀ (nM)	cAMP (%max)
GIP _{1-30NH₂}	3.75 ± 0.55	0.230 ± 0.039	100
[Ppa ¹]GIP _{1-30NH₂}	4.85 ± 1.33	0.930 ± 0.143	107.4 ± 7.7
[D-Tyr ¹]GIP _{1-30NH₂}	29.30 ± 6.8*	13.600 ± 0.7*	113.6 ± 4.8
[D-Glu ³]GIP _{1-30NH₂}	3.84 ± 0.55	0.469 ± 0.126	103.5 ± 7.1
[L-Pro ³]GIP _{1-30NH₂}	25.10 ± 3.6*	ND	36.0 ± 6.4*
[L-(N-Me)Glu ³]GIP _{1-30NH₂}	23.60 ± 2.8*	ND	10.5 ± 0.4*
[D-Ala ⁴]GIP _{1-30NH₂}	30.70 ± 6.6*	ND (~1 μM)	50.9 ± 7.6*
Cyclo[Lys ¹⁶ ,Asp ²¹]GIP _{1-30NH₂}	94.90 ± 7.9*	3.110 ± 1.43*	112.7 ± 14.7

IC₅₀ and EC₅₀ values were determined by nonlinear regression analysis (n = 3–7).

ND = Not determined (half maximal stimulation was not achieved).

*Value differs from responses to GIP_{1-30NH₂} by at least $p < 0.05$ as determined by one way ANOVA.

sistant GIP analogues with minor effects on receptor activity reported here and elsewhere (Kühn-Wache *et al.*, 2000) are unlikely to exhibit dramatically improved potencies *in vivo*. Preliminary studies on [L-Ser²]GIP in rats indicated that this peptide is moderately more potent than native GIP when measuring reductions in glycemic excursions following an oral glucose tolerance test with a concurrent subcutaneous bolus peptide injection (S. Hinke, unpublished data).

Cyclic analogs of small peptide hormones generated by lactam bridge formation between endogenous or substituted Lys and Asp or Glu residues have been reported in the literature (Bitar *et al.*, 1994; Parker *et al.*, 1998; Barbier *et al.*, 2000; Ahn *et al.*, 2001). To our knowledge the current study is the first report of a cyclic GIP analog; this peptide has allowed testing of the effects of further structural constraints on the molecule. By creating a lactam bridge between Lys¹⁶ and Asp²¹, a derivative of GIP was generated with a six amino acid ring, spanning the predicted hinge-domain within the alpha-helical binding core. Remarkably, this peptide was able to stimulate maximal cyclic AMP production, with an increase in EC₅₀ of one order of magnitude relative to native hormone (Figure 1, Table 1). In contrast, the binding affinity of cyclo[Lys¹⁶,Asp²¹]GIP was disproportionately reduced, resulting in an IC₅₀ value for cyclized GIP that was 25.3-times higher than the unmodified peptide (Figure 2, Table 1). Results from cyclo[Lys¹⁶,Asp²¹]GIP have shown the feasibility of using cyclic incretin analogs, as well as confirming hypotheses generated from previous structure-activity relationship studies of GIP. It was shown earlier that the high affinity binding domain resides in the predicted core α -helical domain between residues 6 and 30 of GIP; however, two dissociable bioactive domains were discovered: GIP₁₋₁₄ and GIP₁₉₋₃₀, both of which also possessed weak binding ability (Hinke *et al.*, 2001). Solution structure data for related hormones have shown the presence of a common 16–20 amino acid helical core, either as a continuous helix or with a two amino acid hinge region (Hinke *et al.*, 2001 and references therein). Hence, the lactam bridge in cyclo[Lys¹⁶,Asp²¹]GIP may interfere with receptor binding (Figure 2, Table 1) either by disrupting helix formation or interfering with the flexibility of the hinge domain, without causing proportional reductions in bioactivity (Figure 1).

In a recently published report, using GIP receptor null mice, it was demonstrated that chronic removal of GIP action prevented obesity (Miyawaki *et al.*, 2002), fueling interest in development of GIP antagonists. Our previously published structure-activity studies of GIP extensively examined synthetic peptide antagonists of the GIP receptor, which may exert anti-obesity effects in other experimental models. In general, antagonist potency was directly proportional to ligand binding affinity, and inversely proportional to maximal cAMP production (Gelling *et al.*, 1997; Hinke *et al.*, 2001, 2002). As such, peptides with high receptor affinity but low cAMP stimulating potency may be used as receptor antagonists, such as the DP IV

resistant analogs [Tyr¹-Ala² Ψ (CH₂NH)]GIP, [L-Pro³]GIP and [L-(N-Me)Glu³]GIP (Kühn-Wache *et al.*, 2000; and the current report). Indeed, during preparation of this manuscript, the research group of Flatt and O'Harte reported the use of [L-Pro³]GIP as an antagonist of the GIP receptor (Gault *et al.*, 2002), however, it should be emphasized that these peptides are not pure antagonists, but rather weak full agonists or partial agonists (Figure 1, Table 1) with significant biological activity at high concentrations (11–36% maximal cAMP production); under appropriate conditions they may serve as weak competitive receptor antagonists. Hence, high affinity GIP peptide fragments showing little or no cAMP production are likely more suitable for *in vivo* antagonism studies (Tseng *et al.*, 1996; Gelling *et al.*, 1997; Hinke *et al.*, 2001, 2002) than the low potency/high affinity agonists described here; furthermore, we have shown that the GIP receptor antagonist, GIP_{7-30NH₂} (Tseng *et al.*, 1996), is also a substrate for DP IV (Kühn-Wache *et al.*, 2000), and thus GIP_{6-30NH₂} would likely serve as a more potent antagonist for *in vivo* studies (Gelling *et al.*, 1997).

In conclusion, we have reported the characteristics of a series of synthetic GIP analogs with modifications in the N-terminal 1–4 amino acids, and additionally a cyclic GIP derivative. These peptides all showed variable resistance to enzymatic degradation *in vitro*. The only completely resistant peptides, [L-Pro³]GIP and [L-(N-Me)Glu³]GIP, unfortunately displayed poor bioactivity, but may function as weak competitive receptor antagonists. Hence, only the moderately enzyme-resistant [Ppa¹]GIP and [D-Glu³]GIP, with minor receptor potency alterations, have any promise for *in vivo* studies, with similar unpredictable *in vivo* potency as [Gly²], [Ser²] and [Val²] substituted GIP analogs.

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References

- Ahn, J.M., Gitu, P.M., Medieros, M., Swift, J.R., Trivedi, D., and Hruby, V.J. (2001). A new approach to search for the bioactive conformation of glucagon: positional cyclization scanning. *J. Med. Chem.* 44, 3109–3116.
- Barbier, J.R., MacLean, S., Morley, P., Whitfield, J.F., and Willick, G.E. (2000). Structure and activities of constrained analogues of human parathyroid hormone and parathyroid hormone-related peptide: implications for receptor-activating conformations of hormones. *Biochemistry* 39, 14522–14530.
- Bitar, K.G., Somogyvari-Vigh, A., and Coy, D.H. (1994). Cyclic lactam analogues of ovine pituitary adenylate cyclase activat-

- ing polypeptide (PACAP): discovery of potent type II receptor antagonists. *Peptides* 15, 461–466.
- Fehmann, H.-C., and Göke, B. (1995). Characterization of GIP(1–30) and GIP(1–42) as stimulators of proinsulin gene transcription. *Peptides* 16, 1149–1152.
- Fehmann, H.-C., Göke, R., and Göke, B. (1995). Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocrine Rev.* 16, 390–410.
- Fischer, G., Heins, J., and Barth, A. (1983). The conformation around the peptide bond between the P₁- and P₂-positions is important for catalytic activity of some proline-specific proteases. *Biochim. Biophys. Acta* 742, 452–462.
- Gault, V.A., O'Harte, F.P., Harriot, P., and Flatt, P.R. (2002). Characterization of the cellular and metabolic effects of a novel enzyme-resistant antagonist of glucose-dependent insulinotropic polypeptide. *Biochem. Biophys. Res. Commun.* 290, 1420–1426.
- Gelling, R.W., Coy, D.H., Pederson, R.A., Wheeler, M.B., Hinke, S., Kwan, T., and McIntosh, C.H.S. (1997). GIP_{6–30amide} contains the high affinity binding region of GIP and is a potent inhibitor of GIP_{1–42} action *in vitro*. *Regul. Pept.* 69, 151–154.
- Hinke, S.A., Manhart, S., Pamir, N., Demuth, H.-U., Gelling, R.W., Pederson, R.A., and McIntosh, C.H.S. (2001). Identification of a bioactive domain in the amino-terminus of glucose-dependent insulinotropic polypeptide. *Biochim. Biophys. Acta* 1547, 143–155.
- Hinke, S.A., Gelling, R.W., Pederson, R.A., Manhart, S., Nian, C., Demuth, H.-U., and McIntosh, C.H.S. (2002). Dipeptidyl peptidase IV-resistant [D-Ala²]glucose-dependent insulinotropic polypeptide (GIP) improves glucose tolerance in normal and obese diabetic rats. *Diabetes* 51, 652–661.
- Holst, J.J. (1999). Glucagon-like peptide-1, a gastrointestinal hormone with a pharmaceutical potential. *Curr. Med. Chem.* 6, 1005–1017.
- Holst, J.J., and Ørskov, C. (2001). Incretin hormones - an update. *Scand. J. Clin. Lab. Invest. (Suppl.)* 234, 75–85.
- Holst, J.J., Gromada, J., and Nauck, M.A. (1997). The pathogenesis of non-insulin dependent diabetes mellitus involves a defective expression of the GIP receptor. *Diabetologia* 40, 984–986.
- Kieffer, T.J., and Habener, J.F. (1999). The glucagon-like peptides. *Endocrine Rev.* 20, 876–913.
- Kieffer, T.J., McIntosh, C.H.S., and Pederson, R.A. (1995). Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. *Endocrinology* 136, 3585–3596.
- Kühn-Wache, K., Manhart, S., Hoffmann, T., Hinke, S.A., Gelling, R., Pederson, R.A., McIntosh, C.H.S., and Demuth, H.-U. (2000). Synthesis of analogs of glucose-dependent insulinotropic polypeptide with increased dipeptidyl peptidase IV resistance. *Adv. Exp. Med. Biol.* 477, 187–195.
- Lynn, F.C., Pamir, N., Ng, E.H.C., McIntosh, C.H.S., Kieffer, T.J., and Pederson, R.A. (2001). Defective glucose-dependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 50, 1004–1011.
- Mellor, S.L., Wellings, D.A., Fehrentz, J.A., Paris, M., Martinez, J., Ede, N.J., Bray, A.M., Evans, D.J., and Bloomberg, G.B. (2000). Synthesis of modified peptides. In: *Fmoc Solid Phase Peptides Synthesis - A Practical Approach*, W. Chan and P. White, eds. (New York, USA: Oxford University Press), pp. 137–181.
- Mentlein, R., Gallwitz, B., and Schmidt, W.E. (1993). Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1 (7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* 214, 829–835.
- Miyawaki, K., Yamada, Y., Ban, N., Ihara, Y., Tsukiyama, K., Zhou, H., Fujimoto, S., Oku, A., Tsuda, K., Toyokuni, S. *et al.* (2002). Inhibition of gastric inhibitory polypeptide signalling prevents obesity. *Nature Med.* 8, 738–742.
- O'Harte, F.P.M., Mooney, M.H., Kelly, C.M.N., and Flatt, P.R. (2000). Improved glycaemic control in obese diabetic ob/ob mice using N-terminally modified gastric inhibitory polypeptide. *J. Endocrinol.* 165, 639–648.
- Parker, J.C., Andrews, K.M., Rescek, D.M., Masefski, W.J., Andrews, G.C., Contillo, L.G., Stevenson, R.W., Singleton, D.H., and Suleske, R.T. (1998). Structure-function analysis of a series of glucagon-like peptide-1 analogs. *J. Peptide Res.* 52, 398–409.
- Pauly, R.P., Demuth, H.-U., Rosche, F., Schmidt, J., White, H.A., Lynn, F., McIntosh, C.H.S., and Pederson, R.A. (1999). Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor ile-thiazolidide. *Metabolism* 48, 385–389.
- Pauly, R.P., Rosche, F., Wermann, M., McIntosh, C.H.S., Pederson, R.A., and Demuth, H.-U. (1996). Investigation of GIP_{1–42} and GLP-1 7–36 degradation *in vitro* by dipeptidyl peptidase IV (DPIV) using Matrix-Assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS): a novel kinetic approach. *J. Biol. Chem.* 271, 23222–23229.
- Pederson, R.A. (1994). Gastric inhibitory polypeptide. In: *Gut Peptides: Biochemistry and Physiology*, J. Walsh and G. Dockray, eds. (New York, USA: Raven Press Ltd.), pp. 217–259.
- Pederson, R.A., White, H.A., Schlenzig, D., Pauly, R.P., McIntosh, C.H.S., and Demuth, H.-U. (1998). Improved glucose tolerance in Zucker fatty rats by oral administration of the dipeptidyl peptidase IV inhibitor isoleucine thiazolidide. *Diabetes* 47, 1253–1258.
- Tseng, C.C., Kieffer, T.J., Jarboe, L.A., Usdin, T.B., and Wolfe, M.M. (1996). Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP). Effect of a specific glucose-dependent insulinotropic polypeptide receptor antagonist in the rat. *J. Clin. Invest.* 98, 2440–2445.
- Wang, Y., Montrose-Rafizadeh, M., Adams, L., Raygada, M., Nativ, O., and Egan, J.M. (1996). GIP regulates glucose transporters, hexokinases, and glucose induced insulin secretion in RIN 1046–38 cells. *Mol. Cell Endocrinol.* 116, 81–87.

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