Biological Effects and Metabolic Rates of Glucagonlike Peptide-1 7–36 Amide and Glucagonlike Peptide-1 7–37 in Healthy Subjects Are Indistinguishable

CATHRINE ØRSKOV, ANDRÉ WETTERGREN, AND JENS J. HOLST

The biological effects and the metabolism of the intestinal hormone glucagonlike peptide-1 7–36 amide and glucagonlike peptide-1 7–37 were studied in normal healthy subjects. GLP-1 7–36 amide and GLP-1 7–37 equipotently stimulated insulin secretion (integrated hormone response 0–60 min, 631 ± 211 vs. 483 ± 177 pmol/h × L^{-1}) and C-peptide secretion (integrated hormone response 9064 ± 1804 vs. 9954 ± 2031 pmol/h × L^{-1}) and equipotently lowered plasma glucose (integrated decrease 48.3 ± 5.7 vs. 46.2 ± 8.4 mmol/h × L^{-1}) and plasma glucagon (integrated decrease 80.4 ± 24.3 vs. 156.0 ± 34.6 pmol/h × L^{-1}). Both GLP-1 7–36 amide and GLP-1 7–37 lowered the plasma concentration of free fatty acids significantly. The plasma half-lives of GLP-1 7–36 amide and GLP-1 7–37 were 5.3 ± 0.4 vs. 6.1 ± 0.8 min, and the metabolic clearance rates of the two peptides also were similar (14.6 ± 2.4 vs. 12.2 ± 1.0 pmol/kg × min). In conclusion, COOH-terminal amidation is neither important for the metabolism of GLP-1 nor for its effects on the endocrine pancreas. *Diabetes* 42:658–61, 1993

The posttranslational processing of PG in the small intestine gives rise to GLP-1, which has been shown to have profound effects on the endocrine pancreas in many species (1–5). GLP-1 is secreted in humans in response to physiological stimuli, e.g., a mixed meal (4). The exact structure of GLP-1 has been a matter of some debate. A GLP-1 molecule corresponding to PG 78–107 amide has been isolated from human small intestine and sequenced (6). This peptide has been designated GLP-1 7–36 amide. As in other peptides, the COOH-terminal amide is derived from a Gly residue positioned next to the COOH-terminus, catalyzed by the so-called amidation enzyme, PAM (7,8). Because COOH-terminal Gly-extended intermediates frequently are found together with the amidated peptides, it is conceivable that smaller amounts of a Gly-extended GLP-1 corresponding to PG 78–108, also called GLP-1 7–37, may be found in humans. Thus, in the rat intestine, 66% of the intestinal GLP-1 immunoreactivity has been shown by chromatography to correspond to GLP-1 7–36 amide and 33% to GLP-1 7–37 (9).

Both GLP-1 7–36 amide and the Gly-extended GLP-1 7–37 have been shown previously to stimulate insulin secretion in humans (2,4,10–13). Furthermore, GLP-1 7–36 amide has been shown to inhibit glucagon secretion in humans (4,13), whereas the effect of GLP-1 7–37 on glucagon is unknown. Because of the effects on insulin secretion and glucagon secretion, both peptides have been suggested as a possible treatment for NIDDM (11–14).

A direct comparison of the biological effects and the metabolism of the two peptides, however, has not been made. We therefore studied the effects and the metabolism of GLP-1 7–36 amide and GLP-1 7–37 in healthy volunteer subjects.

RESEARCH DESIGN AND METHODS

The study was approved by the local ethical committee of Copenhagen on 19 February 1992. Six healthy volunteer subjects, 4 men and 2 women, participated in the study. They were 26 ± 2 yr of age and weighed 62 ± 2 kg (mean ± SE). Written informed consent was obtained from all subjects before the study.

Peptides. GLP-1 7–36 amide and GLP-1 7–37 were purchased from Peninsula (code 7241 and 7123, Mer-

From the Department of Clinical Biochemistry and the Department of Gastrointestinal Surgery C, Rigshospitalet; and the Institute of Medical Physiology C, Panum Institute, Copenhagen, Denmark.

Address correspondence and reprint requests to Dr. Cathrine Ørskov, Department of Medical Anatomy B, Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark.

Received for publication 29 October 1992 and accepted in revised form 23 December 1992.

GLP-1, glucagonlike peptide-1; PG, proglucagon; FFA, free fatty acid; NIDDM, non-insulin-dependent diabetes mellitus; RIA, radioimmunoassay; ANOVA, analysis of variance.

MPI EXHIBIT 1052 PAGE 1

seyside, St. Helens, UK). The peptides were dissolved in 0.9% saline containing 1% human serum albumin (Novo Nordisk, Gentofte, Denmark), subjected to sterile filtration, and kept at −20°C until use.

The volunteer subjects were studied after an overnight fast. Cannulas (Venflon, Viggo Products, Helsingborg, Sweden) were placed in antecubital veins—one for infusion and one for blood sampling. The cannulas were kept patent with saline. The two peptides were infused on separate days at least 1 wk apart. After a 20-min basal period, a 60-min continuous infusion of either GLP-1 7–36 amide or GLP-1 7–37, 1.5 pmol/kg per min, was started. Blood was drawn in the basal state, during the infusion, and up to 2 h after termination of the infusion into heparinized tubes, 15000 IE/L for insulin measurements and into tubes containing EDTA, 3.9 mM, and aprotinine 500,000 KIU/L, for glucagon, GLP-1, C-peptide, FFAs, and plasma glucose measurements.

RIAs. GLP-1 immunoreactivity was measured with either synthetic GLP-1 7–36 amide or GLP-1 7–37 as standards (Peninsula), ^{125}I-labeled GLP-1 7–36 amide, and antiserum 2135 as described previously (15). Pancreatic glucagon was measured using antiserum 4305, which recognizes the COOH-terminus of glucagon, purified porcine glucagon (Novo, Bagsværd, Denmark), and ^{125}I-labeled glucagon (gift from Novo) as described previously (16). Insulin was measured as described previously (17). C-peptide was determined with a commercial RIA kit (C-peptide kit, Novo Nordisk a/s, Bagsværd, Denmark). FFAs were determined with a commercial kit (Nefa C kit, Wako Chemicals GmbH, Germany). Plasma glucose was determined by the glucose oxidase method. The plasma half-lives of the two GLPs were calculated after linear transformation of the elimination curves in a semilogarithmic system. The metabolic clearance rates were calculated as metabolic clearance rate = infusion rate (pmol/kg × min)/increase in GLP-1 concentration above basal (pmol/ml).

Statistical analysis. Data are presented as means ± SE for $n = 6$ unless otherwise stated. The significance of differences was evaluated by Wilcoxon's matched-pairs signed-ranks test for paired data. $P < 0.05$ was considered significant. Integrated incremental responses were determined by calculation of the area under the curve with the trapezoidal rule. ANOVA followed by Newman-Keul's Multiple Range test was used for evaluation of changes as function of time.

RESULTS

The two peptides were infused in approximately equimolar amounts as estimated by RIA of infusion samples (232 ± 21 [GLP-1 7–36 amide] vs. 193 ± 16 [GLP-1 7–37] nM, $P > 0.05$), which resulted in similar elevations of the plasma concentration of the two peptides (Fig. 1). The biological effects of the peptides also were extremely similar (Fig. 2, Table 1). Both GLP-1 7–36 and GLP-1 7–37 significantly stimulated insulin and C-peptide secretion and significantly decreased plasma glucose and the glucagon concentrations (Fig. 2). Neither the integrated insulin responses nor the integrated C-peptide responses (Table 1) to the two different synthetic GLP-1 molecules differed significantly. Likewise, the effect on plasma glucose and glucagon (Table 1) was not statistically different. Both GLP-1 7–36 amide and GLP-1 7–37 significantly lowered the concentrations of FFAs (Fig. 3). The half-lives of GLP-1 7–36 amide and GLP-1 7–37 were 5.3 ± 0.4 vs. 6.1 ± 0.8 min ($P > 0.05$). The metabolic clearance rates of GLP-1 7–36 amide and GLP-1 7–37 were 14.6 ± 2.4 and 12.2 ± 1.0 pmol/(kg × min ($P > 0.05$).

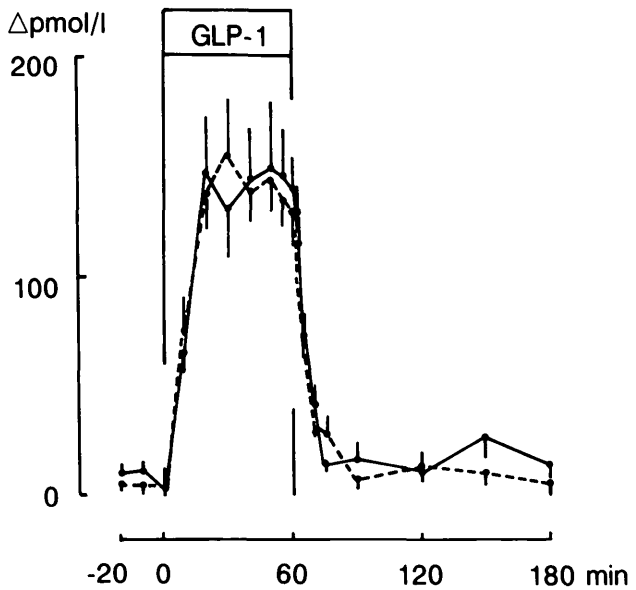

FIG. 1. Incremental plasma concentrations of GLP-1 during and after infusion of synthetic GLP-1 7–36 amide (———) or GLP-1 7–37 (– – – –). Incremental concentrations (pM) are plotted against time ($n = 6$).

DISCUSSION

In this study we found that both the metabolic rate and the biological effects of GLP-1 7–36 amide and GLP-1 7–37 in normal subjects are identical. The question of whether human intestinal GLP-1 is amidated or not has

TABLE 1
Comparison of integrated incremental hormone responses (time 0 to 60 min) to GLP-1 7–36 NH_2 and GLP-1 7–37

	GLP-1 7–36 NH_2	GLP-1 7–37	*P* values*
Glucose (mmol/h × 1^{-1})	48.3 ± 5.7	46.2 ± 8.4	NS
Insulin (pmol/h × 1^{-1})	631 ± 211	483 ± 177	NS
C-peptide (pmol/h × 1^{-1})	9064 ± 1804	9954 ± 2031	NS
Glucagon (pmol/h × 1^{-1})	80.4 ± 24.3	156.0 ± 34.6	NS

Data are means ± SE. $n = 6$.
*$P > 0.05$.

MPI EXHIBIT 1052 PAGE 2

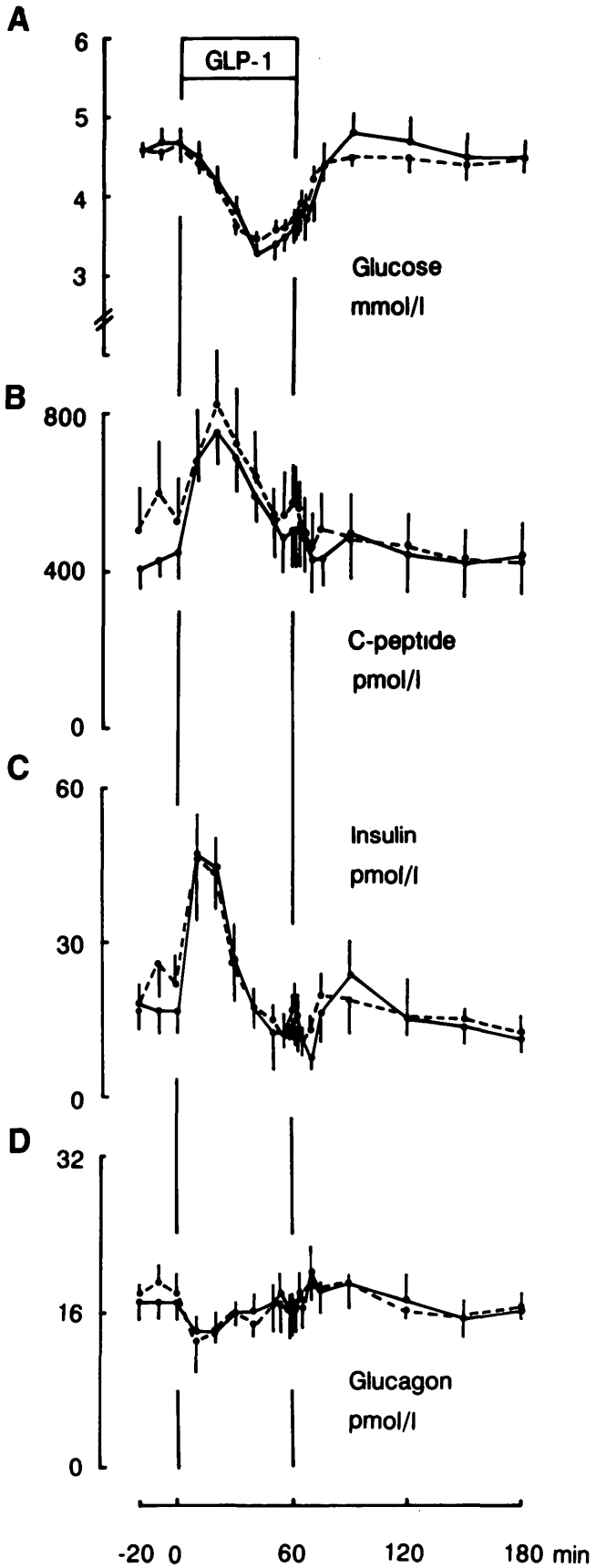

FIG. 2. Plasma concentrations of glucose (*A*), C-peptide (*B*), insulin (*C*), and glucagon (*D*) before, during, and after intravenous infusion of either GLP-1 7–36 amide (———) or GLP-1 7–37 (– – – –). Mean concentrations (mM ± SE or pM ± SE) are plotted against time (n = 6). The framed area shows the infusion period.

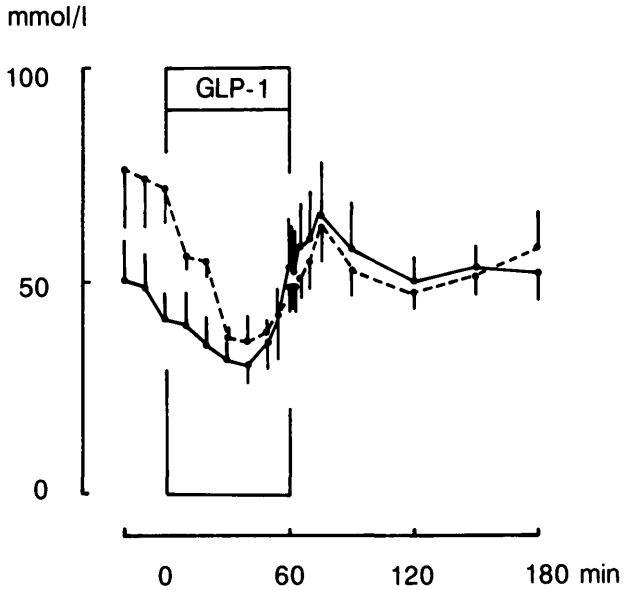

FIG. 3. Plasma concentrations of FFAs before, during, and after intravenous infusion of either GLP-1 7–36 amide (———) or GLP-1 7–37 (– – – –). Mean concentrations (mM ± SE) are plotted against time (n = 6).

been a matter of debate. The background for this is the structure of the GLP-1 precursor, PG. In PG, the GLP-1 sequence is flanked at its COOH-terminus by a pair of basic amino acids, potential posttranslational cleavage sites (1). However, the dibasic amino acids are preceded by the amino acid, Gly, a known amidation signal (18). It has been shown previously that amidation of peptides proceeds via Gly-extended precursors (7). Furthermore, enzymes capable of converting Gly-extended peptides to amidated products have been identified (8,18,19). For most peptide systems in which amidation occurs, small amounts of Gly-extended precursors remain unprocessed (20). For instance, 5–10% of the gastrin molecules stored in the porcine antrum have been shown to be Gly extended (21). Mojsov et al. (3) reported that up to 33% of the GLP-1 immunoreactivity in a rat small intestine chromatographically coeluted with Gly-extended GLP-1. It is not yet known, however, how much Gly-extended GLP-1 is produced in the human small intestine. Presumably, both peptides are present with a preponderance of the amidated form.

The amounts of GLP-1 infused in the healthy subjects caused an increase in the plasma concentration of ~150 pM. This increase is three- to fourfold larger than the increase in plasma concentration seen after a mixed meal in normal subjects (4,12). However, we chose to infuse this slightly supraphysiological dose to facilitate the determination of the plasma half-lives and the metabolic clearance rates of the two peptides.

The effects of GLP-1 7–36 amide and GLP-1 7–37 on

MPI EXHIBIT 1052 PAGE 3

insulin secretion, C-peptide, and glucagon were identical. The inhibitory effect of GLP-1 7–37 on glucagon secretion has not been reported previously in humans.

Both GLP-1 7–36 amide and GLP-1 7–37 decreased the plasma glucose concentration significantly and with equal strength. We noted that the plasma glucose did not drop below an average of 3.3 mM in spite of continued infusion of supraphysiological amounts. The reason for this seems to be that the effect of GLP-1 on the secretion of pancreatic glucoregulatory hormones is glucose dependent. The glucose dependency of GLP-1 7–37 in humans has been described previously by Andersen et al. (22) and Nathan et al. (11). Thus, the insulinotropic effect of GLP-1 is potentiated at increasing blood glucose levels, whereas the glucagon inhibitory effect is more marked at lower blood glucose levels. If GLP-1 is considered as a possible future treatment for diabetes, this is interesting, because it suggests that the risk that GLP-1 administration leads to dangerous hypoglycemia is likely to be extremely small (10–13).

Both GLP-1 7–36 amide and GLP-1 7–37 decreased FFA levels significantly. This has not been reported previously. The basal levels of FFAs, however, were somewhat different on the two study days, making it difficult to directly compare the responses with the two GLP-1 molecules. Faulkner et al. (23) have shown previously that GLP-1 7–36 amide lowered FFA concentrations in fasted sheep. Furthermore, Mérida et al. (24) have reported the presence of specific GLP-1 7–36 amide receptors on isolated human fat cell membranes.

In this study, we found that the half-life of GLP-1 7–36 amide was 5.3 ± 0.4 min, which is similar to the half-life of 4 min reported previously by Kreymann et al.(4) and similar to the half-life of the related hormone glucagon. The half-life of GLP-1 7–37 was 6.1 ± 0.8 min, which is very similar to that of GLP-1 7–36 amide. The half-life of GLP-1 7–37 in humans has not been reported previously. The metabolic clearance rate of GLP-1 7–36 amide was 14.6 ± 2.4 ml/(kg × min), which is consistent with the value of 12 ml/(kg × min) reported previously by Kreymann et al. (4) and with the value of 13 ± 3 ml/(kg × min by Scholdager et al. (25). A similar clearance rate for GLP-1 7–37 was found in this study (12.2 ± 1.0 ml/(kg × min). The clearance rate of GLP-1 7–37 has not been reported previously.

In conclusion, the COOH-terminal amidation of GLP-1 7–36 amide is neither crucial for the effect nor for the metabolism of GLP-1 in humans.

ACKNOWLEDGMENTS

This study was supported by the Danish Medical Research Council.

We thank Lisbet Mardrup for excellent technical assistance.

REFERENCES

1. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC: Exon duplication and divergence in the human preproglucagon gene. *Nature* (Lond) 304:368–71, 1983
2. Holst JJ, Ørskov C, Nielsen OV, Schwartz TW: Truncated glucagon-like peptide-1, an insulin-releasing hormone from the distal gut. *FEBS Lett* 211:169–74, 1987
3. Mojsov S, Weir GC, Habener JF: Insulinotropin: glucagon-like peptide-I (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 79:616–19, 1987
4. Kreymann B, Ghatei MA, Williams G, Bloom SR: Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* 2:1300–303, 1987
5. Komatsu R, Matsuyama T, Namba M: Glucagonostatic and insulinotropic action of glucagonlike peptide-I-(7–36)-amide. *Diabetes* 38:902–905, 1989
6. Ørskov C, Bersani M, Johnsen AJ, Højrup P, Holst JJ: Complete sequences of glucagon-like peptide-1 from human and pig small intestine. *J Biol Chem* 264:12826–29, 1989
7. Suchanek G, Kreil G: Translation of melittin messenger RNA in vitro yields a product terminating with glutaminyl-glycine rather than with glutamineamide. *Methods Enzymol* 106:218–23, 1977
8. Eipper BA, Mains RE, Glembotski CC: Identification in pituitary tissue of a peptide α-amidation activity that acts on glycine-extended peptides and requires molecular oxygen, copper, and ascorbic acid. *Proc Natl Acad Sci USA* 80:5144–48, 1983
9. Mojsov S, Kopczynski MG, Habener JF: Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. *J Biol Chem* 265:8001–8008, 1990
10. Andersen DK, Meneilly GS, Sclater A: The insulinotropic effect of GIP: a dose response comparison and comparison to glucagon-like peptide-1 (7–37) amide (GLP) (Abstract). *Diabetes* 39 (Suppl. 1):59, 1990
11. Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF: Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects. *Diabetes Care* 15:270–76, 1992
12. Gutniak M, Ørskov C, Holst JJ, Ahrén B, Efendic S: Anti-diabetogenic effect of glucagon-like peptide-1 (7–36) amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326: 1316–22, 1992
13. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W: Preserved incretin activity of glucagon-like peptide-1 (GLP-1) 7–36 amide but not of synthetic human gastric inhibitory polypeptide (GIP) in patients with type II diabetes mellitus. *J Clin Invest*. In press
14. McAloon-Dyke M, Fukagawa NK, Habener JF, Andersen DK, Elahi D: The insulinotropic effect of GIP and GLP-1 in healthy and diabetic subjects (Abstract). *Regul Pept* 40:205, 1992
15. Ørskov C, Jeppesen J, Madsbad S, Holst JJ: Proglucagon products in plasma in non-insulin-dependent diabetics and non-diabetic controls in the fasting state and following oral glucose and intravenous arginine. *J Clin Invest* 87:415–23, 1990
16. Holst JJ: Evidence that glicentin contains the entire sequence of glucagon. *Biochem J* 187:337–43, 1980
17. Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME: Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem*. In press
18. Yoo OJ, Powell CT, Agarwal KL: Molecular cloning and nucleotide sequence of full-length cDNA coding for porcine gastrin. *Proc Natl Acad Sci USA* 79:1049–53, 1982
19. Bradbury AF, Finnie MDA, Smyth DG: Mechanism of C-terminal amide formation by pituitary enzymes. *Nature* (Lond) 298:686–88, 1982
20. Bradbury AF, Smyth DG: Biosynthesis of the C-terminal amide in peptide hormones. *Biosci Rep* 7:907–16, 1987
21. Hilsted L: Review: Glycine-extended gastrin precursors. *Regul Pept* 36:323–43, 1991
22. Andersen DK, Meneilly GS, Sclater A, Wong G, McAloon-Dyke M, Mojsov S, Habener JF, Elahi D: The insulinotropic effect of GIP: a dose response comparison and comparison to glucagon-like peptide-1 (7–37) amide (GLP) (Abstract). *Diabetes* 39 (Suppl. 1):142A, 1990
23. Faulkner A, Pollock HT: Effects of truncated glucagon-like peptide-1 on the responses of starved sheep to glucose. *J Endocrinol* 129: 55–58, 1991
24. Mérida E, Delgado E, Molina LM, Villanueva-Penacarrillo E, Valverde I: Presence of glucagon and GLP-1 (7–36) amide specific receptors in human fat membranes (Abstract). *Diabetologia* 35 (Suppl. 1):A76, 1992
25. Scholdager BTG, Mortensen PE, Christiansen J, Ørskov C, Holst JJ: GLP-1 (glucagon-like peptide-1) and truncated GLP-1, fragments of human pro-glucagon, inhibit gastric acid secretion in man. *Dig Dis Sci* 35:703–708, 1989

MPI EXHIBIT 1052 PAGE 4