Incretin Hormone Expression in the Gut of Diabetic Mice and Rats

Peter Berghöfer, Richard G. Peterson, Kerstin Schneider, Hans C. Fehmann, and Burkhard Göke

To elucidate the question of whether production of the insulinotropic gut hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) is altered by a diabetic metabolic state, their intestinal expression pattern was evaluated. Two rodent models for diabetes mellitus were used, non-obese diabetic (NOD) mice as a model for insulin-dependent diabetes and Zucker diabetic fatty (ZDF) rats for non–insulin-dependent diabetes mellitus (NIDDM). Expression of both incretin hormones followed typical patterns, which were similar in both animals and unaltered by the diabetic state. The GIP gene was greatly expressed in the duodenum, jejunum, and ileum, with a continuous decrease from the upper to lower intestines. This pattern was observed in both NOD mice and ZDF rats regardless of the diabetic state. This expression data was corroborated by radioimmunoassay (RIA) analysis of the gene product GIP. Expression of the proglucagon gene encoding GLP-1 had an opposite appearance. The highest expression was seen in the large bowel and the ileum. RIA analysis of the gene product GLP-1 mirrored these data. Although the distribution pattern was similar in both animal models, in contrast to diabetic NOD mice, a regulated expression was found in diabetic ZDF rats. Compared with lean nondiabetic controls, fatty hyperglycemic animals showed an increased expression of the proglucagon gene in the colon and a concomitant reduction in the small intestine. This was mirrored by the GLP-1 content of the colon and ileum. Overall, basal GLP-1 plasma levels were increased in ZDF rats (17.0 ± 2.8 pmol) compared with lean Zucker rats (12.4 ± 1.8 pmol). In conclusion, incretin hormone expression (GIP and GLP-1) follows specific patterns throughout the gut and is unaltered by the diabetic state. In ZDF rats, regulation of proglucagon expression occurs mainly in the large intestine.
Copyright © 1997 by W.B. Saunders Company

THE FUNCTIONAL CONNECTION between the gastrointestinal tract and endocrine pancreas was proven in classic studies in which the insulin response to oral or intravenous glucose—resulting in nearly identical plasma glucose levels—was compared.[1,2] A higher insulin secretion was found after oral glucose. It was calculated that up to 50% of insulin release after oral glucose is triggered by the enteroinsular axis.[3] Several gut hormones were discussed as incretin hormones, but had to be discounted for various reasons.[4,5] Today, we know that glucose-dependent insulinotropic polypeptide (GIP) together with glucagon-like peptide-1 (GLP-1) are incretin hormones with powerful insulinotropic effects.[6] Recent studies indicate that GIP and GLP-1 may explain the full incretin effect, especially since both incretins might additively interact.[7,8] However, since several other incretin hormone candidates exist, the participation of additional factors cannot be excluded.

Although the release and consecutive effects of incretin hormones on endocrine pancreatic function were extensively studied,[6] only a few studies address the intestinal regulation of genes encoding incretin hormones. This is surprising, since the adaptation of gut hormones to nutrients or disease could be of pathophysiological interest. One study has demonstrated that lipid and glucose meals are able to stimulate duodenal GIP gene expression.[9,10] Recently, it has been reported that enteral nutrient intake stimulates small-bowel proglucagon expression.[11] Furthermore, few studies report the plasma levels of GIP and GLP-1 in diabetes mellitus,[6,12,13] and little is known about incretin hormones at the expression level. With the availability of specific probes, we have therefore sought to answer the question of whether the production of GIP and GLP-1 is altered by a diabetic metabolic state by analyzing their intestinal expression pattern. Two rodent models for diabetes mellitus were used, non-obese diabetic (NOD) mice as a model for insulin-dependent diabetes[14,15] and Zucker diabetic fatty (ZDF) rats for non–insulin-dependent diabetes mellitus (NIDDM).[16-19]

MATERIALS AND METHODS

Materials

GLP-1 (7-36) amide was purchased from Saxon Biochemicals (Hanover, Germany). ^{125}I-labeled GLP-1 (7-36) amide was prepared as described previously.[20] The specific activity of the tracer was approximately 1,500 Ci/mmol. Formamide, formaldehyde, 50× Denhardt solution, and sonicated herring sperm DNA were obtained from Sigma (Deisenhofen, Germany). Multiprime labeling system, Hybond N membrane, and α-^{32}P-dCTP (specific activity, 110 TBq/mmol) were from Amersham (Braunschweig, Germany). For autoradiography, gels were exposed to X-OMAT AR x-ray film (Eastman Kodak, Rochester, NY) at −80°C.

Animals

NOD mice and ZDF rats were kept at 21°C in a temperature- and light-controlled room and were fasted overnight before each experiment. To avoid diurnal variation, all experiments were performed between 8 AM and 12 noon. In all experiments, the principles of laboratory animal care (National Institutes of Health Publication No. 86-23, revised 1985) and the German law on the protection of animals were fulfilled.

Protocol

NOD/Lt mice were originally obtained from the Jackson Laboratory (Bar Harbor, ME) at the age of 4 weeks. NOD mice were identified as prediabetic when the normal fasting blood glucose level was accompa-

From the Clinical Research Unit for Gastrointestinal Endocrinology, Philipps University, Marburg, Germany; the Department of Anatomy, Indiana University School of Medicine, Indianapolis, IN; and the Department of Internal Medicine, Justus Liebig University, Gießen, Germany.

Submitted May 1, 1996; accepted September 23, 1996.

Supported by the Deutsche Forschungsgemeinschaft (Bonn-Bad Godesberg).

Address reprint requests to Burkhard Göke, MD, Clinical Research Unit for Gastrointestinal Endocrinology, Philipps University, Baldinger Str., 35033 Marburg, Germany.

Copyright © 1997 by W.B. Saunders Company

0026-0495/97/4603-0006$03.00/0

nied by pancreatic insulitis. Previous observations in this NOD strain ensured that this was the case in 95% of mice aged 12 weeks and older irrespective of sex. When fasting hyperglycemia (>7.8 mmol/L) was determined on 3 different days in NOD mice, they were considered diabetic. Such 12-week-old mice were then consecutively characterized by an intravenous glucose tolerance test and pancreas histology as described elsewhere.[21,22] Briefly, glucose 0.5 g/kg body weight was injected intravenously, and blood glucose was determined (Beckman Glucose Analyzer, Munich, Germany) at 2, 4, 6, 8, 10, 15, 20, and 30 minutes thereafter. From the glucose concentrations, the K value was calculated as percent per minute. Pancreatic sections fixed in Bouin's solution were examined for lymphocytic islet infiltration (insulitis).[21] Mice (n = 6 per group) were killed by decapitation, and the entire intestine was dissected and trimmed free of all mesenteric attachments. It was rapidly cut into defined segments from proximal to distal according to the method reported by Evers et al,[23] shock-frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

A colony of partially inbred obese ZDF rats (ZDF/Gmi-*fa*) in which all male (*fa/fa*) members become diabetic by 10 weeks of age were used as a model of NIDDM as described previously.[16] Because sex-matched nondiabetic obese Zucker fatty rats do not exist, we used as controls age-matched lean male heterozygotes (*fa/+*). The animals were anesthetized at 26 weeks of age by intraperitoneal injection of pentobarbitol sodium, blood was obtained by puncture of the aorta for plasma preparation, and the intestines were rapidly removed, washed with iced 0.9% saline, and immediately shock-frozen in liquid nitrogen after separation into aliquots of defined segments.

Gut tissue sample collection. For radioimmunoassay (RIA) analysis of tissue hormones, defined gut segments were washed with ice-cold saline (0.9%) and shock-frozen in liquid nitrogen. After homogenization by an Ultra-Turrax device (Janke and Kunkee, Staufen, Germany), samples were boiled in acetic acid and ethanol-extracted according to established procedures.[24] The liquid supernatant was lyophilized and analyzed in at least three different assay buffer dilutions in the RIAs.

Assays

Assays were performed with samples from corresponding gut segments of diabetic or control animals to achieve comparability of the data.

RNA isolation and slot-blot and Northern transfer analysis. Total RNA from tissue slices was isolated after rapid homogenization by the Ultra-Turrax device as indicated previously.[9,25] A 300-bp fragment of cDNA coding for rat proglucagon (donated by P.K. Lund, University of North Carolina, Chapel Hill, NC) and a 648-bp fragment encoding for rat GIP (donated by R.A. Liddle, Duke University, Winston-Salem, NC) were radioactively labeled. RNA (20 μg per lane) was either immobilized onto nitrocellulose using a slot-blot apparatus[26] or fractionated on 1% agarose gels containing 2.2 mol/L formaldehyde, transferred to Hybond N membranes, and immobilized by UV cross-linking. RNA quantity was verified by reversibly staining membranes with methylene blue before hybridization. Hybridization was performed as described previously,[9,25] and for quantification of Northern blots, migration positions of the signals were calculated as compared with RNA markers (28s rRNA and β-actin cDNA control probes; Clontech, Palo Alto, CA). The final washing was performed in 0.1%× SSC/0.1%× sodium dodecyl sulfate at 42°C. The hybridization signals were quantified by laser densitometry and computer-assisted integration of the autoradiographic images.[10] Levels were expressed as mRNA/28s rRNA in percent total specific mRNA.

RIAs. Immunoreactive GLP-1 was analyzed by a competitive RIA with the specific polyclonal antibody GA 1178 (Affinity Research, Nottingham, UK). It exhibits 100% reactivity with GLP-1 (1-36) amide and the truncated GLP-1 (7-36) amide. The sensitivity is 0.4 pmol/L. Further characterization of the antibody in our laboratory showed no cross-reaction with GIP, pancreatic glucagon, glicentin, oxyntomodulin, GLP-2, and the GLP-1 agonist exendin-4. ^{125}I-GLP-1 (7-36) amide (specific activity, ~74 TBq/mmol) was prepared as described previously.[20] Interassay and intraassay coefficients of variation are 10.2% and 5.4%, respectively. Gastric inhibitory polypeptide tissue levels were analyzed by means of a commercially available assay system (Peninsula, St. Helens, UK). Peptide levels in tissue were expressed as picograms per gram frozen sample as a percent of the total amount in picograms.

Glucose, insulin, and hemoglobin A_{1c}. Insulin was analyzed by RIA using rat insulin I and II as standard (Biermann, Bad Nauheim, Germany). The assay sensitivity was 30 pmol/L, interassay variance 11.8%, and intraassay variance 9.7%. Plasma glucose was determined by a glucose oxidase method (Boehringer, Ingelheim, Germany) using an automated glucose analyzer and hemoglobin A_{1c} (HbA_{1c}) as indicated previously.[16]

Statistics

Statistical significance was determined with the two-tailed unpaired Student's *t* test (two groups).

RESULTS

After functional studies, NOD mice clearly showed a diabetic metabolic situation; these animals and the nondiabetic animals were further processed for analysis of incretin hormone expression (Table 1). Histologic control studies (not shown) fully corroborated the functional data correlating apparent insulitis with increased plasma glucose, decreased glucose disappearance rate, and glucosuria in the diabetic mice (Table 1 and Fig 1A and B). Diabetes in ZDF rats was controlled by analysis of plasma levels of HbA_{1c}, glucose, and insulin (Fig 2A to C). Typically, insulin-resistant fatty animals were hyperglycemic and had an increased HbA_{1c}. The insulin levels corresponded to those of controls, as shown previously,[16] thereby indicating exhaustion of the β cell.

Expression of both incretin hormones followed typical patterns, which were similar in both animal models and unaltered by the diabetic state. The GIP gene was expressed greatly in the duodenum and jejunum and to a lesser extent in the proximal ileum, with a continuous decrease from the upper to lower intestines. This pattern was observed in both NOD mice (Table 2) and ZDF rats (Fig 3) regardless of the diabetic state. These expression data were corroborated by RIA analysis of the gene product GIP. In mice, GIP levels (in nanomoles per liter) in the duodenum were 21.0 ± 3.2 (diabetic) versus 18.0 ± 2.4 (nondiabetic), in the jejunum, 14.7 ± 2.7 versus 15.0 ± 2.3, in

Table 1. Functional Data From Diabetic and Nondiabetic NOD Mice (mean ± SD)

Parameter	Diabetic	Nondiabetic
Blood glucose (mg/dL)		
Fasting	145 ± 48* (n = 12)	69 ± 5 (n = 12)
Postprandial	429 ± 16* (n = 12)	88 ± 3 (n = 12)
After intravenous glucose tolerance test (t = 30 min)	320 ± 58* (n = 6)	208 ± 24 (n = 6)
Glucose disappearance rate	−1.38 ± 0.2* (n = 6)	−4.04 ± 0.5 (n = 6)
Glucosuria	≥5%	Not detected

*$P < .005$.

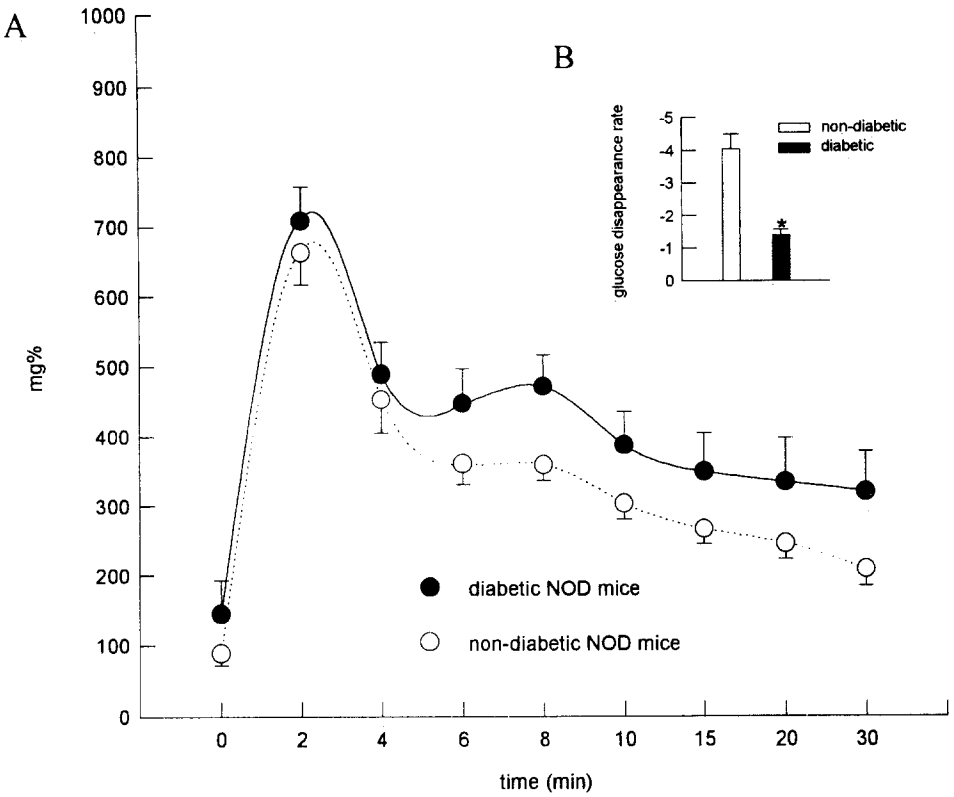

Fig 1. Glucose tolerance in NOD mice. (A) Results of an intravenous glucose challenge (from 6 to 30 minutes, single values were significantly different between nondiabetic and diabetic mice, $P < .05$). (B) Calculated glucose disappearance rates in diabetic and nondiabetic animals (n = 6 per group, $*P < .005$).

the ileum, 8.8 ± 1.2 versus 12.9 ± 1.5, in the colon, 2.6 ± 1.1 versus 4.5 ± 1.7, and in the rectum, 1.6 ± 1.0 versus 1.8 ± 1.0. In rats, corresponding values were found, with no apparent differences between diabetic animals and controls (not shown).

Expression of the proglucagon gene encoding GLP-1 had an opposite appearance. The highest expression was seen in the large bowel, the ileum contained only approximately 20% of total proglucagon mRNA, and low expression was found in the upper intestine. RIA analysis of the gene product GLP-1 mirrored these data. Although the distribution pattern was similar in both animal models, in contrast to diabetic NOD mice (Fig 4), a regulated expression was found in diabetic ZDF rats (Fig 5A). Compared with lean nondiabetic controls, fatty hyperglycemic animals showed an increased expression of the proglucagon gene in the colon and a concomitant reduction in the small intestine, whereas GIP expression remained unaltered in diabetic animals (Fig 5B). The expression data for proglucagon were mirrored by the GLP-1 content of the colon and ileum (Fig 6). Overall, basal GLP-1 plasma levels were slightly increased in fatty diabetic ZDF rats (17.0 ± 2.8 pmol/L), compared with lean ZDF rats (12.4 ± 1.8 pmol/L). The effects observed are shown in Fig 7. A clear increase of GLP-1 expression in colon segments was observed.

DISCUSSION

In man, the number of GLP-1–positive cells increases from the duodenum to the distal jejunum and ileum, and again from the proximal to the distal colon, reaching maximum cell density in the rectum.[27] In rats, the number of L cells follows a similar pattern from the proximal small bowel to the ileum, where a maximal cell number is found.[27] L cells are also present in the rat colon with an increasing abundance from the proximal to the

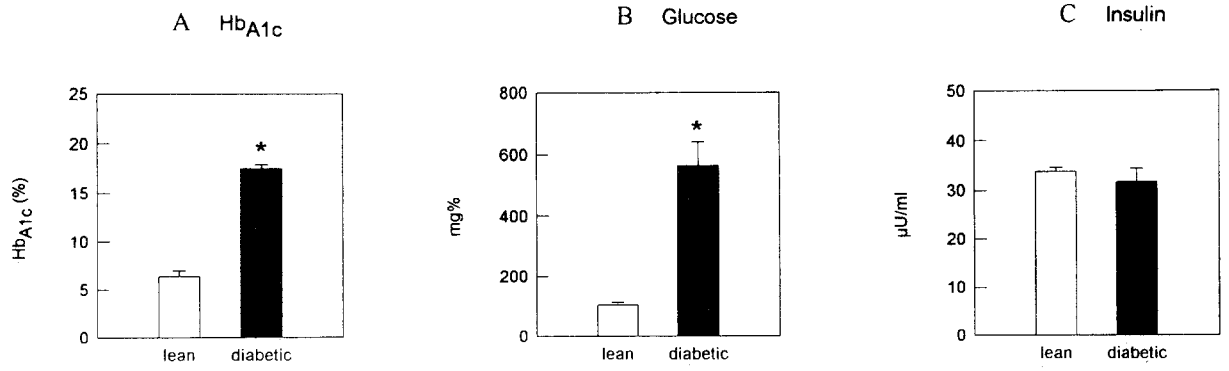

Fig 2. Metabolic parameters in ZDF rats. Plasma levels of HbA_{1c}, glucose, and insulin in diabetic rats were greater than in controls ($*P < .005$).

Table 2. GIP Gene Expression and Tissue Distribution in the Gut of NOD Mice

Segment	% GIP mRNA		% GIP Protein	
	Diabetic	Nondiabetic	Diabetic	Nondiabetic
Duodenum	34.04 ± 5.67	26.64 ± 1.38	37.32 ± 2.27	31.29 ± 3.01
Jejunum	30.50 ± 4.98	28.33 ± 8.67	28.38 ± 2.73	24.27 ± 1.79
Ileum	20.66 ± 2.72	20.83 ± 4.82	21.97 ± 1.74	25.42 ± 1.85
Colon	8.00 ± 2.39	13.20 ± 1.98	9.94 ± 2.18	14.91 ± 3.93
Rectum	6.80 ± 1.74	11.00 ± 2.94	2.39 ± 0.92	4.11 ± 2.08

distal parts. GLP-1 is colocalized in L cells with other proglucagon-derived peptides and, at least partly, with PYY.[27] Interestingly, GLP-1–producing cells were also found in the human anal canal, where the vast majority of PYY-immunoreactive cells labeled also for GLP-1.[28]

Previous studies have analyzed the release of GIP and GLP-1 into the circulation.[29-32] GIP secretion was generally reported to react sensitively to both short- and long-term changes of the diet, particularly changes in dietary fat content.[33] However, few studies have examined the biosynthesis of these incretin hormones, especially under the metabolic situation of hyperglycemia or diabetes mellitus.

Increased levels of GIP were found in obese human subjects and in a subgroup of patients with NIDDM. Ross et al[34] found an exaggerated increase in plasma GIP levels following an oral glucose challenge in NIDDM patients. They suggested that in such patients, GIP could contribute to hypersecretion of glucagon because of its glucagonotropic effect. Interestingly, in a large series of NIDDM patients, a bimodal distribution of GIP secretion was found, after oral glucose,[35] thus demonstrating the occurrence of hyposecretion and hypersecretion of GIP in NIDDM. It was proposed that the phenomenon of GIP hypersecretion might be caused at least in part by an insensitivity of K cells to insulin resulting in an impaired feedback regulation between insulin and GIP release.[4] Similar findings were made in studies with obese mice. Interestingly, these animals are characterized by a hyperplasia of the K cells.[31] Still, most studies emphasize that GIP levels are not elevated in diabetics.[4,31,32] Often, factors that impact gut hormone secretion also are likely to influence the expression of consecutive genes. The complexity of the interaction between gut hormones and the response of

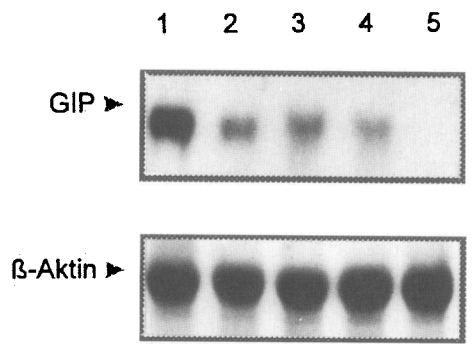

Fig. 3. Representative Northern blot hybridization of GIP mRNA in the intestine of ZDF rats. Lanes 1 to 5, distribution pattern of GIP mRNA for duodenum (1), upper and lower jejunum (2 and 3), proximal ileum (4), and colon (5). β-actin was used to correct for gel loading.

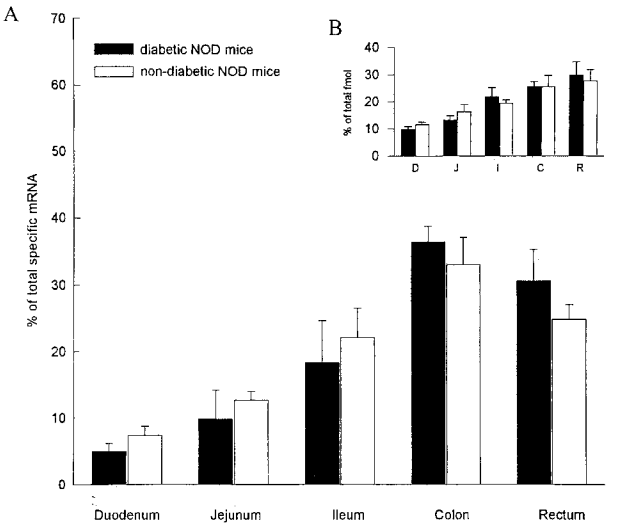

Fig. 4. Relative distribution pattern of specific proglucagon mRNA (A) and of immunoreactive GLP-1 tissue content (B) in the intestines of NOD mice. Results were obtained by slot-blot hybridization of samples from gut segments from identical sites in diabetic animals and controls.

pancreatic hormones and the heterogeneity of NIDDM make gene-level studies difficult but highly interesting.

So far, only Tseng et al[9,10] have presented data on the regulation of intestinal GIP expression. They showed that GIP expression in the duodenum increases after a lipid-rich meal[9] and is also stimulated by a glucose-rich drink.[10] In our study, we did not find regulated GIP expression in NOD mice (hyperglycemic and insulin-deficient) or ZDF rats (hyperglycemic and insulin-resistant). In contrast to Tseng et al, who studied GIP mRNA generation after an short-term nutrient challenge, we were interested in evaluating whether a chronic increase of glucose levels in the circulation plays a role. This clearly more closely mimicks the pathophysiological situation. In examining our negative result for GIP expression, one could argue that, in any case, increased GIP expression does not provide a means for the organism to counteract diabetic metabolism. Accordingly, it was previously demonstrated that even administration of exogenous GIP fails to affect insulin secretion or blood glucose levels in type II diabetics.[36] In contrast to these observations, the insulinotropic effects of GLP-1 were preserved.[36,37] GLP-1 plasma levels were found to be slightly elevated in patients with NIDDM,[38] which was not corroborated in mild non-obese type II diabetes.[13] Furthermore, intestinal GLP-1 content was increased in streptozotocin-treated rats, but 3 weeks after induction of this insulin-deficient diabetes, no differences in proglucagon mRNA levels in the intestine were detected.[39,40]

Proglucagon mRNA levels were not different between diabetic and nondiabetic NOD mice. Furthermore, the distribution pattern of expression of these genes along the gastrointestinal tract remained unchanged. The dominant functional characteristics of this animal model are insulin deficiency and hyperglycemia. Recently, considerable advances have been made in understanding insulin-regulated gene expression.[41] So far, we

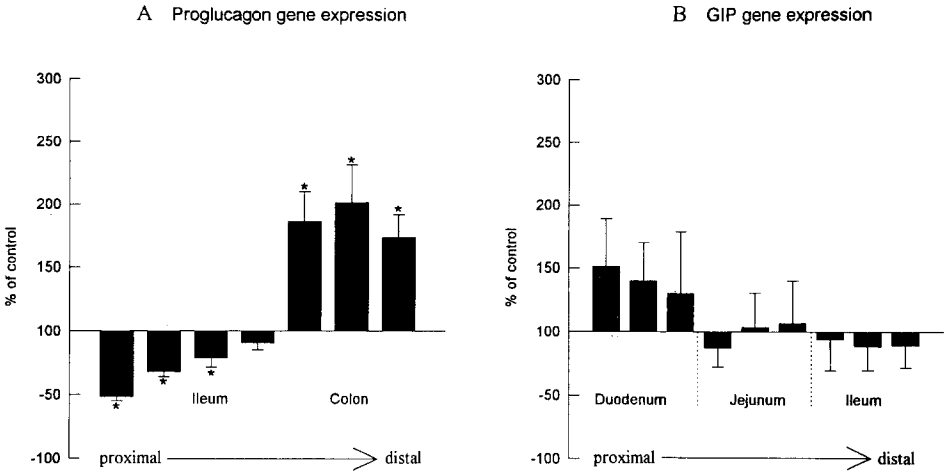

Fig 5. Incretin hormone expression in the intestines of diabetic (n = 6) and lean (control, n = 4) ZDF rats. Data are calculated as percent of control (*$P < .005$).

know that insulin affects the expression of more than 100 genes.[41,42] Although the principles of regulation of gene transcription, protein synthesis, and mRNA stability by insulin were studied in detail,[41,42] little is known about insulin-related effects on gut hormone expression. However, the proglucagon gene in pancreatic α cells is regulated by insulin[41]: insulin inhibits its transcription. Nothing is known so far about the action of insulin on proglucagon expression in intestinal L cells. In any case, our findings argue against an effect of insulin deficiency on in vivo proglucagon expression, at least in mice with experimental type I diabetes.

The situation seems different in the hyperglycemic, normoinsulinemic, but insulin-resistant obese ZDF rat. Although the distribution pattern of incretin hormone expression was not changed, a regulated expression of proglucagon resulting in elevated GLP-1 plasma levels was found. This was obviously the result of an increased upregulation of proglucagon expression in the colon, although it was accompanied by a diminished

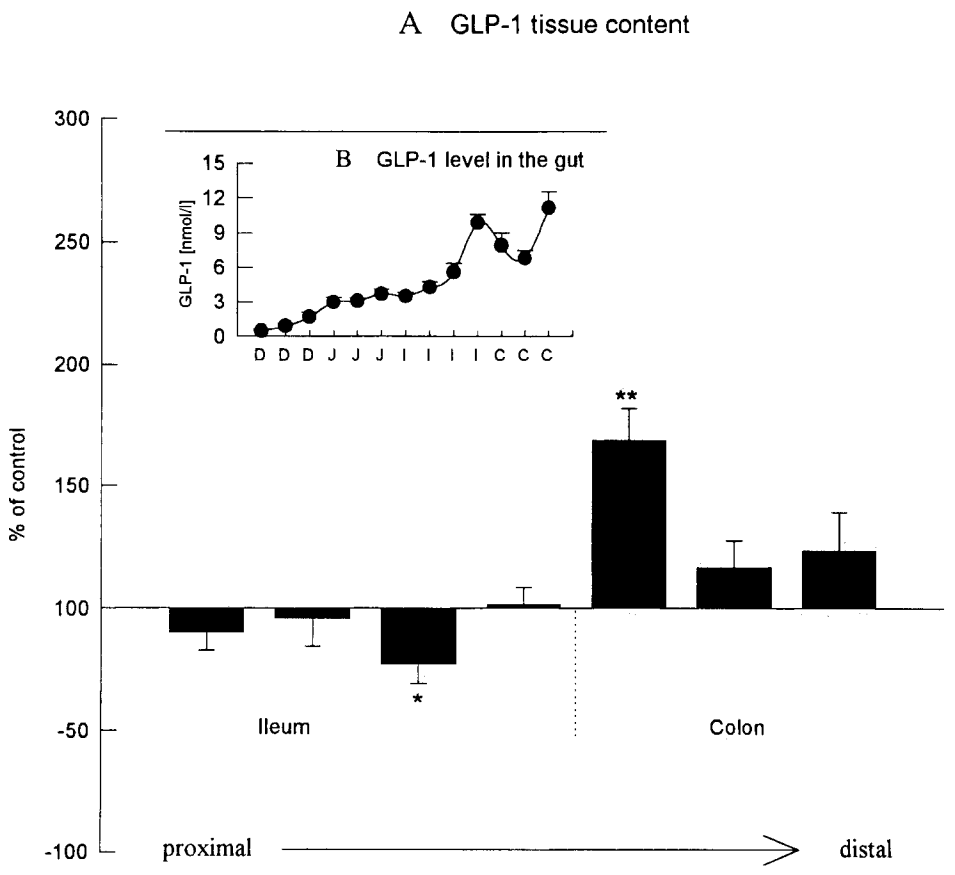

Fig 6. Immunoreactive GLP-1 tissue content in the ileum and colon of diabetic ZDF rats, calculated as percent of control values. *$P < .05$, **$P < .005$. Insert, the pattern expressed as absolute GLP-1 levels of the analyzed segments.

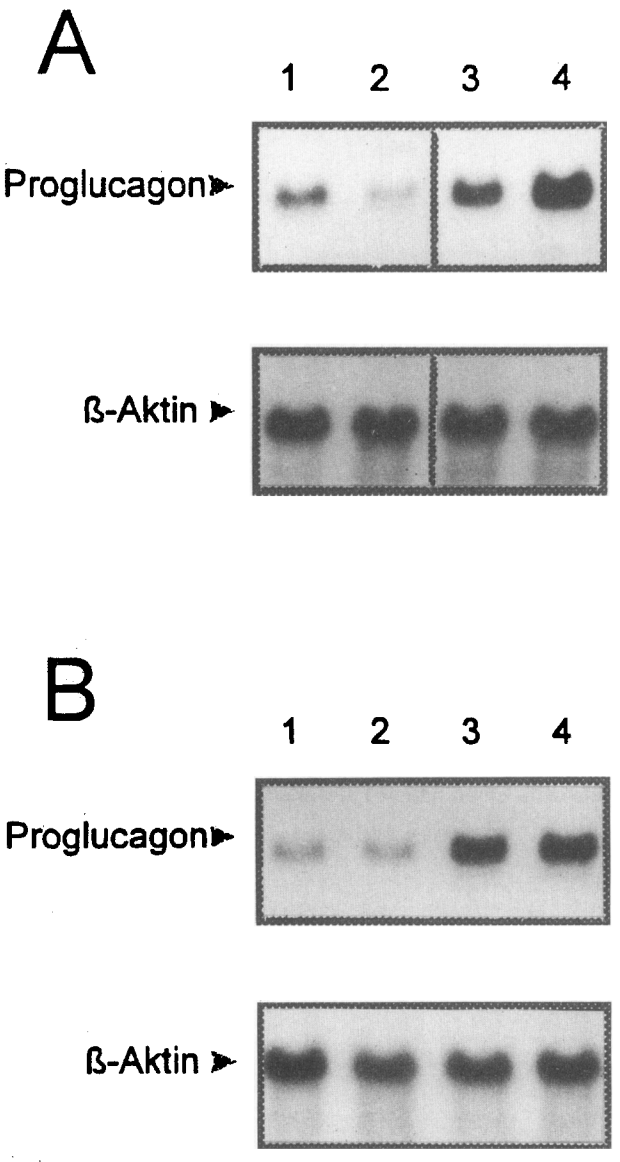

Fig 7. Proglucagon mRNA analyzed in the intestine of diabetic and lean ZDF rats. (A) Ileum (lanes 1 and 2, lean and diabetic ZDF rats) and colon (lanes 3 and 4, lean and diabetic ZDF rats). (B) Colon of lean controls (lanes 1 and 2) and diabetics (lanes 3 and 4).

expression in the lower small gut. Interestingly, recent data from our laboratory showed that stimulated GLP-1 release from an isolated perfused rat colon preparation quantitatively exceeded by far that of the perfused ileum (C. Hermann-Rinke and B. Göke, unpublished data, April 1996). This indicates that upregulation of proglucagon expression could be of functional significance. This is further supported by a recent report on the full processing of proglucagon in the colon, yielding bioactive GLP-1.[43] In this context, it is of interest that it has been recently shown that the slowing of nutrient absorption (ie, carbohydrate) by therapeutic means (glucosidase inhibitors such as acarbose or voglibose) mobilizes the endogenous GLP-1 reserve.[44-46] Prolonged glucosidase inhibitor treatment resulted in depressed GIP plasma levels, but was accompanied by increased GLP-1 plasma levels. The putative effects of such a maneuver on GIP and proglucagon gene expression are currently under investigation.

It seems that hyperglycemia has a contrasting impact on the different gut sections; however, it is still unexplained. Actually, the detrimental effects of chronic hyperglycemia on endocrine cells in diabetes mellitus recently received attention.[47] Best known in this context is the impaired glucostimulatory effect on insulin secretion; the term "glucose toxicity" was coined in reference to this defect. We have learned that the plasma glucose level, acting through a negative-feedback loop, regulates insulin secretion directly and modulates insulin output via the large number of hormones, nutrients, and neurotransmitters that stimulate insulin secretion.[5,6,12] Obviously, chronic hyperglycemia is harmful to this adaptive system, but details are only recently beginning to emerge.[47]

Still, one important problem is that we do not fully understand the regulation of postprandial GLP-1 secretion from the gut. There are solid data that the incretin hormone is promptly released after meals.[29,30] At least the rapid first phase of GLP-1 secretion cannot be explained by glucose being in direct contact with lower-intestinal L cells. From experiments with isolated perfused gut segments, we have learned that several (neuro?) peptidergic signals may account for an enhancement of GLP-1 secretion,[48] but only in rodents was GIP shown to be a potent stimulant in vivo.[49] It is now necessary to define the various factors impacting GLP-1 secretion from the different gut sections, since, as in other organs, such factors likely also regulate proglucagon expression.

Interestingly, in a recent study, an increased number of enteroglucagon-immunoreactive cells were found in the large intestine of diabetic (db/t) mice.[50] This strain is used as a model for NIDDM. This supports our findings in the ZDF rat NIDDM model. Our data further support the idea that in NIDDM, at least to some extent, proglucagon expression is upregulated to counteract hyperglycemia. To achieve this, a residual presence of insulin is necessary.

ACKNOWLEDGMENT

This report is part of the doctoral thesis of P.B.

REFERENCES

1. McIntyre N, Holdsworth CD, Turner DS: New interpretation of oral glucose tolerance. Lancet 2:20-21, 1964

2. Perley MJ, Kipnis DM: Plasma insulin responses to oral and intravenous glucose: Studies in normal and diabetic subjects. J Clin Invest 46:1954-1962, 1967

3. Unger RH, Eisentraut AM: Entero-insular axis. Arch Intern Med 123:261-266, 1969

4. Creutzfeldt W, Ebert R: New developments in the incretin concept today. Diabetologia 28:565-573, 1985

5. Ensinck JW, D'Alessio DA: The entero-insular axis revisited—A novel role for an incretin. N Engl J Med 326:1352-1353, 1992

6. Fehmann HC, Göke R, Göke B: Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr Rev 16:390-410, 1995

7. Fehmann HC, Göke B, Göke R, et al: Synergistic stimulatory effect of glucagon-like peptide-I (7-36) amide and glucose-dependent insulin-releasing polypeptide on the endocrine rat pancreas. FEBS Lett 252:109-112, 1989

8. Kolligs F, Fehmann HC, Göke R, et al: Reduction of the incretion effect in rats by the glucagon-like peptide I receptor antagonist exendin (9-39) amide. Diabetes 44:16-19, 1995

9. Tseng CC, Jarboe LA, Landau SB, et al: Glucose-dependent insulinotropic peptide: Structure of the precursor and tissue-specific expression in rat. Proc Natl Acad Sci USA 90:1992-1996, 1993

10. Tseng CC, Jarboe LA, Wolfe MM: Regulation of glucose-dependent insulinotropic peptide gene expression by a glucose meal. Am J Physiol 226:G887-G891, 1994

11. Hoyt EC, Lund PK, Winesett DE, et al: Effects of fasting, refeeding, and intraluminal triglyceride on proglucagon expression in jejunum and ileum. Diabetes 45:434-439, 1996

12. Brown JC: Entero-insular axis. Baillieres Clin Endocrinol Metab 2:359-373, 1988

13. Fukase N, Manaka H, Sugiyama K, et al: Response of truncated glucagon-like peptide-1 and gastric inhibitory polypeptide to glucose ingestion in non–insulin dependent diabetes mellitus. Acta Diabetol 32:165-169, 1995

14. Kikutani H, Makino S: The murine autoimmune diabetes model: NOD and related strains. Adv Immunol 51:285-322, 1992

15. Tochino Y, Rosenthal AS, Matteo M: The NOC mouse as a model of type I diabetes. Crit Rev Immunol 8:49-85, 1987

16. Peterson RG, Shaw WN, Neel MA, et al: Zucker diabetic fatty rat as a model for non–insulin-dependent diabetes mellitus. ILAR News 32:16-19, 1990

17. Sturis J, Pugh WL, Tang J, et al: Alterations in pulsatile insulin secretion in the Zucker diabetic fatty rat. Am J Physiol 267:E250-E259, 1994

18. Jia X, Elliott R, Kwok YN, et al: Altered glucose dependence of glucagon-like peptide I (7-36)–induced insulin secretion from the Zucker (fa/fa) rat pancreas. Diabetes 44:495-500, 1995

19. Friedmann JE, De Venté JE, Peterson RG, et al: Altered expression of muscle glucose transporter GLUT-4 in diabetic fatty Zucker rats (ZDF/Drt-fa). Am J Physiol 261:E782-E788, 1991

20. Göke R, Conlon JM: Receptors for glucagon-like peptide-I (7-36) amide on rat insulinoma-derived cells. J Endocrinol 116:357-362, 1988

21. Fujino-Kurihara H, Fujita H, Hakura A, et al: Morphological aspects on pancreatic islets of non-obese diabetic (NOD) mice. Virchows Arch 49:107-120, 1985

22. Linn T, Schneider K, Göke B, et al: GLP-1 improves glucose sensitivity in B-cells of NOD mice. Acta Diabetol 33:19-24, 1996

23. Evers BM, Rajaraman S, Chung SH, et al: Differential expression of the neurotensin gene in the developing rat and human gastrointestinal tract. Am J Physiol 265:G482-G490, 1993

24. Lüttichau HR, Van Solinge WW, Nielsen FC, et al: Developmental expression of the gastrin and cholecystokinin genes in rat colon. Gastroenterology 104:1092-1098, 1993

25. Jin SLC, Hynes MA, Lund PK: Ontogeny of glucagon messenger RNA and encoded precursor in the rat intestine. Regul Pept 25:117-131, 1990

26. Kanayama S, Liddle RA: Regulation of intestinal cholecystokinin and somatostatin mRNA by bombesin in rats. Am J Physiol 261:G71-G77, 1991

27. Eissele R, Göke R, Willemer S, et al: Glucagon-like peptide 1 (7-36) amide-cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283-291, 1992

28. Hörsch D, Fink T, Göke B, et al: Distribution and chemical phenotypes of neuroendocrine cells in the human anal canal. Regul Pept 54:527-542, 1995

29. Herrmann C, Göke R, Fehmann HC, et al: Glucagon-like peptide 1 and glucose-dependent insulin-releasing polypeptide secretion in response to nutrients. Digestion 56:117-126, 1995

30. Schirra J, Katschinski M, Weidmann C, et al: Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 97:92-103, 1996

31. Petersen RA: Gastric inhibitory polypeptide, in Walsh JH, Dockray GJ (eds): Gut Peptides: Biochemistry and Physiology. New York, NY, Raven, 1994, pp 217-259

32. Creutzfeldt W, Nauck MA: Gut hormones and diabetes mellitus. Diabetes Metab Rev 8:149-177, 1992

33. Morgan LM: The metabolic role of GIP: Physiology and pathology. Biochem Soc Trans 24:585-591, 1996

34. Ross SA, Brown JC, Dupre J: Hypersecretion of gastric inhibitory polypeptide following oral glucose in diabetes mellitus. Diabetes 26:525-529, 1977

35. Ebert R, Creutzfeldt W: Hypo- and hypersecretion of GIP in maturity-onset diabetes. Diabetologia 19:271-272, 1980

36. Nauck MA, Heimesaat MM, Orskov C, et al: 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 2 diabetes mellitus. J Clin Invest 30:301-307, 1993

37. Gutniak M, Orskov C, Holst JJ, et al: Antidiabetogenic effect of glucagon-like peptide-1(7-36)amide in normal subjects and patients with diabetes mellitus. N Engl J Med 326:1316-1322, 1992

38. Orskov C, Jeppesen J, Madsbad S, et al: Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. J Clin Invest 87:415-423, 1991

39. Kreymann B, Yiangou Y, Kanse S, et al: Isolation and characterization of GLP-1 7-36 amide from rat intestine: Elevated levels in diabetic rats. FEBS Lett 242:167-170, 1988

40. Brubaker PL, So DCY, Drucker DJ: Tissue-specific differences in the levels of proglucagon-derived peptides in streptozotocin-induced diabetes. Endocrinology 124:3003-3009, 1989

41. O'Brien RM, Granner DK: Gene regulation, in LeRoith D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus. Philadelphia, PA, Lippincott-Raven, 1996, pp 234-242

42. O'Brien RM, Granner DK: The regulation of gene expression by insulin. Physiol Rev 76:1109-1161, 1996

43. Deacon CF, Johnson AH, Holst JJ: Human colon produces fully processed glucagon-like peptide-I (7-36)amide. FEBS Lett 372:269-272, 1995

44. Fukase N, Takahashi H, Igarashi M, et al: Differences in glucagon-like peptide 1 and GIP responses following sucrose ingestion. Diabetes Res Clin Pract 15:187-195, 1992

45. Göke B, Fuder H, Wieckhorst G, et al: Voglibose (AO-128) is an efficient α-glucosidase inhibitor and mobilizes the endogenous GLP-1 reserve. Digestion 56:493-501, 1995

46. Göke B, Herrmann C, Göke R, et al: Intestinal effects of α-glucosidase inhibitors: Absorption of nutrients and enterohormonal changes. Eur J Clin Invest 24:25-30, 1994 (suppl 3)

47. Leahy JL: Detrimental effects of chronic hyperglycemia on the pancreatic β-cell, in LeRoith D, Taylor SI, Olefsky JM (eds): Diabetes Mellitus. Philadelphia, PA, Lippincott-Raven, 1996, pp 103-113

48. Herrmann-Rinke C, Vöge A, Hess M, et al: Regulation of glucagon-like peptide-1 (GLP-1) secretion from rat ileum by neurotransmitters and peptides. J Endocrinol 147:25-31, 1995

49. Brubaker PL: Regulation of intestinal proglucagon-derived peptide secretion by intestinal regulatory peptides. Endocrinology 128:3175-3182, 1991

50. Pinto HC, Portella-Gomes GM, Grimelius L, et al: The distribution of endocrine cell types of the gastrointestinal mucosa in genetically diabetic (db/db) mice. Gastroenterology 108:967-974, 1995