Preservation of the Incretin Effect After Orthotopic Pancreas Transplantation in Inbred Rats

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To establish whether the incretin effect is under neural control, insulin, C-peptide, and glucose-dependent insulinotropic peptide (GIP) responses and hepatic insulin clearance were investigated after oral and "isoglycemic" intravenous glucose in 12 inbred rats after denervation of the pancreas by orthotopic transplantation with portal venous drainage (Tx group) and in 12 laparotomized controls (sham group). Effective pancreas denervation was documented by a decreased pancreatic polypeptide (PP) response to insulin-induced hypoglycemia and by decreased levels of norepinephrine and calcitonin gene–related peptide (CGRP) in pancreatic tissue. Basal and incremental arterial plasma glucose integrated over 180 minutes did not differ between oral and intravenous glucose, but the integrated insulin response (mean ± SEM) was significantly greater with oral versus intravenous glucose (Tx group, 104.9 ± 22.0 *v* 31.0 ± 4.9 nmol · L^{-1} · min, $P < .01$; sham group, 79.5 ± 10.6 *v* 36.6 ± 5.8 nmol · L^{-1} · min, $P < .01$). The integrated response of C-peptide was similar during both tests (Tx group, 105 ± 14 *v* 79 ± 8 pmol · mL^{-1} · min; sham group, 112 ± 10 *v* 121 ± 12 pmol · mL^{-1} · min). Hepatic insulin clearance was significantly decreased in both groups by oral compared with intravenous glucose administration (Tx group, 1.3 ± 0.2 *v* 3.3 ± 0.6 mmol/mmol, $P < .01$; sham group, 1.6 ± 0.1 *v* 3.9 ± 0.6 mmol/mmol, $P < .02$). The incretin effects for insulin (Tx group, 5.6 ± 2.7; sham group, 3.0 ± 0.8) and C-peptide (Tx group, 1.4 ± 0.2; sham group, 1.1 ± 0.2), calculated as the ratio of the integrated oral response and integrated intravenous response, and GIP responses to oral and intravenous glucose were not significantly different between the two groups. We conclude that there is preservation of the incretin effect in rats with orthotopically transplanted and hence extrinsically denervated pancreas, thus ruling out the possibility that the autonomic nervous system substantially contributes. Hepatic insulin clearance and insulinotropic hormones such as GIP appear to be more important.

IT IS WELL KNOWN that peripheral plasma insulin levels are significantly higher after oral versus intravenous glucose administration, producing identical plasma glucose profiles. This phenomenon has been interpreted as due to increased insulin secretion from pancreatic β cells, and is termed the incretin effect.[1] The increased release of insulin after glucose ingestion has been attributed mainly to gut hormones that are released after a meal and have known insulinotropic activity. Since the enteroinsular axis contains gut hormones, as well as nerves,[2] and since it is well known that the endocrine pancreas is richly innervated,[3,4] it seems reasonable to assume that pancreatic nerves are involved in the mediation of the incretin effect. However, all available studies on the incretin effect following denervation of the pancreas, whether animal experiments or investigations in humans, were compromised by one of the following: neglect of the cephalic phase by administration of glucose via the intragastric route[5-8]; use of heterotopic pancreas transplantation, which does not take into account interference with the recipient's own pancreas, and which involves systemic venous drainage that abolishes first-pass hepatic insulin extraction[9]; and immunosuppression, producing insulin resistance.[10] Furthermore, it has been shown that a decrease in the hepatic extraction of insulin also contributes to the incretin effect.[11-13] In the present study, we investigated the incretin effect after orthotopic pancreas transplantation in inbred rats. We chose this species and surgical preparation because it has been shown that the cephalic phase is highly important for intact glucose homeostasis in rats.[14] This animal model enabled us to determine whether nerves and hepatic extraction of insulin are involved in the mediation of the incretin effect.

MATERIALS AND METHODS

All studies were in conformity with the National Institutes of Health Principles of Laboratory Animal Care (Publication No. 86-23, Revised 1985) and current German law on the protection of animals, and all were approved by the Governmental Animal Care and Use Committees.

Experimental Groups

Highly inbred male Wistar-Lewis rats weighing between 270 and 330 g (Charles-River Breeding Laboratories, Sulzfeld, Germany) and aged 12 weeks on entering the study were used. They were housed in an environmentally controlled room with a 12-hour light/dark cycle and had free access to standard rat chow (Altromin, Lage, Germany) and water. Organ transplants among these rats are uniformly accepted without rejection. All operations were done under ether anesthesia. The animals were randomly assigned to two groups: Tx group (orthotopic pancreas transplant), rats pancreatectomized before receiving an orthotopic pancreas graft from an age-matched donor rat (n = 12); and sham group (control), laparotomized rats (n = 12).

Pancreas Transplantation

A pancreaticoduodenal graft was obtained from the donor together with a segment of portal vein, common bile duct, and aorta including the celiac and superior mesenteric arteries. In the recipient, the pancreas was completely resected, with excision of the bile duct from the liver hilus to within a few millimeters of its junction with the duodenum, leaving the blood supply to the spleen and duodenum intact. The graft was transplanted with end-to-side anastomoses made between the graft portal vein segment and the host portal vein and between the graft aortic segment and the host aorta. The graft duodenum was then removed, and the continuity of the bile duct was restored by joining the respective ends using two Teflon catheters (Vasofix Braunüle, 1.0 mm/20G OD; Braun, Melsungen, Germany).

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Implantation of Long-Term Indwelling Catheters

Thirteen weeks after the first operation, silastic catheters (Thomafluid-Silikon-Hochtemperatur-Chemieschlauch HighFlexible, 0.5 mm ID × 0.9 mm OD; Reichelt Chemietechnik, Heidelberg, Germany) were inserted in the left and right jugular vein and the left carotid artery.[15] They were advanced until the tips reached the right cardiac atrium and the aortic arch, respectively. An oral catheter was implanted by first making a small incision in the skin of the right cheek and then passing a silastic catheter through the cheek wall and into the oral cavity until the tip was 5 mm from the wall of the cheek.[16] The catheters were exteriorized through subcutaneous tissue to the posterior neck of the animal with the aid of a trochar. The catheters were kept in place by a cuff consisting of Silastic Medical Adhesive Silicone Type A (Dow Corning, Midland, MI) and glued to the catheters 1 cm proximal to the site of vascular access, which was used to anchor the catheters in the subcutaneous tissue. Every other postoperative day, the catheters were flushed with isotonic saline and filled with physiological saline containing chymotrypsin (225 U/mL) and gentamycin (20 mg/mL).[17] The external orifice of the catheters was closed with a small polyethylene plug.

Processing of Blood Samples

All samples were collected into prechilled tubes containing 2.5 mg EDTA and 500 U Trasylol per 1 mL blood, kept on ice, and centrifuged immediately.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed 1 week after insertion of the catheters, when the rats returned to a normal body weight (BW) gain, with BW between 400 and 470 g. All tests were performed in the evening 12 hours after food withdrawal. Before the test, extension tubes were attached to the catheters and suspended overhead by a pulley system. Throughout the procedure, the rat was allowed to move freely within the confines of a metabolic cage. Heparin 100 IU was administered per rat, and 20 to 30 minutes later, the glucose load was applied. After obtaining two basal blood samples, 2 g/kg BW glucose (50% glucose solution) was infused into the intraoral catheter over 1.5 minutes by a Precidor pump (infusion pump model 5003; Precidor Infors, Basel, Switzerland). The rats usually ate all of the proffered glucose spontaneously and rested calmly throughout the experiment. Blood samples for plasma glucose, insulin, and C-peptide determinations were obtained from the arterial line at time points −10, 0, 5, 10, 20, 30, 40, 60, 90, 120, 150, and 180 minutes, and for plasma glucose-dependent insulinotropic peptide (GIP) at time points −10, 5, 20, 60, 120, and 180 minutes. To prevent blood volume depletion, an equivalent volume of washed erythrocytes resuspended in normal saline was administered intravenously after each blood sample. The mean hematocrit obtained at the beginning and end of each study was as follows: sham: 47 ± 1 and 50 ± 1 for OGTT and 43 ± 1 and 48 ± 1 for IVGTT; Tx: 47 ± 1 and 47 ± 1 for OGTT and 44 ± 2 and 46 ± 2 for IVGTT.

Isoglycemic Intravenous Glucose Tolerance Test

One week after the OGTT, 20% glucose was infused intravenously using the Precidor pump at a rate necessary to produce the plasma glucose pattern observed after the oral glucose load in the same rat ("isoglycemic" glucose infusion). Blood samples were taken at the same time points and for the same variables as in the OGTT.

Pancreatic Polypeptide Release After Insulin-Induced Hypoglycemia

The cephalic phase of insulin release is vagus-mediated. To ensure that vagal denervation was effective in the transplanted pancreas, hypoglycemia was induced in two rats of each group 1 week after the intravenous glucose tolerance test (IVGTT). The conditions were the same as for the glucose tolerance tests. Regular human insulin (2 U/kg BW) was administered intravenously through a jugular cannula. Blood samples for plasma glucose and pancreatic polypeptide (PP) determinations were obtained from the arterial line at time points −10 and 30 minutes.

Pancreatic Tissue Content of Norepinephrine and CGRP

Seventeen weeks after pancreas transplantation or sham operation, food was withheld for 12 hours overnight and the animals were laparotomized and exsanguinated from the aorta, and the pancreas was harvested. The pancreas was immediately frozen in liquid nitrogen and stored at −30°C until extraction for determination of the tissue content of norepinephrine and CGRP.

Analyses

The plasma glucose level was measured using the glucose oxidase method (Glucose Analayzer 2; Beckman Instruments, Munich, Germany). Plasma immunoreactive insulin and C-peptide levels were measured by radioimmunoassay using rat insulin and C-peptide as a standard.[18,19] PP in plasma was measured by radioimmunoassay using a bovine assay[20]; a sufficient degree of cross-reactivity with rodent PP was obtained in the middle portion of the standard curve for PP levels of the rat in fasting and post–insulin load plasma to be read. Immunoreactive GIP in plasma was analyzed as described by Kuzio et al[21] in samples obtained after pooling plasma from two randomly chosen rats of the same group; serial dilutions of rat plasma extracts yielded curves parallel to those obtained with the porcine GIP standard reference. In a series of eight animals (four Tx and four sham), tissue specimens from the dorsal part of the pancreas were thawed at 4°C, immersed in ice-cold 6 mol/L perchloric acid, and homogenized. After incubation and centrifugation (20,000 × *g*) for 15 minutes at 4°C, respectively, the supernatant was stored at −80°C after mixture with sodium phosphate buffer (0.5 mol/L, pH 6.5) until determination of the norepinephrine content by high-performance liquid chromatography.[22] In specimens from the ventral and dorsal pancreas of 10 rats (five Tx and five sham) extracted by boiling in 0.5 mol/L acetic acid as described by Byrant and Bloom,[23] CGRP was measured by radioimmunoassay (RIK 6006, CGRP (rat); Peninsula Laboratories, Belmont, CA). The total protein level was measured in the respective pancreatic tissue extracts using the Coomassie Plus Protein assay reagent (Pierce, Rockford, IL).

Calculations and Statistics

The integrated responses of plasma glucose, insulin, and C-peptide to the glucose loads (oral and intravenous glucose) were calculated over 180 minutes by summing the product of the plasma concentration at each time point multiplied by the minutes in the period, and subtracting the product of the basal value and the total minutes after the load.[24] The incretin effect was calculated for insulin and C-peptide using the formula, integrated incremental response (oral)/integrated incremental response (intravenous).[10] Hepatic insulin clearance was calculated from the molar ratio of the integrated responses of C-peptide and insulin after oral and intravenous glucose.[13] However, the interpretation of these data is limited by the use of integrated responses in which the response remained above baseline and did not represent the total amount of insulin and C-peptide in response to the secretory glucose stimulus.

Results are presented as the mean ± SEM. The significance of differences was tested using Student's *t* test for paired and unpaired observations or nonparametric tests (Wilcoxon signed-rank test for paired and Mann-Whitney *U* test for unpaired observations) as appropriate.

RESULTS

In response to intravenous injection of insulin, arterial plasma glucose decreased from 5.1 ± 0.3 mmol/L to 1.9 ± 0.1 mmol/L after 30 minutes. The ΔPP level, ie, PP(t30) minus PP(t0), was 742 and 894 pg/mL in two sham animals, respectively, and 222 and 273 pg/mL in two Tx rats, respectively. The much smaller PP response in Tx animals was considered to indicate effective vagal denervation after transplantation. The norepinephrine content of the pancreas in Tx rats was significantly lower than in sham rats (121 ± 17 *v* 245 ± 28 ng/μg protein, $P < .01$, n = 4, respectively). Also, the CGRP content of the Tx versus sham pancreas was significantly decreased (dorsal pancreas, 149 ± 37 *v* 293 ± 22 pg/mg protein, $P < .05$, n = 5; ventral pancreas, 148 ± 31 *v* 323 ± 17 pg/mg protein, $P < .01$, n = 5). Thus, the tissue content of the mediator of the adrenergic nervous system and of CGRP—one mediator of the peptidergic nervous system—was approximately halved after transplantation. These findings were taken as proof of sufficient denervation.

Plasma glucose levels during oral and intravenous glucose loading in Tx and sham rats are presented in Fig 1. In sham and Tx rats, glucose concentrations attained a peak value within 20 minutes and thereafter reached a postprandial plateau within 180 minutes. The plasma glucose concentrations achieved by adjusting the intravenous infusion were identical to those measured during the OGTT. The integrated incremental response of glucose over 180 minutes was similar after oral and intravenous glucose (Table 1). The mean total glucose load infused intravenously was similar in the two groups (Tx,

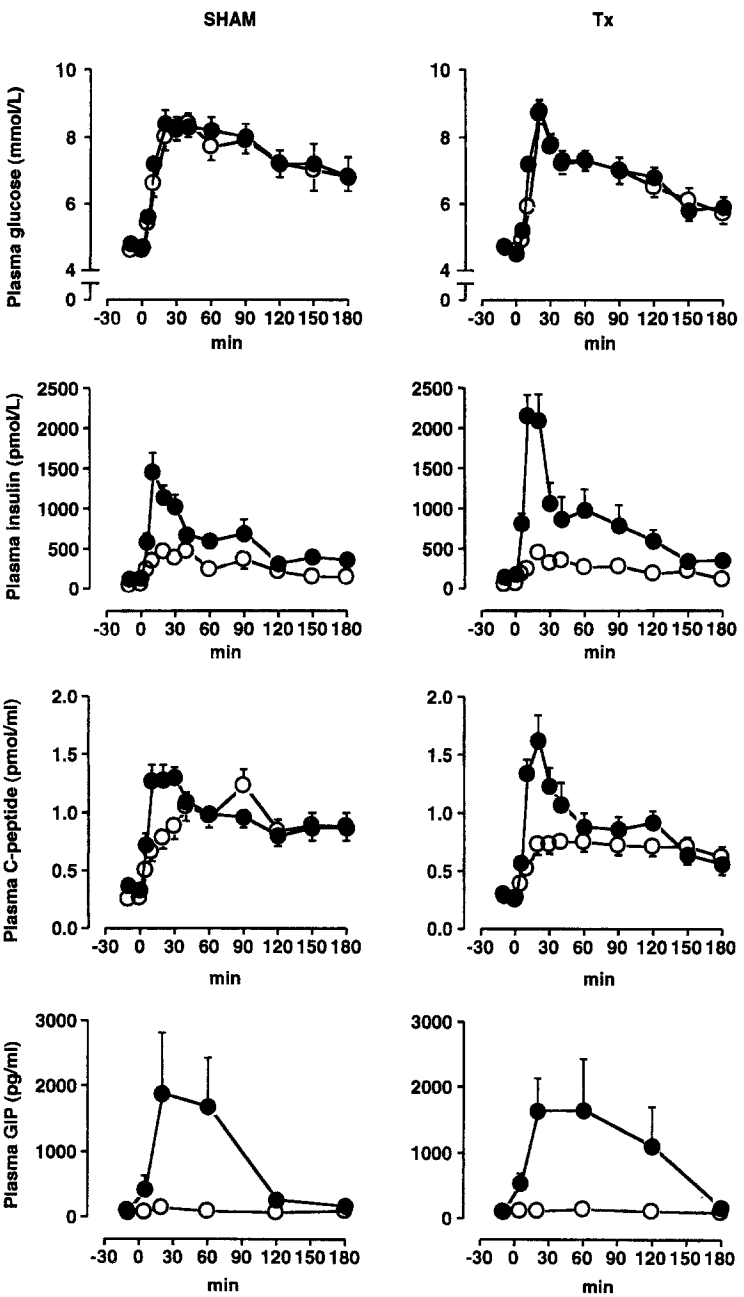

Fig 1. Plasma glucose, insulin, C-peptide, and GIP response to oral glucose (2 g/kg) or intravenous glucose administered at rates mimicking arterial plasma glucose concentrations resulting from oral glucose loading. Sham rats, n = 12; Tx rats, n = 12. Data are the mean ± SEM. (●) Oral; (○) intravenous.

1.9 ± 0.2 mmol; sham, 2.1 ± 0.3 mmol), but was only about half the oral glucose load (Tx, 4.8 ± 0.1 mmol; sham, 4.7 ± 0.1 mmol). In both sham and Tx groups, the difference between oral and intravenous glucose was significant ($P < .01$).

Basal insulin concentrations were similar and plasma insulin during the OGTT was uniformly higher than the level measured during the IVGTT in both groups (Fig 1). Consequently, the integrated incremental response of plasma insulin was also significantly greater after oral versus intravenous glucose (sham and Tx; Table 1). In contrast to the findings for insulin secretion, C-peptide concentrations in both groups were higher only for the first 30 minutes during the OGTT compared with the respective values during the IVGTT (Fig 1). Consequently, the integrated incremental response of C-peptide calculated over 180 minutes was similar for both glucose loads (sham and Tx).

The mean incretin effects on insulin and C-peptide did not differ between Tx and sham rats. However, the mean incretin effect was significantly greater for insulin versus C-peptide. Insulin clearance was significantly decreased after oral versus intravenous glucose in both sham and Tx rats (Table 1).

Fasting GIP values were similar in both groups. Oral glucose stimulated GIP, whereas intravenous glucose did not (sham and TX; Fig 1).

DISCUSSION

Following orthotopic transplantation of a whole pancreas with portal venous drainage, arterial plasma insulin concentrations are still higher after oral versus intravenous glucose despite comparable arterial glucose concentrations. Our finding that the transplanted pancreas remained vagally denervated is in accordance with the observation of Gooszen et al,[25] who found the PP response to be virtually abolished after meal stimulation 12 weeks post–pancreas denervation in dogs. Sufficient adrenergic denervation of the transplanted pancreas in our study was shown by the significantly decreased norepinephrine content. This is in line with the findings from Alm et al,[26] who found no remaining adrenergic nerves in rat pancreas after extirpation of the celiac and superior mesenteric ganglion, and only minute amounts of norepinephrine in the pancreatic tissue. As it has been shown that pancreatic CGRP afferent nerves stem from dorsal root ganglia[27] and that upper-abdominal sympathectomy removed almost all CGRP-IR nerve fibers, suggesting an extrinsic origin of CGRP-IR nerves,[28] the significant reduction of the CGRP content in the transplanted pancreas in our study implies effective denervation. It may therefore be concluded that also in the rat, the incretin effect for insulin was preserved after denervation of the pancreas. It follows that either extrinsic pancreatic nerves do not mediate the incretin effect or their absence can easily be compensated for in the rat. However, the higher arterial insulin levels during the OGTT were only partly due to enhanced insulin secretion, since C-peptide concentrations were higher only for the first 30 minutes, and the integrated incremental response of C-peptide was similar after oral and isoglycemic intravenous glucose administration. The explanation for these findings is that during stimulation of insulin secretion by oral glucose, hepatic extraction of insulin is reduced. This can be assumed because the C-peptide to insulin molar ratio of the integrated response was significantly lower after oral versus intravenous glucose loading in both sham and Tx animals.

Table 1. Integrated Incremental Response (over 180 minutes) for Plasma Glucose, Insulin, and C-Peptide and Molar Ratio of Integrated Incremental Response for C-Peptide to Integrated Incremental Response of Insulin (hepatic insulin clearance) in Sham and Tx Rats After Oral Glucose (2 g/kg) and Isoglycemic Intravenous Infusion (mean ± SEM)

Parameter	Sham	Tx	*P*
Glucose (mmol · L^{-1} · min)			
Oral glucose	513.1 ± 55.4	410.1 ± 39.7	.14
IV glucose	512.3 ± 57.1	380.1 ± 31.6	.05
P	.95	.45	
Insulin (nmol · L^{-1} · min)			
Oral glucose	79.5 ± 10.6	104.9 ± 22.0	.30
IV glucose	36.6 ± 5.8	31.0 ± 4.9	.47
P	<.01	<.01	
C-peptide (pmol · ml^{-1} · min)			
Oral glucose	112 ± 10	105 ± 14	.70
IV glucose	121 ± 12	79 ± 8	.01
P	.60	.10	
Hepatic insulin clearance			
Oral glucose	1.6 ± 0.1	1.3 ± 0.2	.28
IV glucose	3.9 ± 0.6	3.3 ± 0.6	.47
P	<.02	<.01	
Incretin effects			
Insulin	3.0 ± 0.8	5.6 ± 2.7	.68
C-peptide	1.1 ± 0.2	1.4 ± 0.2	.17
P	<.01	<.01	

Abbreviation: IV, intravenous.

Our finding that oral glucose tolerance was normal after whole pancreas transplantation contrasts with results reported by Siegel et al.[14] They performed OGTTs and IVGTTs after intraportal transplantation of pancreatic islets in streptozotocin-diabetic rats; whereas intravenous glucose tolerance was comparable in both transplanted and control animals, oral glucose tolerance could only be normalized by injection of a preabsorptive insulin bolus mimicking nerve-mediated cephalic-phase insulin secretion. One possible explanation for the differences between the findings of Siegel et al[14] and our results in terms of glucose tolerance may be a disruption of the intrinsic innervation by islet isolation and transplantation in the experiments by Siegel et al.

The influence of extrinsic pancreas denervation on the incretin effect has been shown in different animal species and in man, but conflicting results have been reported.[5-10] In a study by Jakob,[5] venous insulin levels were similar after intragastric and intravenous glucose administration in dogs with duodenopancreatic allografts. However, the results were compromised by the fact that ulcerating inflammation of the transplanted duodenum may have reduced the glucose resorption and disturbed the release of incretin factors.

In contrast, Köhler et al[7] found the incretin effect for insulin to be preserved after orthotopic autotransplantation of pancreas in dogs, which is in line with our own observations. However, due to the chosen glucose-matching, ie, the use of venous instead of arterial glucose concentrations during oral and isoglycemic intravenous glucose administration, the incretin effect shown in their study may represent an artifact. Since the arteriovenous difference in plasma glucose is larger after oral

versus intravenous glucose administration, matching the venous glucose concentrations will lead to higher arterial glucose concentrations during oral versus intravenous glucose loading. In turn, the higher arterial glucose concentration that regulates insulin and C-peptide secretion by the pancreatic β cells will increase insulin secretion during an OGTT, and could lead to a pseudo-incretin effect.[12,29]

Two studies in the pig also showed preservation of the incretin effect after pancreas denervation.[6,8] In the study by Jensen et al,[6] venous glucose concentrations were also matched and, in addition, the systemic venous drainage of the duodenopancreas transplant led to hyperinsulinemia and impaired glucose tolerance, thus complicating the interpretation of the results. Such problems were avoided in the study by Nauck et al,[8] in which venous drainage of the denervated pancreas was established via the portal vein and arterial glucose concentrations were matched during glucose infusion.

Our present study in the rat differs from all other incretin studies in various animal species yet published, in one important aspect: we administered glucose orally, whereas the other studies used glucose intragastrically. This means that in our study, the influence of pancreatic denervation on the cephalic phase of insulin secretion could also be assessed; in the former studies, intragastric glucose administration circumvented stimulation of the nerves of the cephalic reflex arch. This latter procedure is unphysiological and places a bias on the results favoring the influence of gastrointestinal hormones.

Two incretin studies in patients undergoing heterotopic pancreas transplantation reported a preserved[9] and an abolished[30] incretin effect, respectively. However, after heterotopic pancreas transplantation with systemic venous drainage, the impact of hepatic insulin clearance on the incretin effect cannot be interpreted, because first-pass extraction of insulin by the liver is abolished. In a study by Clark et al[10] showing a preserved incretin effect after paratopic pancreas transplantation with portal venous drainage, based on the matching of venous glucose concentrations during glucose tolerance tests, there was a trend for an artifactually increased incretin effect. Furthermore, the above-mentioned transplantation studies in humans appear to be compromised by the accompanying immunosuppression, which interferes with the insulin sensitivity of tissues.[31] The need for immunosuppression was avoided in our study by using inbred rats. Another difference between our experiments and the others is the measurement of C-peptide in animals with intact kidneys—in contrast to studies with impaired renal function due to longstanding diabetes—so the metabolic clearance of C-peptide can be assumed to be normal in our animals. Measurement of C-peptide enabled us to estimate the contribution of hepatic insulin clearance to the incretin effect. The decreased hepatic insulin clearance after oral glucose contributed substantially to the incretin effect in our study. This is in line with the findings by Shuster et al,[13] who found that the incretin effect is mediated by both increased secretion and decreased clearance of insulin. Dupré et al[32] suggested that the decreased hepatic extraction of insulin after oral glucose was related to a higher secretion rate of insulin itself and was not a result of the enteroinsular axis following glucose absorption from the gut. However, in our study, C-peptide concentrations were higher only for the first 30 minutes after oral glucose in comparison to the intravenous isoglycemic glucose infusion, and the resulting integrated incremental response over 180 minutes was similar. Therefore, it can be concluded that the insulin secretion rate was higher only for the first 30 minutes, whereas thereafter, the higher insulin levels were likely due to decreased hepatic extraction alone.

The factors that mediate the latter phenomenon are unknown, and may include blood glucose supplied to the liver, factors associated with increased hepatic blood flow, and factors released by intestinal glucose absorption. It is known that GIP and glucagon-like peptide-1 (GLP-1) are important incretin hormones.[33-38] The two hormones together are thought to account for the majority of the intestinal stimulation of insulin secretion.[39] In our study, only GIP was measured. As the plasma GIP concentration in our study reached a maximum within 20 minutes and returned to baseline within 180 minutes, its course paralleled the oral glucose–related changes in plasma insulin. Therefore, GIP may well have accounted for the increased secretion of insulin, as evidenced by the higher C-peptide concentrations within the first 30 minutes after oral glucose ingestion. Roberge and Brubaker[40] have suggested an enteroendocrine loop between GIP released from duodenal K cells and intestinal proglucagon-derived peptide released from ileal L cells, which may account for early increases in GLP-1 secretion after duodenal fat but not after duodenal glucose application in rats. This implies for our study that the initial higher insulin secretion can be mostly attributed to GIP, because only glucose, which is unable to activate the enteroendocrine loop, was administered. Since it has been shown that ileal glucose directly stimulates intestinal proglucagon-derived peptide secretion,[40] GLP-1 may have been secreted in our study when glucose reached the ileum. Therefore, one may speculate that GLP-1 could have modulated the hepatic extraction of insulin, which likely accounted for the higher insulin levels in the absence of elevated C-peptide levels from 30 minutes onward after glucose ingestion. The slightly higher incretin effect in Tx rats, despite interruption of the vagally mediated hepatopancreatic reflex of GLP-1[41,42] by denervation of the transplanted pancreas, may be explained by the fact that the suppression of insulin secretion is neurally mediated.[43,44]

In conclusion, our results show that the incretin effect for insulin is preserved after extrinsic denervation of the pancreas. Gastrointestinal hormones such as GIP and a decrease in hepatic insulin clearance seem to be of more importance than pancreatic innervation for the increase in insulin after oral glucose administration.

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REFERENCES

1. Zunz E, La Barre J: Contributions à l'étude des variations physiologiques de la sécrétion interne du pancréas relations entre les sécrétions externe et interne du pancréas. Arch Int Physiol Biochim 31:20-44, 1929

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

3. Ahrén B, Taborsky GJJ, Porte DJ: Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. Diabetologia 29:827-836, 1986

4. Miller RE: Pancreatic neuroendocrinology: Peripheral neural mechanisms in the regulation of the islets of Langerhans. Endocr Rev 2:471-494, 1981

5. Jakob A, Largiadèr F, Froesch ER: Glucose turnover and insulin secretion in dogs with pancreatic allografts. Diabetologia 6:441-444, 1970

6. Jensen SL, Nielsen OV, Kühl C: The enteral insulin-stimulation after pancreas transplantation in the pig. Diabetologia 12:617-620, 1976

7. Köhler H, Schröter Printzen I, Nustede R, et al: Endocrine response to intragastric and intravenous glucose challenge in the denervated dog pancreas. Int J Pancreatol 11:117-124, 1992

8. Nauck M, van Hoorn W, Gubernatis G, et al: Preserved incretin effect after complete surgical denervation of the pancreas in young pigs. Res Exp Med (Berl) 185:291-298, 1985

9. Nauck MA, Busing M, Orskov C, et al: Preserved incretin effect in type 1 diabetic patients with end-stage nephropathy treated by combined heterotopic pancreas and kidney transplantation. Acta Diabetol 30:39-45, 1993

10. Clark JDA, Wheatley T, Brons IGM, et al: Studies of the entero-insular axis following pancreas transplantation in man: Neural or hormonal control? Diabet Med 6:813-817, 1989

11. Gibby OM, Hales CN: Oral glucose decreases hepatic extraction of insulin. BMJ 286:921-923, 1983

12. Hampton SM, Morgan LM, Tredger JA, et al: Insulin and C-peptide levels after oral and intravenous glucose. Diabetes 35:612-616, 1986

13. Shuster LT, Go VLW, Rizza RA, et al: Incretin effect due to increased secretion and decreased clearance of insulin in normal humans. Diabetes 37:200-203, 1988

14. Siegel EG, Trimble ER, Renold AE, et al: Importance of preabsorptive insulin release on oral glucose tolerance: Studies in pancreatic islet transplanted rats. Gut 21:1002-1009, 1980

15. Popovic V, Popovic P: Permanent cannulation of aorta and vena cava in rats and ground squirrels. J Appl Physiol 15:727-728, 1960

16. Hara E, Saito M: A method for studying the enteroinsular axis in unanesthetized and unrestrained rats. Endocrinol Jpn 27:457-461, 1980

17. Palm Ú, Boemke W, Bayerl D, et al: Prevention of catheter-related infections by a new, catheter-restricted antibiotic filling technique. Lab Anim 25:142-152, 1991

18. Herbert V, Lau K-S, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965

19. Akpan JO, Weide LG, Gingerich RL: A specific and sensitive radioimmunoassay for rat C-peptide. Int J Pancreatol 13:87-95, 1993

20. Chance RE, Moon NE, Johnson MG: Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP), in Jaffe BM, Behrman HR (eds): Methods of Hormone Radioimmunoassays. New York, NY, Academic, 1979, pp 657-672

21. Kuzio M, Dryburgh JR, Malloy KM, et al: Radioimmunoassay for gastric inhibitory polypeptide. Gastroenterology 66:357-364, 1974

22. Oka K, Kojima K, Togari A, et al: An integrated scheme for the simultaneous determination of biogenic amines, precursor amino acids, and related metabolites by liquid chromatography with electrochemical detection. J Chromatogr 308:43-53, 1984

23. Byrant MG, Bloom SR: Measurement in tissues, in Bloom SR, Long RG (eds): Radioimmunoassay of Gut Regulatory Peptides. London, UK, Saunders, 1982, pp 36-41

24. Ebert R, Illmer K, Creutzfeldt W: Release of gastric inhibitory polypeptide (GIP) by intraduodenal acidification in rats and humans and abolishment of the incretin effect of acid by GIP-antiserum in rats. Gastroenterology 76:515-523, 1979

25. Gooszen HG, Van Der Burg MPM, Guicherit OR, et al: Crossover study on effects of duct obliteration, celiac denervation, and autotransplantation on glucose- and meal-stimulated insulin, glucagon, and pancreatic polypeptide levels. Diabetes 38:114-116, 1989 (suppl 1)

26. Alm P, Liedberg G, Owman C: Gastric and pancreatic sympathetic denervation in the rat. Technique and results. Scand J Gastroenterol 6:307-312, 1971

27. Won MH, Park HS, Jeong YG, et al: Afferent innervation of the rat pancreas: Retrograde tracing and immunohistochemistry in the dorsal root ganglia. Pancreas 16:80-87, 1998

28. Su HC, Bishop AE, Power RF, et al: Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. J Neurosci 7:2674-2687, 1987

29. 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

30. Gibby OM, Loke M, Sarson DL, et al: Disruption of the entero-insular axis in pancreatic transplantation. Regul Pept 1:42A, 1980 (suppl 2, abstr)

31. Rizza RA, Mandarino LJ, Gerich JE: Cortisol-induced insulin resistance in man: Impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. J Clin Endocrinol Metab 54:131-138, 1982

32. Dupré J, Behme MT, Hramiak IM, et al: Hepatic extraction of insulin after stimulation of secretion with oral glucose or parenteral nutrients. Metabolism 42:921-927, 1993

33. Holst JJ: Enteroglucagon. Annu Rev Physiol 59:257-271, 1997

34. Göke B, Fehmann H-C, Schirra J, et al: Das Darmhormon Glucagon-like peptide-1 (GLP-1): aus dem Experiment in die Klinik. Z Gastroenterol 35:285-294, 1997

35. Orskov C, Wettergren A, Holst JJ: Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. Scand J Gastroenterol 31:665-670, 1996

36. Fehmann H-C, Gherzi R, Göke B: Regulation of islet hormone gene expression by incretin hormones. Exp Clin Endocrinol Diabetes 103:156-165, 1995 (suppl 2)

37. Wang Z, Wang RM, Owji AA, et al: Glucagon-like peptide-1 is a physiological incretin in rat. J Clin Invest 95:417-421, 1995

38. Kreyman B, Ghatei MA, Williams G, et al: Glucagon-like peptide-1 7-36: A physiological incretin in man. Lancet 2:1300-1304, 1987

39. Nauck MA, Bartels E, Orskov C, et al: Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1 (7-36) amide infused at near-physiological insulinotropic and glucose concentrations. J Clin Endocrinol Metab 76:912-917, 1993

40. Roberge JN, Brubaker PL: Regulation of intestinal proglucagon-derived peptide secretion by glucose-dependent insulinotropic peptide in a novel enteroendocrine loop. Endocrinology 133:233-240, 1993

41. Nakabayashi H, Nishizawa M, Nakagawa A, et al: Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. I. Am J Physiol 271·E808-E813, 1996

42. Nishizawa M, Nakabayashi H, Uchida K, et al: The hepatic vagal nerve is receptive to incretin hormone glucagon-like peptide-1, but not to glucose-dependent insulinotropic polypeptide, in the portal vein. J Auton Nerv Syst 61:149-154, 1996

43. Luzi L, Battezzati A, Perseghin G, et al: Lack of feedback inhibition of insulin secretion in denervated human pancreas. Diabetes 41:1632-1639, 1992

44. Boden G, Chen X, DeSantis R, et al: Evidence that suppression of insulin secretion by insulin itself is neurally mediated. Metabolism 42:786-789, 1993