TPN-evoked dysfunction of islet lysosomal activity mediates impairment of glucose-stimulated insulin release

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Salehi, Albert, Bo-Guang Fan, Mats Ekelund, Gunnar Nordin, and Ingmar Lundquist. TPN-evoked dysfunction of islet lysosomal activity mediates impairment of glucose-stimulated insulin release. Am J Physiol Endocrinol Metab 281: E171–E179, 2001.—We examined the relation between nutrient-stimulated insulin secretion and the islet lysosome acid glucan-1,4-α-glucosidase system in rats undergoing total parenteral nutrition (TPN). During TPN treatment, serum glucose was normal, but free fatty acids, triglycerides, and cholesterol were elevated. Islets from TPN-infused rats showed increased basal insulin release, a normal insulin response to cholinergic stimulation but a greatly impaired response when stimulated by glucose or α-ketoisocaproic acid. This impairment of glucose-stimulated insulin release was only slightly ameliorated by the carnitine palmitoyltransferase 1 inhibitor etomoxir. However, in parallel with the impaired insulin response to glucose, islets from TPN-infused animals displayed reduced activities of islet lysosomal enzymes including the acid glucan-1,4-α-glucosidase, a putative key enzyme in nutrient-stimulated insulin release. By comparison, the same lysosomal enzymes were increased in liver tissue. Furthermore, in intact control islets, the pseudotetrasaccharide acarbose, a selective inhibitor of acid α-glucosidase, has also been shown to impair the glycogenolytic activity in isolated islets (22). Hence, we have hypothesized that the acid glucosidase activity is a particular desensitization of the β-cell to glucose-stimulated insulin secretion (9, 40). Glucose-stimulated insulin release itself is composed of a complex cascade of events (23, 42), the details of which are far from elucidated. We (14–20, 31–36) have proposed that one of these multiple signals, which regulates insulin release stimulated by glucose and other nutrient secretagogues such as leucine and α-ketoisocaproic acid (KIC), is transduced through the vacuolar system involving the activation of the lysosomal acid glucan-1,4-α-glucosidase. This α-glucosidase inhibitor type of enzyme produces nonphosphorylated free glucose and preferentially cleaves α-1,4-linked glucose polymers such as glycogen (15, 17, 26). It has been known for a long time that the islets of Langerhans contain glycogen (8, 22). This glycogen level is fairly constant at a wide range of blood glucose concentrations (22), suggesting that the major part of it is not integrated into the metabolic pool of glucose phosphorylation processes in the cytoplasm but rather is restricted to a vacuolar pool of signal glycogen. In this context, it should be noted that the phosphorolytic breakdown of glycogen in vitro in islet tissue is known to be very slow (22). Hence, we have hypothesized that the acid glucan-1,4-α-glucosidase might attack certain pools of islet vacuolar glycogen to produce high compartmentalized concentrations of glucose, which in turn could act as a further transducer, e.g., cybernetic, metabolic, or osmotic, in the multifactorial process of insulin release (14–20, 31–36). In fact, recent data emphasize the role of compartimentalization and acidification in the final stages of exocytosis (1, 21, 41). In addition, the activated enzyme might have the ability to modify membrane glycoproteins with α-1,4-linked glucose residues of importance for the exocytic process. In accordance with this idea, a series of recent in vitro studies revealed a close relationship between islet acid glucan-1,4-α-glucosidase activity and nutrient-stimulated in-

DESPITE INTENSIVE RESEARCH, the mechanisms behind pancreatic β-cell dysfunction in patients with non-insulin-dependent diabetes mellitus (NIDDM) remain largely unclear. There is general agreement that multiple deficiencies are probably involved, among which is a particular desensitization of the β-cell to glucose-stimulated insulin secretion (9, 40). Glucose-stimulated insulin release itself is composed of a complex cascade of events (23, 42), the details of which are far from elucidated. We (14–20, 31–36) have proposed that one of these multiple signals, which regulates insulin release stimulated by glucose and other nutrient secretagogues such as leucine and α-ketoisocaproic acid (KIC), is transduced through the vacuolar system involving the activation of the lysosomal acid glucan-1,4-α-glucosidase. This α-glucosidase inhibitor type of enzyme produces nonphosphorylated free glucose and preferentially cleaves α-1,4-linked glucose polymers such as glycogen (15, 17, 26). It has been known for a long time that the islets of Langerhans contain glycogen (8, 22). This glycogen level is fairly constant at a wide range of blood glucose concentrations (22), suggesting that the major part of it is not integrated into the metabolic pool of glucose phosphorylation processes in the cytoplasm but rather is restricted to a vacuolar pool of signal glycogen. In this context, it should be noted that the phosphorolytic breakdown of glycogen in vitro in islet tissue is known to be very slow (22). Hence, we have hypothesized that the acid glucan-1,4-α-glucosidase might attack certain pools of islet vacuolar glycogen to produce high compartmentalized concentrations of glucose, which in turn could act as a further transducer, e.g., cybernetic, metabolic, or osmotic, in the multifactorial process of insulin release (14–20, 31–36). In fact, recent data emphasize the role of compartimentalization and acidification in the final stages of exocytosis (1, 21, 41). In addition, the activated enzyme might have the ability to modify membrane glycoproteins with α-1,4-linked glucose residues of importance for the exocytic process. In accordance with this idea, a series of recent in vitro studies revealed a close relationship between islet acid glucan-1,4-α-glucosidase activity and nutrient-stimulated in-

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sulin release at different Ca^{2+} concentrations as well as in the presence and absence of various selective α-glucosidase inhibitors such as the pseudotetrasaccharide acarbose, the deoxynojirimycin derivatives miglitol and emiglitate, and the indolizine alkaloid castanospermine, whereas receptor-activated insulin secretion induced, for example, by a cholinergic stimulus was independent of this enzyme activity (19, 20, 30–36). Furthermore, previous in vivo experiments disclosed a surprisingly good correlation between glucose-stimulated insulin release and islet acid glucan-1,4-α-glucosidase activity both in normal mice and in the insulin-hypersecreting ob/ob mouse, an animal model with certain similarities to the obese type of human NIDDM (15–19).

Because patients with NIDDM often display abnormalities not only in glucose metabolism but also in lipid metabolism, the most prominent being hypertriglyceridemia with elevated free fatty acid (FFA) levels (2, 24, 29), and because such abnormalities have been suggested to greatly impair β-cell function (2, 24, 37, 43), the question arose as to whether elevated levels of triglycerides and FFA would have any impact on the insulin-secretory signal transduced through the lysosome acid glucan-1,4-α-glucosidase system in the β-cell. It has been known for a long time that, similar to other nutrients such as glucose and certain amino acids, FFA can acutely stimulate insulin release both in vitro and in vivo (3, 29, 39). In contrast, recent data have shown that long-term elevation of FFA either infused into rats for 48 h or added to isolated islets during long-term culture, greatly impair Δβ-cell function (2, 24, 29, 39, 43, 44). Such an impairment was reportedly further exaggerated in the obese, prediabetic Zucker (ZDF) rat, where islet triglyceride content was markedly increased, probably as a consequence of increased plasma FFA levels (39).

The aim of the present investigation was to characterize, in a rat model of total parenteral nutrition (TPN), where the TPN solution was infused for 12 days in healthy, normal Sprague-Dawley rats, the effects of long-term elevation of plasma levels of FFA and triglycerides on insulin release induced by two nutrient insulin secretagogues, glucose and KIC, and one receptor-activating secretagogue, the cholinergic muscarinic agonist carbachol, in relation to the activity of the islet lysosome acid glucan-1,4-α-glucosidase system. For comparison, measurements of different lysosomal enzyme activities in liver tissue after TPN infusion were also performed. Finally, to directly test the function of the islet lysosomal/vacuolar system, we investigated, in islets isolated from both TPN-infused and freely fed control rats, the ability of the pseudotetrasaccharide acarbose, a potent and selective inhibitor of islet acid glucan-1,4-α-glucosidase and glucose-stimulated insulin release (36), to enter into the lysosomal system and modulate enzyme activity and glucose-induced insulin secretion.

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats (B&K Universal, Sol lentuna, Sweden) weighing 225–235 g at the start of the infusion experiments were used. All animals were housed in metabolic cages. The temperature was maintained at a constant level, and a 12:12-h light-dark cycle was provided.

Drugs and chemicals. Collagenase (CLS 4) was purchased from Worthington Biochemical (Freehold, NJ). Methylumbiferyl-coupled substrates, KIC, and carbachol were obtained from Sigma Chemical (St. Louis, MO). Etomoxir 2-[b-(4-chlorophenox)-hexyloxirane-2-carboxylate was purchased as the sodium salt (RBJ Research Biochemicals International, Natick, MA). Bovine serum albumin (BSA) was from ICN Biomedicals (High Wycombe, UK). The pseudotetrasaccharide acarbose was generously supplied by Bayer (Le verkusen, Germany). All other drugs and chemicals were from British Drug Houses (Poole, UK) or Merek (Darmstadt, Germany). The radioimmunoassay (RIA) kits for insulin determination were obtained from Novo Nordisk (Bagsvaerd, Denmark) or Diagnostika (Falkenberg, Sweden).

Experimental procedures: TPN. Rats were anesthetized intraperitoneally with chloral hydrate and operated on under sterile conditions. The neck of the rat was gently washed with an iodine solution. A silicon-rubber catheter, 0.37 in. OD (Silastic, Dow Corning, Midland, MI), was inserted into the right jugular vein. The catheter was transferred to the skull subcutaneously and connected to a swivel via a protective coil attached to the skin of the skull. Immediately after surgery, all rats in the TPN group were infused with a 5% glucose solution at 2.0 ml/h for 12 h, followed by TPN at 200 mg/kg−1·day−1. Every 2nd day, the catheters were flushed with 100 U/kg of low molecular weight heparin (Fragmin; Pharmacia, Uppsala, Sweden). No oral intake, including water, was allowed during the infusion period. Control animals underwent the same operative procedure, including insertion of a catheter, but no TPN infusion was performed. The catheters were similarly flushed with 100 U/kg of Fragmin every 2nd day. The control animals were allowed free access to a standard pellet diet (B&K Universal) and tap water ad libitum. Details of the methodology as well as the composition of the TPN solution were recently described (28). The TPN infusion experiments lasted 12 days. All animals were housed in metabolic cages with a constant temperature. A 12:12-h light-dark cycle was provided. No significant differences in body weights were detected between the TPN and the control groups at the end of the experiments.

Determination of serum lipids. Concentrations of FFA, triglycerides without free glycerol (TG), and cholesterol in serum were determined enzymatically with kits from Wako Chemicals (Neuss, Germany) and Boehringer Mannheim (Indianapolis, IN). High-density lipoprotein (HDL) cholesterol was determined as cholesterol in the supernatant after precipitation with polyethylene glycol 6000. Sera were stored at −20°C until analyzed.

Insulin secretion from isolated islets. Insulin secretion studies were performed with freshly isolated islets. The islets were isolated directly after the TPN infusion device was disconnected, with the exception of one series of experiments (illustrated in Fig. 1, D-F), where the TPN rats (and the controls) were fasted for 12 h after disconnection. After decapitation, preparation of isolated pancreatic islets from TPN-infused and control rats was performed by retrograde injection of a collagenase solution via the bile-pancreatic duct (4). The islets were then preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, supplemented with 10 mmol/l HEPES, 0.1% BSA, and 1 mmol/l...
glucose. Each incubation vial was gassed with 95% O₂-5% CO₂ (vol/vol) to obtain constant pH and oxygenation. After preincubation, the buffer was changed to a medium containing different glucose concentrations and test agents, and the islets were incubated for 60 min. All incubations were performed at 37°C in an incubation box. Immediately after incubation, aliquots of the medium were removed and frozen for subsequent RIA of insulin (7). In the experiments with acarbose, the islets were first preincubated (with or without acarbose) for 60 min (to allow for acarbose uptake) at 1 mmol/l glucose (36). After this preincubation, the buffer was changed to a medium containing 1 or 16.7 mmol/l glucose with or without acarbose, and then the islets were incubated for 120 min (36).

Determination of lysosomal enzyme activities and neutral α-glucosidase in isolated islets and liver. For determination of lysosomal enzyme activities, 150–200 isolated islets (35 islets in the acarbose incubation experiments) were thoroughly washed in a glucose-free Hanks' solution and collected and stored in acetate-EDTA buffer (1.1 mmol/l EDTA and 5 mmol/l acetate, pH 5.0) at −20°C. After thawing in an ice bath and subsequent sonication, the islet homogenates were analyzed for enzyme activities as previously described in detail (13, 15, 17, 30). Similarly, the determination of enzyme activities in liver tissue has previously been described (15, 30). Protein was analyzed according to Lowry et al. (11).

RESULTS
Basal metabolic characteristics of TPN-infused rats and their controls. Table 1 shows the basal metabolic status as reflected in serum of the TPN-infused rats.
and their freely fed controls on days 4, 8, and 12 after start of the treatment. It was seen that the TPN rats displayed greatly elevated serum levels of FFA, TG, and cholesterol, whereas HDL-cholesterol was modestly decreased. There was no difference in the serum glucose levels between the two groups (Table 1). Furthermore, in ancillary experiments, we found no difference in the basal plasma levels of either insulin or thermore, in ancillary experiments, we found no difference in the basal plasma levels of either insulin or glucose recorded on Fig. 1

**Effect of TPN on the insulin secretory response stimulated by glucose, KIC, or carbachol.** Figure 1A shows the effect of low (1 mmol/l) and high (16.7 mmol/l) glucose on insulin release from islets isolated either from freely fed controls or from TPN rats directly after the infusion of TPN was stopped. It is seen that insulin secretion from TPN islets was increased at low glucose. At high glucose, however, the increase in insulin release from control islets was 14-fold above basal, whereas the increase from TPN islets was only approximately fourfold. Figure 1B illustrates the effect of another nutrient secretagogue, KIC (10 mmol/l), on insulin release from isolated islets after TPN treatment. This series of experiments was performed in a glucose-free KRB medium. It is seen that insulin secretion from TPN islets, in the absence of glucose, was increased twofold above the release observed in control islets. Addition of KIC had practically no effect on insulin release in TPN islets but induced a fivefold increase in control islets. Figure 1C shows that insulin secretion at a more physiological glucose concentration (4 mmol/l) was unaffected in islets isolated from TPN rats. Moreover, cholinergic receptor-activated stimulation of insulin release by the cholinergic muscarinic agonist carbachol (20 μmol/l) was of the same magnitude in TPN and control islets (Fig. 1C).

**Effect of a 12-h normalization period after TPN treatment cessation.** To elucidate whether the TPN-induced impairment of glucose-stimulated insulin release was rapidly and readily reversible, we performed a series of experiments with islets isolated at 12 h after the TPN infusion was stopped. During this 12-h period, all animals were allowed drinking water but no food. Figure 1, D and E, shows that the impairment of glucose-induced insulin release from isolated islets of the TPN rats compared with the control group was still very obvious. Insulin secretion at 1 or 4 mmol/l glucose, as well as insulin release stimulated by carbachol, was similar to that of the controls. To test whether the poor insulin release in response to glucose was also present in the in vivo situation an intravenous glucose load (4.4 mmol/kg) was given to both groups of animals. Figure 1F shows that the glucose-induced in vivo insulin response in the TPN rats was also reduced. The glucose tolerance curve was slightly impaired at 15 and 30 min in the TPN group, but the area under the curve was not significantly different from that of the controls (data not shown).

**Influence of etomoxir on glucose-induced insulin release in TPN-treated islets.** We next investigated whether the profound impairment of glucose-induced insulin release after TPN treatment could be explained by a direct influence of the elevated serum FFA on islet glucose metabolism, because long-term exposure to FFA has been reported to inhibit glucose-stimulated insulin secretion through a glucose-fatty acid cycle (29, 43). To this purpose, etomoxir, a mitochondrial carnitine palmitoyltransferase 1 inhibitor, was added to the test tubes. Figure 2 shows that glucose-induced insulin release was suppressed by ~55% in TPN-treated islets. Only a minor fraction of this inhibition was reversed by etomoxir treatment. Etomoxir did not influence basal (1 mmol/l) or glucose-stimulated (16.7 mmol/l) insulin release in control islets but slightly increased basal insulin secretion in TPN-treated islets (Fig. 2).

**Lysosomal enzyme activities in isolated islets and liver tissue.** To test whether the elevated serum lipids might influence the lysosomal system, we performed an analysis of islet and liver lysosomal enzyme activities after TPN. Directly after the TPN infusion was stopped, pancreatic islets were isolated, and liver specimens were removed for analysis of the activity of the acid glucan-1,4-α-glucosidase as well as analysis of other lysosomal enzymes and the neutral α-glucosidase (an enzyme attributed to the endoplasmic reticulum). Figure 3A shows the activities of the different lysosomal enzymes recorded in islet tissue. It is seen that the activities of the glycogen-hydrolyzing enzyme acid glucan-1,4-α-glucosidase (~45%) and acid α-glucosidase (~30%) were significantly reduced. Likewise, the activ-

### Table 1. Concentrations of serum glucose and serum lipids in freely fed controls and TPN rats during TPN infusion

<table>
<thead>
<tr>
<th></th>
<th>Glucose, mmol/l</th>
<th>Free Fatty Acids, mmol/l</th>
<th>Triglycerides, mmol/l</th>
<th>Cholesterol, mmol/l</th>
<th>HDL-Cholesterol, mmol/l</th>
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<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>6.0 ± 0.3</td>
<td>0.81 ± 0.05</td>
<td>0.87 ± 0.04</td>
<td>2.10 ± 0.12</td>
<td>1.44 ± 0.14</td>
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<tr>
<td>TPN</td>
<td>5.3 ± 0.4</td>
<td>1.22 ± 0.13</td>
<td>1.65 ± 0.16</td>
<td>2.97 ± 0.20</td>
<td>1.08 ± 0.08</td>
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<tr>
<td>Day 8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>6.4 ± 0.4</td>
<td>0.72 ± 0.05</td>
<td>1.26 ± 0.10</td>
<td>1.86 ± 0.13</td>
<td>1.39 ± 0.14</td>
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<tr>
<td>TPN</td>
<td>5.3 ± 0.4</td>
<td>1.89 ± 0.32†</td>
<td>2.32 ± 0.25</td>
<td>3.59 ± 0.15‡</td>
<td>0.85 ± 0.03†</td>
</tr>
<tr>
<td>Day 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.7 ± 0.5</td>
<td>0.88 ± 0.08</td>
<td>1.25 ± 0.19</td>
<td>1.82 ± 0.10</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>TPN</td>
<td>6.6 ± 0.5</td>
<td>1.96 ± 0.21‡</td>
<td>2.15 ± 0.16†</td>
<td>3.04 ± 0.24‡</td>
<td>0.83 ± 0.06</td>
</tr>
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Values are mean ± SE of 6 rats in each group. TPN, total parenteral nutrition. Blood samples were drawn on days 4, 8, and 12 of the infusion period. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. controls.
The activity of the proteolytic lysosomal enzyme cathepsin D, however, was unaffected, as was the nonlysosomal neutral α-glucosidase.

The influence of TPN infusion on lysosomal enzyme activities in liver tissue is illustrated in Fig. 3B. In sharp contrast to the suppressive effect observed in islet tissue, the liver tissue of TPN-infused rats displayed markedly increased levels of lysosomal enzyme activities. Thus the activities of cathepsin D, N-acetyl-β-D-glucosaminidase and acid α-glucosidase were increased by 70% and acid phosphatase and acid glucan-1,4-α-glucosidase by 15–45%. β-Glucuronidase was not affected, and the nonlysosomal neutral α-glucosidase was suppressed (–20%). When the lysosomal enzyme activities in the islets and the liver of the control Sprague-Dawley rats are compared, it can be seen that acid phosphatase, N-acetyl-β-D-glucosaminidase, and cathepsin D were of approximately the same magnitude in both tissues, whereas β-glucuronidase was eightfold greater in the liver. Moreover, and most notably, the activity of the acid glucan-1,4-α-glucosidase in isolated islets was extremely high (50-fold higher) compared with the liver. The acid α-glucosidase was fivefold higher in the islets than in the liver, and the nonlysosomal neutral α-glucosidase displayed the same activity in both tissues. In this context, it should be recalled that both acid α-glucosidases display α-1,4-spliiting properties but that the acid glucan-1,4-α-glucosidase is mainly a glycogen-hydrolyzing enzyme, whereas the acid α-glucosidase prefers maltose and oligosaccharides as substrate (15–17, 26).

**Effects of acarbose.** To elucidate whether the TPN islets might suffer from a general dysfunction of the lysosomal/vacuolar system, we performed a series of experiments with the the selective acid glucan-1,4-α-glucosidase inhibitor acarbose, a pseudotetrasaccharide, which is known to enter the cell through endocytosis (12, 19, 30, 31, 36). Figure 4 shows the dose-response relationship for the effect of acarbose on lysosomal enzyme activities after direct addition to islet homogenates as well as the effect of acarbose on glucose-induced insulin release in isolated islets. Acid glucan-1,4-α-glucosidase (Fig. 4A) and acid α-glucosidase (Fig. 4B) activities were dose dependently inhibited by acarbose in islet homogenates from both controls and TPN rats. However, compared with controls, the ED_{50} for the inhibitory effect of acarbose on acid glucan-1,4-α-glucosidase activity in TPN islet homogenates was clearly shifted to the right by more than one order of magnitude. The acarbose inhibition curve for acid α-glucosidase was also shifted to the right, although it was less pronounced. Other lysosomal enzyme activities in freely fed control and TPN islets were not affected by acarbose (Fig. 4, E and F). Neutral α-glucosidase activity was reduced only at very high concentrations of acarbose and equally for both control and TPN islets (Fig. 4D). In parallel with the effects of acarbose on islet acid α-glucosidasehydrolase activities,
the glucose-stimulated insulin release from islets of control rats was dose dependently and markedly suppressed by the pseudotetrasaccharide (Fig. 4C). In contrast, acarbose displayed no effect at all on glucose-stimulated insulin release from islets isolated from TPN-rats (Fig. 4C). To directly assess the effect of acarbose on the lysosomal enzyme activities in intact islets and thus whether acarbose would have access to the islet lysosomal/vacuolar system, isolated islets from freely fed control rats and TPN rats were incubated at both low (1 mmol/l) and high (16.7 mmol/l) glucose in the absence and presence of a high concentration of acarbose (10 mmol/l). Figure 5 shows that incubation with acarbose inhibited acid glucan-1,4-α-glucosidase activity modestly at low (1 mmol/l) and markedly at high (16.7 mmol/l) glucose (Fig. 5A). Acid α-glucosidase activity was inhibited only at high glucose (Fig. 5C). In contrast, no significant effects of acarbose were observed on acid α-glucosidase-hydrolase activities in islets from TPN rats (Fig. 5, A and C), suggesting that not only were the catalytic activities of the acid α-glucosidases impaired but also the function of the lysosomal/vacuolar system itself. Compared with control islets, other lysosomal enzyme activities, i.e., acid phosphatase (Fig. 5D) and N-acetyl-β-d-glucosaminidase (Fig. 5E), were increased in TPN islets in both the absence and the
presence of acarbose. Thus acarbose had no effect on these enzymes in islets isolated from either freely fed control or TPN rats. Neutral α-glucosidase was unaffected by both TPN and acarbose (Fig. 5F). Moreover, glucose-stimulated insulin release from control islets was severely impaired (Fig. 5B). In contrast, glucose-stimulated insulin release from TPN islets was not significantly inhibited by acarbose, although a slight tendency could be noted. Basal insulin release at low glucose was slightly inhibited by acarbose in control islets but was unaffected in TPN islets (Fig. 5F). Finally, it should be noted that, in contrast to the homogeneous pattern of decreased lysosomal enzyme activities compared with controls, in TPN islets isolated directly “ex vivo” (Fig. 3A), the present results obtained after incubation of intact islets in vitro showed increased activities of the classical lysosomal enzymes acid phosphatase and N-acetyl-β-d-glucosaminidase (Fig. 5, D and E) in islets taken from TPN rats compared with freely fed controls, whereas the acid glucan-1,4-α-glucosidase activity was markedly decreased at both high and low glucose and acid α-glucosidase at high glucose (Fig. 5, A and C).

**DISCUSSION**

Acute stimulation of insulin release by FFA at basal glucose has been well established for a long time (3, 27). However, the mechanisms of action of FFA in stimulating the β-cell secretory machinery are still open to debate and have been suggested to include, for example, increased mitochondrial oxidation of FFA, increased Ca\(^{2+}\) influx, and increased long-chain acyl-CoA esters, which in turn activate protein kinase C (39). In contrast, direct demonstration of long-term effects of elevated serum FFA on β-cell function, thus in a way mimicking the situation in obese NIDDM, has received less attention. However, very recently, Shimabukuro et al. (37) and Unger (39) demonstrated, by investigating the ZDF (fa/fa) rat (having a mutated leptin receptor), that the β-cell failure in this type of obesity-associated diabetes with high plasma FFA was correlated with excessive accumulation of fat within the islet tissue due to an increased capacity to esterify, and a decreased capacity to oxidize, FFA.

The present study was initially encouraged by an early article by Greenberg et al. (5), showing that TPN treatment in human subjects for 25 days greatly reduced the insulin response to a standardized meal without any change in gut hormone release, and by more recent studies by Sako and Grill (29) and Zhou and Grill (43, 44) in the normal rat. They observed that 48 h of fat infusion or islet culture with elevated FFA in the medium greatly impaired the insulin secretory capacity in response to glucose and that the decreased insulin response in their experiments could be explained largely by an FFA-induced decrease in islet glucose oxidation and decreased pyruvate dehydrogenase activity. However, in contrast to these studies (29, 43) we found in our long-term experiments that the TPN-induced suppression (~55%) of glucose-stimulated insulin release was only slightly restored by exposure to the carnitine palmitoyltransferase 1 inhibitor etomoxir. Indeed, when the elevated release of basal insulin from TPN islets was taken into account, the inhibitory effect of glucose-stimulated release exerted by TPN, when calculated as ΔIRI (i.e., increase of insulin above basal), was found to be 66%, of which only 11% was reversed by etomoxir. Thus only a minor part of the inhibition could be explained by an inhibitory influence of FFA on glucose oxidation. Moreover, in contrast to Roth et al. (28) and Zhou and Grill, we found that the insulin-releasing action of KIC, a keto acid directly metabolized in the mitochondria, was totally abolished by TPN treatment. Because this nutrient is oxidized directly in the citric acid cycle without involvement of pyruvate dehydrogenase, it appears that the major inhibitory effect on insulin release stimulated by glucose and KIC in TPN islets probably is exerted distally to glucose oxidation and pyruvate dehydrogenase activity. However, in this context, it should be noted that it cannot be excluded that part of the impairing effect of our long-term infusion of TPN on nutrient-stimulated insulin release could be elicited not only by FFA but also by other (lipid?) components of the TPN solution.

The pattern of stimulated insulin release from the islets of TPN-infused rats was strikingly similar to that previously seen after selective blockade of islet acid glucan-1,4-α-glucosidase. That is, nutrient-stimulated insulin release was markedly suppressed, whereas receptor-mediated insulin secretion induced by phospholipase C-protein kinase C activating agents was unaffected (20, 31–36). In accord with this, we observed a marked inhibition of acid glucan-1,4-α-glucosidase activity in islets isolated from TPN-treated rats assayed directly ex vivo. Moreover, the activities of other lysosomal enzymes were also greatly reduced. In contrast, liver tissue taken from the same animals displayed a marked increase in the various lysosomal enzyme activities. These results thus reveal a surprisingly great variability among different lysosomal enzyme activities in islets and liver, respectively, after TPN treatment. It has been known for a long time that a normal physiological variability in lysosomal enzyme activities may exist among different tissues as well as within a given tissue (25). The total pattern of the lysosomal enzyme activities in islets and liver of TPN-treated rats is also suggestive of enzyme heterogeneity among the lysosomes and/or that lysosomal enzyme activities in these tissues can be modulated relatively independently of one another. Furthermore, it has previously been reported that catabolic, destructive, and degenerative processes in a given tissue are accompanied by increased levels in the activities of classical lysosomal enzymes (38). Such a reaction pattern was clearly evident in liver tissue and was most likely due to an excessive stimulation of the lysosomal/vacuolar system by fat overloading during the long-standing TPN infusion. In contrast, the lysosomal enzyme activities in the islets of Langerhans were all highly suppressed by the same treatment. It may be that the
liver, by virtue of its important physiological role in lipid metabolism, is liable to take up comparatively more fat and, when being heavily “overloaded,” its lysosomal/vacuolar system is activated. The islets, on the other hand, are not primarily a fat-storing organ and thus are affected differently by the abnormal fat loading, resulting in a state of suppressed activity of the whole lysosomal/vacuolar system. It is also possible that specialized subpopulations of lysosomal organelles with differing physiological functions are reacting differently in the islets and the liver, respectively. Indeed, our present data on islet lysosomal enzyme activities after in vitro incubation of intact islets (Fig. 5) suggest a different action of the TPN infusion on different organelles of the islet lysosomal/vacuolar system, because the α-glucosidehydro-lase activities were strongly suppressed, as was glucose-stimulated insulin release, whereas the activities of other lysosomal enzymes were markedly increased. It is known from previous data recorded in other tissues that fat overloading may affect lysosomal enzyme activities differently. Thus aortic cells taken from cholesterol-fed rabbits displayed markedly increased activities of acid cholesteryl esterase, N-acetyl-β-D-glucosaminidase, and β-galactosidase (6). On the other hand, addition of oxidized low-density lipoproteins to cultured J-774 cells (murine macrophage cell line) resulted in a significant decrease of the total activities of N-acetyl-β-D-glucosaminidase and cathepsin L (10).

Our results from the present acarbose experiments provide the evidence to assume that, in the TPN islets, it is both the acid glucan-1,4-α-glucosidase activity itself and the whole lysosomal/vacuolar system that were malfunctioning. In previous experiments, we had observed that the pseudotetrasaccharide acarbose, a potent inhibitor of islet acid α-glucosidasehydro-lases (36), when incubated together with mouse islets, was taken up and exerted a marked inhibitory effect on both acid glucan-1,4-α-glucosidase activity and glucose-stimulated insulin release. In contrast, a close acarbose analog, the tetrasaccharide maltotetraose, which is devoid of enzyme-inhibitory properties, did not affect either enzyme activity or insulin release (36). It should be emphasized that, because acarbose is a selective α-glucosidehydro-lase inhibitor, our data strongly suggest a direct cause-effect relationship between inhibition of islet acid glucan-1,4-α-glucosidase activity on the one hand and suppression of glucose-stimulated insulin release on the other. Indeed, the present results revealed that, in the presence of acarbose in the control islets, the inhibition curve for glucose-stimulated insulin release was dose dependent and similar in shape to the inhibition curve for acid glucan-1,4-α-glucosidase activity. In contrast, no inhibitory effect by acarbose was observed in intact, incubated islets from TPN-infused rats, although a slight tendency to inhibition was found at a very high concentration of acarbose (10 mM). These data suggested that, in the TPN islets, relevant amounts of acarbose were not given access to its target, the acidic lysosomal/vacuolar compartment in the β-cells. This is in accord with very recent data (30) showing that dysfunction of the islet lysosomal/vacuolar system in the spontaneously diabetic Goto-Kakizaki rat may be an important factor involved in the impaired glucose-stimulated insulin release of this animal NIDDM model.

In conclusion, the data of the present study suggest, but do not directly prove, that a TPN-induced general-ized suppression of the islet lysosomal/vacuolar system and its acid glucan-1,4-α-glucosidase activity is associated with an impairment of glucose-stimulated insulin secretion. Our in vitro observations further suggest that this defective insulin response to nutrient secre-tagogues in TPN islets may be referred to both as a selective suppression of the acid glucan-1,4-α-glucosidase activity and as a dysfunction of the whole lysoso-mal/vacuolar system possibly conveyed, for example, by a hitherto unrecognized lipid-induced impairment of the membrane function of the acidic organelles involved and/or other regulatory factors modulating the acid glucan-1,4-α-glucosidase activity.

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