Fatty Acids Decrease IDX-1 Expression in Rat Pancreatic Islets and Reduce GLUT2, Glucokinase, Insulin, and Somatostatin Levels*

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IDX-1 (islet/duodenum homeobox-1) is a transcription factor expressed in the duodenum and pancreatic beta and delta cells. It is required for embryonic development of the pancreas and transactivates the Glut2, glucokinase, insulin, and somatostatin genes. Here we show that exposure of isolated rat pancreatic islets to palmitic acid induced a ~70% decrease in IDX-1 mRNA and protein expression as well as 40% and 65% decreases in the binding activity of IDX-1 for its cognate cis-regulatory elements of the Glut2 and insulin promoters, respectively. The inhibitory effect of palmitic acid required its mitochondrial oxidation since it was prevented by the carnitine palmitoyltransferase I inhibitor bromopalmitic acid. The palmitic acid effect on IDX-1 was correlated with decreases in GLUT2 and glucokinase expression of 40 and 25%, respectively, at both the mRNA and protein levels. Insulin and somatostatin mRNA expression was also decreased by 40 and 60%, whereas glucagon mRNA expression was not modified. After 48 h of exposure to fatty acids, total islet insulin, somatostatin, and glucagon contents were decreased by 85, 55, and 65%, respectively. At the same time, total hormone release was strongly stimulated (13-fold) for glucagon, whereas its was only marginally increased for insulin and somatostatin (1.5- and 1.7-fold, respectively). These results indicate that elevated fatty acid levels 1) negatively regulate Idx-1 expression; 2) decrease the expression of genes transactivated by IDX-1 such as those for GLUT2, glucokinase, insulin, and somatostatin; and 3) lead to an important increase in glucagon synthesis and secretion. Fatty acids thus have pleiotropic effects on pancreatic islet gene expression, and the negative control of Idx-1 expression may be an initial event in the development of these multiple defects.

One of the metabolic abnormalities associated with diabetes mellitus in both human and animals is a pronounced hyperlipidemia with an increase in plasma free fatty acids (1, 2). These defects may already be observed in the prediabetic state, and the increase in circulating free fatty acids may participate in the pathogenesis of diabetes. For instance, in male Zucker diabetic (fa/fa) rats, plasma free fatty acids levels become elevated before the onset of diabetes, whereas in female rats, which do not develop diabetes, no such elevation occurs even though they display the same hypertriglyceridemia as male rats (3). High levels of free fatty acids have been shown to induce a state of insulin resistance in cardiac and skeletal muscles by inducing a decrease in glucose metabolism via a fatty acid/glucose inhibitory cycle (4). In pancreatic beta cells, fatty acids have been shown to have a stimulatory effect on glucose-induced insulin secretion over short-term exposure (1–3 h), but an inhibitory effect after longer periods of treatment (6–24 h) (5–8). The long-term effects correlate with a decrease in pyruvate dehydrogenase activity and an increase in pyruvate dehydrogenase kinase activity, which together lead to impaired glucose oxidation (9, 10). In INS-1 insulinoma cells, fatty acids induce a decrease in acetyl-CoA carboxylase gene expression (11) and an increase in carnitine palmitoyltransferase I gene expression (12), leading to an increase in long-chain fatty acyl-CoA oxidation. The mechanisms by which fatty acids modify gene expression in beta cells, either directly or following their metabolism, is not yet known, but may rely on activation of fatty acid-regulated transcription factors such as the peroxisome proliferator-activated receptors (13). Fatty acids can also have a direct inhibitory effect on insulin secretion by activating ATP-sensitive K⁺ channels, as recently shown in HIT-T15 insulinoma cells (14).

Islet/duodenum homeobox-1 is a homeobox gene that was simultaneously cloned from different species and called IUF-1 (insulin upstream factor-1) in man (15), STF-1 (somatostatin-transactivating factor-1) or IDX-1 (islet/duodenum homeobox-1) in rat (16, 17), and IPF-1 (insulin promoter factor-1) in mouse (18). It is a 283-amino acid transcription factor protein that contains a central homeodomain implicated in IDX-1/DNA interaction and two N- and C-terminal proline-rich regions (19) implicated in gene transactivation. The IDX-1 C terminus may be involved in homodimerization with another IDX-1 molecule (19), whereas the N terminus may participate in synergistic transactivation of genes by interacting with other trans-acting factors such as E47, which binds to the E1 region of the insulin promoter (20, 21). IDX-1 was first described as a transactivator of the insulin (18, 22) and somatostatin (16) genes that recognized a TAAT/T/G sequence present in their promoters. More recently, it was also shown to transactivate the Glut2 (23) and glucokinase (24) genes by recognizing similar cis-regulatory elements present in the promoters of these genes.

Idx-1 gene expression is restricted to the duodenum and pancreatic beta and delta cells. During embryogenesis, it is expressed starting at embryonic day 8.5 in the duodenum and pancreatic bud, before the appearance of glucagon or insulin cells (18). IDX-1 importance in pancreas development was further highlighted when IDX-1−/− homozygous knockout mice were shown to fail to develop pancreases, leading to early postnatal death (25, 26). More recently, identification of mutations in the human IDX-1 gene was also shown to be responsi-
ble for lack of pancreas organogenesis in man (27). It was therefore hypothesized that, during development, all endocrine and exocrine pancreatic cells originate from IDX-1-expressing cells and that only beta and delta cells would retain its expression in mature islets.

We previously reported that dexamethasone and palmitic acid treatments of isolated rat pancreatic islets lead to a decrease in Glut2 expression and a decrease in glucose-induced insulin secretion (28). Whereas the dexamethasone effect was due to increased post-translational degradation of GLUT2, the fatty acid effects took place at a pretranslational stage. We now report that, following palmitic acid treatment, IDX-1 mRNA and protein expression is also markedly reduced.

The reduction in protein expression is correlated with a decrease in IDX-1 binding activity for its cognate cis-regulatory sequence of the insulin and Glut2 gene promoters. The expression of the Glut2, glucokinase, insulin, and somatostatin genes, but not that of the glucagon gene, is also reduced under these conditions. Moreover, incubation of islets in the presence of fatty acids leads to a 13-fold increase in total glucagon release, with little change in insulin or somatostatin secretion. These data show that fatty acids can modulate the expression of the Idx-1 gene, which correlates with a reduced level of expression of pancreatic genes under the control of IDX-1. This phenomenon may be important in the pathogenesis of diabetes mellitus.

**EXPERIMENTAL PROCEDURES**

**Materials**—Male Sprague-Dawley rats were purchased from Biological Research Laboratories Ltd., palmitic acid (sodium salt) and bromopalmitic acid (free acid) from Fluka, collagenase (type IV) from Worthington, and Ficoll DL-400 and bovine serum albumin (BSA); fraction V, essentially fatty acid-free) from Sigma. For RNA analysis, GeneScreen nylon membranes were obtained from NEN Life Science Products, and the random-primer DNA labeling kit was from Boehringer Mannheim. For protein analysis, Protran nitrocellulose membranes were obtained from Schleicher & Schuell, the BCA protein assay was from Pierce, and Enhance Chemi (ECL) and horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin antibody were from Amersham Corp. Finally, antibodies used in the insulin radioimmunoassay as well as the radioimmunoassay for glucagon detection were from Linco Research Inc. The Somostatin radioimmunoassay reagents were purchased from Elcser Corp.

**Islet Isolation**—Islets were isolated from Sprague-Dawley rats weighing ~200 g by collagenase digestion of the total pancreas and subsequent separation on discontinuous Ficoll DL-400 gradients as described previously by Gotoh and colleagues (29).

**Islet Culture**—Islets were kept in culture in a humidified atmosphere containing 5% CO₂. At the end of the isolation step, islets in batches of 150–200 were placed in 10-cm tissue culture dishes and cultured for 24 h in RPMI 1640 medium (containing 11 mM glucose) supplemented with 10% fetal calf serum, 10 mM Hapes, pH 7.4, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol. On the next day, the medium was changed to RPMI 1640 medium containing 2.8 mM glucose, and the culture was continued for another 24 h. Finally, islets were incubated with different concentrations of palmitic acid in the presence of 2.8, 5.6, or 30 mM glucose for different periods of time.

Palmitic acid (sodium salt) was prepared as an 8 mM solution in Hapes-buffered Krebs-Ringer bicarbonate buffer, pH 7.4, containing 10% BSA (essentially fatty acid-free; final fatty acid/BSA molar ratio = 57). Fatty acid was then allowed to equilibrate overnight with BSA at 37 °C and filtered before use. Bromopalmitic acid (free acid) was first submitted to saponification by addition of a final 0.1% concentration of NaOH to a 100 mM bromopalmitic acid solution. The precipitated bromopalmitic acid sodium salt was then recovered by filtration, lyophilized, and prepared as described above for palmitic acid. In every experiment, control islets were exposed only to glucose plus 500 μM of palmitic acid vehicle (Krebs-Ringer bicarbonate buffer containing 10% BSA).

**RNA Extraction and Northern Blot Analysis**—Total RNA was extracted from 80 islets (120 islets for glucokinase mRNA detection) by the guanidinium thiocyanate/phenol/chloroform extraction method (30) using 20 μg of yeast tRNA as carrier. Total RNA was separated on 1.4% agarose gels containing 2% formamide and transferred to nylon membranes. After transfer, the membranes were UV-cross-linked and prehybridized for 2 h at 42 °C in hybridization buffer (50% formamide, 5× SSC (SSC = 150 mM NaCl and 15 mM sodium citrate, pH 7.0), 0.1 mM NaPO₄, pH 6.5, 10 mM EDTA, 1% sodium dodecyl sulfate, 5× Denhardt’s solution (Denhardt’s solution = 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), and 0.1 mg/ml yeast tRNA). The buffer was then changed, and hybridization was conducted overnight with 10^6 cpm of a 32P-labeled probe/ml. CDNA fragments were used as probes for GLUT2, γ-actin, and insulin mRNA detection as described previously (28). The CDNA fragment used as probe for IDX-1 mRNA detection was a 1.4-kilobase BamHI fragment from IDX-pBJ5 (31); that for rat glucokinase was a 1-kilobase EcoRI-Sphi fragment from the glucokinase-pGEM4z plasmid (gift from C. B. Newgard); that for glyceraldehyde-3-phosphate dehydrogenase mRNA was a polymerase chain reaction fragment derived with primers corresponding to 5′-GCGTCTACACCCACTGGAGA-3′ (sense; base pairs 294–313 of the human glyceraldehyde-3-phosphate dehydrogenase gene) and 5′-CGGCCATACGCGCCACAGTTT-3′ (antisense; base pairs 593 to 574 of the human glyceraldehyde-3-phosphate dehydrogenase gene); that for rat somatostatin was a 0.4-kilobase HindIII-KpnI fragment from the pGEM-4-zs plasmid (gift from J. Philip); and finally, that for rat glucagon was a 1.1-kilobase PstI-SacI fragment from the pGEM-3-g-gluc plasmid (32).

**Protein Extraction and Western Blot Analysis**—150–200 islets were lysed in 80 mM Tris, pH 6.8, 5% SDS, 5 mM EDTA, 2 mM N-ethylmaleimide, and 2 mM phenylmethylsulfonyl fluoride and sonicated for 1.5 min in the cup of a sonicator. Proteins were quantitated with the BCA protein assay (33).

**In vitro Translation and Western Blot Analysis**—In vitro translation was performed using a rabbit reticulocyte-free lysate (Promega). Translation products were fractionated on 7.5% polyacrylamide gels and transferred to nitrocellulose membranes. Detection of GLUT2 was performed as described previously (28). IDX-1 was detected with a rabbit antibody generated against a peptide corresponding to amino acids 164–283 of the rat IDX-1 protein (31). For supershift assays, both this and a polyclonal anti-mouse IDX-1 antibody (gift from H. Edlund) (25) were used. Glucokinase was detected with a sheep anti-glucokinase antibody generated against a glutathione S-transferase-glucokinase fusion protein (gift from M. Magnuson). The α-subunit of the Na⁺/K⁺-ATPase was detected using an antibody directed against the protein from Bufo marinus as described (28). The second antibodies used were a horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin antibody to detect the Na⁺/K⁺-ATPase (sense) and a polyclonal antibody to detect the Na⁺/K⁺-ATPase (antisense). For supershift assays, 1 μl of preimmune or anti-IDX-1 serum was added to the reaction before the EMSA.

**Pulse-labeling and Pulse-Chase Experiments**—For pulse-labeling experiments, 150–200 islets were incubated for 48 h in 30 mM glucose in the presence or absence of 0.6 mM palmitic acid. After washing twice with PBS, the islets were incubated for 30 min in RPMI 1640 medium depleted of methionine and supplemented with 10% dialyzed fetal calf serum. Pulse labeling was for 5 min at 37 °C with 50 μCi of [35S]methionine. Islets were then washed twice with ice-cold PBS, lysed in 100 μl of 1% SDS in PBS containing 2 mM N-ethylmaleimide and 2 mM phenylmethylsulfonyl fluoride, and further diluted with 400 μl of 1.25% Triton X-100 in PBS plus the same protease inhibitors. For pulse-chase experiments, islets were first labeled for 2 h with [35S]methionine and, after washing twice with PBS, either lysed as described above or returned to normal medium supplemented with 2 mM unlabeled methionine for 23.5 h before being lysed. GLUT2 was then immunoprecipitated from equal amounts of proteins and analyzed as described previously (28).

**Insulin, Glucagon, and Somatostatin Dosages**—Insulin, somatostatin, and glucagon were quantitated using radioimmunoassays.
RESULTS

Regulation of IDX-1 Expression by Fatty Acids—The effect of fatty acids on IDX-1 mRNA levels was first investigated by Northern blot analysis. For this purpose, islets were cultured for different periods of time in the presence of 30 mM glucose and in the absence or presence of different concentrations of palmitic acid. Total RNA was then extracted from 80 islets and analyzed by Northern blotting. The results are shown in Fig. 1A and are expressed as the IDX/actin mRNA ratio. Normalization was also done relative to glyceraldehyde-3-phosphate dehydrogenase mRNA and gave similar results (data not shown). In dose-response experiments, IDX-1 mRNA levels were maximally decreased to 43 ± 6% of the control value (n = 5) in the presence of 0.6 mM palmitic acid. In time-course experiments, in the presence of 0.6 mM palmitic acid, the maximal effect was already reached after 10 h of incubation. At that time, IDX-1 mRNA levels were decreased to 50 ± 7% of the control value (n = 3) (Fig. 1B), which was not significantly different from the level reached at the end of the 48-h incubation (39 ± 6%).

To evaluate whether oxidation of fatty acids was needed for the negative effect on IDX-1 mRNA to be observed, islets were exposed for 24 h to 0.6 mM bromopalmitic acid in the presence or absence of 0.6 mM palmitic acid. In islets exposed to bromopalmitic acid alone, the IDX-1 mRNA level was increased to 162 ± 13% of the control value, whereas in the presence of equimolar concentrations of bromopalmitic and palmitic acids, IDX-1 mRNA levels were similar to the control value (99 ± 14%) (Fig. 1C).

To investigate whether the palmitic acid effect on IDX-1 mRNA was glucose-dependent, islets were cultured for 48 h in the presence of 2.8, 5.6, or 30 mM glucose in the presence or absence of palmitic acid. At a low glucose concentration (2.8 mM), no significant effect of palmitic acid on IDX-1 mRNA was observed (128 ± 23% of the control value (n = 3)). The same negative effect of palmitic acid on IDX-1 mRNA levels was observed, however, in the presence of 5.6 or 30 mM glucose (58 ± 11% versus 54 ± 7% of control values, respectively (n = 3 for each experiment)) (Fig. 2).

Regulation of the IDX-1 protein level by fatty acids was determined by Western blot analysis. The results of Fig. 3 show that IDX-1 protein expression in islets exposed to palmitic acids was decreased to 29 ± 5% of the control value (n = 6).

To determine whether the decrease in IDX-1 protein expression was correlated with a decrease in the binding activity of the transcription factor for its cis-regulatory elements of the insulin and Glut2 gene promoters, EMSAs were performed using nuclear extracts of palmitic acid-treated and control islets. Fig. 4 (A and B) shows the results of the EMSA measurements, and Fig. 4C shows the quantitation of these results. The binding activity for the IDX-1 cis-element of the Glut2 promoter was decreased to 35 ± 8% of control values in nuclear extracts of palmitic acid-treated islets. In the same extracts, the binding activity for the IDX-1 cis-element of the insulin promoter was reduced to 63 ± 3% of control values. The binding activity of nuclear extracts of palmitic acid-treated islets for the GTHII sequence of the Glut2 promoter or for the major late transcription factor-binding sequence was not altered. To determine that the bands observed in these EMSAs using the IDX-1 oligonucleotides were indeed due to binding to IDX-1, supershift assays were performed with antibodies to rat and mouse IDX-1. Fig. 4D shows the almost complete disappearance of the shifted band in the presence of both antisera, but not in the presence of preimmune serum. Similar data were obtained using the IDX-1-binding oligonucleotides of the Glut2 promoter sequence (data not shown).

Regulation of GLUT2 and Glucokinase Expression—in agreement with previous observations (28), exposure of isolated rat pancreatic islets to palmitic acid induced a decrease in GLUT2 mRNA expression to 52 ± 6% of the control value (n = 6) (Fig. 5A). Fig. 5B shows that GLUT2 protein steady-state levels were decreased to 63 ± 10% of the control value (n = 6).
The effect of palmitic acid on GLUT2 protein biosynthesis and stability was also analyzed. Five-minute pulse-labeling experiments performed with islets preincubated for 48 h in the presence or absence of 0.6 mM palmitate showed a reduction in the GLUT2 translation rate to 58 ± 5% of the control value (n = 3) (Fig. 5C), consistent with the extent of decrease in GLUT2 mRNA. To assess the GLUT2 degradation rate, islets were pulse-labeled for 2 h and then either directly lysed or lysed after an additional 23.5-h chase period, and GLUT2 was immunoprecipitated and analyzed by gel electrophoresis and scanning densitometry of the autoradiograms. The GLUT2 protein half-life was similar in palmitic acid-treated and control islets, ~20 h (data not shown). This value is as previously reported (28).

Regulation of glucokinase expression in palmitic acid-treated islets was investigated by Northern and Western blot analyses. Fig. 6A shows that glucokinase mRNA levels were decreased to 76 ± 7% of the control value in treated islets (n = 3). Fig. 6B shows that the glucokinase protein level was decreased to 76 ± 7% of the control value (n = 8) in palmitic acid-treated islets.

As a control, the level of expression of the α-subunit of the Na+/K+-ATPase in control or palmitic acid-treated islets was evaluated by Western blot analysis. Fig. 7 shows that the level of expression of this membrane protein was not affected by the treatment.

Pancreatic Islet Hormone Gene Expression, Cellular Content, and Secretion—The effect of palmitic acid treatment on the expression of insulin and somatostatin was first analyzed by Northern blotting. Fig. 8 shows that the presence of palmitic acid for 48 h induced a decrease in insulin and somatostatin mRNA levels to 59 ± 7% (n = 7) and 41 ± 4% (n = 5) of the control values, respectively. In contrast, glucagon mRNA levels were not significantly affected (110 ± 15% of the control value (n = 9)).

Total islet hormone content and secretion following a 48-h incubation with or without palmitic acid were then analyzed (Table I). In the presence of palmitic acid, insulin, somatostatin, and glucagon islet contents were decreased to 14 ± 7, 45 ± 13, and 54 ± 19% of the control values, respectively (n = 8). Insulin and somatostatin release in the medium was only marginally, although significantly, increased (1.5 ± 0.4-fold (p < 0.05) and 1.7 ± 0.4-fold, respectively). In contrast, under the same conditions, glucagon secretion was strongly stimulated (13 ± 3-fold (n = 8)). The total of intracellular plus secreted insulin and somatostatin was slightly increased over the 48-h incubation period in the presence of palmitic acid. For glucagon, this was more than doubled (Table I). When measured only in the second 24-h period of the 48-h incubation, secreted glucagon was increased by 8 ± 3-fold.

DISCUSSION

Here we demonstrated that palmitic acid treatment of isolated pancreatic islets induced an important decrease in IDX-1 mRNA and protein expression as well as in IDX-1 binding activity for its cis-regulatory elements of the insulin and Glut2 genes. This was correlated with the decreased expression of several important pancreatic islet genes normally transactivated by IDX-1, i.e. the Glut2, glucokinase, insulin, and somatostatin genes.

Palmitic acid induced a dose- and time-dependent decrease in IDX-1 mRNA, with a maximum decrease observed in the presence of 0.6 mM fatty acid and already reached after 10 h of incubation. This effect was also observed at the protein level and correlated with a proportional decrease in binding of IDX-1 to its cognate cis-regulatory elements of the insulin and Glut2 gene promoters. The effect of palmitic acid was glucose-dependent since it was observed only in the presence of 5.6 or 30 mM glucose, but not in the presence of 2.8 mM glucose. Mitochondrial oxidation was also required to observe the palmitic acid effect since bromopalmitate, an inhibitor of carnitine palmitoyltransferase I, suppressed the inhibitory effect of palmitic acid. Interestingly, when added alone, bromopalmitate increased IDX-1 mRNA levels, but was without effect when an equimolar concentration of palmitic acid was added at the same time to the islet culture medium. This suggests that, in the normal culture medium, which contains 10% fetal calf serum, a basal level of fatty acids may be present and have an inhibitory effect on Idx-1 expression. This inhibitory effect may be prevented by the addition of bromopalmitate.

Previous experiments have described a regulation of IDX-1 DNA binding activity by glucose (34). High glucose concentrations stimulated phosphorylation of the transcription factor, which increased its binding activity as measured by EMSA. In our study, glucose concentrations were high (30 mM), and fatty acids were able to induce a decreased binding activity. This therefore indicates that the inhibitory effect of fatty acids may be dominant over the stimulatory effect of glucose. A recent report describes the regulation of Idx-1 expression and DNA binding activity by glucocorticoids in beta cell lines (35). Together with these previously published data, our present studies indicate that multiple regulatory mechanisms control Idx-1 expression in pancreatic beta cells.

IDX-1 was described as a transactivator of at least four islet genes: Glut2 and glucokinase, which are key elements of the
IDX-1 DNA binding activity is decreased in nuclear extracts of palmitic acid-treated islets. A, islets were treated for 48 h with 30 mM glucose in the presence or absence of 0.6 mM palmitic acid. Nuclear extracts were then prepared, and binding activity for the GLUT2-specific IDX-1 cis-element and for the GTII- and major late transcription factor (MLTF)-binding sequences was analyzed by EMSA. B, the same experiment as described for A was carried out, but using the insulin-specific IDX-1 cis-element as a probe. C, quantitations are shown of the EMSA analysis. Left panel, GLUT2 IDX-1 cis-element; right panel, insulin IDX-1 sequence. The results are presented as mean ± S.E. (n ≥ 3). D, the same experiment as described for B was performed, but preimmune serum or anti-rat (antibody 1) or anti-mouse (antibody 2) IDX-1 antibodies were added to the EMSA. Both anti-IDX-1 antibodies, but not the preimmune antisemrum, induced the disappearance of the shifted band. In the presence of the immune serum, part of the radioactivity remained in the loading well (band at the top of the gel).
beta cell glucose sensor, and insulin and somatostatin (16, 18, 22–24). We therefore investigated the expression of these genes in palmitic acid-treated islets, where IDX-1 expression was shown to be reduced by 70%.

GLUT2 mRNA levels were indeed decreased in the presence of fatty acids, and a proportional reduction in the GLUT2 protein synthesis rate and steady-state levels was observed, with an unchanged GLUT2 protein degradation rate. We had GLUT2 and actin mRNAs were analyzed by Northern blot analysis. Quantitation of the GLUT2/actin ratio is presented in the histogram as mean ± S.E. (n = 6).

Fig. 5. Decrease in GLUT2 mRNA and protein expression in palmitic acid-treated islets. A, total RNA was extracted from islets treated for 48 h in the presence or absence of 0.6 mM palmitic acid. Quantitation of the GLUT2/actin ratio is presented in the histogram as mean ± S.E. (n = 6). B, Western blot analysis of GLUT2 protein in the total cellular lysate of islets treated as described for A. Quantitation of six different experiments is presented in the histogram as mean ± S.E.

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previously described that dexamethasone induced a decrease in GLUT2 protein expression in isolated rat pancreatic islets through the activation of a post-translational degradation system, with no change in GLUT2 mRNA levels (28). These different effects of fatty acids and dexamethasone on GLUT2 down-regulation may combine to induce the strong reduction in transporter expression observed in animal models of diabetes (36).

Glucokinase mRNA and protein steady-state levels were also affected by palmitic acid treatment, although to a lesser extent than GLUT2. However, as glucokinase is the rate-limiting step in glycolysis in beta cells, even a small decrease in glucokinase protein expression can have an important impact on glucose metabolism and therefore on glucose-induced insulin secretion (37). This is indeed supported by the finding that mutations in the glucokinase gene that lead to a decrease in enzyme activity cause abnormal glucose-induced insulin secretion and MODY2 (maturity-onset diabetes of the young), a particular form of non-insulin-dependent diabetes mellitus (38, 39).

It is noteworthy that pancreatic alpha cells have recently been shown to also express glucokinase (40). If the presently observed decrease in glucokinase is due to a primary regulation of *Idx-1* by fatty acids, the levels of glucokinase in alpha cells should not be altered since these cells do not express this transcription factor. The actual decrease in glucokinase expression in beta cells may thus be more important than indicated by the measurements performed on whole islets.

The observed decreased insulin and somatostatin but unchanged glucagon mRNA levels in palmitic acid-treated islets are consistent with the reported trans-acting functions of *IDX-1* on insulin and somatostatin, but not on glucagon. Analysis of the pancreatic islet hormone content and release at the end of the palmitic acid treatment showed interesting modifications. First, insulin content was reduced by 85%, with an apparent increase in insulin secretion of 150%. The increased release over the 48-h incubation period could be due to the initial (<3 h) stimulatory effect of palmitic acid on insulin secretion (8) even though after prolonged exposure (>6 h) to fatty acids, insulin secretion by beta cells is inhibited (5–7). Total somatostatin content was also decreased to 55% of the control value, with a total release increased by 170%. Interestingly and unexpectedly, glucagon release over this period of time was increased by 13-fold compared with the control, with a reduction of content to 45% of controls. This strong increase in glucagon release could not result from acute alpha cell death induced by fatty acids since glucagon release over the second 24-h period of the incubation in the presence of fatty acid was still 8-fold increased compared with control islets. Moreover, after the 48 h of incubation with palmitic acid, glucagon mRNA was still present at the same level, and total cellular and secreted glucagon was more than doubled, indicating preserved cellular integrity. Fatty acids seem then to stimulate both glucagon production and secretion. This might result from a direct effect of fatty acids on glucagon protein expression and alpha cell secretory activity. Alternatively, this effect may be mediated indirectly through the decreased expression of insulin and somatostatin, which normally inhibit glucagon gene transcription and release (41, 42). However, as the present results were obtained under static culture conditions in which the total concentration of insulin and somatostatin was instead increased in the presence of fatty acids, it may be more likely that the dominant cause of glucagon hypersecretion is a direct effect of fatty acids on alpha cells. In addition, since these experiments were performed in the presence of 30

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**Fig. 7.** Western blot analysis of the α-subunit of the Na⁺/K⁺-ATPase. Islets were incubated in the presence or absence of 0.6 mM palmitic acid for 48 h. The level of expression of the α-subunit of the Na⁺/K⁺-ATPase was then assessed by Western blot analysis. Palmitic acid treatment did not modify the expression level of this protein.

**Fig. 8.** Reduced insulin and somatostatin but unchanged glucagon mRNAs levels in palmitic acid-treated islets. *A*, Northern blot analysis of insulin, glucagon, somatostatin, and actin mRNAs in islets treated for 48 h with or without 0.6 mM palmitic acid; *B*, quantitation of the insulin/actin, glucagon/actin, and somatostatin/actin ratios. Results are expressed as mean ± S.E. (*n* ≥ 5).
Fatty Acid Regulation of IDX-1 in Pancreatic Beta Cells

TABLE I
Insulin, glucagon, and somatostatin islet secretion and content in the presence and absence of palmitic acid

|                          | Control                  | 0.6 mM palmitate          | % of control |
|--------------------------|--------------------------|---------------------------|--------------|
| **Secretion**            |                          |                           |              |
| Insulin (ng/10 islets/48 h) | 877 ± 119                | 1382 ± 144                | 146 ± 36     |
| Glucagon (pg/10 islets/48 h) | 1553 ± 208               | 20,296 ± 2392             | 1307 ± 329   |
| Somatostatin (pg/10 islets/48 h) | 259 ± 35                 | 452 ± 38                  | 175 ± 38     |
| **Content**              |                          |                           |              |
| Insulin (ng/10 islets)    | 193 ± 33                 | 27 ± 11                   | 14 ± 8       |
| Glucagon (pg/10 islets)   | 10,443 ± 1633            | 5641 ± 1112               | 54 ± 19      |
| Somatostatin (pg/10 islets) | 145 ± 55                | 66 ± 8                    | 46 ± 13      |
| **Secretion**            |                          |                           |              |
| Glucagon (pg/10 islets/24 h) | 1250 ± 312               | 9942 ± 786                | 795 ± 261    |

a Secretion of 10 islets over a 48-h incubation period.
b Content of 10 islets at the end of the 48-h incubation period.
c Secretion of 10 islets over the second 24-h of the 48-h incubation period.

md glucose, fatty acids must have a dominant effect over the inhibition of glucagon secretion induced by high glucose. Analysis of purified alpha cells should provide important information on the role of fatty acids in glucagon synthesis and secretion.

The presently observed regulation of Idx-1 expression by fatty acids provides new important information about the role of this class of nutrients in beta cell functions. It has indeed been known that fatty acids have long-term deleterious effects on the functions of beta cells. These were correlated not only with metabolic effects or short-term allosteric regulation of enzyme activity, but also with the capability of these nutrients to act as both positive and negative regulators of gene expression. In beta cells, the gene for acetyl-CoA carboxylase was shown to be inhibited by elevated fatty acid concentrations, whereas transcription of the carnitine palmitoltransferase I gene was strongly and rapidly increased. Carnitine palmitoyltransferase I is the first step of fatty acyl-CoA oxidation, and acetyl-CoA carboxylase catalyzes the formation of malonyl-CoA, which is an inhibitor of carnitine palmitoyltransferase I. Together, these previously published data demonstrate that fatty acids modulate enzyme expression so as to increase fatty acid oxidation in mitochondria. In our present work, we further extended these observations by showing that fatty acids modulate the expression of the beta cell-specific transcription factor IDX-1, which is a transactivator of glucl2 and glucokinase genes, key regulators of glucose entry into the glycolytic pathway, as well as a transactivator of the insulin and somatostatin genes. The decrease in glucose oxidation due to activation of fatty acid oxidation by an increase in carnitine palmitoyltransferase I and a decrease in acetyl-CoA carboxylase activity is thus further aggravated by the down-regulation of GLUT2 and glucokinase expression. Whether the observed decreases in GLUT2 and glucokinase mRNA levels are due to the decreased IDX-1 expression and/or to a direct inhibition of the expression of these genes by fatty acids is not yet known. The fact that IDX-1 is required for both endocrine and exocrine pancreas development and that, in mature pancreatic islets, it is restricted to beta and delta cells indicates that its expression is of critical importance for the function of these cells. A decreased expression of this transcription factor may thus be a key pathogenic event in diabetes mellitus. Recently, the genes responsible for MODY1 and MODY3 have been identified as the genes for the transcription factors hepatic nuclear factor-4α and hepatic nuclear factor-1α, respectively (43, 44). This demonstrates that a reduction in transcription factor expression may lead to diabetes. Furthermore, as hepatic nuclear factor-4α is a transactivator of hepatic nuclear factor-1α, it may also be possible that these factors regulate Idx-1 gene expression and that an original regulation of one or both of these genes by fatty acids might regulate Idx-1 expression.

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