Expression Profiling of Palmitate- and Oleate-Regulated Genes Provides Novel Insights Into the Effects of Chronic Lipid Exposure on Pancreatic β-Cell Function

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Chronic lipid exposure is implicated in β-cell dysfunction in type 2 diabetes. We therefore used oligonucleotide arrays to define global alterations in gene expression in MIN6 cells after 48-h pretreatment with oleate or palmitate. Altogether, 126 genes were altered ≥1.9-fold by palmitate, 62 by oleate, and 46 by both lipids. Importantly, nine of the palmitate-regulated genes are known to be correspondingly changed in models of type 2 diabetes. A tendency toward β-cell de-differentiation was also apparent with palmitate: pyruvate carboxylase and mitochondrial glycerol 3-phosphate dehydrogenase were downregulated, whereas lactate dehydrogenase and fructose 1,6-bisphosphatases were induced. Increases in the latter (also seen with oleate), along with glucosamine-phosphate N-acetyl transferase, imply upregulation of the hexosamine biosynthesis pathway in palmitate-treated cells. However, palmitate also increased expression of calcyclin and 25-kDa synaptosomal-associated protein (SNAP25), which control distal secretory processes. Consistent with these findings, secretory responses to noncarbohydrate stimuli, especially palmitate itself, were upregulated in palmitate-treated cells (much less so with oleate). Indeed, glucose-stimulated secretion was slightly sensitized by chronic palmitate exposure but inhibited by oleate treatment, whereas both lipids enhanced basal secretion. Oleate and palmitate also induced expression of chemokines (MCP-1 and GRO1 oncogene) and genes of the acute phase response (serum amyloid A3). Increases in transcriptional modulators such as ATF3, CCAAT/enhancer binding protein-β (C/EBPβ), C/EBPδ, and c-fos were also seen. The results highlight links between regulated gene expression and phenotypic alterations in palmitate versus oleate-pretreated β-cells. *Diabetes* 51:977–987, 2002

C irculating nutrients regulate the function of pancreatic β-cells at multiple levels: they acutely stimulate insulin secretion, modulate insulin biosynthesis, and, over the longer term, bring about adaptive changes in gene expression (1). Dysregulation of any of these aspects of the nutrient response and/or alterations in β-cell differentiation and survival are potentially implicated in the onset of type 2 diabetes. This disease is associated with peripheral insulin resistance but only develops in conjunction with a failure of β-cell compensation, which otherwise counteracts the insulin resistance (2–5). Type 2 diabetic patients display reductions in β-cell mass as compared with insulin-resistant nondiabetic individuals, as well as a number of clearly defined secretory abnormalities. The latter include both an elevated fasting secretion and a selective loss of sensitivity to glucose (2).

Although ongoing hyperglycemia is undoubtedly a major contributor to β-cell decompensation, it is apparent that chronic elevations in circulating fatty acids (FAs) also accompany the progression to type 2 diabetes (3–5). Since FAs themselves stimulate insulin secretion, a key role in secretory compensation has been ascribed to their elevation (4,6). Carried to extremes, however, or in association with an underlying genetic predisposition, prolonged exposure to FAs could lead to "overstimulation" or "β-cell exhaustion" (5). Moreover, chronic exposure of β-cells to elevated FAs, both in vivo and in vitro, leads to an elevated basal secretion and a blunted response to glucose, both of which defects are reminiscent of the diabetic state (3,5,7). Furthermore, basal hypersecretion in some animal models of diabetes is ablated by reversal of the alterations in β-cell lipid metabolism that also characterize those models (3,8). Finally, elevated FAs might also contribute to the reduction in β-cell mass that accompanies diabetes (9). Although initial studies of β-cell dysfunction due to chronic lipid exposure focused on metabolic alterations (5,7), more recent emphasis has been on changes in β-cell gene expression. Around a dozen genes have now been identified as being lipid-regulated in β-cells (10–14).

At present, however, there is no comprehensive documentation of the global alterations in gene expression that would occur in lipid-treated β-cells. In contrast, a host of glucose-regulated genes have recently been identified by transcript profiling of β-cells using high-density oligonucleotide arrays (15). That study made use of the highly

differentiated and glucose-responsive cell line MIN6 as a source of homogenous β-cells (16). Our present aim was to undertake a comparable analysis of lipid-regulated genes. Transcript profiling of expressed genes revealed novel and multiple effects of the major circulating FAs oleate and palmitate, including induction of pro-inflammatory genes and, at least with palmitate, a partial β-cell de-differentiation. In addition, a palmitate-induced upregulation of genes encoding a number of exocytotic and signaling enzymes suggested a previously unappreciated sensitization to noncarbohydrate stimuli, which was confirmed functionally. Most importantly, palmitate exposure was sufficient to reproduce many changes in β-cell gene expression that have been previously shown to accompany the progression of diabetes.

RESEARCH DESIGN AND METHODS

Materials. Culture media, TRIzol, and the Superscript Choice system were from Gibco BRL (Gaithersburg, MD). Falcon plasticware was obtained from Becton Dickinson (Franklin Lakes, NJ). The molecular biological reagents used (and their sources) were: RNeasy mini kits (Qiagen, Melbourne, Australia), Wizard PCR preps (Promega, Madison, WI), High Yield RNA transcript labeling kits (Enzo Biochem, New York), Faststart DNA Master kit and SYBR-Green II (Roche Diagnostics, Castle Hill, Australia). U74A murine genome microarrays and T2 test arrays were purchased from Affymetrix (Santa Clara, CA). Kits for radioimmunoassay of rat insulin were obtained from Linco Research (St. Louis, MO). Nonesterified FA 1C kits for assaying free FAs were from Wako (Osaka, Japan). Diazoxide, 98% FA-free BSA, palmitate, oleate, and all other biochemicals and specialized reagents were from Sigma Aldrich (St. Louis, MO).

Cell culture and treatment. MIN6 cells were passaged in 75-cm^2 flasks with 20 ml Dulbecco's modified Eagle's medium (DMEM) containing 25 mmol/l glucose, 24 mmol/l NaHCO$_3$, 10 mmol/l HEPES, 10% (vol/vol) FCS, 50 IU/ml penicillin, and 50 μg/ml streptomycin. Cells were seeded at 3×10^5 per well in 0.5 ml DMEM in a 24-well dish for secretory experiments, and they were seeded at 5×10^6 per 25-cm flask for transcript profiling or RT-PCR. At 48 h before the experiment (24 h after seeding), the medium was replaced with DMEM (as above but with 6 mmol/l glucose) and supplemented with either BSA alone or BSA coupled to palmitate or oleate. In some experiments, diazoxide (0.1 mmol/l) was added to maintain cellular insulin contents during culture. For FA coupling, 18.4% BSA was dissolved in DMEM (25 mmol/l glucose) by gentle agitation at room temperature for 3 h. Palmitate or oleate (8 mmol/l) was then added as Na$^+$ salts, and the mixture was agitated overnight at 37°C. The pH was then adjusted to 7.4, and then, after sterile filtering, FA concentrations were verified using a commercial kit, and aliquots were stored at −20°C. Similar couplings were made using glucose-free modified Krebs-Ringer bicarbonate (KRB) buffer containing 5 mmol/l NaHCO$_3$, 1 mmol/l CaCl$_2$, 0.5% (wt/vol) BSA, and 10 mmol/l HEPES (pH 7.4) instead of DMEM. This procedure generated BSA-coupled FA in a molar ratio of 3:1 (generally, 0.4 mmol/l to 0.92% BSA, final).

Secretory assays. Cultured cells were washed once (twice for diazoxide-treated cells) in modified KRB buffer (see above) containing 2.8 mmol/l glucose, and then they were preincubated for a further 30 min in 0.5 ml of the same medium at 37°C. This buffer was then replaced with 0.5 ml of prewarmed KRB containing other additions as indicated, and it was incubated for a further 60 min at 37°C. An aliquot was then removed for analysis of insulin content by radioimmunoassay. The cell monolayers were washed twice in PBS and then extracted for measurement of total insulin content by lysis in 0.5 ml H$_2$O per well, followed by sonication.

Transcript profiling. RNA (25–40 μg) was isolated using RNeasy columns, and total and double-stranded cDNA was synthesized using the Superscript Choice system with an high-performance liquid chromatography–purified T7-(dT)24 primer. This was converted to biotin-labeled, double-stranded cRNA using a high yield RNA transcript labeling kit according to the manufacturer's instructions. After purification on RNeasy columns, the cRNA was fragmented at 94°C for 35 min in 100 mmol/l potassium acetate, 30 mmol/l magnesium acetate, and 40 mmol/l Tris-acetate, pH 8.1. After quality verification using Test2 arrays, the fragmented cRNA was hybridized to oligonucleotide microarrays, according to the Affymetrix instructions, in 1 mol/l NaCl, 20 mmol/l EDTA, 0.01% Tween, 100 mmol/l 2-[N-morpholino]ethanesulfonic acid (MES), pH 6.6, for 16 h at 45°C. Mouse genome U74A version 2 arrays were used, except for the first palmitate experiment, which was conducted with

the original version U74A arrays, now known to contain some faulty probe sets. The arrays were then washed on an Affymetrix fluidics station and stained with streptavidin-phycoerythrin according to the manufacturer's instructions. Probe sets were visualized on a Hewlett-Packard GeneArray scanner and then quantified for intensity and comparison between experimental groups using Affymetrix Genechip software. This raw data were further analyzed using Phenzomix, a data management tool developed in-house for use with these microarrays. Based on FileMaker Pro 5.3, this tool facilitated cluster formation based on both inputs from the arrays (mRNA intensity, fold change, and present/absent calls) and known functionality. For the current study, we assembled clusters of palmitate- or oleate-regulated genes satisfying the following criteria: *1*) genes were called as "changed" by the Genechip software and showed alterations of at least 1.9-fold by lipid treatment, *2*) those called "increased" or "marginally increased" were also described as "present" in the experimental group, and *3*) those called "decreased" or "marginally decreased" were also "present" in the control group. Gene clusters satisfying these conditions in two independent experiments for each lipid were saved and combined in multiple-comparison statements, using Phenzomix to define the smaller dataset of genes coregulated in both experiments. These genes were classified using Swiss Prot and TrEMBL databases.

Real-time RT-PCR. Total RNA was prepared using RNeasy or Trizol, and 0.2 μg per reaction was converted to cDNA using a Superscript cDNA kit according to the manufacturer's instructions. Real-time PCR was undertaken on a LightCycler (Roche) with a commercial kit containing 10 mmol/l MgCl$_2$ and 1 μl cDNA in a PCR of 45–55 cycles (annealing temperature of 55°C for all primers). Standards for each transcript were prepared using appropriate primers in a conventional PCR and purified using Wizard PCR preps. For experimental analyses, PCR products were quantified fluorometrically using SYBR-Green II. Concentrations were then calculated from standard curves as copies per microliter. These PCR products of known concentration for each primer pair were then used in the corresponding LightCycler PCR to provide standards for these reactions. β-Actin expression was calculated in parallel as a control (this was not altered by lipid pretreatment). The following primers were used (forward and reverse): AAT CCT GTG GCA TCC ATG AAA C and CGC AGC TCA GTA ACA GTC CG (β-actin); TGG CAA GGA AGG TGA CAA GCA C and CGG TCC CAT TTT ATT TCA GAG C (calcyclin); AAC CTG TAT GGG ATT TCG GGG and TCC AGA GGG TCA AAG CAA ACC (fructose 1,6-bisphosphatase [FBPase]); TCC TTT GTC GGC AAT CCC TAC and CCC TCC TTG ACT TTC TCT TCA TTC C (phosphatidic acid phosphohydrolase 2 [PAP2]); and TGG ATG GGG AGC AGA TG and GGT TTT GTT GGA GTC AGC CTT CTC (SNAP25).

Data analysis. Unless otherwise indicated, results are expressed as the means ± SE, with the number of observations in brackets. Statistical significance was determined with Students' t test.

RESULTS

As a prerequisite to analysis of gene expression, it was first essential to establish that lipid-treated MIN6 cells showed secretory defects similar to those seen in other β-cell models. To preclude potentially confounding influences due to chronically elevated glucose, cells were cultured at a substimulatory (6 mmol/l) glucose concentration during the 48-h period of lipid treatment. As shown in Fig. 1*A*, prior exposure to either palmitate or oleate dramatically reduced the subsequent secretory response to high glucose as compared with control cells. However, consistent with earlier islet studies (7,10,17), an accompanying loss of cellular insulin content caused by the chronic FA treatment (Fig. 1*A*, insert) may have contributed to the ablated glucose response. Recalculating the results as a percentage of cell content revealed that basal secretion was actually increased two- or fourfold, respectively, after exposure to oleate or palmitate (Fig. 1*B*). In addition, the fold stimulation due to 16.7 mmol/l glucose was reduced from 14 times basal in control cells to four- to fivefold in cells pretreated with either lipid. The increased basal secretion and diminished fold increase to high glucose are comparable to numerous studies using lipid-pretreated islets or β-cell lines (7,11,12,17–20). However, our results additionally suggested that oleate and palmitate were not
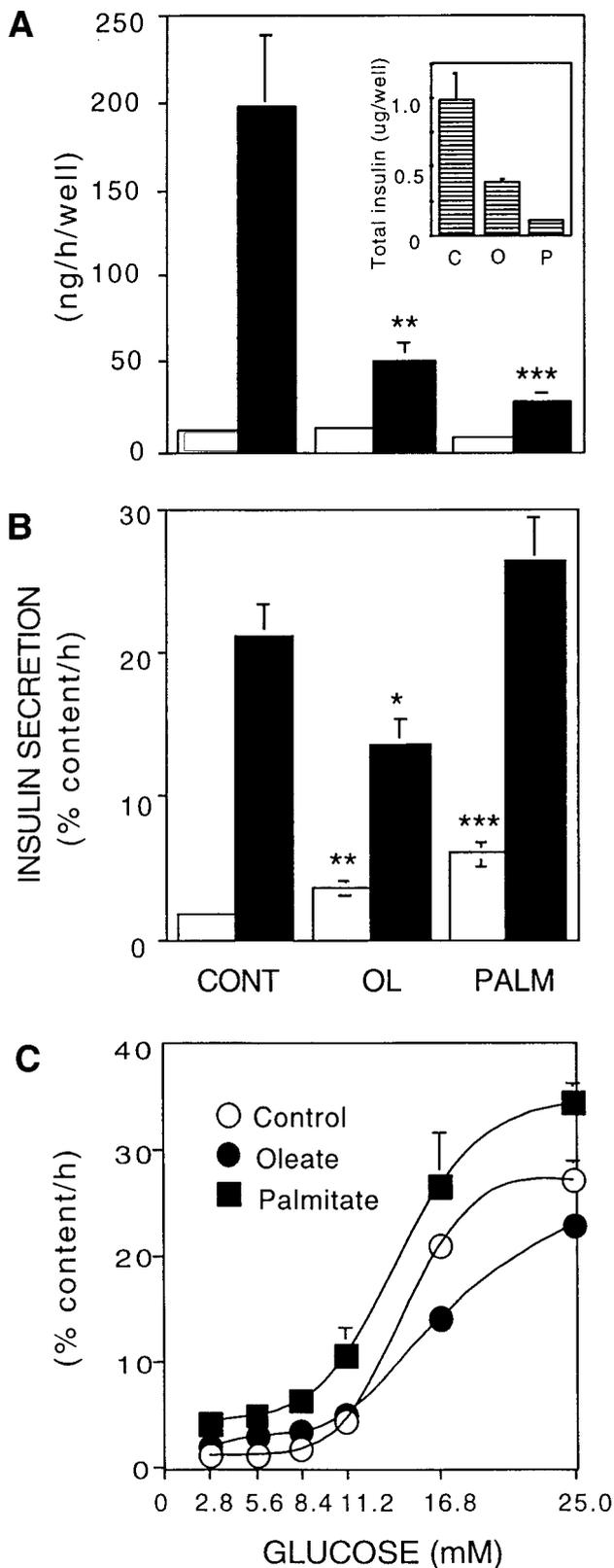
FIG. 1. Effect of 48-h pretreatment with oleate or palmitate on basal and glucose-stimulated insulin secretion. *A*: MIN6 cells grown in 24-well plates in DMEM with 6 mmol/l glucose were treated for 48 h with 0.92% BSA (CONT) or 0.4 mmol/l oleate (OL) or palmitate (PALM) with 0.92% BSA (molar ratio 3:1). Then, after a 30-min preincubation in KRB medium containing 2.8 mmol/l glucose, the cells were incubated in KRB medium containing either 2.8 mmol/l glucose (□) or 16.8 mmol/l glucose (■) for 1 h. Medium was taken to determine levels of insulin secretion, and total cellular insulin content was determined in cell lysates (insert) as described in RESEARCH DESIGN AND METHODS. *B* and *C*: Insulin secretion expressed as relative to total cellular insulin content. Results are means ± SE of three separate experiments performed in quadruplicate. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the equivalent response in control treated cells. In *C*, all values were at least $P < 0.05$ vs. corresponding glucose control, except for oleate at 2.8 and 11.2 mmol/l and palmitate at 16.8 mmol/l glucose.

entirely equivalent in their effects. This is more apparent in Fig. 1*C*, in which palmitate is shown to augment insulin secretion slightly over a range of glucose concentrations. In contrast, oleate pretreatment tended to flatten the glucose dose-dependency by both elevating responses at low glucose and inhibiting those at high glucose.

We next undertook transcript profiling, using microarrays to characterize alterations in β-cell gene expression caused by 48-h exposure to palmitate and oleate. As with the secretory experiments, a FA-to-BSA ratio of 3:1 was used, which was previously shown as half-maximal for alterations in expression of some β-cell genes (12). This corresponds to a circulating concentration of ~2 mmol/l, which is within the low physiologically elevated range. Of the 12,500 genes or expressed sequence tags represented on the microarray, ~4,700 were present in the control MIN6 cells. The expression of 40 genes was increased ≥1.9-fold in common by both palmitate and oleate; 66 were augmented solely by palmitate, but only 4 were augmented by oleate and not by palmitate. Of the genes decreased by ≥1.9-fold by lipid pretreatment, only 2 were coregulated by both palmitate and oleate, with 18 responding solely to palmitate and 12 solely to oleate. These altered genes are listed in Table 1 according to function. The changes in expression of four genes not previously known to be regulated by FAs were also examined using RT-PCR (Fig. 2). Comparing these results with those in Table 1, it is apparent that the two methodologies gave highly equivalent values for expression of all of the chosen genes: FBPase2, calcyclin, PAP2, and SNAP25.

The largest group of genes regulated by FAs encoded metabolic enzymes. Of the five genes relating specifically to lipid metabolism, all except fatty acyl elongase are involved in β-oxidation. Some genes implicated in steroid metabolism were also increased. Those grouped under amino acid metabolism encoded either transporters or enzymes controlling polyamine metabolism. Many genes relating to carbohydrate metabolism were also altered, generally in a manner that might be expected to disrupt the tight coupling between glycolysis and oxidative phosphorylation, which is a hallmark of β-cell metabolism (21–23). The biggest change was a decrease in the glucose transporter GLUT2, which was much more pronounced with palmitate than oleate. Also decreased were mitochondrial glycerol 3-phosphate dehydrogenase (mGPDH), which forms part of the glycerol phosphate shuttle, and pyruvate carboxylase (PC), which controls a critical step in the anaplerotic pathway (21–23). Consistent with general belief (22,23), FBPase and lactate dehydrogenase (LDH) were absent in untreated β-cells. However, the FAs induced expression of both muscle (type 2) and liver (type 1) forms of FBPase, and palmitate also upregulated LDH. Increases in FBPase, which reverses an early step of glycolysis, could augment metabolic flux through branch pathways such as the hexosamine biosynthesis pathway

979

TABLE 1
Transcripts differentially regulated in MIN6G cells treated for 48 h with palmitate oroleate

Function	Accession no.	Gene name	Palmitate	Oleate
METABOLISM				
Lipid	AF017175	Carnitine palmitoyl transferase 1 (CPT1)	+6.2 ± 0.1	+5.4 ± 0.6
	AI849271	Homologous to 3-ketoacyl-CoA thiolase (general purpose)	+2.6 ± 0.2	+3.2 ± 0.2
	AW122523	Fatty acyl elongase	+2.2 ± 0.3	
	AB017112	Mitochondrial carnitine acylcarnitine translocase (mCAC)	+1.8 ± 0.2	+2.1 ± 0.3
	D29639	3-hydroxyacyl CoA dehydrogenase (SCHAD)	−2.5 ± 0.6	−1.3 ± 0.0
Steroid	M55413	Group-specific component	+6.1 ± 0.0	+3.8 ± 0.4
	AA824102	3-βhydroxy-δ 5-C27-steroid oxidoreductase	>5.0*	>2.0
	AF064635	Putative steroid dehydrogenase (KIK-1)	+3.8 ± 1.6	—
	AW120882	Homology to retinal short-chain dehydrogenase/reductase	+2.8 ± 0.5	—
	AW122260	Cyt p450 51 lanosterol 14-α demethylase	+2.7 ± 0.4	—
	AF057368	7-Dehydrocholesterol reductase	+2.0 ± 0.1	—
	AW050387	Retinaldehyde dehydrogenase 3	—	>2.0
Amino acid	U70859	Cationic amino acid transporter CAT3	+6.6 ± 1.3	+2.4 ± 0.6
	M12330	Ornithine decarboxylase	+5.4*	+3.5 ± 1.0
	L10244	Spermine/spermidine N1-acetyl transferase	>2.0	—
	L42115	Insulin-activated amino acid transporter	+2.0 ± 0.6	—
	Z49976	GAD 67 Glutamic acid decarboxylase 1	—	−2.0 ± 0.1
	L03290	Cationic amino acid transporter CAT2, low affinity	−2.7 ± 0.3	—
Carbohydrate	D42083	Fructose 1,6-bisphosphatase 2 (FBPase 2)	>5.0	>5.0
	AI790931	Fructose 1,6-bisphosphatase 2 (FBPase 1)	>5.0	>2.0
	AW123026	Glucosamine-phosphate N-acetyl transferase (GPAT)	+3.1 ± 0.7	—
	M17516	Lactate dehydrogenase (LDH) A4	>2.0	—
	D50430	Glycerol-3-phosphate dehydrogenase (mGPDH)	−2.1 ± 0.1	—
	L09192	Pyruvate carboxylase (PC)	−2.2 ± 0.2	−1.8 ± 0.2
	X15684	GLUT2	−12 ± 0.6	−2.9 ± 0.2
Electron transport	U08439	Balb/c cytochrome C oxidase subunit VIaH	>5.0	—
	AI839690	Homology to NADH-cytochrome B5 reductase	+2.8 ± 0.1	—
	U12961	NAD(P)H menadione oxidoreductase 1	—	−2.2 ± 0.2
GROWTH/GENE REGULATION				
Transcription	X61800	CCAAT/enhancer binding protein (C/EBP δ)	+9.2*	+5.0 ± 0.1
	M31885	Inhibitor of DNA-binding 1 (Id1)	+8.3 ± 4.4	+5.3 ± 2.6
	V00727	c-fos (FBJ ostesarcoma oncogene)	+7.5*	+3.8 ± 0.3
	U19118	LRG-21 (ATF-3)	+4.7 ± 0.2	—
	X89749	mTGIF	+4.3 ± 0.6	+2.4 ± 0.3
	U20344	Gut-enriched Kruppel-like factor	+2.7 ± 0.1	—
	M61007	CCAAT/enhancer binding protein (C/EBPβ)	+2.6 ± 0.7	—
	AA614971	Possibly MAIL (IκBζ)	+2.2 ± 0.1	—
	D14485	DNA-binding protein A	+2.1 ± 0.2	—
	M60285	cAMP responsive element modulator (CREM)	>2.0	—
	U47008	Ngfi-binding protein 1 (EGR-I binding protein 1)	+2.0 ± 0.1	—
	AW121012	Homologous to ring finger protein 19	−2.0 ± 0.0	—
	Y07688	Nuclear factor 1, X-type (NfiX1)	−2.2 ± 0.3	—
	L08074	Glial and testis specific homeobox gene (Nkx6.2)	<−2	—
	AF072546	Brachyury the second (T2) gene	<−2	—
Growth	AF056187	IGF-1 receptor	+5.7 ± 0.5	+3.1 ± 0.1
	L41352	Amphiregulin	>5.0	>2.0
	AJ009862	TGF-β1	>2.0	—
Cell cycle	U09507	Cyclin-dependent kinase inhibitor 1A (p21)	+3.7 ± 0.3	—
	AF059567	Cyclin-dependent kinase inhibitor 2B (p15)	+2.2 ± 0.3	—
	U22399	p57 Cyclin-dependent kinase inhibitor 1C	—	−2.3 ± 0.2
INFLAMMATORY RESPONSE				
	X03505	Serum amyloid A3	>20	>10
	X81627	24p3 Neutrophil gelatinase-associated lipocalin	>20*	>10
	M19681	Monocyte chemoattractant protein-1 (MCP-1)	>10	>5.0
	L12029	Stromal cell derived factor 1	+5.9*	+2.9 ± 0.6
	J04596	GRO1 oncogene	>5.0	>5.0
	AF049124	Neuronal pentraxin 2	+3.1 ± 0.8	—
	X59769	Interleukin-1 receptor type II	—	+2.5 ± 0.2
STRUCTURAL				
Extracellular	X13986	Minopontin	+20 ± 0.4	+8.2 ± 2.4
	M77196	Biliary glycoprotein 1	+2.5 ± 0.1	—

TABLE 1
(Continued)

Function	Accession no.	Gene name	Palmitate	Oleate
	U16162	Prolyl 4-hydroxylase alpha (I) subunit	+2.5 ± 0.5	+2.0 ± 0.3
	AF039663	AC133 antigen homolog (prominin)	+2.3 ± 0.1	+1.5 ± 0.1
	X67809	Peptidylpropyl isomerase C-associated protein	+2.2 ± 0.2	+1.8 ± 0.0
	L25274	DM-GRASP (CD166 antigen)	+2.1 ± 0.2	—
	U87948	Epithelial membrane protein 3	>2.0	>2.0
	U91513	Ninjurin (nerve injury–induced protein 1)	>2.0	—
	L24430	Osteocalcin-related protein	>2.0	—
	AV087000	Cell surface protein L6 antigen	—	<−2.0
	AF064749	Type IV collagen α 3-subunit	−2.2 ± 0.2	—
Major histocompatibility complex	X00496	Ia-associated invariant chain	+3.5 ± 0.9	—
	X01838	b-2 microglobulin	+2.2 ± 0.2	—
	AF016309	Histocompatibility 2	>2.0	—
Cytoskeleton	M18775	Microtubule-associated protein tau	−1.9 ± 0.0	—
	AI641895	Shroom, PDZ domain actin-binding protein	—	−2.0 ± 0.0
	AJ228865	CLIP-115 (cytoplasmic linker protein)	—	−2.5 ± 0.0
SIGNALLING				
Ca^{2+} binding	X66449	Calcyclin	>5.0	>2.0
	AI841303	Homologous to GAP43	+2.8 ± 0.1	+1.8 ± 0.1
	U41341	Calgazzarin	+2.7 ± 0.8	+2.7 ± 0.8
	M16465	Calpactin I light chain	+2.4 ± 0.3	—
	AW124113	BASP1 (NAP-22)	−2.7 ± 0.4	−1.6 ± 0.1
Phospholipid	AF083497	Phospholipase D1 (PLD1)	+5.6 ± 2.8	+5.4 ± 1.9
	D84376	Phosphatidic acid phosphatase 2 (PAP2)	+3.5 ± 0.3	—
	AI845798	Group XII-1 phospholipase A2 (PLA2)	+3.3 ± 0.1	—
Channels	U72680	Ion channel homolog RIC	>10	—
	U04294	Long QT syndrome 2	>2.0	—
	AF033017	Inward rectifying potassium channel protein TWIK-1	+2.0 ± 0.1	—
Phosphorylation	AI845584	Related to dual specificity phosphatase 6 (MKP-3)	>5.0	>5.0
	M12056	Proto-oncogene tyrosine-protein kinase lck	+3.7 ± 0.7	—
	U28423	Protein kinase inhibitor p58	+2.3 ± 0.1	+1.9 ± 0.1
	AI849305	Protein tyrosine phosphatase ζ precursor	>2.0	
	L35236	JNK3		+2.1 ± 0.2
	AW122076	Protein phosphatase inhibitor 1A	−2.1 ± 0.2	−1.9 ± 02
	AF011908	Apoptosis-associated tyrosin kinase (AATYK)	−2.1 ± 0.1	—
G-protein	AW121294	GTP-binding protein like 1	+2.4 ± 0.3	—
	X95761	New-Rhobin (oncogene *lfc*)	+2.1 ± 0.1	—
	AB004315	Regulator of G-protein signalling 4 (RGS4)	>2.0	+3.8 ± 0.7
	U10551	GTP-binding protein (*ras*-like protein KIR)	>2.0	—
	U20238	GTPase-activating protein III (GapIII)	−2.1 ± 0.1	−1.8 ± 0.1
	AW049573	*rap*2 interacting protein x	−4.6*	−2.2 ± 0.1
	AW228792	Melanophilin	<−5.0	—
SECRETION				
Exocytosis	M22012	SNAP25	+2.0 ± 0.1	—
Secreted hormones	AF026537	Prodynorphin	+10 ± 7.2	+1.9 ± 0.5
	M18208	Pancreatic polypeptide	+9.0 ± 4.3	+2.2 ± 0.2
	D17584	Tachykinin 1	>5.0	—
	Z46845	Glucagon	+4.0 ± 1.4	+5.2 ± 0.2
	U22516	Angiogenin	+2.5 ± 0.5	+2.1 ± 0.2
	AF031035	Stanniocalcin 2	>2.0	—
	U77630	Adrenomedullin	>2.0	—
	AF045887	Angiotensinogen	>2.0	>2.0
Secreted proteins	D00073	Transthyretin	+2.8 ± 0.5	+2.4 ± 0.5
	AI182588	Carboxypeptidase-N	+2.8 ± 0.4	+2.2 ± 0.2
	M38337	Milk fat globule-EGF factor 8 protein	>2.0	—
	M10114	Casein-κ	>2.0	+3.6 ± 0.4
PROTEIN SYNTHESIS				
Processing/export	AW122851	Homology to FK506-binding protein precursor	+19 ± 7.0	+10 ± 2.4
	AW120711	DNAJ b/9	+2.6 ± 0.4	—
	AA879709	Translocon-associated protein	+2.4 ± 0.4	—
	AW122551	Endoplasmic reticulum–associated DNAJ	+2.1 ± 0.2	—
	AA755004	Similar to protein disulfide isomerase–related protein	+2.1 ± 0.1	+1.9 ± 0.1
	AI642389	Similar to KDEL endoplasmic reticulum receptor 3	>2.0	+4.9 ± 1.9
	Y00884	Protein disulfide isomerase A4	>2.0	+2.0 ± 0.1

TABLE 1
(Continued)

Function	Accession no.	Gene name	Palmitate	Oleate
Glycosylation	X98014	α-2,8-sialyltransferase V		>2.0
	AI153959	α-2,3-sialyltransferase IV	>2.0	—
	AB030836	α-2,6-sialyltransferase	−2.8 ± 0.6	—
Proteolysis	M64086	Serine protease inhibitor 2-2	>10	>5.0
	M25529	Serine protease inhibitor 1-2, α1 antitrypsin 1-2	>2.0	—
	AF010254	Complement component 1 inhibitor	>2.0	<2.0
	X06086	Cathepsin-L	+1.9 ± 0.0	—
Redox	X91864	Glutathione peroxidase 2, pseudogene 1	+2.8 ± 0.1	—
	AI843119	Glutathione S-transferase homolog	>2.0	—
NOT GROUPED				
	AJ222586	NEFA protein	+4.3 ± 0.4	+2.8 ± 0.5
	AI197161	Homologous to RNA polymerase II elongation factor	+2.6 ± 0.4	—
	AB008553	LIMP II	+2.1 ± 0.2	+1.7 ± 0.1
	AJ250490	Receptor activaty modifying protein 2 (RAMP2)	>2.0	—
	X67644	Gly96	>2.0	—
	AB023957	Ethanol induced gene	>2.0	>2.0
	L76193	Paraoxonase 3	>2.0	—
	Z31362	(Balb/C) TX01	+2.0 ± 0.1	—
	AB015790	LR11 sortilin-related receptor	—	>2.0
	AI316859	Homologous to BRL bromodomain-containing protein	—	−2.0 ± 0.3
	M18775	Microtubule-associated protein tau	<−2.0	—
	D29763	Seizure-related gene 6	<−2.0	—
	U43085	Interferon-induced protein with tetratricopeptide repeats	—	<−2.0
	AF099977	Schlafen4	—	<−2.0

Transcripts differentially regulated in MIN6 cells treated for 48 h with palmitate. Listed are mRNAs with expression found to be changed ≥1.9-fold in MIN6 cells exposed for 48 h to BSA-coupled palmitate or oleate (molar ratio 3:1) by Affymetrix microarray analyses. Some oleate genes showing smaller changes are also included where the corresponding change for palmitate is ≥1.9-fold. Two independent analyses were performed for each lipid treatment using MIN6 cells separated by eight passages (palmitate) or six passages (oleate). dRNA-samples for hybridization were prepared as described in RESEARCH DESIGN AND METHODS. Mouse U74A arrays were used for the first palmitate replicate and U74A version 2 arrays for the remaining experiments. Genes marked as * in the palmitate column represent those which were unrecognized in the first palmitate replicate by probes sets subsequently defined by Affymetrix to be faulty, but which were called as changed in both of the oleate experiments and in the second palmitate experiment as determined with the corrected chips. Data are mean fold-change ± range for the gene with the listed accession number. For genes not detectable in control cells, but induced in lipid-treated cells, increases in the range of 2- to 4-fold as calculated by the Affymetrix software are indicated by >+2.0. Similarly, >+5.0 indicates estimated fold change between 5 and 10, >+10 indicates between 10 and 20, and >+20 indicates estimated change over 20-fold. For genes present in control cells, but decreased to absent upon treatment, calculated changes in the 2.0- to 10-fold range are indicated as <−2.0. The most conservative change in each of the duplicate experiments is reported.

(HBP). Indeed, expression of another enzyme in the HBP, glucosamine-phosphate N-acetyl transferase (GPAT), was also increased by palmitate.

There were also alterations in 15 genes controlling transcription; 4 of these genes were decreased by palmitate and are probable transcription factors. One of these, Nkx6.2, is closely related Nkx6.1, a transcription factor specific to differentiated β-cells (24). Conversely, of the 11 transcriptional genes showing increased expression due to palmitate, as many as 7 potentially act as repressors, including ATF3 (25), Id1 (26), mTGIF (27), and EGR-binding protein 1 (28). CCAAT/enhancer binding protein-β (C/EBPβ) can either inhibit or stimulate transcription (29,30). Finally, the upregulated CREM family member is probably the inducible cAMP early repressor (ICER), the sole member documented in β-cells (31). Of these potential repressors, only Id-1 was co-induced by oleate. Both lipids upregulated the transcriptional factors C/EBPδ and c-*fos*. They also increased expression of two genes that would be expected to stimulate cell growth: the IGF-1 receptor and amphiregulin, a close relative of β-cellulin, which binds to the EGF family of receptors. Oleate and palmitate exerted conflicting effects on genes controlling the cell cycle, with upregulation of p21 and p15 by

palmitate expected to favor growth arrest, as opposed to downregulation of p57 by oleate, which would facilitate cell cycle progression.

An unexpected category of regulated genes was that mediating inflammatory responses. Most of these genes were absent in control cells and markedly upregulated by both lipids. Three genes (MCP-1, stromal cell-derived factor 1, and GRO1 oncogene) encode chemokines, whereas pentraxins are cell surface proteins potentially implicated in proinflammatory responses. Lipocalin-2 is a secreted factor promoting leukocyte apoptosis (32). Serum amyloid A3 is a secreted lipoprotein whose induction is indicative of the acute phase response, or activation of the innate immune system (33). Interestingly, other features of this response include upregulation of some antiproteolytic enzymes and cell matrix proteins (33), which also occurred in β-cells exposed to palmitate.

Many genes encoding signal transduction enzymes were also lipid-regulated, including categories involved in protein phosphorylation and G-protein function. The observed upregulation of phospholipase D1 (PLD1) and PAP is of potential interest because these enzymes catalyze the two-step formation of diacylglycerol from precursor phospholipids. This implies increased activity of the diacylglyc-
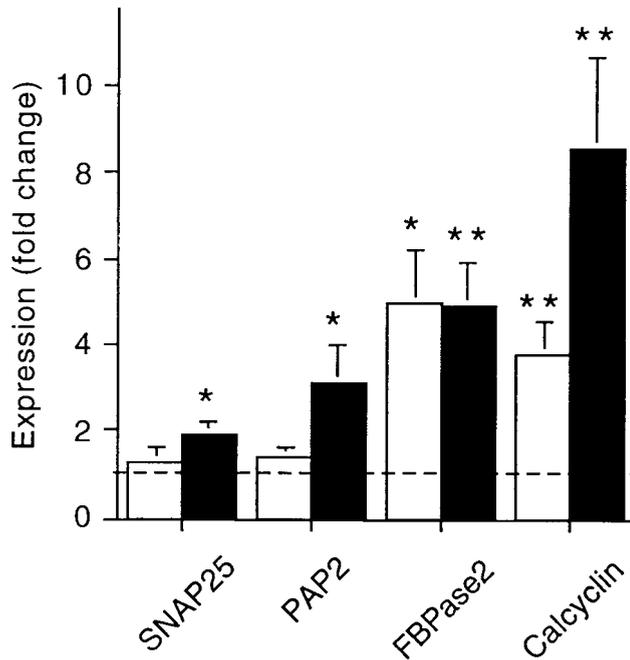
FIG. 2. Confirmation by real-time PCR of lipid-regulated genes identified by microarray analysis. MIN6 cells were exposed for 48 h to either BSA alone (– – –) or BSA coupled to oleate (□) or palmitate (■) as described in the legend for Fig. 1. Total RNA was extracted, cDNA was prepared, and real-time PCR of selected genes was performed using LightCycler technology as described in RESEARCH DESIGN AND METHODS. The following genes were selected: PAP2, calcyclin, FBPase2, and SNAP25. RNA was prepared in duplicate from cells of four different passages of MIN6 cells. *$P < 0.05$ and **$P < 0.01$ vs. control with BSA alone.
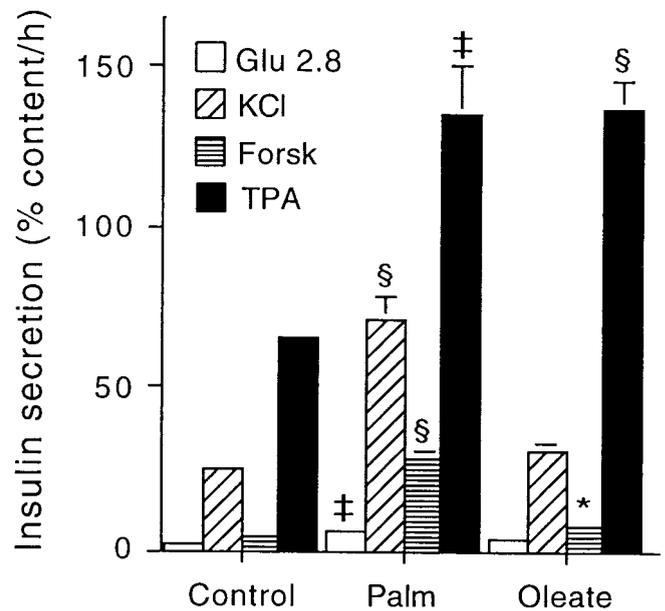

FIG. 3. Palmitate-induced sensitization of insulin secretion caused by pharmacological secretagogues at basal glucose concentrations. MIN6 cells were grown in DMEM, 6 mmol/l glucose for 48 h with BSA alone (Control), or BSA coupled to oleate or palmitate (Palm) as indicated (lipid-to-BSA molar ratio of 3:1). The cells were washed and, after a 30-min preincubation in KRB at 2.8 mmol/l glucose, were exposed for 1 h to either KRB with 2.8 mmol/l glucose (Glu) alone or 2.8 mmol/l glucose with 30 mmol/l KCl, 100 nmol/l TPA, or 50 μmol/l forskolin (Forsk) as specified. Insulin secretion was measured as described in RESEARCH DESIGN AND METHODS. Results are the means ± SE of five experiments performed in quadruplicate. *$P < 0.05$, ‡$P < 0.005$, and §$P < 0.001$ compared with the corresponding value in control cells not chronically lipid-treated.

erol/protein kinase C (PKC) pathway in lipid-treated cells. There was also a (generally) increased expression of genes for Ca^{2+} and phospholipid-binding proteins. One of these, calcyclin, whose expression was most sensitive to palmitate exposure, has been directly implicated in the control of insulin secretion (34,35). SNAP25, an essential component of the exocytotic machinery (36), was also increased in the lipid-treated cells. Increased expression of calcyclin and SNAP25 suggests that the distal exocytotic pathway might be upregulated in cells pretreated with palmitate. Such a possibility would also be consistent with enhancement of genes relating to protein processing and export, such as chaperone proteins, disulphide isomerases, and an endoplasmic reticulum translocon protein. Indeed, there was also a general upregulation of genes for secreted products and hormones. The increased expression of islet hormones, such as pancreatic polypeptide and glucagon, which are not normally found in β-cells, might point to a relative loss of cellular differentiation induced by FA exposure.

To address the prediction that lipid pretreatment would lead to a sensitization of distal secretory pathways, functional responses to a variety of nonnutrient secretagogues were next examined. As shown in Fig. 3, pretreatment with palmitate for 48 h markedly enhanced secretion due to agents acting at the level of activation of PKC (the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate [TPA]), the cAMP-dependent protein kinase (forskolin), and Ca^{2+}-dependent secretion elicited with a depolarizing concentration of KCl. In cells correspondingly pretreated with oleate, only TPA-induced secretion was augmented to an extent equiva-

lent to that seen with chronic palmitate treatment, although the forskolin response was very slightly sensitized relative to control. Ca^{2+}-dependent secretion was not sensitized at all by oleate pretreatment. These observations would be consistent with the finding that whereas oleate and palmitate both elevated PLD1, expression of SNAP25 and calcyclin were more markedly upregulated by palmitate.

Palmitate-pretreated cells appeared to show a much larger enhancement in their subsequent responses to the pharmacological agents (Fig. 3) than was seen with glucose (Fig. 1), possibly because of a negative effect on carbohydrate metabolism, which might partially counteract the upregulation of distal secretory processes. We therefore used palmitate as both an acute and chronic stimulus to determine whether a physiological, but noncarbohydrate, secretagogue was capable of bypassing such a proximal defect. In cells not preexposed to FAs, acute addition of palmitate doubled insulin secretion at low glucose and markedly potentiated the response to 16.7 mmol/l glucose (Fig. 4*A*). In cells chronically exposed to palmitate, the responses to either high or low glucose alone were slightly increased, consistent with the findings of Fig. 1. In contrast, there was a marked sensitization to palmitate, most obvious at low glucose (nearly 10 times the palmitate response in control cells), but also observed with the combined stimuli of 16.7 mmol/l glucose and palmitate. To exclude that the accompanying depletion of insulin stores might somehow contribute to this sensitization, the studies were repeated using MIN6 cells cultured during the pretreatment period in the presence of diazox-
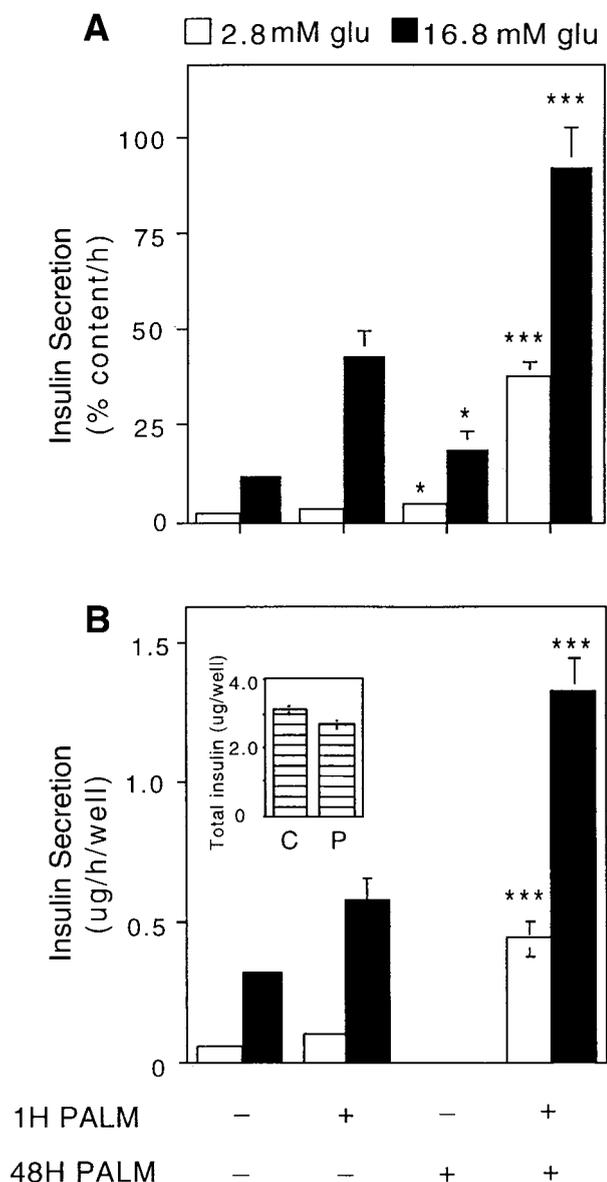
A

B

FIG. 4. Palmitate-induced sensitization of palmitate-induced insulin secretion at basal or stimulatory glucose concentrations. *A*: MIN6 cells were exposed for 48 h to BSA alone or BSA coupled to palmitate, as described in the legend for Fig. 1. After a 30-min preincubation in KRB at 2.8 mmol/l glucose, cells were incubated in KRB at 2.8 or 16.8 mmol/l glucose (glu) for 1 h in the absence or presence of 0.4 mmol/l palmitate. During all incubations, the medium contained 0.92% BSA (both in the absence and presence of palmitate). Insulin secretion and total cellular insulin levels were determined as described under RESEARCH DESIGN AND METHODS. *B*: Incubations and experimental analysis were performed as described in *A*, except that the cells were exposed to 0.1 mmol/l diazoxide during the 48-h preincubation, which prevented palmitate-induced depletion of intracellular insulin stores (insert). Responses to glucose alone in pretreated cells were not assessed in this analysis. Results are the means ± SE of five (*A*) or three experiments (*B*) performed in quadruplicate. *$P < 0.05$ and ***$P < 0.001$ vs. the corresponding value in nonpretreated cells.

ide, a specific inhibitor of insulin secretion. Although under these conditions there was minimal depletion of insulin content due to palmitate-pretreatment (Fig. 4*B*, insert), a marked sensitization was still observed.

DISCUSSION

We have made use of the highly differentiated and glucose-responsive cell line MIN6 (16) to define β-cell genes

regulated by palmitate and oleate. These cells possess the advantage over primary islets of being a homogeneous cellular population and have been previously used in a similar approach to define genes sensitive to glucose (15). The suitability of MIN6 cells for these studies is underscored by our findings that when cultured in the presence of elevated FAs, they display secretory alterations broadly similar to those observed using isolated islets (7,10,16,19) and other β-cell lines (11,12,18,20). However, our detailed analyses point to additional secretory changes that have not been previously described, probably because of difficulties in obtaining sufficient pancreatic islets to conduct comparable studies and because of the poor glucose-responsiveness of many other β-cell lines. Thus, we have shown that oleate and palmitate, though both enhancing basal secretion, are far from equivalent in other respects. Oleate pretreatment inhibited secretion due to maximal glucose concentrations and, with the exception of TPA, barely sensitized the cells to stimuli acting on distal secretory processes. In contrast, palmitate-treated cells were sensitized to all secretagogues tested, including glucose and palmitate, as well as the pharmacological activators. However, the sensitization to glucose was less apparent than that seen with other secretagogues. This is potentially explained by an additional effect of lipid pretreatment to disrupt the coupling between glucose metabolism and proximal signaling pathways, which might partially counter the upregulation of distal secretory processes. In the case of oleate, a similar disruption of glucose signaling might be more obvious because upregulation of the distal pathways is less apparent.

Should this modest augmentation of glucose-induced insulin secretion in palmitate-treated cells be viewed as a sensitization or, alternatively, as a desensitization if the maximal response, is calculated as the diminished fold increase over an elevated baseline? In our view, this question is no longer especially relevant, since it is based on a rather artificial protocol in which β-cells are pretreated with FAs and then challenged with glucose in the absence of FAs. In contrast, our studies suggest that under physiological conditions of constant exposure to FAs, the degree of resultant sensitization to the combined stimuli would far outweigh any small effect seen on the response to glucose alone. Such a conclusion would be consistent with the findings of one earlier study, in which the effect of a combined challenge of glucose plus FA was enhanced in lipid-pretreated islets (17). However, the experimental design of that study did not allow for a full appreciation of the multiple influences at work. On the other hand, oleate-pretreated INS-1 cells did not show an enhanced acute response to a subsequent challenge with oleate (20), but this might represent a difference in the potency of palmitate versus oleate in the sensitization process. Moreover, our findings are supported by in vivo evidence that secretory responses to nonglucose stimuli were upregulated in fat-fed mice and in several animal models of diabetes (37–39). However, it is uncertain whether these enhanced responses, and hence the palmitate-induced sensitization described here, are ultimately beneficial in terms of compensatory insulin secretion, or whether they are implicated in a pathophysiological overstimulation of the β-cell, and hence progression to diabetes. In the latter context, it

needs to be stressed that there was a depletion of intracellular insulin content in MIN6 cells pretreated with either oleate or palmitate. This is consistent with previous studies using islets, and the knowledge that lipids are unable to stimulate transcriptional or translational pathways for insulin synthesis to compensate for chronically stimulated secretion (7,10,17).

The further utility of the MIN6 cell model for transcript profiling is witnessed by the fact that four genes, GLUT2, carnitine palmitoyl transferase 1 (CPT1), c-fos, and PC, previously known to be lipid-regulated in β-cells, were also detected in our screen (10,11,13,40). Similarly, expression of the β-cell–specific transcription factor pancreatic duodenal homeobox-1 (PDX-1), known to be downregulated in islets by palmitate treatment (10), was also inhibited in the MIN6 cells by 1.4 ± 0.1-fold. Although this decrease was too small to be included in Table 1, it is likely to be functionally significant. Many other genes known to be altered during β-cell dysfunction were also shown here to be regulated by palmitate (see below). However, we failed to detect changes in some other β-cell genes previously reported to be lipid-regulated, including: acetyl CoA carboxylase (12) and nur-77 (13) (not present on the microarrays), glucokinase (10), inducible NO synthase (9), and uncoupling protein 2 (14), as well as insulin (10). The latter was expressed so highly in MIN6 cells that it saturated even the "mismatch" probe sets on the array and was therefore paradoxically called as absent. Glucokinase is downregulated by only 25% by palmitate in islets (15), which was probably too a small a difference to be detected here. Uncoupling protein 2 was well expressed in MIN6 cells, but its levels were unaltered by lipid pretreatment (not shown), in contrast to a previous study using INS-1 cells (14). The discrepancy is possibly due to differences in treatment times (18 vs. 48 h here) and the fact that we used a much lower effective FA concentration (molar FA-to-BSA ratio of 3:1, in contrast to 6:1 in the previous study). We were also unable to demonstrate upregulation of NO synthase by lipid, but this has not been previously shown using homogenous β-cells. Macrophages present in the islet might be important for induction of this gene in situ.

Our results provide novel insights into the effects of chronic FA exposure on β-cell function in terms of the distal sensitization (described here) as well as the enhancement of basal secretion and disruption of glucose-dependent stimulus-secretion coupling that have been previously reported. The increased expression of SNAP25, calcyclin, and PLD in palmitate-treated cells is consistent with the observed sensitization to distally acting stimuli, especially those activating Ca^{2+}-dependent secretion. SNAP25 is essential for targeting insulin secretory granules to the sites of exocytosis, and inhibition of its function blocks insulin secretion (36). Similarly, calcyclin is also required for Ca^{2+}-dependent insulin secretion, and, crucially, its overexpression is sufficient for upregulation of the secretory response (34,35). Enhanced PLD signaling should also be stimulatory, either by activation of PKC or at the level of vesicular trafficking. Most importantly, the relative capacities of palmitate versus oleate to alter these genes is consistent with their observed effects on distal secretory pathways. Thus, oleate pretreatment only sensitized the MIN6 cells to PKC-dependent secretion, in keeping with the accompanying increases in PLD1 expression, relatively modest effects on calcyclin, and no effect at all on SNAP25. However, causative relationships between these alterations in gene expression and secretory function will need to be substantiated in future experiments.

Upregulation of SNAP25, calcyclin, and PLD1 might also contribute to the enhancement of basal secretion that accompanies lipid pretreatment of β-cells. However, the observed decrease in the expression of short chain L-3-hydroxyacyl CoA dehydrogenase (SCHAD) is also of particular interest because an inactivating mutation in that enzyme has very recently been causally associated with basal insulin hypersecretion (41). On the other hand, downregulation of GLUT2, PC, and mGPDH might be expected to contribute specifically to the decoupling of glucose metabolism from proximal signaling pathways (21–23). Moreover, the unexpected induction of two forms of FBPase, by both palmitate and oleate, might be particularly relevant to the loss of glucose sensitivity because these enzymes could divert glycolytic flux into alternative metabolic pathways. Indeed, upregulation of one of these branch pathways, the HBP, is sufficient to inhibit glucose-stimulated insulin release (42). Other evidence implicates the HBP in the β-cell apoptosis that accompanies prolonged exposure to high glucose concentrations (43). It is noteworthy that another enzyme in the HBP pathway, GPAT, was also upregulated in palmitate-pretreated MIN6 cells. Interpreting these earlier observations in the light of our new findings, it is conceivable that augmented flux through the HBP could contribute both to the secretory defects and the apoptotic response that accompany chronic β-cell exposure to lipids.

Importantly, our results also demonstrate that palmitate pretreatment is sufficient to alter β-cell gene expression in a similar fashion to that occurring in models of type 2 diabetes. The coregulated genes include GLUT2 (3,44), mGPDH (44,45), LDH (44), PC (45), SNAP25 (46,47), p21 (48), ATF3 (25), ICER (49), and C/EBPβ (50). The fact that highly abundant β-cell genes like GLUT2, PC, and mGPDH were downregulated after lipid treatment, whereas poorly expressed ones like LDH and FBPase (as well as those for hormones other than insulin) were induced, is suggestive of a loss of β-cell differentiation. Upregulation of C/EBPβ might be important in this context (50,51) because this also occurs with supraphysiological glucose levels in vitro and is causally associated with decreased expression of PDX-1 (30). Indeed, a primary role for C/EBPβ in de-differentiating β-cells has been postulated as an underlying cause of type 2 diabetes (51). Our results would be consistent with this hypothesis, but they suggest that other transcriptional regulators are also involved. ATF3 is induced in β-cells as a result of oxidative stress, and its ectopic overexpression leads to abnormal islet development (25). Likewise, induction of ICER correlates with transcriptional repression of the insulin gene in vitro (31), and expression of ICER is increased in the islets of diabetic GK rats relative to nondiabetic controls (49).

Another major finding was the upregulation by both lipids of proinflammatory genes encoding chemokines and mediators of the acute phase response. Although the latter is known to be triggered by cellular stress, its induction in β-cells by the physiologically relevant FA concentrations

used here is surprising. On the other hand, type 2 diabetes is associated with increases in circulating markers of the acute phase response, and a causative role of a hypersensitive innate immune system in the progression of the disease has been hypothesized (52). According to this hypothesis, however, β-cells are passive victims of this response and not, as our results might now suggest, themselves playing a more active role. Whether this, along with chemokine release, could form part of self-destructive loop will obviously require further investigation. However, the fact that binding elements for C/EBPβ are found in the promoters of many acute-phase genes (29) suggests, at the very least, that β-cell dysfunction and the acute phase response share a common etiology.

In conclusion, we have demonstrated that palmitate and oleate induce profound, but differing, alterations in β-cell gene expression. Our results suggest novel explanations, such as induction of FBPase and upregulation of the HBP, for the secretory defects that also occur in lipid-treated islets. Moreover, palmitate pretreatment was sufficient to reproduce many of the alterations in gene expression previously documented in models of β-cell dysfunction. This is in marked contrast to those genes regulated by glucose, as assessed in a comparable study (25). However, palmitate pretreatment also increased expression of genes controlling distal secretory processes and was shown functionally to sensitize β-cells to secretory stimuli. Our study highlights the contribution of regulated gene expression to the phenotypic alterations induced in β-cells after FA exposure, but our results additionally demonstrate that these phenotypic alterations are multiple and complex. The balance between them is likely to be influenced by genetic factors. Allelic variation in some of the lipid-regulated genes that we have documented here might therefore predispose to type 2 diabetes.

REFERENCES

1. Deeney JT, Prentki M, Corkey BE: Metabolic control of β-cell function. *Semin Cell Dev Biol* 11:267–275, 2000
2. Porte D Jr: Banting lecture 1990: β-Cells in type II diabetes mellitus. *Diabetes* 40:166–180, 1991
3. Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications (Review). *Diabetes* 44:863–870, 1995
4. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM (Review). *Diabetes* 46:3–10, 1997
5. Grill V, Bjorklund A: Dysfunctional insulin secretion in type 2 diabetes: role of metabolic abnormalities. *CMLS Cell Mol Life Sci* 57:429–440, 2000
6. McGarry JD Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 2000
7. Zhou Y-P, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J Clin Invest* 93:870–876, 1994
8. Zhou Y-T, Shimabukuro M, Wang M-Y, Lee Y, Higa M, Milburn JL, Newgard CB, Unger RH: Role of peroxisome proliferator-activated receptor α in disease of pancreatic β cells. *Proc Natl Acad Sci U S A* 95:8898–8903, 1998
9. Shimabukuro M, Zhou Y-T, Levi M, Unger RH: Fatty acid-induced β cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 95:2498–2502, 1998
10. Gremlich S, Bonny C, Waeber G, Thorens B: Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. *J Biol Chem* 272:30261–30269, 1997
11. Assimacopoulos-Jeannet F, Thumelin S, Roche E, Esser V, McGarry JD, Prentki M: Fatty acids rapidly induced the carnitine palmitoyltransferase I gene in the pancreatic β-cell line INS-1. *J Biol Chem* 272:1659–1664, 1997
12. Brun T, Assimacopoulos-Jeannet F, Corkey BE, Prentki M: Long-chain fatty acids inhibit acetyl-CoA carboxylase gene expression in the pancreatic β-cell line INS-1. *Diabetes* 46:393–400, 1997
13. Roche E, Buteau J, Aniento I, Reig JA, Soria B, Prentki M: Palmitate and oleate induce the immediate early response genes c-*fos* and *nur*-77 in the pancreatic β-cell line INS-1. *Diabetes* 48:2007–2014, 1999
14. Lameloise N, Muzzin P, Prentki M, Assimacopoulos-Jeannet F: Uncoupling protein 2: a possible link between fatty acid excess and impaired glucose-induced insulin secretion. *Diabetes* 50:803–809, 2001
15. Webb GC, Akbar MS, Zhao C, Steiner DF: Expression profiling of pancreatic β-cells: glucose regulation of secretory and metabolic pathway genes. *Proc Natl Acad Sci U S A* 97:5773–5778, 2000
16. Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K: Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. *Endocrinology* 127:126–132, 1990
17. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ: Chronic exposure to free fatty acid reduces pancreatic β cell insulin content by increasing basal secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. *J Clin Invest* 101:1094–1101, 1998
18. Liang Y, Buettger C, Berner DK, Matshinsky FM: Chronic effect of fatty acids on insulin release is not through the alteration of glucose metabolism in a pancreatic beta-cell line (βHC9). *Diabetologia* 40:1018–1027, 1997
19. Liu YQ, Tornheim K, Leahy JL: Fatty acid-induced β cell hypersensitivity to glucose: increased phosphofructokinase activity and lowered glucose-6-phosphate content. *J Clin Invest* 101:1870–1875, 1998
20. Segall L, Lameloise N, Assimacopoulos-Jeannet F, Roche E, Corkey P, Thumelin S, Corkey BE, Prentki M: Lipid rather than glucose metabolism is implicated in altered insulin secretion caused by oleate in INS-1 cells. *Am J Physiol* 277:E521–E528, 1999
21. MacDonald MJ: Elusive proximal signals of β-cells for insulin secretion (Review). *Diabetes* 39:1461–1466, 1990
22. Matschinsky FM: Banting Lecture 1995: A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* 45:223–241, 1996
23. Prentki M: New insights into pancreatic β-cell metabolic signalling in insulin secretion. *Eur J Endocrinol* 134:272–286, 1996
24. Rudnick A, Ling TY, Odagiri H, Rutter WJ, German MS: Pancreatic beta cells express a diverse set of homeobox genes. *Proc Natl Acad Sci U S A* 91:12203–12207, 1994
25. Allen-Jennings AE, Hartman MG, Kociba GJ, Hai T: The roles of ATF3 in glucose homeostasis: a transgenic mouse model with liver dysfunction and defects in endocrine pancreas. *J Biol Chem* 276:29507–29514, 2001
26. Wice BM, Bernal-Mizrachi E, Permutt MA: Glucose and other insulin secretagogues induce, rather than inhibit, expression of Id-1 and Id-3 in pancreatic islet beta cells. *Diabetologia* 44:453–463, 2001
27. Wotton D, Massague J: Smad transcriptional corepressors in TGF beta family signalling Curr. *Top Microbiol Immunol* 254:145–164, 2001
28. Bernal-Mizrachi E, Wice BM, Inoue H, Permutt MA: Activation of serum response factor in the depolarization induction of Egr-1 transcription in pancreatic islet β-cells. *J Biol Chem* 275:25681–25689, 2000
29. Himes-Lekstrom J, Xanthopoulos KG: Biological role of the CCAAT/enhancer-binding protein family of transcription factors. *J Biol Chem* 273:28545–28548, 1998
30. Lu M, Seufert J, Habener JF: Pancreatic β-cell-specific repression of insulin gene transcription by CCAAT/enhancer-binding protein β. *J Biol Chem* 272:28349–28359, 1987
31. Hussain MA, Daniel PB, Habener JF: Glucagon stimulates expression of the inducible cAMP early repressor and suppresses insulin gene expression in pancreatic β-cells. *Diabetes* 49:1681–1690, 2000
32. Devireddy LR, Teodoro JG, Richard FA, Green MR: Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. *Science* 293:829–834, 2001

33. Uhlar CM, Whitehead AS: Serum amyloid A, the major vertebrate acute-phase reactant. *Biochem J* 265:501–523, 1999
34. Okazaki K, Niki I, Iino S, Kobayashi S, Hidaka H: A role of calcyclin, a Ca^{2+}-binding protein, on the Ca^{2+}-dependent insulin release from the pancreatic β cell. *J Biol Chem* 269:6149–6152, 1994
35. Niki I, Hidaka H: Roles of intracellular Ca^{2+} receptors in the pancreatic β-cell in insulin secretion. *Mol Cell Biochem* 190:119–124, 1999
36. Sadaul K, Lang J, Montecucco C, Weller U, Regazzi R, Catsica S, Wollheim CB, Halban PA: SNAP-25 is expressed in islets of Langerhans and is involved in insulin release. *J Cell Biol* 128:1019–1028, 1995
37. Simonsson E, Ahren B: Potentiated β-cell response to non-glucose stimuli in insulin-resistant C57BL/6J mice. *Eur J Pharmacol* 350:243–250, 1998
38. Zawalich WS, Kelley GG: The pathogenesis of NIDDM: the role of the pancreatic beta cell. *Diabetologia* 38:986–991, 1995
39. Chen N-G, Rosmos DR: Persistently enhanced sensitivity of pancreatic islets from ob/ob mice to PKC-stimulated insulin secretion. *Am J Physiol* 272:E304–E311, 1997
40. Yoshikawa H, Tajiri Y, Sako Y, Hashimoto T, Umeda F, Nawata H: Effects of free fatty acids on β-cell functions: a possible involvement of peroxisome proliferator-activated receptors α or pancreatic/duodenal homeobox. *Metabolism* 50:613–618, 2001
41. Clayton PT, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, Datta V, Malingre HEM, Berger R, van den Berg IET: Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of β-oxidation in insulin secretion. *J Clin Invest* 108:457–465, 2001
42. Kaneto H, Xu G, Song K-H, Suzuma K, Bonner-Weir S, Sharman A, Weir GC: Activation of the hexosamine pathway leads to deterioration of pancreatic β-cell function through induction of oxidative stress. *J Biol Chem* 276:31099–31104, 2001
43. Liu K, Paterson AJ, Chin E, Kudlow JE: Glucose stimulates protein modification by *O*-linked GlcNAc in pancreatic β cells: linkage of *O*-linked GlcNAc to β cell death. *Proc Natl Acad Sci U S A* 97:2820–2825, 2000
44. Jonas J-C, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC: Chronic hyperglycemia triggers loss of pancreatic β-cell differentiation in an animal model of diabetes. *J Biol Chem* 274:14112–14121, 1999
45. MacDonald MJ, Tang J, Polonsky KS: Low mitochondrial glycerol phosphate dehydrogenase and pyruvate carboxylase in pancreatic islets of Zucker diabetic fatty rats. *Diabetes* 45:1626–1630, 1996
46. Chan CB, MacPhail RM, Sheu L, Wheeler MB, Gaisano HY: β-cell hypertrophy in *fa/fa* rats is associated with basal glucose hypersensitivity and reduced SNARE protein expression. *Diabetes* 48:997–1005, 1999
47. Nagamatsu S, Nakamichi Y, Yamamura C, Matsushima S, Watanabe T, Ozawa S, Furukawa H, Ishida H: Decreased expression of t-SNARE, syntaxin 1, and SNAP-25 in pancreatic β-cells is involved in impaired insulin secretion from diabetic GK rat islets: restoration of decreased t-SNARE proteins improves impaired insulin secretion. *Diabetes* 48:2367–2373, 1999
48. Kaneto H, Kajimoto Y, Fujitani Y, Matsuoka T, Sakamoto K, Matsuhisa M, Yamasaki Y, Hori M: Oxidative stress induces p21 expression in pancreatic islet cells: possible implication in beta-cell dysfunction. *Diabetologia* 42:1093–1097, 1999
49. Inada A, Yamada Y, Someya Y, Kubota A, Yasuda K, Ihar Y, Kagimoto S, Kuroe A, Tsuda K, Seino Y: Transcriptional repressors are increased in pancreatic islets of type 2 diabetic rats. *Biochem Biophys Res Commun* 253:712–718, 1998
50. Seufert J, Weir GC, Habener JF: Differential expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein β and transactivator islet duodenum homeobox-1 in rat pancreatic β-cells during the development of diabetes mellitus. *J Clin Invest* 101:2528–2539, 1998
51. Weir GC, Sharma A, Zangen DH, Bonner-Weir S: Transcription factor abnormalities as a cause of beta cell dysfunction in diabetes: a hypothesis. *Acta Diabetol* 34:177–184, 1997
52. Pickup JC, Crook MA: Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 41:12241–1248, 1998