Overexpression of Repressive cAMP Response Element Modulators in High Glucose and Fatty Acid-treated Rat Islets

A COMMON MECHANISM FOR GLUCOSE TOXICITY AND LIPOTOXICITY?

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The hyperlipidemia and hyperglycemia of the diabetic state accelerate β-cell dysfunction, yet the mechanisms are not fully defined. We used rat islet-specific oligonucleotide arrays (Metabolex Rat Islet Genechips) to identify genes that are coordinately regulated by high glucose and free fatty acids (FFA). Exposure of rat islets to FFA (125 μM for 2 days) or glucose (27 mM for 4 days) reduced glucose-stimulated insulin secretion by 70 ± 5 and 40 ± 4%, respectively, relative to control-cultured islets. These treatments also substantially reduced the insulin content of the islets. Islet Genechips analysis revealed that the mRNA levels of cAMP response element modulator (CREM)-17X and inducible cAMP early repressor were significantly increased in both 27 mM glucose- and FFA-treated islets. Removing FFA or high glucose from the culture medium restored glucose-stimulated insulin secretion and the mRNA levels of the two CREM repressors to normal. Northern blot analysis revealed a 5-fold increase in the abundance of CREM-17X mRNA and a concomitant 50% reduction in the insulin mRNA in FFA-treated islets. Transient transfection of the insulin-secreting βHC9 cells with CREM-17X suppressed rat insulin promoter activity by nearly 50%.

Overexpression of CREM-17X in intact islets via adenovirus infection decreased islet insulin mRNA levels and resulted in a significant decrease in glucose- or KCl-induced insulin secretion. Taken together, these data suggest that up-regulation of CREM repressors by either FFA or high glucose exacerbates β-cell failure in type 2 diabetes by suppressing insulin gene transcription.

Type 2 diabetes is characterized by a progressive loss of β-cell function throughout the course of the disease (1, 2). There is a loss of first phase insulin secretion in early stages, followed by a decreasing maximal capacity of glucose to stimulate and potentiate insulin secretion. A significant proportion of the patients eventually develop major β-cell failure requiring supplements of exogenous insulin. None of the current remedies, sulfonylureas, metformin, and insulin, alone or in combination, appear to halt or reverse such a progressive loss of β-cell function/mass in type 2 diabetes (3), with the exception of thiazolidinediones, which may have some subtle beneficial effects on β-cell function in certain patients (4).

Over the past decade, studies in animals and man have suggested that long term hyperglycemia and hyperlipidemia from the diabetic milieu contribute to the deterioration of β-cell function and lead to the concepts of β-cell glucose toxicity (5) and lipotoxicity (6). Blocking β-cell glucose toxicity and lipotoxicity represent a novel potential therapeutic approach for type 2 diabetes. Therefore it is essential to clarify the mechanism by which the detrimental effects of excessive glucose and FFA lead to loss of β-cell function.

It is noteworthy that islets treated with high glucose and FFA share multiple pathophysiological features, such as increased basal and reduced glucose-stimulated insulin secretion (GSIS), increased activity of hexokinase, and reduced glucose oxidation (7–10). At the level of gene expression, excessively elevated glucose or FFA down-regulates several important β-cell genes including pancreatic duodenal homeobox-1 (PDX-1), glucose transporter-2, glucokinase, and insulin (11–13). Most of these genes are also reduced in islets from spontaneous or experimentally induced diabetic models (14, 15). The similarities between glucotoxicity and lipotoxicity and the coordinated changes of multiple genes imply that some of the same master transcription factors are involved in both pathological processes.

We have recently developed custom islet-specific Affymetrix oligonucleotide microarrays (Metabolex Islet Genechips) that can simultaneously detect at least 90–95% of the genes and their splice variants expressed in pancreatic islets. The rat Islet Genechips, a set of three arrays, contain 22,787 probe sets representing ~12,000 known genes or expressed sequence tags present in an adult rat islet cDNA library. In this study we used the rat Islet Genechips to examine changes in the expression of β-cell transcription factors in long term high glucose- and FFA-treated rat islets. We found that the expression of two repressive forms of the cyclic AMP response element modulator (CREM), CREM-17X, and the inducible cAMP early repressor (ICER-1) (16), were significantly increased in both models. Overexpression of CREM-17X in insulin-secreting cells directly suppresses the transcriptional activation of the insulin gene promoter (RIP) and GSIS. Overexpression of CREM repressors therefore may represent a com-

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mon mediator of islet lipotoxicity and glucotoxicity in type 2 diabetes.

EXPERIMENTAL PROCEDURES

Islet Isolation—Pancreatic islets were isolated from fed 9–12-week-old Sprague-Dawley rats (Harlan, Indianapolis, IN) by collagenase digestion in conjunction with Ficoll gradient separation as previously described (17). The islets were cultured overnight in RPMI 1640 medium (11 mM glucose) before experimental treatment.

Treatment of Islets and Experimental Design—Islets were exposed to either 27 mM glucose for 4–8 days or 125 μM FFA (a 1:1 mixture of palmitate and oleate) for 2–4 days during tissue culture in RPMI 1640 medium supplemented with 10% fetal calf serum. Additionally, a recovery group that was first treated with glucose or FFA and then cultured another 2–4 days in regular medium was included in both studies (Table I). These treatments were compared with islets cultured at 11 mM glucose alone (glucose study) or with 0.5% ethanol (FFA study) for the same time period.

GSIS and Islet Insulin Content—GSIS was determined by the 1-h static incubation of islets in Krebs-Ringers’ bicarbonate medium at 37 °C as described (18). The Kreb-Ringer’s bicarbonate medium contains 143.5 mM Na+ , 5.8 mM K+ , 2.5 mM Ca2+ , 1.2 mM Mg2+ , 1.2 mM PO4 3− , 1.2 mM SO4 2− , 25 mM CO3 2− , and 10 mM Hepes, 2 mg/ml bovine serum albumin, and 2 mM glucose (pH 7.4). Insulin was measured in aliquots of the incubation buffer by radioimmunoassay with a commercial kit (Linco Research, St. Charles, MO). Islet insulin content was measured in islet homogenate after acid ethanol (0.18 M HCl in 70% ethanol) extraction. Aliquots of the islet homogenates were also used for islet RNA quantitation using SYBR green I nucleic acid gel stain reagent (Molecular Probes, Eugene, OR).

Metabolex Islet Genecips and RNA Hybridization—Custom rat islet-specific Affymetrix Genecips (Metabolex Islet Genecips) were used in RNA hybridization experiments with Affymetrix standard protocols for chip hybridization (www.affymetrix.com/support/technical/). Briefly, 5 μg of total RNA extracted from 200–300 islets with TRIzol Reagent (Invitrogen) was used to synthesize double-stranded cDNA (Superscript Choice System; Invitrogen), followed by an in vitro transcription reaction to yield biotin-labeled cRNA. Ten μg of cRNA was used to hybridize each individual chip. The Affymetrix Genechip Analysis Suite (version 3.2) was used to convert the image signal of each probe set to an average difference (AD) value. The AD value is the mean of fluorescence intensity differences between the 20 pairs of perfectly matched and mismatched features (probes). The expression levels of each gene or expressed sequence tag in control and glucose- or FFA-treated islets were measured as the means of the AD values from the three to five replicate samples for each group.

Northern Blot and Taqman Quantitative PCR—RNA blot analysis of CREM-17X and insulin was performed with an Ambion Northern blot kit (Ambion, Austin, TX) by following the manufacturer’s instructions. The same blot was also probed for β-actin to correct for sample loading and transferring efficiency. The intensity of the hybridization signal with each probe was quantified with a Storm™ PhosphorImager scanner (Molecular Dynamics).

The mRNA levels of NeuroD1, PDX-1, and insulin were quantified by real time PCR using the SYBR® Green I dye and an ABI Prism 7700 sequencer (Applied Biosystems, Foster City, CA) and were normalized to β-actin level in each sample. The sequences of the gene-specific primers were as follows: NeuroD1, 5′-TGGCTGCTCGTCTGGCA-3′ (sense) and 5′-GGCGATGTCCGGATTCG-3′ (antisense); PDX-1, 5′-AAAAAGCCGGCTCTGTTCA AAA-3′ (sense) and 5′-GCAAGCTCTGCGGTTCCAC-3′; insulin, 5′-CTGCCGCGCTTTTGTGCA-3′ (sense) and 5′-CCCCACACAGGTGAGC-3′ (antisense); β-actin, 5′-CGTGAAGATGACAGGATGCA-3′ (sense) and 5′-CACAGCCTGGATGCGTACTG-3′ (antisense). The fold changes were calculated by using the comparative threshold cycle method.

Plasmid Constructs and Transfection—The RIP-βGal reporter vector was made by replacing the CMV promoter of the pcDNA3.1/Hygro/βGal/Z DNA (Invitrogen) with a 0.7-kb fragment of the rat insulin II promoter (-695 to 1 of the insulin gene) (20). The full-length cDNA of CREM-17X was subcloned into the pcDNA4 vector (Invitrogen). The insulin-secreting pH9C9 cells were cultured in DMEM (25 mM glucose) medium supplemented with 10% fetal calf serum and antibiotics as described previously (21). Transient transfection of the pH9C9 cells with RIP-βGal and CREM17X or empty pcDNA4 vectors (at 1:2 ratio) were carried out with the DMRIE reagent (Invitrogen). Cellular βGal activity was measured 48 h later with Gal-F beta-galactosidase detection kit (Sigma).

Adenovirus-mediated Overexpression of CREM-17X in Islets—Recombinant adenoviruses expressing enhanced green fluorescence protein (eGFP) alone (Ad-eGFP, encoded by a gene incorporated into the viral backbone) or CREM17X plus eGFP (Ad-CREM17X) was generated using the AdEasy system as described (22). Batches of 50 freshly isolated rat islets were exposed to 50 × 106 plaque-forming units of the virus in 1.5 ml of Adeno infection medium (RPMI1640 medium with 5% heat-inactivated fetal calf serum) for 1.5 h. The islets were then cultured for 4 days before being used for GSIS, islet insulin content assays. Pilot study with the Ad-eGFP control virus demonstrated that this protocol of infection induced GFP expression (visualized under fluorescence microscope with filter set of excitation of 435 nm and emission of 465 nm) in ~85% of islet cells, and the virus infection itself did not affect GSIS.

For the islet perfusion study, the islets were exposed to the viruses in Adeno infection medium for 16 h and then cultured in regular RPMI medium for another 48 h. An extra group of islets were treated with 125 μM FFA in regular medium as described earlier in this section to serve as positive controls. After the viral infection or FFA exposure (6 h in total), the islets (25 of each) were perfused with Kreb-Ringer’s bicarbonate medium in parallel mini-chamber, as previously described (22), to measure insulin secretory responses to glucose and 30 mM KCl. Similarly treated islets were also subjected to Taqman quantitative PCR analysis for insulin mRNA levels.

Statistical Analysis—All of the data were expressed as the means ± S.E. A Student’s t test was used to determine the significance of differences between groups.

RESULTS

Insulin Secretion and Islet Insulin Content in High Glucose or FFA-treated Rat Islets—Exposing rat islets to 27 mM glucose (G27) for 4 days increased the basal insulin secretion at 2 mM glucose by 3-fold (6.4 ± 0.3 versus 2.3 ± 0.2 ng/5 islets/h, p < 0.01) and decreased insulin secretory responses to 20 mM glucose by 40% (51 ± 4.8 versus 73 ± 9.7 ng/5 islets/h, n = 4, p < 0.05) relative to control cultured islets (Fig. 1A). The insulin content in G27-treated islets was reduced by 35% (31 ± 4 versus 48 ± 5 ng/islet, p < 0.05; Fig. 1B). The changes of insulin secretion and insulin content in G27-treated islets were completely reversed by the 4-day recovery culture at 11 mM glucose. When expressed as a percentage of islet insulin content released during the 1-h incubation (Fig. 1C), the basal secretion in G27-treated islets increased by 5-fold, whereas GSIS was comparable with that of control (32 ± 3% in G27-treated islets versus 30 ± 4.2% in control islets). These results indicate that GSIS and islet insulin content were reduced comparably in G27-treated islets.

GSIS in islets exposed to 125 μM FFA for 48 or 96 h was reduced by 70–80% (11 ± 2 and 8 ± 1.2 versus 38 ± 4 ng/5 islets/h, n = 3, p < 0.01), along with a 50–60% reduction in islet insulin content. An additional 48-h culture of the islets in medium without FFA restored GSIS and insulin content to ~80% of the values of islets never exposed to FFA (Fig. 1, D and E). The percentage of insulin content secreted at basal levels (2 mM glucose) was again significantly higher in FFA-treated islets than in control.
islets than control. Unlike G27-treated islets, the fractional insulin secretion stimulated by 20 mM glucose was also markedly reduced in FFA-treated islets (Fig. 1F). These results for insulin secretion and insulin content in glucose- and FFA-treated rat islets are largely consistent with the previous studies of others and us (7–12).

Expression Levels of Major Islet Transcription Factors in Glucose and FFA-treated Islets—Insulin gene expression and CREM and β-Cell Dysfunction
the maintenance of normal islet function in adult islets depend on the action of a complex network of transcription factors (24, 25). To evaluate the potential role of these factors in the induction of glucose toxicity and lipotoxicity, we examined changes in the expression of islet transcription factors in G27- and FFA-treated islets.

The Islet Genechip study showed that the expression of PDX-1 and NeuroD1/Beta2 were significantly suppressed in FFA-treated islets (Fig. 2A). Quantitative PCR measurement confirmed the reductions of PDX-1 and NeuroD1/BETA2 mRNA in FFA-treated rat islets (Fig. 2B, n = 2 for each). No significant reduction of PDX-1 and NeuroD1 was found in G27-treated islets by the Genechip (Fig. 2B) and Taqman PCR study (Fig. 2C).

Other transcription factors that were found not changed in the chip experiments were Nkx6.1, PAX6, hepatocyte nuclear factor (HNF)-1α, HNF-3β, HNF-3γ, Hes-2, Hes-3, Islet-brain 1, neurogenin-3, and Pan-1. The rat Islet Genechips were unable
treated islets and the effects of CREM overexpression on rat insulin secretion. When measured in a static incubation, GSIS in AdCREM17X-infected islets was ~35% lower than that in Ad-eGFP-treated islets (313 ± 20 versus 463 ± 41 pg/ngDNA, n = 3, p < 0.05). The adenovirus-mediated CREM-17X expression in islet cells also reduced islet insulin content by ~30% (2.3 ± 0.2 versus 3.5 ± 0.2 ng/ng DNA, n = 3, p < 0.01; Fig. 5D).

The suppression of insulin secretion by CREM-17X overexpression was also documented in islet perfusion experiments performed 64 h after the viral infection. The reduction of GSIS in Ad-CREM17X-infected islets occurred mainly in the first phase of the insulin secretion process (Fig. 5E). Thus, the average insulin secretion during the first 5 min of glucose stimulation was reduced by 50% (1.4 ± 0.4 versus 2.8 ± 0.5 ng/25 islets/min, p < 0.01, n = 3) in Ad-CREM17X-infected islets relative to Ad-eGFP-infected islets, whereas the second phase secretion (between the 6th and 20th min of glucose stimulation) was not significantly different between the two groups of islets (1.3 ± 0.08 in the Ad-CREM17X and 1.5 ± 0.15 ng/25 islets/min in Ad-eGFP islets). Notably, the decrease of GSIS in FFAtreated islets was seen in both first phase (by 33%, average insulin secretion 1.9 ± 0.2 ng/25 islets/min) and more profoundly the second phase (by 50%, 0.7 ± 0.03 ng/25 islets/min). Furthermore, the insulin secretory responses to the glucose-independent membrane depolarization induced directly by 30 mM KCl was also significantly reduced in Ad-CREM17X- and FFAtreated islets (Fig. 5E). A similar conclusion could be reached when the area under the curve was used to detect cAMP-responsive element-binding protein, Hes-1, HNF-1β, HNF-3α, HNF-4α, and MASH1 (data not shown), perhaps because of either low expression level or a lack of sensitivity of these probes.

**Overexpression of CREM Repressors in FFAtreated Islets**—The most striking change in the expression of transcription factors in G27- or FFAtreated islets was the up-regulation of CREM-17X and ICER-1, two repressor forms of CREM. As shown in Fig. 2, the expression of CREM-17X and ICER-1 was increased by 3–5-fold in both FFAtreated and G27treated islets relative to control cultured islets.

The up-regulation of CREM repressors was also the most prominent change observed across the entire DNA array. Of all probe sets representing ~12,000 known genes on the rat islet microarray, only 15 of them were changed significantly (p < 0.05, 12 up and 3 down) in all islet groups with reduced GSIS (cultured with G27 for 4 or 8 days and FFA for 2 or 4 days) and restored to normal in islets that had gone through the recovery culture. Notably, 3 of the 12 increased probe sets represent CREM-17X or ICER-1 (Table II).

The changes in the gene expression of CREM-17X and ICER-1 were tightly coupled with the islet function. Thus, withdrawal of G27 and FFA from the culture medium restored both GSIS and the AD values of CREM-17X and ICER-1 to normal levels (Fig. 3). The changes in the expression of CREM-17X and ICER-1 are apparently specific to treatment by G27 or FFA, because no change in the expression of β-actin and GAPDH tiled on the chips was seen in either of the treatment groups (data not shown).

**Overexpression of CREM Impairs Insulin Gene Transcription and GSIS**—To evaluate the potential roles of overexpression of CREM repressors in high glucose- and FFA-induced β-cell dysfunction, we examined insulin mRNA levels in FFAtreated islets and the effects of CREM-17X overexpression on rat insulin promoter activation and GSIS. Northern blot analysis revealed a 5-fold increase in the abundance of CREM-17X mRNA in FFAtreated islets compared with control islets. More importantly, the same blot also showed a 50% reduction of insulin mRNA level in FFAtreated islets (Fig. 4). Removing FFA from the culture medium largely restored the mRNA levels of CREM-17X and insulin to normal.

To investigate the effect of CREM repressors on insulin gene transcription, βHC9 cells, a mouse insulin secreting β-cell line, were transiently co-transfected with RIP-βGal and CREM-17X. The ectopically expressed RIP retained ample glucose responsiveness in βHC9 cells. Thus, increasing glucose concentrations from 0.1 to 25 mM during the 48-h culture after the transfection enhanced RIP activity (measured by βGal expression driven by the promoter) nearly 3-fold when it was co-transfected with a second empty vector (Fig. 5A). However, co-expression of CREM-17X in the cells inhibited the activation of RIP by glucose, especially at the level of 25 mM glucose (by 44 ± 5%, p < 0.05, n = 4; Fig. 5B).

The effects of CREM overexpression on insulin secretion and islet insulin content were investigated by infecting intact rat islets with an adenovirus expressing CREM-17X (Ad-CREM17X) followed by GSIS assay 2–4 days after the infection. The infection by the Ad-CREM17X virus of islets was confirmed by visualizing GFP expression under a fluorescence microscope (data not shown). As shown in Fig. 5C, infecting the islets with the Ad-eGFP control virus did not affect insulin secretion. When measured in a static incubation, GSIS in AdCREM17X-infected islets was ~35% lower than that in Ad-eGFP-treated islets (313 ± 20 versus 463 ± 41 pg/ngDNA, n = 3, p < 0.05). The adenovirus-mediated CREM-17X expression in islet cells also reduced islet insulin content by ~30% (2.3 ± 0.2 versus 3.5 ± 0.2 ng/ng DNA, n = 3, p < 0.01; Fig. 5D).

**Table II.** Genes changed reversibly in both 27 mM glucose- and FFAtreated islets

| Probe set on array | Gene name | NCBI number |
|--------------------|-----------|-------------|
| Down-regulated genes | IP3 receptor subtype 3 | L06096 |
| MBXRATISL09987 Insulin receptor-related receptor | M90661 |
| RNTSX Testis-specific gene | X99797 |
| Up-regulated genes | Brain creatine kinase | M14400 |
| MBXRATISL11524 ICER-I | S66024 |
| MBXRATISL11825 ICER-I | S66024 |
| MBXRATISL10690 CREM-17X (CREMΔC-G) | Z15158 |
| MBXRATISL11565 Gene 33 polypeptide | X07266 |
| MBXRATISL11657 Gene 33 polypeptide | X07266 |
| MBXRATISL10759 Glutamic acid dehydrogenase | X57573 |
| MBXRATISL18113 MAP kinase phosphatase | AF013144 |
| MBXRATISL10704 Phosphoglycerate dehydrogenase | X97772 |
as a measurement of insulin secretion in these experimental conditions.

To investigate to what extent the impaired insulin secretion in CREM-17X overexpressing islets was linked to the expression of insulin gene, we went on to measure the insulin mRNA levels in AdCREM17X-treated islets by quantitative reverse transcription-PCR. As shown in Fig. 5, the relative abundance of insulin mRNA in AdCREM17X-infected islets was ~60% lower than that of Ad-eGFP-infected islets. The 64-h exposure to FFA caused a 50% reduction in insulin mRNA levels in this study.

**DISCUSSION**

The genes responsible for islet glucose toxicity and lipotoxicity are yet to be discovered. The results of the present study demonstrate that the overexpression of ICER-1 and CREM-17X are the most prominent changes in high glucose- or FFA-treated rat islets and are tightly associated with the changes of GSIS. Exposing rat islets to G27 for 4–8 days or 125 μM FFA for 2–4 days resulted in a significant reduction of GSIS and a 3–5-fold increase in the mRNA levels of ICER-1 and CREM-17X. Removing high glucose and FFA from the culture medium restored GSIS and the expression levels of the two CREM repressors to normal.

CREM is a member of the ATF/cAMP-responsive element-binding protein family of transcription factors that controls the expression of multiple genes in neuroendocrine cells including insulin. Pancreatic β-cells express multiple forms of CREM originating from alternative splicing or the use of an intronic promoter (16, 26). ICER-1 and CREM-17X are apparently the major isoforms of CREM in primary rat islet cells (26). Both ICER-1 and CREM-17X lack the two glutamate-enriched domains and therefore act as transcriptional repressors. CREM-17X is indistinguishable from a CREM isoform identified earlier called CREMAGC-G (27).

In addition to the tight coupling of overexpression of CREM-17X/ICER-1 with the reduction of insulin secretion, several lines of evidence in this study further support the notion that CREM repressors may play important roles in β-cell dysfunction. First, Northern blot analysis confirmed that increased CREM-17X is associated with reduced expression of the insulin gene in FFA-treated islets. A previous study has shown that the expression of CREM-17X and ICER-1 are increased in the islets of the Goto-Kakizaki rats (26). Second, none of the other islet transcription factors examined by our Genechip experiments were changed in both models of glucose toxicity and lipotoxicity or disturbed to the magnitude of CREM-17X/ICER-1. Third, transient transfection of βIC9 cells with CREM-17X significantly inhibited the activation of RIP by glucose. Most importantly, adenovirus-mediated expression of CREM-17X in rat islets significantly inhibited GSIS and reduced islet insulin content and insulin mRNA levels. The reduction of insulin gene transcription and/or insulin production is probably the main mechanism for the decrease of insulin content in Ad-CREM17X-infected islets, because basal insulin secretion in these islets is reduced rather than increased as seen in G27- or FFA-treated islets.

The virally mediated overexpression of CREM-17X in rat islets mimics some important characteristics of the β-cell dysfunction induced by long term high glucose and FFA. Like high glucose and FFA, the adenovirally mediated overexpression of CREM-17X significantly reduced islet insulin content and GSIS. Both CREM-17X overexpression and long term FFA exposure reduced islet insulin mRNA levels. High glucose treatment did not suppress islet insulin mRNA relative to normal glucose, but given the increased demand for insulin caused by high glucose, transcriptional inhibition by CREM would reduce the capacity of the β-cell to replenish secreted insulin.

Nevertheless, CREM overexpression does not explain all of the effects of hyperglycemia and hyperlipidemia observed in this study. The overexpression of CREM-17X did not change the basal insulin secretion at 2 mM glucose, whereas high glucose and FFA both increased basal insulin secretion. Also, FFA inhibited both the first phase and second phase of insulin secretion in an in vitro islet perfusion system, whereas CREM-17X overexpression mainly suppressed the first phase of secretion, indicating the involvement of more than one mechanism for FFA inhibition of GSIS. These differences may suggest that suppressing insulin gene transcription is not the only mechanism by which CREM-17X exerts its negative effects on insulin secretion. Further studies are needed to determine whether CREM regulates the transcription of other genes pivotal to the regulation of insulin secretion in β-cell.

There are certainly other genes that are important for the development of both β-cell glucose toxicity and lipotoxicity. It is important to clarify that the present study is focused only on the transcription factors that are potentially important for both high glucose-induced (glucose toxicity) and FFA-induced (lipotoxicity) β-cell dysfunction. Genes that are changed by only one of the treatments are not considered in this study. We did not see any differences in the expression level of PDX-1 or other homeobox factors between high glucose and control treated islets in our chip studies, although others have shown that high glucose reduces PDX-1 expression in human (10) and rat islets
using reverse transcription-PCR techniques. The reason we saw only a limited number of gene expression charges in this study is not known at this point. One possibility is that we used 11 mM glucose as control in our culture system. Although this concentration of glucose is traditionally viewed as most favorable (therefore "physiological") to rodent islets when cul-

![Fig. 5. Effect of CREM-17X on RIP activation and insulin secretion. A, glucose-responsiveness of the RIP-LacZ construct. βHC9 cells were transfected by a mixture of the RIP-LacZ and pcDNA4 (empty vector) DNA with DMRIE reagent, and the activity of RIP was then determined by measuring the βGal activity in cell lysates after a 48-h culture in medium with different glucose concentration. B, CREM-17X inhibited the activation of RIP by 25 mM glucose. The cells were co-transfected with RIP-LacZ and CREM-17X DNA, and RIP activation by glucose during the 48-h culture after transfection was measured by βGal activity in cell lysate. The results are expressed as percentages of the RIP activity at 0.1 mM glucose. The data are the means ± S.E. of four separate experiments. *, p < 0.05 when compared with control vector. C, effect of CREM-17X overexpression on GSIS. Freshly isolated rat islets were infected with Ad-CREM17X or Ad-eGFP and cultured in RPMI1640 medium for 4 days. Insulin secretion was measured by static incubation. The rate of insulin secretion was corrected by the islet DNA content. The data are the means ± S.E. of three separate experiments. *, p < 0.05 when compared with Ad-eGFP-infected islets. D, insulin content in Ad-CREM17X-infected islets. Islets incubated at 2 mM glucose during the static incubation in C were retrieved and extracted for insulin content measurement. The same islet homogenates were used to measure islet DNA content with the SYBR green I dye. *, p < 0.01 when compared with Ad-eGFP-infected islets. E, perifusion of Ad-CREM17X- or FFA-treated islets. The islets infected by Ad-eGFP or Ad-CREM17X viruses or treated by 125 μM FFA for 64 h were perfused with Kreb-Ringer’s bicarbonate buffer to measure the insulin responses to 20 mM glucose or 30 mM KCl. The data are the means ± S.E. of three separate experiments. F, quantitative reverse transcription-PCR measurement of insulin mRNA levels in Ad-CREM17X- or FFA-treated islets. Total RNA was isolated from islets treated in a similar way as those described for E. The relative abundance of insulin mRNA and that of β-actin were quantified by real time PCR with the SYBR green dye techniques as described for Fig. 2C. The data are the means ± S.E. of three separate experiments. *, p < 0.05 compared with eGFP control virus-infected islets.
tured in RPMI 640 medium (19, 28), it is much higher than the normal blood glucose level of rodent. Therefore it is possible that we are, in this study, actually comparing “hyperglycemia” with “super-hyperglycemia” or “hyperglycemia” with “hyperglycemia with FFA.”

There are other genes showing expression changes similar to CREM following the exposure to elevated glucose and FFA. Some of them may also contribute to β-cell dysfunction associated with long term treatment with high glucose and FFA. Further exploration of the importance of these genes is beyond the scope of this report. Also, it has to be mentioned that the actual number of genes that fall into this category may well exceed what we present here. This has relevance to the limitations of the Genechip technology and to the degree of the “translation” of the rat transcriptome. As more genes are functionally characterized in the future, we will know the nature of those unknown genes (or expressed sequence tags) that show similar patterns of expression. Having said this, the present study represents a substantive global survey of the known islet expressed genes, and the fact that we have found only a handful of genes that are coordinately expressed in RPMI 640 medium (19, 28), it is much higher than the normal blood glucose level of rodent. Therefore it is possible that we are, in this study, actually comparing “hyperglycemia” with “super-hyperglycemia” or “hyperglycemia” with “hyperglycemia with FFA.”

In conclusion, we have demonstrated that high concentrations of both glucose and FFA up-regulate the expression of ICER-1 and CREM-17X in parallel to the reversible suppression of GSIS. Overexpression of CREM repressors in pancreatic β-cells clearly inhibit insulin gene transcription and insulin secretion. These results suggest that transcriptional repression by CREM variants may contribute to the loss of β-cell function in type 2 diabetes by inhibiting insulin gene transcription.

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