Chronic Exposure to Free Fatty Acid Reduces Pancreatic β Cell Insulin Content by Increasing Basal Insulin Secretion That Is Not Compensated For by a Corresponding Increase in Proinsulin Biosynthesis Translation

L. Cornelius Bollheimer, Robert H. Skelly, Michael W. Chester, J. Denis McGarry, and Christopher J. Rhodes
Gifford Laboratories for Diabetes Research & Department of Internal Medicine and Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-8854

Abstract

The pancreatic β cell normally maintains a stable balance among insulin secretion, insulin production, and insulin degradation to keep optimal intracellular stores of the hormone. Elevated levels of FFA markedly enhance insulin secretion; however, the effects of FFA on insulin production and intracellular stores remain unclear. In this study, twofold elevation in total circulating FFA effected by infusion of lard oil and heparin into rats for 6 h under normoglycemic conditions resulted in a marked elevation of circulating insulin levels evident after 4 h, and a 30% decrease in pancreatic insulin content after a 6-h infusion in vivo. Adding 125 μM oleate to isolated rat pancreatic islets cultured with 5.6 mM glucose caused a 50% fall in their insulin content over 24 h, coupled with a marked enhancement of basal insulin secretion. Both effects of fatty acid were blocked by somatostatin. In contrast to the stimulatory effects of oleate on insulin secretion, glucose-induced proinsulin biosynthesis was inhibited by oleate up to 24 h, but was unaffected thereafter. This result was in spite of a two- to threefold oleate-induced increase in preproinsulin mRNA levels, underscoring the importance of translational regulation of proinsulin biosynthesis in maintaining β cell insulin stores. Collectively, these results suggest that chronically elevated FFA contribute to β cell dysfunction in the pathogenesis of NIDDM by significantly increasing the basal rate of insulin secretion. This increase in turn results in a decrease in the β cell's intracellular stores that cannot be offset by commensurate FFA induction of proinsulin biosynthesis. (*J. Clin. Invest.* 1998. 101:1094–1101.) Key words: pancreatic islet • non-insulin-dependent diabetes mellitus (NIDDM) • free fatty acid • insulin secretion • proinsulin biosynthesis

Introduction

FFA are an important physiological fuel for islets, and act as a supplemental nutrient secretagogue to potentiate insulin release acutely in the presence of glucose (1–7). Moreover, there is emerging evidence that transient elevation of cytosolic long-chain fatty acyl-CoA as a consequence of increased glycolytic flux in the β cell is a critical step for metabolic coupling of glucose-stimulated insulin release (8, 9). However, chronically elevated FFA are believed to play a role in the pathogenesis of certain forms of type II diabetes by both inhibiting insulin-stimulated peripheral glucose uptake and contributing to β cell dysfunction (10–14). In contrast to short-term effects of FFA, prolonged exposure to FFA has detrimental effects for β cell function, including impairment of glucose-induced insulin release (15–17) as well as other metabolic and morphological abnormalities (12, 18–21).

Under normal physiological circumstances, the pancreatic β cell maintains a remarkably stable balance between insulin secretion and insulin production. Whenever glucose stimulates insulin release, there is a rapid and corresponding glucose-induced increase in proinsulin biosynthesis at the translational level that efficiently replenishes intracellular insulin stores (24–27). A similar scenario applies to the vast majority of nutrient secretagogues of the β cell (23). Although short-term exposure to FFA markedly potentiates glucose-induced insulin release (1–5, 28), the effect of FFA on proinsulin biosynthesis remains relatively undefined. Indeed, recent studies have indicated that in contrast to FFA potentiating glucose-induced insulin secretion, FFA inhibit glucose-induced proinsulin biosynthesis (28). It follows that, unlike the stimulus–response coupling pathway for glucose-induced insulin release where FFA play an important signaling role (8, 9), these lipid moieties are unlikely to be involved in the metabolic signal transduction pathway for proinsulin biosynthesis at the translational level. If this is so, the insulin content of the β cell cannot be rapidly replenished after acute stimulation of insulin release by FFA. Under normal circumstances, only a small proportion of the β cell's insulin intracellular store is released after an acute stimulation by a secretagogue (29), so that short-term FFA-induced insulin release would have little adverse effect on the β cell's secretory capacity. However, chronic exposure to FFA could severely deplete the internal insulin stores since there is apparently no biosynthetic backup to compensate for FFA-induced insulin hypersecretion. This study addresses the question as to how maintenance of β cell insulin stores is affected by chronic elevated level of FFA.

Methods

Reagents. L-[^{35}S]Methionine (1,175 Ci mmol^{-1}) and [^{32}P]Cytidine 5′-triphosphate (800 Ci mmol^{-1}) were from Dupont-NEN (Boston, MA). Human [^{125}I]Tyr^{A14}-insulin (374 Ci g^{-1}) was kindly provided by Eli Lilly and Co. (Indianapolis, IN), and radioimmunoassays were performed using a kit from Linco Research Inc. (St. Charles, MO) using rat insulin standards. Collagenase P (1.5 U mg^{-1}) and fatty acid

Address correspondence to Christopher J. Rhodes, Ph.D., Gifford Laboratories for Diabetes Research, Room Y8.222, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-8854. Phone: 214-648-6375; FAX: 214-648-9191; E-mail: rhodes02@utsw.swmed.edu

Received for publication 17 April 1997 and accepted in revised form 14 November 1997.

J. Clin. Invest.

0021-9738/98/03/1094/08 $2.00
Volume 101, Number 5, March 1998, 1094–1101
http://www.jci.org

ultra-free BSA (Fraction V) were from Boehringer Mannheim Biochemicals (Indianapolis, IN). Oleate (*cis*-9-octadecenoic acid) was from Alltech Assoc., Inc. (Deerfield, IL), Pansorbin™ was from Calbiochem Corp. (La Jolla, CA), and Biotrans™ nylon membranes (0.2-m pore size) were from ICN (Costa Mesa, CA). Prime-it II™ random primer labeling kit was from Stratagene Inc. (La Jolla, CA). Histopaque-Ficoll™, dialyzed FBS, bovine insulin antiserum, somatostatin, and all other analytical-grade biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

In vivo fatty acid infusion. It has been previously established that the fatty acid mixture present in lard oil provides a potent stimulus for glucose-induced insulin secretion (7). Preparation of a 20% lard oil emulsion and its infusion into fed 300-g male Sprague-Dawley rats under euglycemic conditions was as previously described (7). Blood samples were taken every hour for analysis of plasma glucose, total FFA, and insulin concentrations as previously described (6, 7). The euglycemic glucose clamp was applied to maintain a constant plasma glucose level of 120–130 mg/100 ml as described (6, 7). At the end of the 6-h infusion period, the animal was killed, and the pancreas was excised for evaluation of insulin content by acid–ethanol extraction and radioimmunoassay (29).

Islet isolation and culture. Pancreatic islets were isolated from male 150-g Sprague-Dawley rats by Collagenase digestion and Histopaque-Ficoll™ density gradient centrifugation as described (27). Batches of 200 islets were maintained for 16 h in 8 ml of RPMI-1640/5.6 mM glucose/10% dialyzed FBS (MW cutoff < 10 kD to remove bovine insulin) at 37°C in a 95% CO_2 atmosphere. After this conditioning phase, islets were further incubated for periods up to 48 h in culture medium that additionally contained 125 μM oleate/0.5% ethanol as previously described (17, 20). Control islets were cultured in the presence of 0.5% ethanol.

Insulin secretion and content analysis during culture. In 24-well plates (Costar Corp., Cambridge, MA) batches of 10 islets were placed in 400 μl of culture medium with or without oleate for different time periods. In some experiments, islets were similarly cultured in the presence or absence of an additional 500 nM of somatostatin. At the end of each incubation period, the medium was collected, centrifuged, and kept frozen at −80°C pending analysis of secreted insulin by radioimmunoassay. To determine the insulin content, islets were washed in 400 μl RPMI-1640 and lysed in 150 μl ice-cold lysis buffer (50 mM Hepes, 0.1%[vol/vol] Triton X-100, 1 μM PMSF, 10 μM E-64, 10 μM pepstatin A, 10 μM TLCK, 100 μM leupeptin; pH 8.0). After sonication (25 W for 20 s) and centrifugation (10,000 *g* for 2 min), the resultant supernatants were stored at −80°C and subsequently analyzed for insulin content by radioimmunoassay (29, 30).

Glucose-induced proinsulin biosynthesis and insulin secretion analysis. Islets were cultured for different time periods with or without oleate. Afterwards, groups of 50 islets were washed and transferred into 300 μl of Krebs-Ringer bicarbonate buffer/2.8 mM glucose/16 mM Hepes (pH 7.4). The buffer for the oleate-treated islets was supplemented with 125 μM oleate precomplexed to 0.1% (wt/vol) BSA (6–8). 0.1% BSA was used for the controls. The samples were preincubated at 37°C with 2.8 mM glucose for 60 min followed by a further 60-min incubation at either 2.8 or 16.7 mM glucose. After this period, islets were spun down at 1,000 *g* for 2 min; 200 μl of the supernatant was removed and stored at −80°C pending analysis for insulin. Islets were resuspended in the remaining 100 μl of buffer and incubated for a further 20 min with 10 μCi L-[^{35}S]methionine. Cells were spun down (1,000 *g*/2 min), placed in 300 μl of lysis buffer as described above, and sonicated (25 W for 20 s). The resulting lysate was then centrifuged (10,000 *g* for 2 min) to remove debris, and the supernatant was stored at −80°C for later analysis. Four aliquots of 5 μl each were taken for determination of total insulin content as well as for measurement of total protein synthesis in the islets by trichloroacetic acid precipitation as previously described (27, 31). The remainder of the radiolabeled lysate (280 μl) was subjected to immunoprecipitation with a specific antiserum against (pro)insulin. The immunoprecipitates were then eluted in 380 mM glycine, 10 M urea, and 50 mM Tris (pH 8.6) at 37°C and analyzed by alkaline-urea gel electrophoresis, fluorography, and denistometric scanning (27, 31).

Northern blot mRNA analysis. Batches of 400 islets were cultured in RPMI-1640/5.6 mM glucose with or without oleate for different time periods as described above, and were subsequently washed and lysed in a 4 M guanidinium isothiocyanate solution. RNA was isolated by cesium chloride gradient centrifugation and precipitation with ethanol as described (32, 33). A total of 3 μg RNA per lane was separated by 1% agarose/formaldehyde gel electrophoresis and transferred to a nylon membrane by capillary elution. After UV cross-linking, the blot was analyzed for preproinsulin mRNA and actin mRNA using the respective random primer–labeled cDNA probes (33).

Statistics. Unless otherwise mentioned, data are presented as means±SE of at least three independent experiments. Statistically

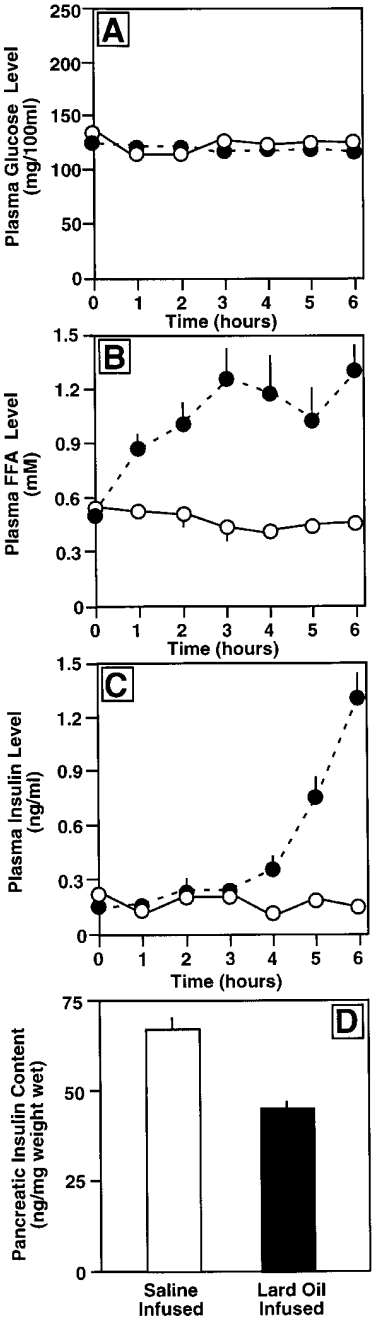

Figure 1. Effect of a 6-h lard oil or saline infusion in normal rats on insulin secretion and pancreatic insulin content under normoglycemic conditions. A 6-h infusion of either lard oil (●) or saline as a control (○) into normal adult rats under euglycemic clamp conditions was performed in duplicate as described in Methods. Levels of plasma glucose (*A*), FFA (*B*), and insulin (*C*) were monitored during the 6-h infusion period. At the end of 6 h, total insulin content of the pancreas was evaluated (*D*). Values are means±range of two experiments.

significant differences were analyzed using appropriate Student's *t* test, where $P \leq 0.05$ was considered significant.

Results

Fatty acid infusion of normoglycemic rats increases insulin secretion and lowers pancreatic insulin content in vivo. It has been shown that lard oil is a potent stimulator of insulin release in vivo (7). In this study, plasma glucose levels were maintained at 120–130 mg/100ml for 6 h in both saline- and lard oil–infused rats (Fig. 1 *A*). As anticipated, total plasma FFA levels increased two to threefold ($P \leq 0.02$) at 1–6 h in lard oil–infused rats compared with saline-infused controls (Fig. 1 *B*). Under the continuous normoglycemic conditions applied, it was found that after 4 h of lard oil infusion there was a twofold increase in circulating insulin levels over saline-infused control rats (Fig. 1 *C*) that increased to fourfold at 5 h and to eightfold by 6 h (Fig. 1 *C*). After 6 h of lard oil infusion, it was found that there was a decrease (29.9%) in pancreatic insulin content compared with saline-infused control animals (Fig. 1 *D*).

Islets cultured with oleate at 5.6 mM glucose show an increased insulin secretory rate and a decrease in β cell insulin content. Batches of 10 islets were cultured at 5.6 mM glucose with or without 125 μM oleate, and both insulin secretion and intracellular insulin content were monitored over a period of 48 h. Islets incubated in the presence of oleate released 4.5-fold more insulin than did control islets ($P \leq 0.01$, from 6 h onwards; Fig. 2 *A*). While in control islets, the intracellular insulin content remained constant throughout the 48-h incubation period (Fig. 2 *B*); in those exposed to oleate it gradually decreased with time to 46±2% ($P \leq 0.05$) that of the control value by 24 h, and remained at this reduced level for the subsequent 24-h period. After a 6-h exposure to oleate in vitro, insulin secretion was increased threefold, and the islet insulin content fell by 25% compared with the control ($P \leq 0.02$). This in vitro decrease in β cell insulin content was compared with that seen in vivo with lard oil infusion (Fig. 1).

Glucose-induced insulin secretory response in isolated islets incubated with oleate. Islets were cultured for 3, 6, 12, 24, or 48 h at 5.6 mM glucose±125 μM oleate, and were subsequently challenged with a basal (2.8 mM) or a stimulatory (16.7 mM) concentration of glucose for 1h. Insulin secretion, insulin content, and proinsulin biosynthesis were then assessed. Basal insulin secretion (at 2.8 mM glucose) in control islets cultured throughout the 48-h period remained stable (120±10 pg insulin/islet/h), but in the presence of oleate, basal insulin secretion was increased two- to fourfold (Fig. 3 *A*; $P \leq 0.05$). When stimulated with 16.7 mM glucose, insulin secretion in both control and oleate-treated islets was significantly elevated (five- to eightfold) compared with the respective 2.8-mM glucose control islets cultured throughout the 48-h period (Fig. 3 *A*; $P \leq 0.05$). However, 16.7-mM glucose-stimulated insulin secretion in oleate-treated islets was not significantly different from that found in equivalent control islets (Fig. 3 *A*). Nonetheless, it follows that because of the two- to fourfold increase in basal insulin secretion in the presence of oleate, the actual magnitude of the glucose-induced insulin secretory response in oleate-treated islets was significantly blunted compared with that of control islets (Fig. 3 *A*; $P \leq 0.05$ for 6, 12, and 48 h).

Islet insulin content was not altered by 1 h of exposure to 16.7 or 2.8 mM glucose, either in control or oleate-treated islets (Figs. 1 and 3 *B*). As previously observed (Fig. 2 *B*), intraislet insulin stores of oleate-treated islets decreased within the first 24 h of incubation, compared with respective control islets (Fig. 3 *B*). The percentage of insulin content secreted at a basal 2.8 mM glucose in control islets remained stable throughout the 48-h incubation (0.3±0.1%/ h). In control islets incu-

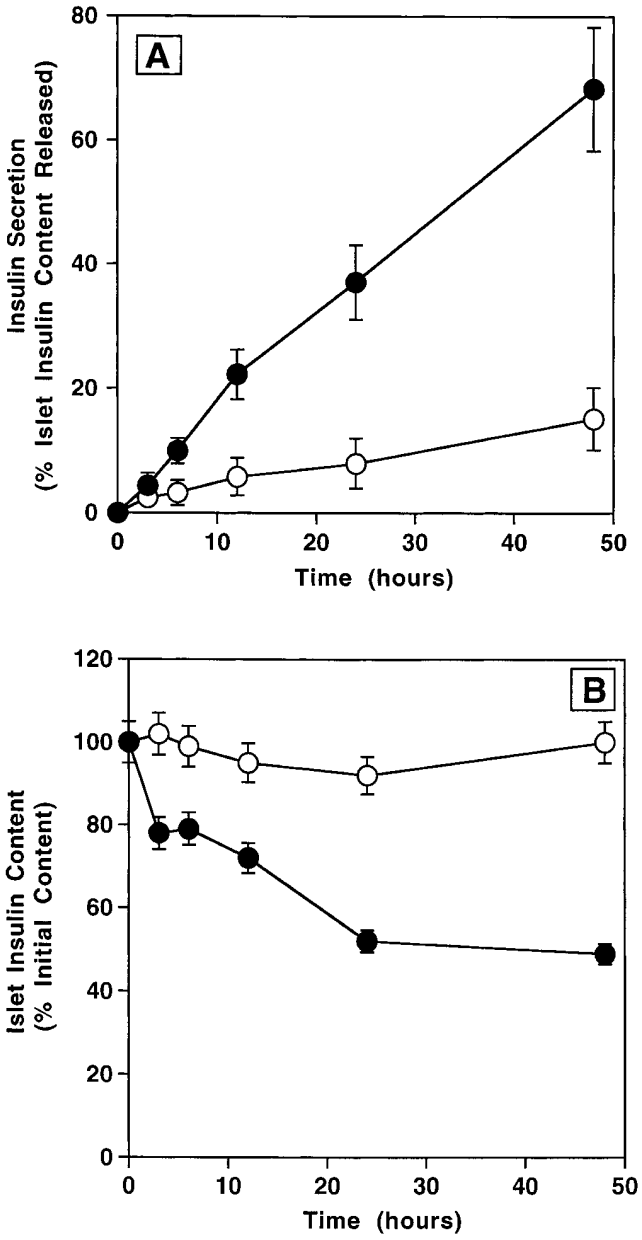

Figure 2. Effect of FFA on insulin secretion and insulin content in a normoglycemic culture of rat islets. Batches of 10 islets each were cultured for various lengths of time in RPMI-1640 tissue culture medium/5.6 mM glucose/10% dialyzed FBS supplemented with (●) or without (○) 125 μM oleate. The amount of insulin released over the culture period was determined by radioimmunoassay of the culture medium (*A*). Intracellular insulin content was assessed after lysis of the islets (*B*). Cumulative insulin secretion and intracellular content are expressed as a percentage of the initial intracellular insulin content at the start of incubation (32±5 ng insulin per islet). Values are means±SE of three independent experiments, each performed in duplicate.

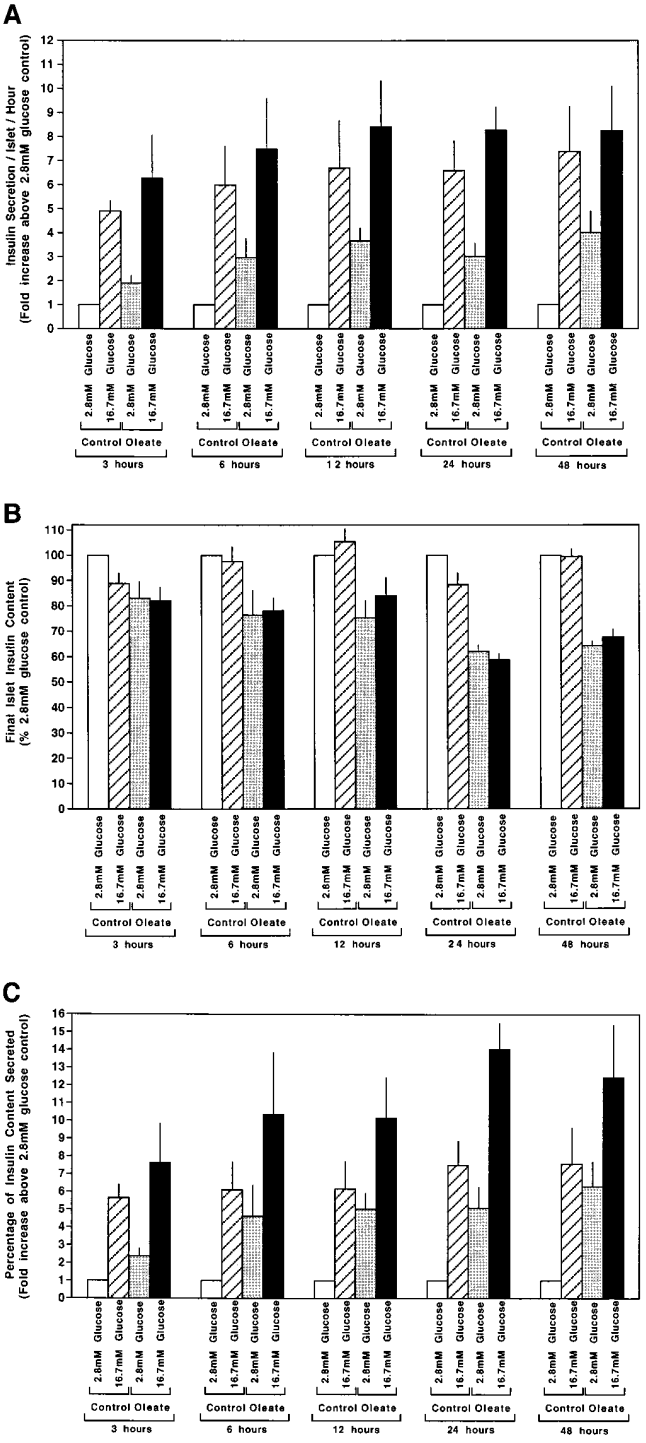

Figure 3. Glucose-induced secretory response in isolated islets after a normoglycemic culture with or without oleate. Islets were cultured for 3, 6, 12, 24, or 48 h in the presence or absence of 125 μM oleate. Afterwards, batches of 50 islets were exposed for 1 h to either 2.8 mM or 16.7 mM glucose in modified Krebs-Ringer-bicarbonate buffer appropriately supplemented with or without oleate. The amount of insulin secreted per islet during the last hour (*A*), the final intracellular insulin content per islet (*B*), and the percentage of insulin secretion relative to content (*C*) were analyzed. Data are expressed as a fold increase or a percentage decrease relative to respective control islets incubated at a basal 2.8 mM glucose. The values represent means±SE of three to five independent experiments. *Control,* islets cultured and challenged in the absence of oleate; *oleate,* islets cultured and challenged in the presence of oleate.

bated at 16.7 mM glucose, the percentage of insulin secreted in 1 h was increased five- to sevenfold (1.7±0.3%/h) compared with those incubated at a basal 2.8 mM glucose (Fig. 3 *C*; $P \leq 0.05$). However, in islets incubated in the presence of oleate between 6 and 48 h, the percentage of islet insulin content secreted at 2.8 mM glucose was significantly increased four- to sixfold compared with control islets (Fig. 3 *C*; $P \leq 0.05$). Oleate also potentiated the percentage of insulin content secreted at 16.7 mM glucose (Fig. 3 *C*) so that at 24 h, oleate-treated islets released 5.0±0.5% of their insulin content per h. This value corresponded to a 14-fold increase above basal insulin secretion in control islets ($P \leq 0.001$) and a 2-fold increase above control islets challenged with 16.7 mM glucose ($P \leq 0.01$). Nonetheless, in spite of oleate-mediated potentiation of the percentage of insulin content secreted (Fig. 3 *C*), the magnitude of response to a 16.7-mM glucose stimulus was significantly blunted ($P \leq 0.05$) in oleate-treated islets (two- to threefold) compared with that of the control islets (five- to sevenfold).

Glucose-dependent regulation of proinsulin biosynthesis in oleate-treated islets. In parallel to the analysis of insulin secretion and content, total protein and specific proinsulin biosynthesis were also assessed in isolated islets incubated in vitro±oleate. As previously observed for isolated islets maintained in vitro (34, 35), the proinsulin biosynthetic response to glucose diminished with increasing time in culture. Nonetheless, in control islets, after 48 h of culture there remained a significant fourfold increase in proinsulin biosynthesis in the 1-h incubation with 16.7 mM glucose vs. 2.8 mM glucose (Fig. 4 *A*, $P \leq 0.05$). However, compared with control islets, glucose-stimulated proinsulin biosynthesis in oleate-treated islets was markedly diminished, especially with shorter term exposure to fatty acid (Fig. 4 *A*). Glucose-induced proinsulin biosynthesis in islets exposed to oleate for 3 h was reduced by 90% ($P \leq 0.05$), by 75% after 6 h ($P \leq 0.01$), by 65% after 12 h, and by 42% after 24h. No effect of oleate on glucose-induced proinsulin biosynthesis was observed after a culture period of 48 h.

As previously observed (27), 16.7 mM glucose-induced total protein biosynthesis in islets was two- to threefold above that at a basal 2.8 mM of glucose (Fig. 4 *B*; $P \leq 0.05$). However, between 3 and 12 h, this glucose-induced increase in total protein biosynthesis was blunted by the presence of oleate (Fig. 4 *B*; $P \leq 0.05$). By correcting glucose-induced proinsulin biosynthesis (Fig. 4 *A*) relative to glucose-induced total biosynthesis (Fig. 4 *B*), specific glucose-regulated proinsulin biosynthesis above that of general protein biosynthesis could be ascertained (Fig. 4 *C*). In control islets, specific stimulation of proinsulin biosynthesis by glucose was maintained throughout culture (Fig. *C*; $P \leq 0.05$ for 3–24 h), although there was a depleted response with increasing culture time (34, 35). In comparison to control islets, specific glucose-induced proinsulin biosynthesis was significantly inhibited during the first 12 h of culture ($P \leq 0.05$), although no significant difference occurred between 24 and 48 h (Fig. 4 *C*). Nonetheless, the inhibitory effect of oleate on glucose-induced proinsulin biosynthesis was in contrast to the potentiating effects on insulin secretion seen in the very same islets (Fig. 3).

Oleate-induced effects on preproinsulin mRNA levels. Batches of 400 islets were cultured at 5.6 mM glucose±125 μM oleate for 0, 3, 12, or 24 h, after which Northern blot analysis for preproinsulin mRNA, actin mRNA, and 28S-rRNA was performed (Fig. 5). As previously observed (33, 36, 37), con-

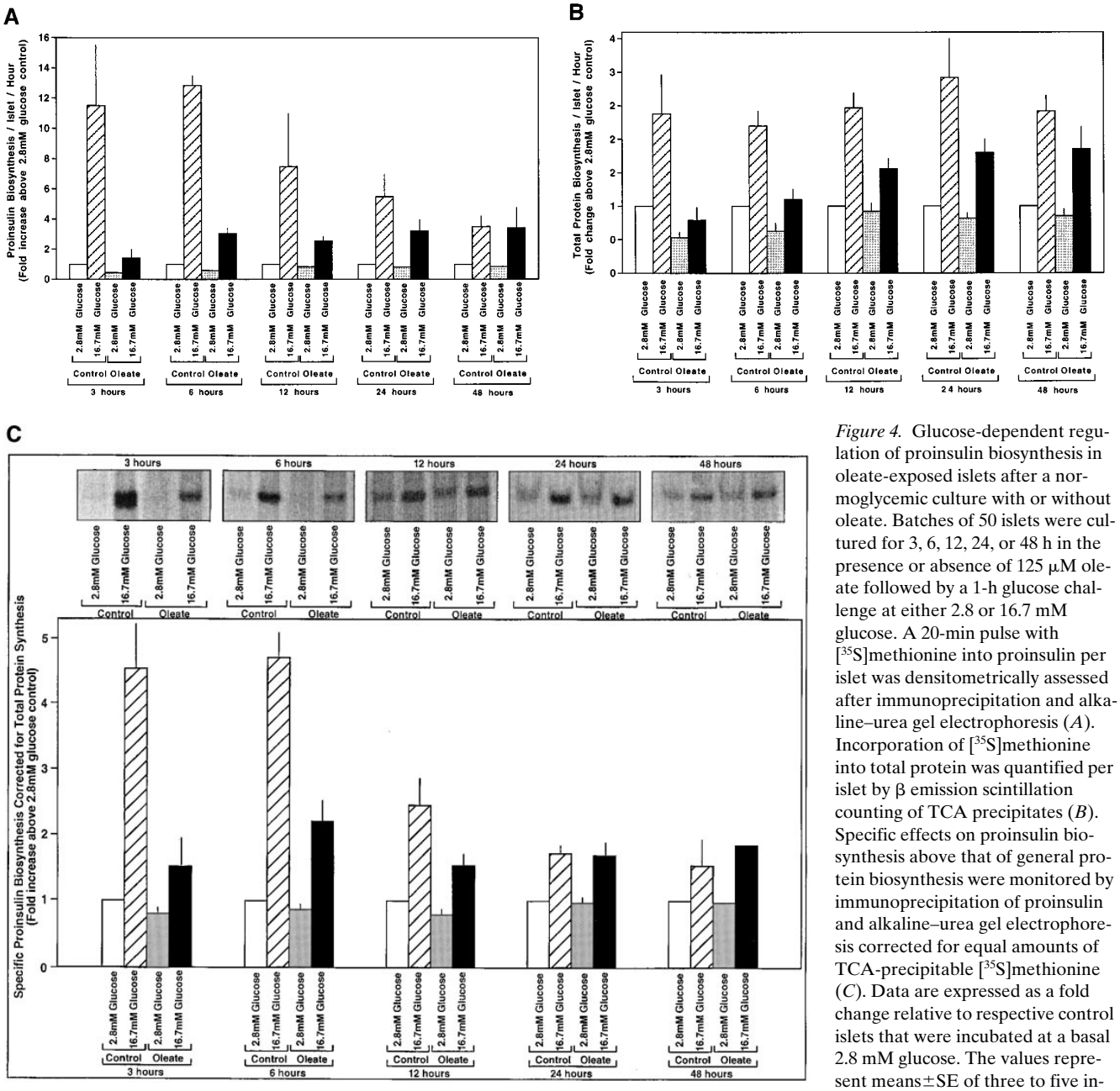

Figure 4. Glucose-dependent regulation of proinsulin biosynthesis in oleate-exposed islets after a normoglycemic culture with or without oleate. Batches of 50 islets were cultured for 3, 6, 12, 24, or 48 h in the presence or absence of 125 μM oleate followed by a 1-h glucose challenge at either 2.8 or 16.7 mM glucose. A 20-min pulse with [^{35}S]methionine into proinsulin per islet was densitometrically assessed after immunoprecipitation and alkaline–urea gel electrophoresis (*A*). Incorporation of [^{35}S]methionine into total protein was quantified per islet by β emission scintillation counting of TCA precipitates (*B*). Specific effects on proinsulin biosynthesis above that of general protein biosynthesis were monitored by immunoprecipitation of proinsulin and alkaline–urea gel electrophoresis corrected for equal amounts of TCA-precipitable [^{35}S]methionine (*C*). Data are expressed as a fold change relative to respective control islets that were incubated at a basal 2.8 mM glucose. The values represent means±SE of three to five independent experiments. Representative fluorographs that were TCA-equilibrated to gain equivalent total protein biosynthesis per observation (so that specific proinsulin biosynthesis was represented) are depicted in the upper part of *C*. *Control*, islets cultured and challenged in the absence of oleate; *oleate,* islets cultured and challenged in the presence of oleate).

trol β cells cultured in vitro at 5.6 mM glucose showed a decline in total preproinsulin mRNA content, while levels of actin mRNA remained stable (Fig. 5). However, islets cultured in the presence of oleate maintained their preproinsulin mRNA levels, resulting in a two- to threefold increase above that in the control islets (Fig. 5). The specific nature of oleate stimulation of preproinsulin mRNA levels was indicated, in that actin mRNA levels were unaffected by exposure to the fatty acid (Fig. 5).

Somatostatin-mediated inhibition of oleate-induced increases in basal insulin secretion maintains intracellular insulin content. Batches of 10 islets were cultured for 24 h at 5.6 mM glucose±125 μM oleate and/or 500 nM somatostatin. In the absence of somatostatin, the intracellular insulin content of oleate-treated islets decreased to 59±4% of that of control islets ($P \leq 0.05$), and insulin secretion was 10-fold higher in oleate-treated islets compared with controls (Fig. 6; $P \leq 0.05$). In the presence of somatostatin, oleate-induced basal insulin secretion was completely inhibited ($P \leq 0.0001$; Fig. 6 *A*). Accordingly, insulin content of islets exposed to oleate was preserved at the same level as control islets (94±8%; Fig. 6 *B*) in the presence of somatostatin. Somatostatin did not affect proinsulin biosynthesis (38; data not shown). Importantly, inhibition of oleate-induced basal insulin secretion and preservation

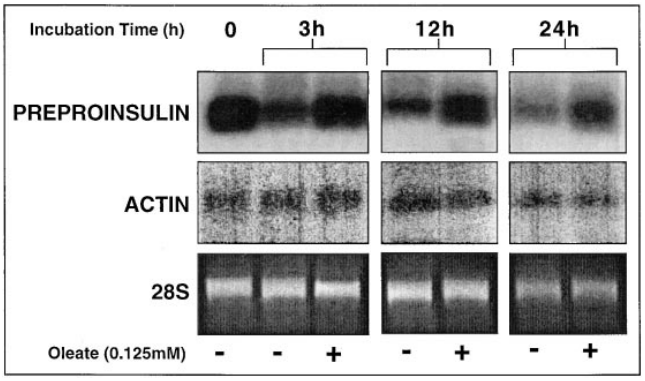

Figure 5. Oleate-induced effects on preproinsulin mRNA levels. Batches of 400 islets were cultured for 0, 3, 12, or 24 h in the presence or absence of 125 μM oleate. Northern blot analysis was performed for preproinsulin mRNA and actin mRNA and corrected for equal loading per lane relative to 28S rRNA. The results shown are representative of those obtained in two independent experiments. +, islet cultures in the presence of oleate; −, indicates islet cultures in the absence of oleate.

of insulin content by somatostatin ruled out any nonspecific detergent effect of the fatty acid in these studies.

Discussion

Acute presentation of long-chain FFA to pancreatic β cells is a potent stimulus for insulin secretion (1–7). This fact has supported the idea that transient elevation of cytosolic long-chain fatty acyl moieties in β cells, as a consequence of either increased glycolytic metabolism and/or an acute increase in exogenous FFA concentration, is important as a metabolic stimulus–response coupling factor for triggering insulin exocytosis (8, 9). Thus, there is a growing body of evidence to suggest that not only the glucose concentration, but also the level of circulating FFA will influence insulin secretion in vivo (6, 7). However, prolonged exposure to elevated FFA levels can cause impairment of pancreatic β cell function (15–17). Given the role that fatty acid moieties might play in triggering insulin release (9), such chronic exposure to FFA could evoke a continual stimulation of insulin release with subsequent hyperinsulinemia that is commonly observed in obesity and NIDDM (13, 39). Indeed, persistent elevated circulating FFA levels have been proposed as a major pathogenic factor for obesity and NIDDM since they can influence both insulin sensitivity and β cell dysfunction (9–14).

In this study, doubling FFA levels under normoglycemic conditions in normal rats over a 6-h period caused a threefold increase in circulating insulin levels and a significant 30% decrease in pancreatic insulin content in vivo. Similar results were obtained when isolated rat pancreatic islets were exposed to oleate in vitro for up to 48 h. Within 24 h, oleate caused a 50% decrease in islet insulin content that correlated with a marked increase in basal insulin secretion. Whereas oleate enhanced insulin release over a 24-h period, it specifically inhibited glucose-regulated proinsulin biosynthesis at the translational level. This result was in spite of an oleate-induced increase in β cell preproinsulin mRNA levels. Thus, the decrease in β cell intracellular insulin triggered by the fatty acid

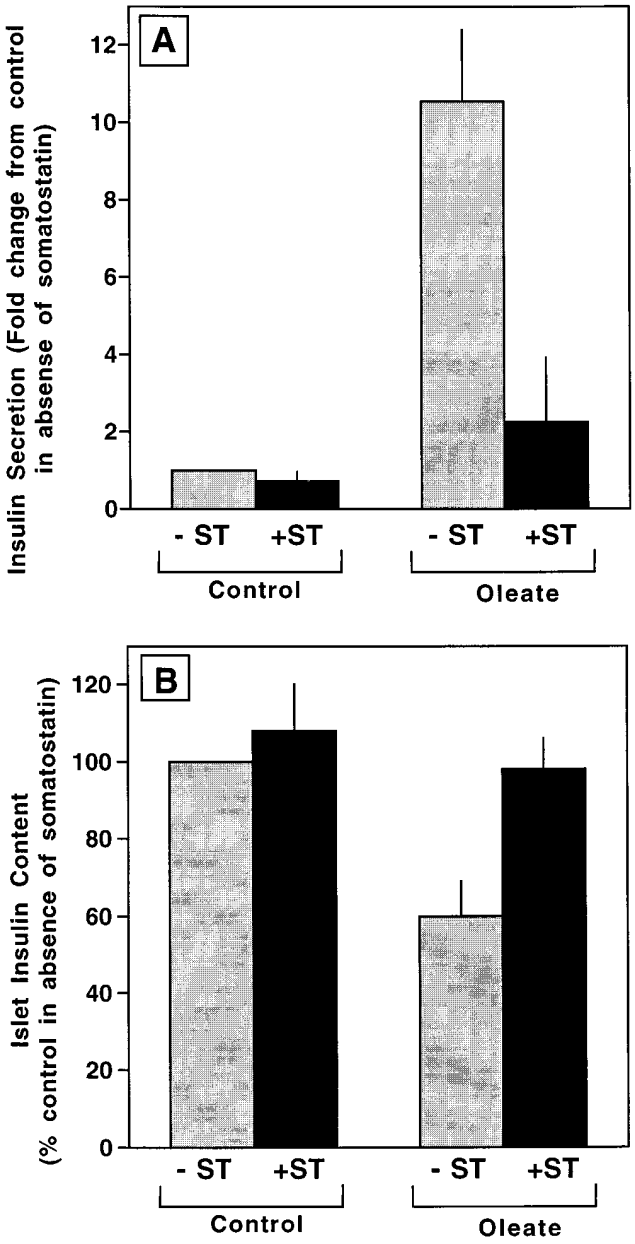

Figure 6. Somatostatin-mediated inhibition of oleate-induced insulin hypersecretion maintains intracellular insulin content. Batches of 10 islets were cultured for 24 h in the presence or absence of 125 μM oleate and/or 500 nM somatostatin. Cumulative insulin secretion (*A*) and intracellular insulin content (*B*) were determined as described in Methods. Data are expressed as a fold change relative to respective control islets cultured in the absence of oleate and somatostatin. Means±SE of three independent experiments, each performed in duplicate, are shown. *Control,* islets cultured in the absence of oleate; *oleate,* islets cultured in the presence of oleate; +ST, islets cultured in the presence of somatostatin; −ST indicates islets cultured in the absence of somatostatin.

was due to an increase in insulin secretion without concomitant FFA-mediated upregulation of proinsulin biosynthesis. This point was reinforced by the observation that when somatostatin blocked FFA-induced basal insulin hypersecretion, intracellular insulin content was maintained at normal levels.

Although somatostatin is a potent inhibitor of FFA-induced insulin release (8), it has no effect on the regulation of proinsulin biosynthesis at the translational level (38).

Functional impairment of the pancreatic β cell after prolonged exposure to elevated concentrations of FFA has been previously suggested (15, 17, 20). In isolated rat pancreatic islets cultured under hyperglycemic and hyperlipacidemic conditions, an apparent blunting of glucose-induced insulin secretion was observed (17). However, in the present study which used hyperlipacidemic and normoglycemic (5.6 mM glucose) culture conditions, no inhibition of glucose-induced insulin secretion within 48 h of exposure to oleate was observed. The amount of insulin secreted per islet at a stimulatory 16.7 mM glucose was similar whether oleate was present or not. However, insulin secretion at basal glucose concentrations was markedly elevated in the presence of the fatty acid, which in turn reduced the apparent amplitude of response to glucose (15, 17). Notwithstanding, it should also be considered that FFA-mediated elevation of basal insulin secretion resulted in a significant reduction in the insulin content of pancreatic islet β cells. Thus, when insulin secretion was considered as a percentage of the intracellular insulin content, it was found that FFA significantly potentiated glucose-induced insulin release as well as elevating basal insulin secretion. Viewed in this way, chronic exposure of pancreatic islets to FFA over a 48-h period in vitro enhanced rather than inhibited glucose-induced insulin release. This result is reminiscent of in vivo studies in which glucose-stimulated insulin secretion remained potentiated after 48 h of exposure to FFA (40).

Within the first 24 h of exposure to oleate in vitro, there was a marked reduction in islet insulin content, but thereafter the content appeared to remain relatively constant at a 50% reduced amount compared with control islets. This leveling out of insulin content after 24 h of exposure to fatty acid occurred despite continued elevation of basal insulin secretion at 5.6 mM glucose (Fig. 2) so that it was not likely to have resulted from fatty acid depletion as the incubation time increased. Thus, there was an apparent adaptation of the β cell to maintain its intracellular insulin content, albeit at a reduced level, to compensate partly for hypersecretion of insulin after 24 h of exposure to oleate. One possible explanation for this observation could be the difference in threshold glucose concentration needed to stimulate proinsulin biosynthesis (2–4 mM) vs. insulin secretion (4–6 mM; 23, 25). At the basal 5.6 mM glucose concentration used in these studies, insulin secretion would be at a nonstimulatory rate in the absence of FFA, whereas translational regulation of proinsulin biosynthesis would actually be enhanced (23, 25). This stimulation of proinsulin biosynthesis at 5.6 mM glucose, although modest, might be sufficient to maintain insulin stores at least at a 50% reduced level after 24 h of FFA-induced insulin hypersecretion. Another consideration could conceivably be a contribution made by the FFA-induced increase of preproinsulin mRNA levels to maintain an adequate preproinsulin mRNA pool available for proinsulin biosynthesis, but this would also be subject to translational regulation in the β cell (23). Notwithstanding, further experiments will be required to define better the mechanism through which a reduced steady-state islet insulin content is sustained in the face of FFA-induced insulin hypersecretion.

Whereas elevated FFA concentrations clearly increase the rate of insulin secretion in vivo and in vitro (6, 7, 40), relatively short-term exposure to oleate (≤ 12 h) resulted in a contrasting inhibition of glucose-stimulated proinsulin biosynthesis at the translational level (Fig. 4). In general, nutrients that stimulate insulin release correspondingly increase proinsulin biosynthesis to effect a balance between (pro)insulin secretion and biosynthesis that maintains β cell insulin stores at an optimal level (23). However, FFA appear to be an exception to this rule. While long-chain FFA moieties are involved in the metabolic stimulus–response coupling pathway for nutrient-stimulated insulin release (6–9), they are apparently not required for the metabolic signal transduction pathway for nutrient-induced stimulation of proinsulin biosynthesis at the translational level (28). In contrast to the inhibitory effects of FFA on proinsulin biosynthesis at the translational level, FFA increased preproinsulin mRNA levels in the β cell at a normoglycemic 5.6-mM glucose concentration (Fig. 5). This observation suggests that FFA are capable of regulating preproinsulin gene expression. Further experiments will be required to establish whether FFA are acting at the level of transcription (41, 42) and/or mRNA stability (36), and indeed whether FFA have a direct effect on transcription/mRNA stability or a secondary effect by enhancing glucose-induced preproinsulin mRNA transcription as in animal models of NIDDM (43). Regardless, it should be noted that in spite of FFA-induced upregulation of preproinsulin mRNA levels, there was no corresponding effect on proinsulin biosynthesis at the translational level. There are essentially two pools of preproinsulin mRNA in β cells: one in a ribosome-free storage compartment in the cytosol, and the other with ribosomes attached, actively undergoing proinsulin biosynthesis translation located mainly on the rough endoplasmic reticulum (23, 26, 44). It follows that FFA-induced increases in preproinsulin mRNA levels were likely contributing to a quiescent cytoplasmic storage pool. Such findings emphasize the importance of translational regulation of proinsulin biosynthesis in maintenance of β cell insulin content.

In obesity, insulin resistance, hyperinsulinemia, and hyperlipacidemia coexist, and may contribute to a clinical state of non-insulin-dependent diabetes mellitus (45). However, in humans, although there is a degree of correlation between obesity and type II diabetes, a good proportion of hyperlipacidemic obese individuals do not present symptoms of diabetes (46). The current work shows that while intracellular insulin stores decreased by 50% with ≥ 24h exposure to FFA, the ability of the β cell to generate an adequate output of insulin in the presence of FFA was not lost relative to control islets. While this result does not rule out a reduction in insulin secretory capacity of the β cell with a more prolonged exposure to FFA (15, 17), it is probable that other factors in addition to hyperlipacidemia contribute to the pathogenesis of NIDDM (10–13, 47). It is well-established that hyperglycemia can also lead to severe β cell dysfunction (48–50). Therefore, a combination of chronic hyperlipacidemia and hyperglycemia more likely leads to reduced insulin secretory capacity, β cell exhaustion, and onset of diabetes (11, 17), which would only be worsened by the additional presence of insulin resistance (9–13).

Acknowledgments

We are grateful to the Eli Lilly Company for the gift of [^{125}I]insulin.

This work was supported by grants from the National Institutes of Health (DK 47919 and DK 50610 to C.J. Rhodes, and DK 18573 to J.D. McGarry), the Juvenile Diabetes Foundation/National Institutes

of Health Diabetes Interdisciplinary Research Program, and from the Deutsche Forschungsgemeinschaft (Bo 1379/1-1).

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