A Key Role for β-Cell Cytosolic Phospholipase A₂ in the Maintenance of Insulin Stores But Not in the Initiation of Insulin Secretion

Shanta J. Persaud, Helen M. Rodrigo-Milne, Paul E. Squires, David Sugden, Caroline P.D. Wheeler-Jones, Phil J. Marsh, Véronique D. Belin, Melanie J. Luther, and Peter M. Jones

Cytosolic phospholipase A₂ (cPLA₂) is a Ca²⁺-sensitive enzyme that has been implicated in insulin secretion, response to agents that elevate β-cell intracellular Ca²⁺ ([Ca²⁺]ᵢ). We generated clones of the MIN6 β-cell line that stably underexpress cPLA₂ by transfection with a vector in which cPLA₂ cDNA had been inserted in the antisense orientation. Reduced expression of cPLA₂ was confirmed by Western blotting. The insulin content of cPLA₂-deficient MIN6 cells was reduced by ~90%, but they showed no decrease in preproinsulin mRNA expression. Measurements of stimulus-dependent changes in [Ca²⁺]ᵢ indicated that reduced expression of cPLA₂ did not affect the capacity of MIN6 cells to show elevations in Ca²⁺ in response to depolarizing stimuli. Perifusion experiments indicated that cPLA₂ underexpressing MIN6 pseudoislets responded to glucose, tolbutamide, and KCl with insulin secretory profiles similar to those of cPLA₂ expressing pseudoislets, but that secretion was not maintained with continued stimulus. Analysis of the ultrastructure of cPLA₂-deficient MIN6 cells by electron microscopy revealed that they contained very few mature insulin secretory granules, but there was an abundance of non–electron-dense vesicles. These data are consistent with a role for cPLA₂ in the maintenance of insulin stores, but they suggest that it is not required for the initiation of insulin secretion in β-cells. Diabetes 51:98–104, 2002

RESEARCH DESIGN AND METHODS

Materials. MIN6 cells were obtained from Dr. Y. Oka and Professor J.-I. Miyazaki (University of Tokyo, Tokyo, Japan). cPLA₂α cDNA, housed in the pSG5 expression vector, was provided by Dr. B. Kennedy (Merck Frosst, Quebec, Canada), and the cPLA₂ polyclonal antibody was obtained from Dr. R. Kramer (Lilly, Indianapolis, IN). Tissue culture reagents, G418 and Moloney Murine Leukemia Virus (MMLV) reverse transcriptase, were obtained from Gibco (Paisley, U.K.). The Dynabeads Oligo(dT)₂₅ kit was from Dynal (Oslo, Norway). Restriction endonucleases were obtained from Promega (Madison, WI), and pcDNA3.1 was from Stratagene Europe (Amsterdam, the Netherlands). PCR primers were prepared in-house (Molecular Biology Unit, King’s College London), and real-time quantitative PCR was performed using a LightCycler rapid thermal cycling system from Roche Diagnostics (Lewes, Sussex, U.K.). General laboratory chemicals, including fura-2-acetoxymethyl-ester (fura-2/AM), 3-aminopropyltriethoxysilane (APES), forskolin, and phorbol myristate acetate (PMA) were purchased from Sigma (Poole, Dorset, U.K.). The Axiovert 135 Research Inverted microscope was obtained from Carl Zeiss (Welwyn Garden City, U.K.), the Axon Imaging Workbench was from Axon Instruments (Foster City, CA), and the ISIS camera was from Photonics Science (Roberts-Bridge, Sussex, U.K.).

Vector construction, transfection of MIN6 cells, and selection of clones. Antisense expression constructs were made by digesting pSG5 to completion with BglII and partially with EcoRI to yield the cPLA₂ sequence on a 1.6-kbp fragment. This was gel-purified and ligated to pcDNA3.1 that had


Address correspondence and reprint requests to Shanta J. Persaud, Room 3.2A, New Hunt’s House, King’s College London, London SE1 1UL, U.K.

E-mail: shanta.persaud@kcl.ac.uk

Received for publication 16 March 2001 and accepted in revised form 4 October 2001.

APES, 3-aminopropyltriethoxysilane; cPLA₂: cytosolic phospholipase A₂; DMEM, Dulbecco’s modified Eagle’s medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MMLV, Moloney Murine Leukemia Virus; PCR, polymerase chain reaction; PI, pseudosiet; PMA, phorbol myristate acetate.

DIADETS, VOL. 51, JANUARY 2002
been cut with EcoRI and BamHI. The DNA was prepared for transfection by
banding plasmid preparations on CsCl according to standard methods. MIN6
cells, grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented
with 15% fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin,
and 2 mM glutamine, were electroporated (2 kV/cm, 3 µF) with HindIII-
linearized cPLA2 antisense vector or with linearized dCDNA3.1 to which cPLA2
antisense cDNA had not been ligated (empty vector). Neo-resistant transfect-
cells were selected by growing in medium supplemented with 1 mg/ml G418.
Colonies and resistant cells were expanded for analysis and functional studies.

**Western blotting.** Extracts of G418-resistant β-cell clones were prepared by
sonication in a lysis buffer (10), and protein concentration was determined by
the Bradford method (11) using bovine serum albumin as standard. Protein
(200 µg) was fractionated on 10% polyacrylamide gels and transferred to
polyvinylidene difluoride membranes. cPLA2 protein was detected by an
enhanced chemiluminescence system using an anti-cPLA2 rabbit polyclonal
primary antibody (1:5,000 dilution) and an anti-rabbit IgG conjugated with
horseradish peroxidase (1:10,000 dilution).

**Insulin secretion.** MIN6 cells in monolayers do not show robust secretory
responses to physiological stimulators of insulin secretion, but their secretory
output improves when they are cultured as three-dimensional aggregates,
which we term “pseudosdots” (Pls) (12). To maximize secretory performance,
we therefore carried out all secretion experiments using MIN6 Pls. Initial
experiments were performed in static incubations, where groups of 10 Pls were
incubated with fresh culture medium supplemented with 50 µM glucose for
30 min. Data were collected every 3 s for multiple treatments. Insulin secretion
was assessed using a multichamber perifusion system at 37°C
supplemented with agents of interest, and insulin secretion was measured by
fluorimetry. MIN6 cells were seeded onto APES-coated glass coverslips and allowed to adhere overnight in DMEM under standard tissue
culture conditions. Agonist-evoked changes in cytosolic Ca²⁺ were examined in
MIN6 cells grouped within monolayer clusters (3–20 cells/cluster), rather
than from single cells in isolation, which are poorly nutrient-responsive (12).
Cell clusters were loaded for 20 min at 37°C with 2.5 µmol/l of the Ca²⁺
fluorophore fura-2/AM. The coverslips were washed and placed in a steel
chamber, the volume of which was ~500 µl. A single 22-mm coverslip formed
the base of the chamber, which was mounted into a heating platform on the
stage of a Nikon Optiphot microscope. Calcium was imaged using an Axon Imaging
Workbench. Emitted light was filtered using a 510-nm long-pass barrier filter and
detected using an EMCCD camera. Changes in the emission intensity of fura-2,
expressed as a ratio of dual excitation were used to indicate changes in [Ca²⁺],
using established procedures (17). Data were collected every 3 s for multiple
regions of interest in any one field of view. All records have been corrected for
background fluorescence (determined from cell-free coverslips).

**Preproinsulin mRNA expression.** Messenger RNA was isolated from control
and cPLA2-deficient MIN6 cells using a Dynabeads Oligo(dT)²⁵ kit
according to the manufacturer’s instructions. Oligo(dT)²⁵ (1 µg) and random
t-mer primer (1 µg) were added to the mRNA (10 µl), and the mixture was heated
(70°C, 5 min) to remove secondary RNA structure then cooled on ice. DTT (10
mM), dATP, dCTP, dGTP, and dTTP (all 0.5 mM); recombinant ribonu-
sease inhibitor (80 µg/ml), RNasin; and MMLV-RT (200 µl) were added to the
mixture. The reactions were incubated at 37°C for 50 min. MMLV-RT was inactivated by heating at 70°C for 15
min. The cDNA was diluted 10-fold with tRNA (10 µg/ml) and used immediately
or stored at −20°C for future use. An aliquot of mRNA was not
reverse-transcribed and was diluted with tRNA and stored at −85°C.

Mouse preproinsulin I and II cDNA sequences (18) were used to design
primers to the preproinsulin and GAPDH primers were
5'-CCC ATC ACC ATC TCC CCC GAG CAG C-3'; antisense primer, 5'-CCA GTG AGC TCC CCG TTG GAG C-3'. The predicted size of the preproin-
sulin PCR product was 267 bp. Forward and reverse glyceraldehyde-3-
phosphate dehydrogenase (GAPDH) PCR primers were as follows: sense
primer, 5'-CCC ATC ACC ATC TCC CCC GAG CAG C-3'; antisense primer, 5'-CCA GTG AGC TCC CCG TTG GAG C-3'. The predicted size of the GAPDH
PCR product was 473 bp.

PCR products amplified by the preproinsulin and GAPDH primers were separated by agarose gel electrophoresis (2% wt/vol) and visualized by
ing staining with ethidium bromide (0.5 µg/ml). The preproinsulin product was
further purified by sequence on an ABI 377 using fluorescent
terminator methods. Concentrations of PCR products were determined
by densitometry by comparison with known amounts of molecular weight
markers. Tenfold serial dilutions were prepared as standards ranging from 1
fg to 100 pg.

Real-time PCR amplification was performed using a LightCycler rapid
thermal cycle system. Reactions were performed in a 10-µl volume contain-
ing nucleotides, Taq DNA polymerase, and buffer (all included in the Light-
Cycler-PCR Master SYBR Green I mix); MIN6 cell cDNA; and 3 mM MgCl₂
and 0.5 µmol/l primers. All PCR protocols included a 10-s denaturation step
and then continued for 40 or 45 cycles consisting of a 95°C denaturation for
0 s, annealing for 10 s at 55°C (GAPDH) or 57°C (preproinsulin), and a 72°C
extension phase for 11 s (preproinsulin) or 19 s (GAPDH). Fluorescence
measurements were taken at 85°C for 2 s to eliminate fluorescence from
primer-dimer formation. The amplification products of both primer pairs were
subjected to melting point analyses and subsequent gel electrophoresis to
ensure specificity of amplification.

**Electron microscopy.** Transmission electron micrographs of glutaraldehyde-
fixed, osmium-stained MIN6 cells were prepared by John Pacy of King’s
College London Electron Microscope Unit using standard techniques.

**Data analysis.** Data are expressed, where appropriate, as means ± SE
and were analyzed statistically using Student’s t test, analysis of variance,
and Bonferroni’s multiple comparison test as appropriate. Differences between
treatments were considered significant at P < 0.05.

**RESULTS**

**Immunodetection of cPLA₂.** Changes in MIN6 β-cell cPLA₂ expression after transfection with a vector coding cPLA₂ in the antisense orientation was assessed by Western blotting of extracts of G418-resistant MIN6 cell populations. It can be seen from Fig. 1 (top panel) that native MIN6 cells expressed a form of cPLA₂ that migrated with an apparent molecular mass of ~110 kDa, consistent with the reported retarded mobility of cPLA₂ on polyacrylamide gels relative to its predicted molecular mass (20). G418-
resistant β-cell clones were expanded and harvested after transfection with the antisense cPLA₂ plasmid, and Fig. 1 shows that antisense clones, termed I, N, and K, showed much reduced expression of cPLA₂. The underexpression was stable, with no recovery of cPLA₂ expression with continued passage. This is clearly shown in Fig. 1 (bottom panel), where the loss of cPLA₂ expression by clone N was still apparent after continued passing for 11 months. This clone was used for functional analysis unless other-
wise indicated in the text or figure legends.
Protein and insulin content of MIN6 cells underexpressing cPLA₂. The protein and insulin contents of PIs were measured in parallel with their use in secretion experiments, and comparisons were made between passage-matched, unselected, nontransfected cells (“controls”); MIN6 cells transfected with an empty vector (“EV”); and MIN6 cells transfected with cPLA₂ antisense cDNA (“antisense”). Protein content of PIs underexpressing cPLA₂ was of a similar level to passage-matched control PIs (antisense N, 262 ± 13.9 ng protein/PI; antisense I, 293 ± 41.7; control, 242 ± 26.2; n = 9; P > 0.2), but insulin content was considerably lower in the cPLA₂-deficient PIs (antisense N, 0.15 ± 0.03 ng insulin/PI; antisense I, 0.13 ± 0.05; control, 1.42 ± 0.04; EV, 1.85 ± 0.17; n = 5–16; P < 0.001, control and EV versus antisense).

Insulin secretion from MIN6 cells underexpressing cPLA₂. Basal insulin secretion (2 mmol/l glucose) from cPLA₂-deficient PIs was significantly less than that from equivalent passage control PIs (control, 0.24 ± 0.02 ng · PT⁻¹ · h⁻¹; antisense N, 0.02 ± 0.005 ng · PT⁻¹ · h⁻¹, mean of four separate experiments; P < 0.001), consistent with their much reduced insulin content. Insulin secretion measurements in static incubations indicated that although the amount of insulin secreted by cPLA₂-depleted PIs was very low, the fold increases in secretion in response to depolarizing stimuli were not significantly different from responses elicited by control cells (20 mmol/l KCl: control, 368 ± 30% basal; antisense N, 417 ± 13% basal; 100 μmol/l tolbutamide: control, 197 ± 16% basal; antisense N, 233 ± 62% basal, mean of four separate experiments; P < 0.05 versus basal; P > 0.1, antisense N versus control). However, the small magnitude of the secretory response of late passage MIN6 cells to glucose precluded meaningful analysis of any possible alterations in nutrient-induced insulin secretion after reduced cPLA₂ expression in static incubation experiments.

Additional secretion experiments were therefore performed in perifusions in which small changes in secretion could be determined readily, and expression of secretion data as a percentage of basal insulin release allows direct comparison of the profile of secretion from the two populations. It can be seen from Fig. 2 that 20 mmol/l glucose caused a significant increase in insulin secretion from control PIs, with a short-lived peak and a sustained plateau. Despite their much-reduced insulin content and insulin output, cPLA₂ underexpressing MIN6 PIs (clones N and I) showed a similar profile of insulin secretion to that seen with control PIs after a glucose challenge (Fig. 2). cPLA₂ underexpressing PIs also showed secretory profiles in response to KCl and tolbutamide that were similar to those of control PIs (Fig. 3; clone N). However, cells underexpressing cPLA₂ showed a much reduced magnitude of response upon exposure to potentiators of insulin secretion after an initial stimulus with glucose, KCl, or tolbutamide. Thus, activation of protein kinase C by PMA or increases in cyclic AMP in response to forskolin resulted in significant potentiation of insulin secretion from control PIs, but secretory output declined with time after depletion of cPLA₂ (Fig. 3). This was not a consequence of defects in signaling systems at the level of effector enzymes because cPLA₂-deficient PIs showed robust secretary responses to forskolin when it was added as the primary stimulus, but secretion again declined with continued stimulation (Fig. 4; clone N), suggesting an inability of the cPLA₂-deficient cells to maintain a prolonged secretory response.

Stimulus-induced changes in intracellular Ca²⁺ in MIN6 cell clusters underexpressing cPLA₂. A real-time fluorescence-based PCR method (LightCycler) was used to determine whether the site of the lesion in cPLA₂-deficient β-cells that resulted in abnormally low insulin content was at the level of preproinsulin mRNA expression. Primers were used to amplify a preproinsulin fragment from cPLA₂-deficient β-cell clones N and I and passage-matched control MIN6 β-cells. Quantiﬁcation was performed by standardizing preproinsulin product against a sequence-verified preproinsulin fragment, and these values were normalized against the content of GAPDH mRNA in the same extracts. The melting point-curves obtained using GAPDH and preproinsulin primers in the LightCycler indicated that single products were amplified (Fig. 6; top panel); the middle panel shows that the products were of the appropriate predicted sizes (267 bp for preproinsulin and 473 bp for GAPDH). The standard
curves generated for the two products were linear (Fig. 6, bottom panel), and quantification of preproinsulin mRNA in the samples using these standard curves indicated that cPLA2-deicient/H9252-cells contained levels of preproinsulin mRNA that were not significantly different \((P > 0.2)\) from passage-matched control cells (control, 16.6 ± 2.8 fg preproinsulin mRNA/fg GAPDH mRNA, \(n = 3\); antisense N, 14.0 ± 2.7, \(n = 4\); antisense I, 17.9 ± 7.6, \(n = 2\)).

Transmission electron micrographs of MIN6 cells underexpressing cPLA2. The morphology of MIN6 cells underexpressing cPLA2 was consistent with the insulin content data. Thus, it is clear from Fig. 7 that cells derived from antisense clone N had considerably less secretory granules than control cells, and they showed altered morphology of the endoplasmic reticulum and Golgi network, with indication of the formation of non–electron-dense vesicle-like organelles. In all other respects, the ultrastructure of the cPLA2-deficient MIN6 cells was indistinguishable from that of native MIN6 cells. An abundance of non–electron-dense vesicles and a scarcity of insulin granules were also observed in micrographs prepared from cells derived from antisense clone I (data not shown).

**DISCUSSION**

We and others have shown that the conventional cPLA2 (cPLA2α) is expressed in islets and β-cells (4–6) and that arachidonic acid, the product of cPLA2 activation, is capable of initiating insulin secretion (reviewed in ref. 21). The sensitivity of cPLA2α to concentrations of \(\text{Ca}^{2+}\) reached in β-cells after \(\text{Ca}^{2+}\) entry in response to nutrient-induced membrane depolarization has made it an attractive candidate as a pivotal transducer of nutrient-generated signals in β-cells, and data obtained using inhibitors of cPLA2 are consistent with its being involved in glucose-induced insulin secretion (8). However, cPLA2 inhibitors lack specificity (3), so we have now examined the effects of stable underexpression of cPLA2 on the
Expanded clones. Maintenance of the altered phenotype requires when assessing nutrient-induced effects of retain their glucose responsiveness with continued passage after transfection are likely to have been established after transfection (22), so clones by cloning from a pancreatic insulinoma (22), so clones.

102 DIABETES, VOL. 51, JANUARY 2002

First, we required a cell line rather than primary islets so that we could create stable transfects and expand the transfected populations so that all cells, whether at the single cell level or in population experiments, were deficient in cPLA2. Second, the MIN6 cell line was established by cloning from a pancreatic insulinoma (22), so clones established after transfection are likely to have been derived from common parental cells. Third, MIN6 cells retain their glucose responsiveness with continued passage when configured as PIs (12), which was an absolute requirement when assessing nutrient-induced effects of expanded clones. Maintenance of the altered phenotype was assessed by immunodetection of cPLA2 expression in clones soon after selection and again after continued culture of the cells in G418-containing medium for 11 months. These measurements consistently showed that cPLA2 expression was much reduced in the G418-resistant clones. The cPLA2 underexpressing β-cells grew on tissue culture plastic as monolayers indistinguishable from cPLA2-expressing control cells, and they formed PIs when plated into gelatin-coated flasks, similar to native MIN6 cells (12).

Functional studies were performed to determine whether depletion of cPLA2 affected the ability of β-cells to respond to agents that elevate intracellular Ca2+ and might be expected to activate this Ca2+-sensitive enzyme. Our Ca2+ microfluorimetry data indicated that depletion of cPLA2 had no effect on the proportion of cells that were able to respond to glucose, KIC, tolbutamide, and KCl or on the amplitudes of the mean basal to peak Ca2+ responses. These data are entirely consistent with cPLA2 being a Ca2+-responsive enzyme that can be placed downstream in the stimulus-response coupling cascade to the initial glucose metabolism, membrane depolarization, and Ca2+ influx events. Thus, it is clear that loss of cPLA2 expression does not affect the capacity of β-cells to recognize and metabolize glucose, close KATP channels, depolarize cells, or permit the entry of Ca2+.

Measurement of the insulin content of cPLA2-deficient PIs revealed that they had only 10% of the content of control cells. The significantly reduced rate of basal insulin secretion by MIN6 cells underexpressing cPLA2 was consistent with their greatly reduced insulin content. However, the depleted insulin content did not markedly affect the capacity of cPLA2-deficient cells to respond to nutrient and non-nutrient secretagogues and to depolarizing stimuli with increases in insulin secretion. Thus, when insulin secretion was expressed relative to the appropriate basal levels of secretion, cPLA2-deficient PIs showed initial secretory profiles that were not significantly different from those obtained with control, cPLA2-containing PIs. However, whereas control PIs showed sustained insulin secretory responses, secretion from β-cells underexpressing cPLA2 was not maintained when an initial stimulus was followed by further stimulation by activators of protein kinase C or A. Experiments in which cPLA2-depleted PIs were initially exposed to forskolin indicated that PKA activity was not compromised and that the inability of the cells to maintain insulin output was independent of the stimulus. The most likely explanation for this, given the reduced insulin content of the cPLA2 underexpressing β-cells, is that their intracellular stores of insulin were sufficient for an initial secretory response but that they soon became depleted and secretion therefore declined with continued stimulus.

Morphological analysis by electron microscopy was consistent with this interpretation, and the micrographs indicated two distinct features of MIN6 cells underexpressing cPLA2. First, they showed a significant reduction in the number of secretory granules per cell that was considerably greater than the natural decline in insulin secretory granule number that occurs with continued passaging of MIN6 cells. Second, and distinct from anything we have observed with prolonged culture of control MIN6 cells, was the increased incidence of non–electron-dense vesicles within cPLA2-deficient cells. This profound change in the ultrastructure of cells underexpressing cPLA2 did not seem to have a generalized deleterious effect on protein synthesis by these β-cells, because their protein content was not reduced compared with controls, but their insulin content was reduced by ~90%. A recent study in which cPLA2 was overexpressed in kidney epithelial cells (23) implicated cPLA2 in intracellular vesicle trafficking processes, and the data obtained in the current

FIG. 5. Stimulus-evoked changes in intracellular Ca2+ in cPLA2-deficient cells. MIN6 cells underexpressing cPLA2 were loaded with fura-2 and exposed to glucose (20 mmol/L), KCl (20 mmol/L), tolbutamide (100 μmol/L), and KIC (10 mmol/L), as shown. The top and middle show responses of clone N cells, and the bottom shows responses of clone I cells. Basal cytosolic Ca2+ was 0.76 ± 0.14 arbitrary units (n = 60 cells) in control cells, 0.69 ± 0.09 (n = 65 cells) in clone N antisense cells, and 0.77 ± 0.01 (n = 48 cells) in clone I antisense cells.

Our Ca2+ responses of clone N cells, and the bottom shows responses of clone I cells. Basal cytosolic Ca2+ was 0.76 ± 0.14 arbitrary units (n = 60 cells) in control cells, 0.69 ± 0.09 (n = 65 cells) in clone N antisense cells, and 0.77 ± 0.01 (n = 48 cells) in clone I antisense cells.
study are consistent with cPLA\textsubscript{2} being required for appropriate architecture of the Golgi apparatus. Disruption of the trans-Golgi network would affect the formation of secretory vesicles, resulting in decreased insulin content despite maintained insulin mRNA levels, as was observed in our experiments.

In summary, the current data indicate that cPLA\textsubscript{2} is not required for pancreatic β-cells to show an initial secretory response to nutrient and non-nutrient secretagogues, suggesting that it is not a pivotal Ca\textsuperscript{2+}-sensitive sensor in β-cell stimulus-secretion coupling. However, our data are consistent with a role for cPLA\textsubscript{2} in the maintained secre-

FIG. 6. Preproinsulin expression in cPLA\textsubscript{2}-deficient β-cells. Real-time fluorescence-based PCR were performed on cDNA prepared from cPLA\textsubscript{2}-deficient MIN6 β-cells (clones N and I) and passage-matched control, cPLA\textsubscript{2}-expressing MIN6 cells. A: Melting point analyses for GAPDH (left) and preproinsulin (right) standards and MIN6 cell samples and indicate that single products were obtained using GAPDH and preproinsulin primer pairs. This was confirmed by agarose gel electrophoresis and ethidium bromide staining, where single products of the appropriate predicted sizes were obtained (B). C: Standard curves generated for GAPDH (left) and preproinsulin (right) from which GAPDH and preproinsulin mRNA levels in the MIN6 cell samples were determined.
FIG. 7. Transmission electron micrographs of MIN6 β-cells. The top shows a typical transmission electron micrograph of control, cPLA2-expressing MIN6 β-cells, passage-matched to those shown in the bottom. The micrograph shows part of the nucleus and numerous insulin secretory granules (black arrows). The bottom shows a transmission electron micrograph of cPLA2-deficient MIN6 cells (clone N). These were characterized by few secretory granules (black arrow) and numerous non-electron-dense vesicles (white arrows).

Acknowledgments

We are grateful to Diabetes U.K. for project grant support (RD97/0001525). P.E.S. was an RD Lawrence Fellow of Diabetes UK (RD97/0001453). V.D.B. is funded by the Eli Lilly International Foundation. We thank Dr. Y. Oka and Professor J.I. Miyazaki (University of Tokyo, Tokyo, Japan) for provision of the MIN6 cells, Dr. B. Kennedy (Merck Frosst, PQ, Canada) for the cPLA2 vector, and Dr. R. Kramer (Lilly, Indianapolis, IN) for the cPLA2 antisera.

References