Volume 303, number 1, 85–90 FEBS 11067 May 1992

Protein kinase C activity affects glucose-induced oscillations in cytoplasmic free Ca^{2+} in the pancreatic B-cell

Henrik Kindmark, Martin Köhler, Suad Efendić, Patrik Rorsman*, Olof Larsson and Per-Olof Berggren

The Rolf Luft Center for Diabetes Research, Department of Endocrinology, Karolinska Institute, Box 60 500, Karolinska Hospital, S-104 01 Stockholm, Sweden

Received 25 March 1992

Acute stimulation of protein kinase C (PKC) inhibited glucose-induced slow oscillations in cytoplasmic free Ca^{2+}-concentration, $[Ca^{2+}]_i$, in mouse pancreatic B-cells. In PKC-depleted cells glucose induced rapid transients in $[Ca^{2+}]_i$, lasting for approximately 10 s, superimposed on the slow oscillations in $[Ca^{2+}]_i$. It was demonstrated that the transients did not occur in the absence of extracellular Ca^{2+}. Each transient typically was preceded by a slow increase in $[Ca^{2+}]_i$, representing the rising phase of an ordinary glucose-induced slow oscillation, and the $[Ca^{2+}]_i$, immediately after a transient was lower than just before the spike. These data further emphasize the interplay between voltage-dependent Ca^{2+}-channels and the phospholipase C system in the regulation of B-cell $[Ca^{2+}]_i$-oscillations.

Pancreatic B-cell: Stimulus-secretion coupling; Ca^{2+}-oscillation; Voltage-dependent Ca^{2}-channel; Phospholipase C-system

1. INTRODUCTION

The insulin-secreting B-cell is equipped with voltage-gated Ca^{2+} channels and even before direct observations of changes in the free cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) were made, it was predicted that $[Ca^{2+}]_i$ should oscillate in a fashion similar to the oscillations in membrane potential [1]. Slow oscillations in $[Ca^{2+}]_i$, with a period of 1–3 min, have previously been demonstrated in single mouse B-cells [2] and in single rat B-cells, purified by autofluorescence-activated cell-sorting [3]. Single mouse B-cells, stimulated with 8–10 mM glucose, exhibit bursts of action potentials separated by repolarized intervals with durations ranging between 1 and 4 min [4]. It is likely that this electrical behaviour accounts for the slow oscillation in $[Ca^{2+}]_i$, which have been reported in isolated single B-cells.

Activation of protein kinase C (PKC) with 10 nM of the phorbol ester TPA inhibits both the formation of inositol 1,4,5-trisphosphate ($InsP_3$), and the $[Ca^{2+}]_i$ increase in response to the muscarinic receptor-agonist carbamylcholine in mouse pancreatic B-cells [5]. Subsequent to down-regulation of PKC, the B-cells demonstrate a pronounced carbamylcholine-induced increase in both the formation of $InsP_3$ and the $[Ca^{2+}]_i$ signal [5]. More efficient activation of the phospholipase C (PLC) pathway, after PKC depletion, has also been reported in clonal, insulin-producing RINm5F cells [6]. These results indicate that PKC can have a restraining influence on PLC-activity in insulin producing cells. In addition to interacting with the PLC system, PKC can also directly modulate Ca^{2+}-handling in these cells. Such a direct modulation involves stimulation of both Ca^{2+} inward transport, through voltage-activated Ca^{2+}-channels [7], and Ca^{2+}-efflux, the latter effect likely to be mediated by activation of the plasma membrane Ca^{2+}-ATPase [8]. In the present study, we were interested in clarify whether PKC is involved in the molecular mechanisms regulating glucose-induced oscillations in $[Ca^{2+}]_i$ in mouse pancreatic B-cells.

Permanent address: Department of Medical Physics, Gothenburg University, Box 330 31, S-400 33 Gothenburg, Sweden.

Correspondence address: P.-O. Berggren, The Rolf Luft Center for Diabetes Research, Department of Endocrinology, Karolinska Institute, Box 60 500, Karolinska Hospital, S-104 01 Stockholm, Sweden. Fax: (46) (8) 303 458.

2. MATERIALS AND METHODS

All reagents were of analytical grade and Millipore-water was used. 12-*O*-Tetradecanoyl phorbol 13-acetate (TPA), ethylen-glycon-bis(β-amino-ethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and bovine serum albumin fracion V were from Sifma, St. Louis, MO, USA. Collagenase was from Boehringer Mannheim, Germany. Fura-2/acetoxymethylester was from Sigma and Boehringer.

2.1. *Animals and preparation of islet cells*

Adult obese hyperglycemic mice (gene symbol *ob/ob*) of both sexes were obtained from a local non-inbred colony [9] and starved for 24 h. The animals were killed by decapitation and the islets isolated by a collagenase technique [10]. The islets of these mice contain more then 90% B-cells [11]. A cell suspension was prepared and washed essentially as previously described [12]. The cells were resuspended in RPMI 1640 culture medium (Flow Laboratories, Scotland, UK), containing 11 mM glucose, supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 60 μg/ml gentamycin. The cell suspension was seeded onto coverslips. The cells were allowed to

attach for 2 h, whereafter the coverslips were placed in Petri dishes and cultured for up to 7 days.

2.2. *Media*

The basal medium used for preparation of cells, as well as experiments, was a HEPES buffer, pH 7.4, with Cl^- as the sole anion [13], containing 1.28 or 2.56 mM Ca^{2+} and 1 mg/ml bovine serum albumin.

2.3. *Measurements of* $[Ca^{2+}]_i$

Coverslips with single B-cells and B-cell aggregates were incubated in basal medium containing 0 mM glucose and 1.5 μM fura-2/AM for 20 min at 37°C. The coverslip with fura-2 loaded cells was then used as part of the bottom of an open chamber designed for microscopic work, covering a centrally located circular hole in the bottom plate. A rubber ring constituted the chamber wall and was pressed to the coverslip by a circular steel plate, with a central opening and by a thin steel ring. Two cannulas penetrated the top piece of the chamber and were connected to a two-channel peristaltic pump (Ismatec), allowing steady superfusion of the cells. A superfusion rate of 300 μl/min was used. The chamber was placed in a holder on the stage of an inverted microscope (Zeiss, Axiovert 35M). The stage itself was thermostatically controlled to maintain a temperature of 37°C in the superfusate inside the chamber. The microscope was equipped with a photon counting photometer and was connected to a SPEX fluorolog-2 CM1T11I system, allowing fluorometry using two excitation wavelengths. The excitation wavelengths (340 and 380 nm) were generated by two monochromators and emitted light was collected through a 510 nm filter inside the microscope. One 340/380 fluorescence ratio was obtained every second. By using a 40 × 0.75 NA objective and by changing the diameter of a variable diaphragm in the microscope, it was possible to select and measure from a single cell.

Transformation of the 340/380 fluorescence ratios into $[Ca^{2+}]_i$-values was done according to the formula reported by Grynkiewicz et al. [14]. The K_d for the Ca^{2+}–fura-2 complex was taken as 220 nM [14]. Values for maximum and minimum fluorescence ratios were obtained in separate experiments using 1 μl drops of a K^+-rich buffer, the ionic composition of which was similar to the intracellular milieu. The drops contained free fura-2 acid and either a saturating Ca^{2+}-concentration, or no Ca^{2+} in the presence of EGTA. Such standard curves obtained in vitro have been reported to be almost identical to standard curves generated in situ [15]. In order to compensate for possible variations in output of light intensity from the two monochromators, the calibration experiments included a fluorescence ratio where both monochromators were set at 360 nm. Each subsequent experiment also included a 360/360 ratio. The calibration parameters as well as every experiment was then normalized by dividing all fluorescence ratios with the corresponding 360/360 ratio. Background fluorescence was estimated and corrected for after quenching the fura-2 fluorescence with manganese.

In some experiments no value for background fluorescence was obtained, since addition of manganese would have made it impossible to measure fura-2 fluorescence from more than one cell on the same coverslip. Figures showing these experiments give $[Ca^{2+}]_i$ only as fluorescence ratio. In all other experiments $[Ca^{2+}]_i$ is given both in nM and as fluorescence ratio. Each figure shows one representative experiment chosen from a group of at least three experiments performed using the same protocol.

3. RESULTS

3.1 *Effects of glucose stimulation on* $[Ca^{2+}]_i$

When single B-cells or small B-cell aggregates were stimulated with 7, 10 or 12 mM glucose, after a 20 min exposure to 0 mM glucose during loading with fura-2/AM, several different types of $[Ca^{2+}]_i$ responses were obtained. In one group of cells a single, large peak of $[Ca^{2+}]_i$ could be observed (Fig. 1A). A second group of cells responded with oscillations in $[Ca^{2+}]_i$, with a period of 2–5 min (Fig. 1B). In many cases nadirs between oscillations reached basal $[Ca^{2+}]_i$ (Fig. 1B), but sometimes oscillations clearly occurred superimposed on a sustained elevated $[Ca^{2+}]_i$ (Fig. 2A). In a third group of cells an initial peak of $[Ca^{2+}]_i$ was immediately followed by a sustained, elevated $[Ca^{2+}]_i$, lower than the peak value (Fig. 2B).

Possibly, the different response types indicate varying sensitivity to glucose among individual B-cells [16]. With regard to oscillations a 'Ca^{2+}-fingerprint' for individual clonal B-cells, responding to stimulation of muscarinic receptors, has previously been reported [17].

The fraction of B-cells that oscillated varied between preparations. In some preparations not a single B-cell oscillated. In other preparations of islet cells, up to 45% of the coverslips contained cells that were found to oscillate. Generally, not more than four cells per coverslip were studied.

3.2. *Effects of acute stimulation of PKC on glucose-induced oscillations in* $[Ca^{2+}]_i$

When a B-cell, or an aggregate of such cells, was found to oscillate in the presence of 10 mM glucose, PKC was activated by adding 10 nM TPA. It is well established that this concentration of the phorbol ester specifically activates PKC [18] and that this is true also for the pancreatic B-cell [19]. PKC activation by 10 nM TPA inhibited the oscillations (Fig. 3A and B) and caused a sustained level of $[Ca^{2+}]_i$, well below the $[Ca^{2+}]_i$ reached at the oscillatory peaks. Subsequent to PKC activation, the cells still responded, with a marked increase in $[Ca^{2+}]_i$, to depolarization with 25 mM K^+ (Fig. 3A), arguing against a non-specific toxic effect by TPA. In experiments performed with buffer containing 1.28 mM Ca^{2+}, only one additional oscillation was observed after addition of TPA (Fig. 3A). When the buffer contained 2.56 mM Ca^{2+}, oscillatory activity seemed to be halted more gradually by PKC-activation (Fig. 3B). TPA was prepared as a 1000× concentrated stock solution in DMSO. When DMSO was added alone there was no effect on the oscillations (Fig. 3C).

3.3. *Effects of glucose stimulation on* $[Ca^{2+}]_i$ *in B-cells subsequent to down-regulation of PKC*

B-Cells were cultured in the presence of 200 nM TPA. This treatment leads to down-regulation of PKC-activity, and after 24 h only 16% of the original PKC-activity remains [19]. When such cells were stimulated with 10 mM glucose, $[Ca^{2+}]_i$ increased according to similar patterns as in non-PKC-depleted B-cells. Fig. 4A shows that the slow $[Ca^{2+}]_i$-oscillations were preserved in PKC-depleted cells. They did, however, occur at more irregular intervals and displayed a more varying duration compared to non-PKC-depleted B-cells. Interestingly, shortlived transients in $[Ca^{2+}]_i$, lasting for about 10 s, were often superimposed on the glucose-induced elevated $[Ca^{2+}]_i$ (Fig. 4A–C). These spikes usually occurred

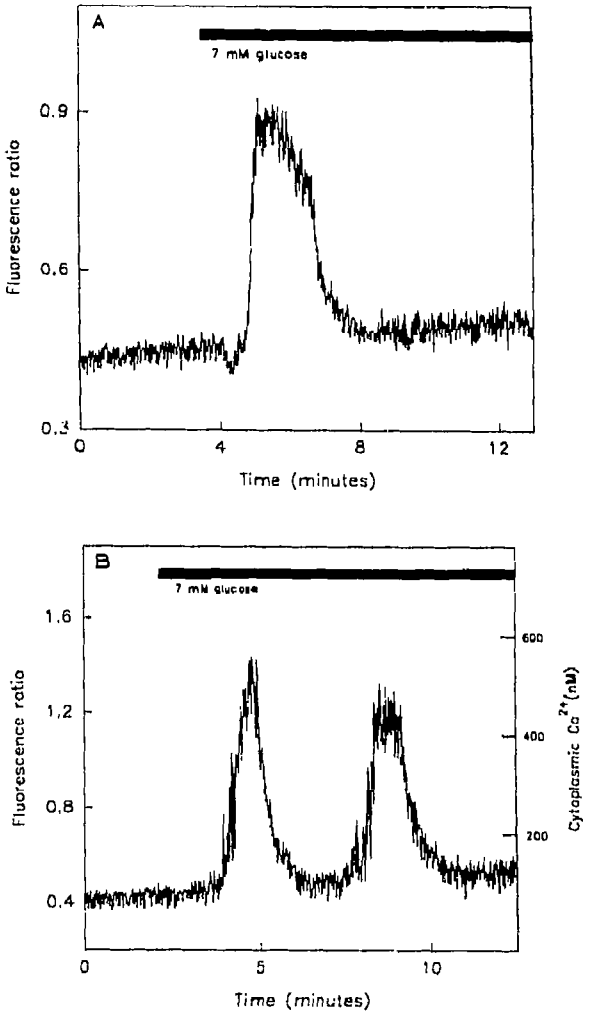

Fig. 1. Effects of sub-maximally stimulating glucose concentrations on $[Ca^{2+}]_i$ in single B-cells. Addition of glucose indicated by filled bars. (A) 7 mM glucose added to a B-cell. $[Ca^{2+}]_i$ given as fluorescence ratio in this case. (B) 7 mM glucose added to a B-cell.

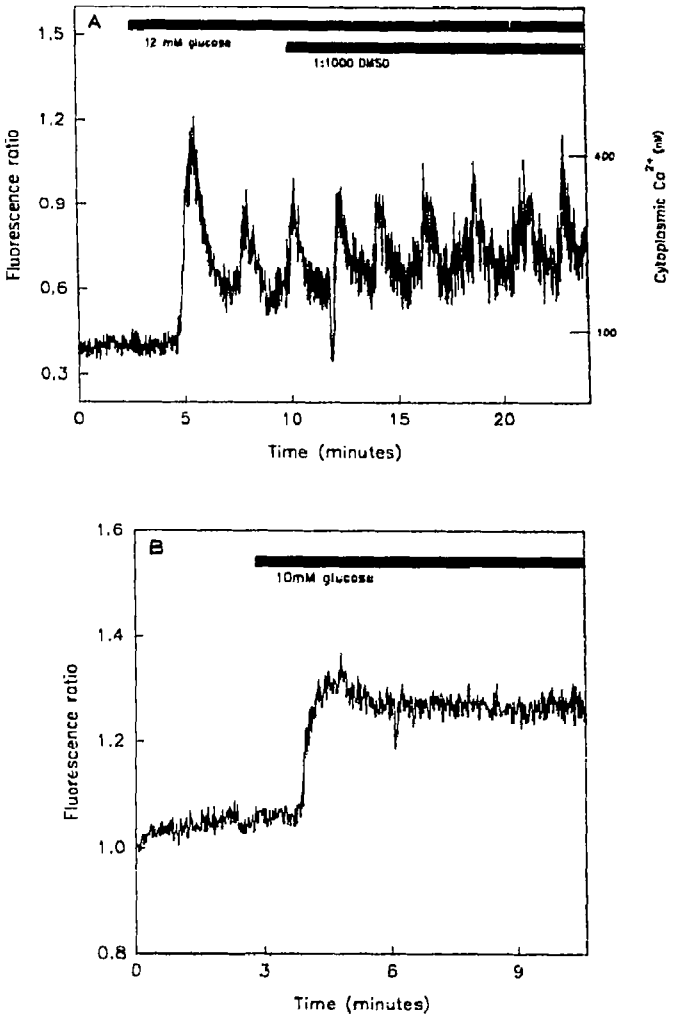

Fig. 2. Effects of sub-maximally stimulating glucose concentrations on $[Ca^{2+}]_i$ in small aggregates of B-cells. (A) 12 mM glucose added to an aggregate of B-cells. DMSO, 0.1%, added as a control to show that this substance did not affect oscillations. (B) 10 mM glucose added to an aggregate of B-cells. $[Ca^{2+}]_i$ given as fluorescence ratio in this case.

just when the rising $[Ca^{2+}]_i$ reached a peak. The $[Ca^{2+}]_i$ immediately after the spike was lower than just before the spike. In approximately half of the cells displaying shortlived Ca^{2+}-transients, one or more additional spikes were seen after the first one, at irregular intervals (Fig. 4C). Attempts were made to elucidate whether or not the $[Ca^{2+}]_i$-spikes were dependent on extracellular Ca^{2+}. The glucose concentration was raised from 0 to 10 mM in the absence of extracellular Ca^{2+} and in the presence of 0.5 mM EGTA (Fig. 4C). $[Ca^{2+}]_i$ remained unaffected under these conditions. When 2.56 mM Ca^{2+} was introduced, however, $[Ca^{2+}]_i$ increased and not only the $[Ca^{2+}]_i$-spikes but also the slow $[Ca^{2+}]_i$-oscillations appeared. The $[Ca^{2+}]_i$-spikes had the same duration and were similar to the transients evoked by GTPγS and $InsP_3$ in a previous study [20].

Cells cultured for at least 24 h in DMSO alone (TPA-solvent) responded normally to stimulation with 10 mM glucose. In such cells slow oscillations in $[Ca^{2+}]_i$, with a period of 2–5 min, could frequently be observed (Fig. 4D). Spikes in $[Ca^{2+}]_i$ did not, however, occur.

4. DISCUSSION

We now demonstrate that the previously reported slow 2–5 min $[Ca^{2+}]_i$-oscillations in single B-cells and small B-cell aggregates from mouse [2] are prevented by activation of PKC. Repolarization is an inlikely explanation to the termination of these oscillations, since PKC-activation has been shown to have no effect on average membrane potential in cell suspensions of glucose stimulated B-cells [19]. Instaed, the inhibition of the slow $[Ca^{2+}]_i$-oscillations by PKC, probably reflects the stimulated efflux of Ca^{2+} from the B-cell, due to activation of the plasma membrane Ca^{2+}-pumps [8]. By

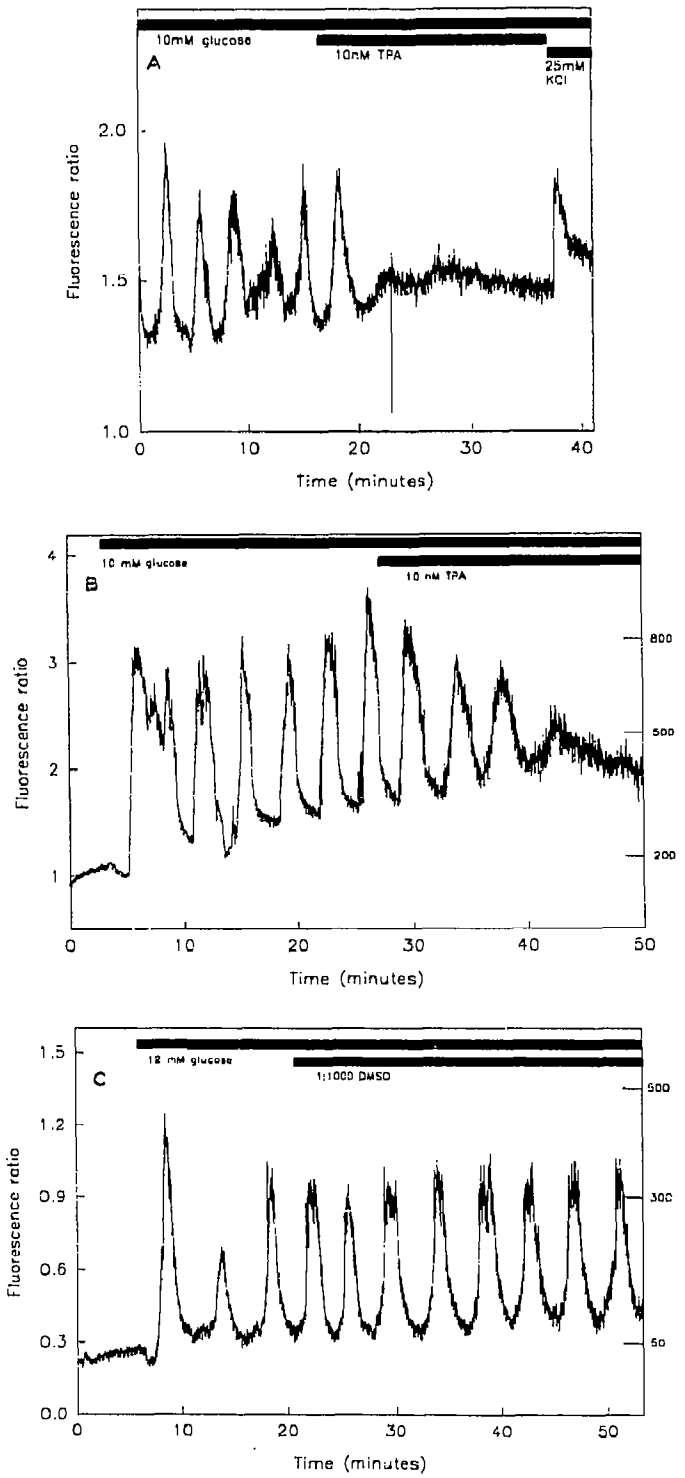

Fig. 3. Effect of 10 nM TPA on oscillations in $[Ca^{2+}]_i$ in aggregates of B-cells or single B-cells. Additions of TPA, K^+ and DMSO indicated by filled bars. (A) Effect on TPA on oscillations in $[Ca^{2+}]_i$ in a single B-cell. 10 mM glucose present throughout the experiment. 25 mM K^+ added as indicated. Experiment performed in the presence of 1.28 mM Ca^{2+}. $[Ca^{2+}]_i$ given as fluorescence ratio. (B) Effect of TPA on oscillations in $[Ca^{2+}]_i$ in an aggregate of B-cells. 10 mM glucose added as indicated. Experiment performed in the presence of 2.56 mM Ca^{2+}. (C) Control experiment demonstrating the effect of 12 mM glucose on $[Ca^{2+}]_i$ in a single B-cell. DMSO, 0.1%, added as indicated.

stimulating plasma membrane Ca^{2+}-pumps, PKC activation keeps $[Ca^{2+}]_i$ close to basal levels and prevents $[Ca^{2+}]_i$-oscillations from occurring. Noteworthy is that the slow $[Ca^{2+}]_i$-oscillations are inhibited also in the absence of extracellular Ca^{2+} or in the presence of blockers of L-type voltage-activated Ca^{2+}-channels [22]. In contrast to what might have been expected, the slow $[Ca^{2+}]_i$-oscillations were much more irregular in PKC-down-regulated cells. The variation in appearance of the slow oscillations under these conditions probably reflects the fact that PKC is also needed in the phosphorylation of the voltage-dependent Ca^{2+}-channels, enabling them to conduct Ca^{2+} influx more efficiently [7].

When mouse B-cells were depleted in PKC large transients in $[Ca^{2+}]_i$, lasting for approximately 10 s, were often superimposed on the slow $[Ca^{2+}]_i$ oscillations. When PKC is down-regulated, the normal inhibition of the PLC-system and as well stimulation of membrane Ca^{2+}-pumps by the enzyme should be suppressed. The resulting enhanced formation of $InsP_3$ [5], in combination with reduced Ca^{2+}-efflux from the cell and maybe reduced intracellular buffering, promote the generation of the large $[Ca^{2+}]_i$-transients. The $InsP_3$- and most likely as well Ca^{2+}-induced $[Ca^{2+}]_i$-spikes [23] will open Ca^{2+}-activated K^+-channels [20]. This leads to repolarization, closure of voltage-activated Ca^{2+}-channels, a decrease in Ca^{2+}-influx and thereby a lowering in $[Ca^{2+}]_i$, explaining why $[Ca^{2+}]_i$ immediately after a spike was lower than just before the transient. The 10 s transients in $[Ca^{2+}]_i$ did not occur in the absence of extracellular Ca^{2+}, reflecting the fact that the glucose-induced activation of the PLC-system in the B-cell is secondary to the rise in $[Ca^{2+}]_i$ [24]. Moreover, this supports the concept of a complex interplay between Ca^{2+}-influx through voltage-activated L-type Ca^{2+}-channels and Ca^{2+}-release from intracellular stores, in the regulation of the B-cell glucose-induced fast $[Ca^{2+}]_i$-transients. When the PLC-system is directly activated by agonists like carbamylcholine and GTPγS, in non-PKC-depleted B-cells, the $[Ca^{2+}]_i$-spikes can be observed even in the absence of extracellular Ca^{2+} [20]. The duration of $[Ca^{2+}]_i$-transients in the present study is similar to both the duration of slow waves in electrical activity [25] and of the rapid $[Ca^{2+}]_i$-oscillations observed in intact pancreatic islets [26]. An interplay between influx of Ca^{2+} through L-type voltage-activated Ca^{2+}-channels and release of Ca^{2+} from intracellular stores could be easily envisaged also in the generation of fast $[Ca^{2+}]_i$-oscillations in the intact islet, since the B-cell under these conditions is exposed to a variety of neuropeptides, other neurotransmitter substances and hormones, which in addition to glucose activate the PLC pathway.

Whereas the glucose-induced fast $[Ca^{2+}]_i$-transients seem to reflect mobilization of intracellularly bound Ca^{2+}, the glucose-induced slow oscillations in $[Ca^{2+}]_i$ are probably generated by other molecular mechanisms. We have preliminary data (Kindmark, H. and

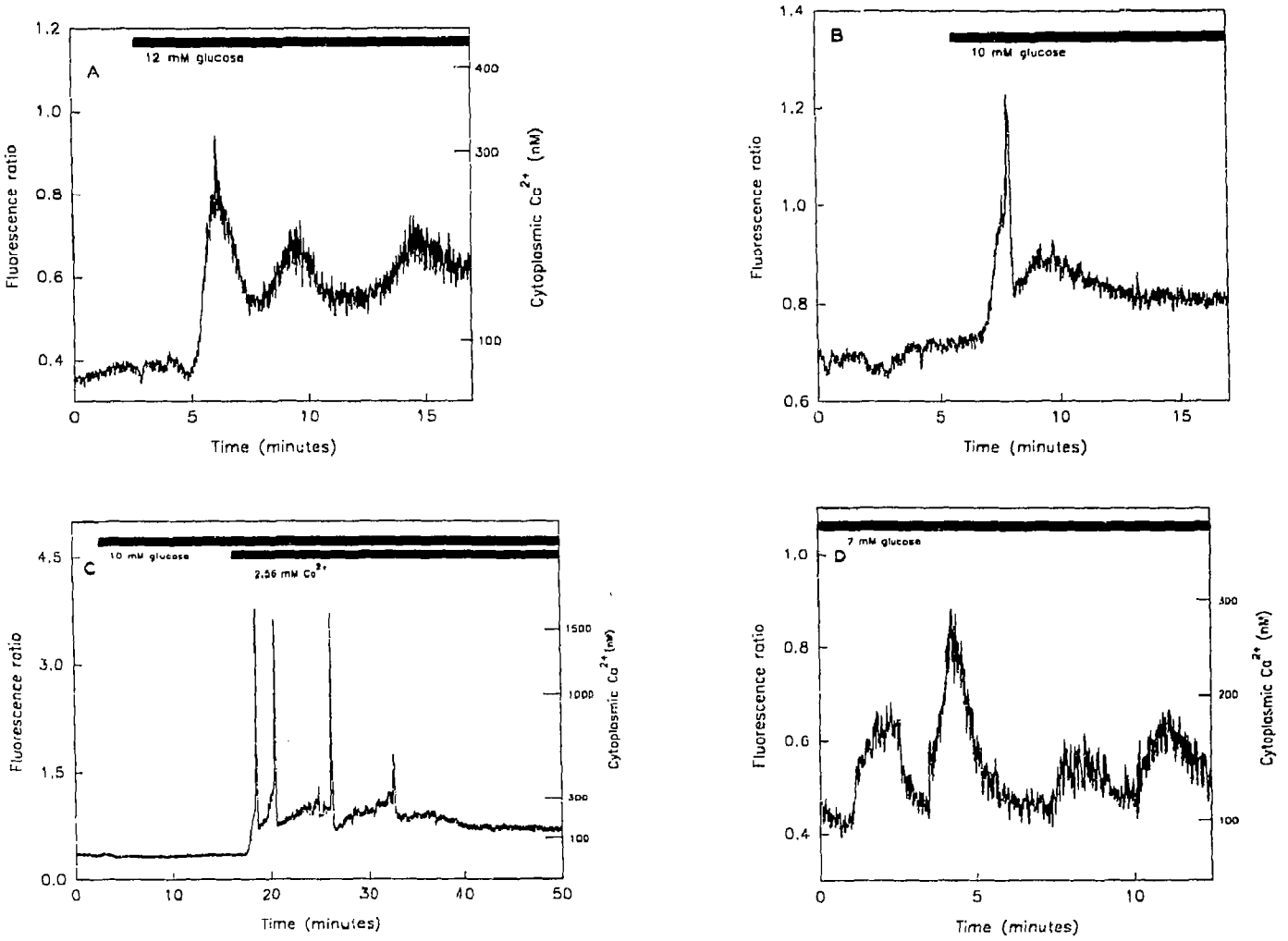

Fig. 4. Effect of 10–12 mM glucose on $[Ca^{2+}]_i$ in single B-cells cultured for more than 24 h in the presence of 200 nM TPA. Addition of glucose and Ca^{2+} indicated by filled bars. (A) Experiment performed in the presence of 1.28 mM Ca^{2+}. (B) Experiment performed in the presence of 2.56 mM Ca^{2+}. $[Ca^{2+}]_i$ given as fluorescence ratio. (C) Glucose added in the presence of 0 mM Ca^{2+} and 0.5 mM EGTA. 2.56 mM Ca^{2+} introduced as indicated. (D) Control experiment demonstrating the effect of 7 mM glucose on $[Ca^{2+}]_i$ in an aggregate of B-cells cultured for more than 24 h in the presence of 0.1% DMSO.

Breggren, P-O, unpublished observations) which are compatible with the concept [27], that the slow oscillations are metabolically driven and hence reflect, inter alia, oscillations in the cytoplasmic ATP/ADP-concentration ratio. Moreover, oscillations in $[Ca^{2+}]_i$ and NADH-fluorescence, with a strikingly similar frequency, have recently been observed in pancreatic B-cells [28]. Oscillations in the cytosolic ATP/ADP-ratio would imply parallel oscillations in the open/closed state of the ATP- regulated K^+-channels. However, glucose-stimulated membrane potential oscillations do not appear to be due to marked changes in the K^+-channel activity as, in one study [4], no increase in K^+-conductance, as compared with that observed during the plateau phase, was observed subsequent to repolarization. This suggests that is the slow oscillations are driven by metabolic variations in the ATP/ADP-ratio, then the effect on the ATP-regulated K^+-channel activity must be minute. Alternatively, such changes could act by affecting the Ca^{2+}-conductance. Indeed, B-cell Ca^{2+}-channel activity has been reported to be metabollicall regulated [29,30].

Acknowledgements: Financial support was obtained from the Swedish Medical Research Council (19x-00034, and 04x-09890), the Bank of Sweden Tercentenary Foundation, the Board for Technical Developments in Sweden, the Swedish Diabetes Association, the Nordic Insulin Foundation, the Swedish Hoechst Diabetes Research Foundation, the Syskonen Svenssons Foundation, Magnus Bergvalls Foundation, Lars Hiertas Memorial Foundation, Aage and Louise-Hansens Memorial Foundations, NOVO Industry, Farmitalia Carlo-Erba, Fredrik and Ingrid Thurings Foundation, the Torsten and Ragnar Söderbergs Foundations, Funds of the Karolinska Institute, the Anders Otto Swärds Foundation, the TAAA Amundsons Fund, the Swedish Society for Medical Research and 'Förenade Liv' Mutual Group Life Insurance Co.

REFERENCES

[1] Chay, T.R. and Keizer, J. (1983) Biophys. J. 42, 181–189.
[2] Grapengiesser, E., Gylfe, E. and Hellman, B. (1988) Biochem. Biophys. Res. Commun. 151, 1299–1304.
[3] Wang, J.-L. and McDaniel, M.L. (1990) Biochem. Biophys. Res. Commun. 166, 813–818.
[4] Smith, P.A., Ashcroft, F.M. and Rorsman, P. (1990) FEBS Lett. 261, 187–190.
[5] Berggren, P.-O., Juntti-Berggren, L., Sjöholm, Å. and Rorsman, P. (1991) Diabetes 40, 698.
[6] Li, G., Regazzi, R., Ullrich, S., Pralong, W.-F. and Wollheim, C.B. (1990) Biochem. J. 272, 637–645.
[7] Rorsman, P., Arkhammar, P. and Berggren, P.-O. (1986) Am. J. Physiol 251, C1-C8.
[8] Berggren, P.-O., Arkhammar, P. and Nilsson, T. (1989) Biochem. Biophys. Res. Commun. 165, 416–421.
[9] Hellman, B. (1965) Ann. NY Acad. Sci. 131, 541–558.
[10] Lacy, P.E. and Kostianovsky, M.K. (1967) Diabetes 16, 35–39.
[11] Nilsson, T., Arkhammar, P., Hallberg, A., Hellman, B. and Berggren, P.-O. (1987) Biochem. J. 248, 329–336.
[12] Lernmark, Å. (1974) Diabetologia 10, 431–438.
[13] Hellman, B. (1975) Endocrinology 97, 392–398.
[14] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
[15] Wahl, M., Lucherini, M.J. and Gruenstein, E. (1990) Cell Calcium 11, 487–500.
[16] Pipeleers, D.G. (1987) Diabetologia 30, 277–291.
[17] Prentki, M., Glennon, M.C., Thomas, A.P. Morris, R.L., Matschinsky, F.M. and Corkey, B.E. (1988) J. Biol. Chem. 263, 11044–11047.
[18] Nishizuka, Y. (1984) Nature 308, 693–698.
[19] Arkhammar, P., Nilsson, T., Welsh, M., Welsh, N. and Berggren, P.-O. (1989) Biochem. J. 264, 207–215.
[20] Ämmälä, C., Larsson, O., Berggren, P.-O., Bokvist, K., Juntti-Berggren, L., Kindmark, H. and Rorsman, P. (1991) Nature 353, 849–852.
[21] Smallwood, J.I., Gügi, B. and Rasmussen, H. (1988) J. Biol. Chem. 263, 2195–2202.
[22] Grapengiesser, E., Gylfe, E. and Hellman, B. (1989) Arch. Biochem. Biophys. 268, 404–407.
[23] Islam, M.S., Rorsman, P. and Berggren, P.-O. (1992) FEBS Lett. 296, 287–291.
[24] Biden, T.J., Peter-Riesch, B., Schlegel, W. and Wollheim, C.B. (1987) J. Biol. Chem. 262, 3567–3571.
[25] Cook, D.L. (1983) Metabolism 32, 681–685.
[26] Valdeolmillos, M., Santos, R.M., Contreras, D., Soria, B. and Rosario, L.M. (1989) FEBS Lett. 259, 19–23.
[27] Longo, E.A., Tornheim, K., Deeney, J.T., Varnum, B.A., Tillotson, D., Prentki, M. and Corkey, B.E. (1991) J. Biol. Chem. 266, 9314–9319.
[28] Pralong, W.-F., Gjinovci, A. and Wollheim, C.B. (1991) Diabetes 40, 317.
[29] Velasco, J.M., Petersen, J.U.H. and Petersen, O.H. (1988) FEBS Lett. 213, 366–370.
[30] Smith, P.A., Rorsman, P. and Ashcroft, F.M. (1989) Nature 342, 550–553.