Dephosphorylation and deactivation of Ca^{2+}/calmodulin-dependent protein kinase II in βTC3-cells is mediated by Mg^{2+}- and okadaic-acid-sensitive protein phosphatases

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The α-toxin-permeabilized βTC3 cell has been utilized as an experimental model for the identification of protein phosphatases responsible for the dephosphorylation and deactivation of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) *in situ*. In this model, the elevation of Ca^{2+} from 0.05 to 10 μM induced the near-total conversion of CaM kinase II into a Ca^{2+}/calmodulin-independent (autonomous) form characteristic of autophosphorylated, activated enzyme. On the removal of Ca^{2+}, the activation state of CaM Kinase II rapidly returned to prestimulated levels. This reversal was slowed, but not prevented, by the inhibitors of protein phosphatase-1 (PP-1) and PP-2A, okadaic acid and calyculin A, and by the selective chelation of Mg^{2+} by the addition of EDTA. Near-complete prevention of enzyme deactivation, however, was observed in the combined presence of both okadaic acid and EDTA. Under these conditions, CaM kinase II phosphatase was more sensitive to calyculin A relative to okadaic acid, characteristic of the involvement of PP-1. CaM kinase II deactivation was not affected by FK-506, eliminating the involvement of PP-2B in this process. These data suggest that CaM kinase II dephosphorylation and deactivation in the pancreatic β-cell is mediated by the combined action of an okadaic-acid-sensitive phosphatase and a Mg^{2+}-dependent phosphatase, such as PP-2C.

INTRODUCTION

On activation by Ca^{2+}/calmodulin, the multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) undergoes rapid intersubunit autophosphorylation on a threonine residue in the auto-inhibitory domain of the enzyme (reviewed in [1,2]). The incorporation of a phosphate moiety at this site induces the conversion of the enzyme into a form that is no longer dependent on Ca^{2+}/calmodulin for activity [3,4]. Autonomous Ca^{2+}/calmodulin-independent activity persists until the phosphate at Thr-286/-287 is removed by the action of a protein phosphatase [5]. Physiologically, this autophosphorylation event may permit CaM kinase II to continue to phosphorylate cellular substrates beyond the restoration of cytoplasmic Ca^{2+} concentrations to basal levels [6], and has implicated CaM kinase II in the regulation of synaptic plasticity and related complex mechanisms of learning and memory [7]. To what extent protein phosphatases regulate the degree of autonomous kinase activity *in situ* is not known.

An increased level of autonomous kinase activity is induced in isolated rat islets stimulated by the physiological stimulator, D-glucose [8,9]. The close correlation of kinase activation with both initial and sustained phases of insulin secretion in perifused islets [9] is consistent with a fundamental role of this enzyme in this process, although this has yet to be substantiated. Interestingly, these parameters are dissociated on the withdrawal of a glucose stimulus, such that the deactivation of CaM kinase II follows a delayed time course relative to the termination of insulin secretion [9], indicative of involvement in a process proximal to exocytosis. The study of this potentially important mechanism requires the identification of the cellular phosphatase responsible for the deactivation of CaM kinase II.

In the current study, the demonstration that the provision or elimination of exogenous Ca^{2+} can control the activation state of CaM kinase II in *Staphylococcus aureus* α-toxin-permeabilized β-cells (βTC3) has provided an opportunity to study the mechanism of enzyme deactivation in a pseudo-physiological setting. Utilizing pharmacological intervention of phosphatase activity or the selective chelation of ionic cofactors, the involvement of known type I and II protein phosphatases in the deactivation of β-cell CaM kinase II has been evaluated.

EXPERIMENTAL

Materials

Culture medium Roswell Park Memorial Institute (RPMI) 1640 was obtained from Mediatech (Herndon, VA, U.S.A). Fetal bovine serum was purchased from Life Technologies (Gaithersburg, MD, U.S.A). α-Haemolysin (*Staph. aureus* α-toxin), ATP (disodium salt), calmodulin and leupeptin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). Okadaic acid (free acid) was purchased from Research Biochemicals International (Natick, MA, U.S.A.). Calyculin A was obtained from LC Laboratories (Woburn, MA, U.S.A.). The calcineurin [protein phosphatase-2B (PP-2B)] inhibitor, FK-506, was obtained from Fugisawa USA, Inc. (Melrose Park, IL, U.S.A.). [γ-^{32}P]ATP was purchased from NEN Research Chemicals (DuPont, Boston, MA, U.S.A.). Autocamtide-2, sequence KKALRRQETVDAL [10], was synthesized by Bio-Synthesis, Inc. (Lewisville, TX, U.S.A.). All other chemicals were of the finest reagent grade available.

Abbreviations used: CaM kinase II, Ca^{2+}/calmodulin-dependent protein kinase II; PP-1/-2A/-2B/-2C, protein phosphatases-1, -2A, -2B and -2C, respectively.

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Culture and permeabilization of βTC3 cells

βTC3 cells, derived from insulinomas generated in transgenic mice carrying a hybrid insulin promoter simian virus-40 tumour antigen gene, were obtained from Dr. Shimon Efrat (Albert Einstein College of Medicine, NY, U.S.A.) [11]. These cells were cultured (passage 32–53) in RPMI 1640 medium supplemented with 2 mM glutamine and 10 % (v/v) fetal bovine serum. Within these culture limits, βTC3 cells retain features of differentiated β-cells, such as the expression of mature insulin in dense core secretory granules [11]. Cells were cleaved from growth flasks using trypsin/EDTA (Life Technologies, Gaithersburg, MD, U.S.A.) and cultured in suspension in RPMI medium for 2 h at 37 °C. Following brief centrifugation (IEC Clinical Centrifuge, 500 ***g***, 1 min) cells were washed with Ca^{2+}-free Krebs–Ringer bicarbonate/Hepes buffer (25 mM Hepes (pH 7.4)/115 mM NaCl/24 mM $NaHCO_3$/5 mM KCl/1 mM $MgCl_2$) containing 1 mM EGTA, 6 mM glucose and 0.1 % (w/v) BSA. Permeabilization was initiated by the addition of *Staph. aureus* toxin, α-haemolysin, to a concentration of 125–200 units/10^6 cells per 0.1 ml Ca^{2+}-free permeabilization buffer (20 mM Hepes (pH 7.0)/140 mM potassium glutamate/5 mM NaCl/4 mM $MgSO_4$/1 mM EGTA/300 μM Na_2ATP). Permeabilization was conducted at 37 °C for 15 min with the efficiency monitored by detecting Trypan Blue accessibility to > 60 %, and then terminated by the addition of ice-cold Ca^{2+}-free permeabilization buffer (washing twice). Cells were resuspended in permeabilization buffer containing 0.05 μM Ca^{2+}, and placed on ice before experimental treatments. Free Ca^{2+} concentrations in incubation buffers were determined using a Ca^{2+} electrode (Orion) calibrated against known standards, as described by Bers [12].

Insulin secretion

For insulin-secretion studies, βTC3 cells were seeded in 96-well plates at an initial density of 10^4 cells/well and cultured for 2–3 days. Cells were permeabilized in monolayers using conditions similar to those described above, except that the buffer contained increased concentrations of EGTA (10.2 mM) and Na_2ATP (2 mM). The insulin content of the medium was determined by enzyme-linked immunoassay (EIA) using rat insulin as the standard [13].

Assay of CaM kinase II activity

CaM kinase II activity was assayed using the determination of ^{32}P incorporation into exogenously added selective peptide substrate, autocamtide-2, by a method described previously [14]. This assay provides a determination of Ca^{2+}-independent kinase II activity of autophosphorylated, activated enzyme. The expression of the ratio of this activity with that obtained in the presence of Ca^{2+}/calmodulin provides a measure of the extent of activation (illustrated in Figures as % autonomy). However, since the catalytic activity of autonomous enzyme is known to be only a fraction (20–80 %) of the native enzyme [2], this ratio is an underestimation of enzyme activation. The activation of CaM kinase II in β-cell homogenates in the presence of excess Ca^{2+}/calmodulin and ATP results in a maximal autonomous-to-Ca^{2+}-dependent activity ratio of 53.3 ± 2.88 %.

Statistical treatment of data

Data are presented as means ± S.E.M. determined from at least 3 independent observations unless otherwise stated.

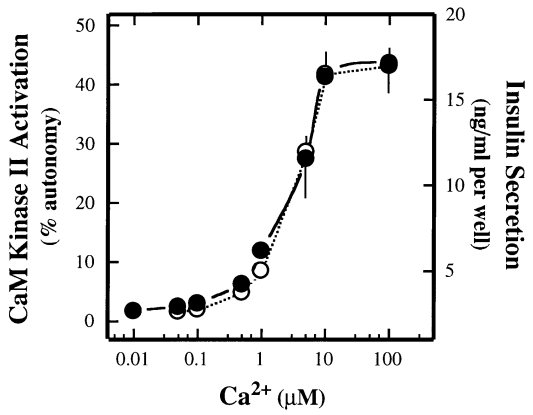

Figure 1 Parallel induction of insulin secretion and CaM kinase II activation by Ca^{2+} in permeabilized βTC3 cells

α-Toxin-permeabilized βTC3 cells were incubated at 37 °C in buffers containing 0.05–10 μM Ca^{2+} for 1 min (○) or 30 min (●) and then assayed for CaM kinase II activation (○) or insulin secretion (●). Autonomous CaM kinase II activity was determined as described in the Experimental section and expressed as a percentage of Ca^{2+}-dependent kinase activity.

RESULTS

Establishment of the experimental model

Staph. aureus α-toxin generates pores of uniform diameter (≈ 2 nm) in mammalian cells accessing the cell cytoplasm to ions such as Ca^{2+} and nucleotides, but with the retention of cellular proteins of > 3 kDa [15]. In this study, βTC3 cells permeabilized with this toxin dose-dependently secreted insulin as the buffer-free Ca^{2+} was incrementally increased from 0.1 to 10 μM (Figure 1), similar to that previously observed for other β-cells (HIT) [16]. Under these conditions, Ca^{2+} also induced the marked activation of CaM kinase II that correlated closely with insulin secretion with respect to ion concentration (Figure 1); half-maximal and maximal activation was achieved at approx. 2 μM and 10 μM respectively, for both parameters. With respect to CaM kinase II, maximal activation was achieved by 1 min and was sustained for at least 30 min, confirming the adequate provision of ATP to support extended enzyme autophosphorylation (Figure 2A). The level of autonomy achieved (≈ 46 %) at 10 μM was determined to represent more than 85 % of total enzyme activation (see the Experimental section). The maintenance of CaM kinase II activation was, however, dependent on the presence of Ca^{2+} since its removal resulted in the rapid return to basal activation levels. Greater than 80 % reversal was achieved within 15–30 s, and complete reversal was evident by 2 min (Figure 2B, triangular symbols and Figure 3). Since the utilized assay of CaM kinase II activation, i.e. % autonomy relative to Ca^{2+}-dependent activity, is determined by autophosphorylation of a threonine residue in the regulatory domain, this reversal represents the dephosphorylation of this site.

These observed phenomena provided a useful model to study the nature of the protein phosphatase(s) involved in the deactivation of CaM kinase II in βTC3 cells. All experiments incorporated a 10 min period in which permeabilized βTC3 cells were preincubated in a buffer containing basal concentrations of Ca^{2+} (0.05 μM) followed by an activation period of 1 min in the presence of stimulatory concentrations of Ca^{2+} (10 μM). The permeabilized cells were then either: (i) harvested and the fraction of CaM kinase II in an activated form determined (zero time); or (ii) returned to a basal Ca^{2+} buffer in the absence and presence of

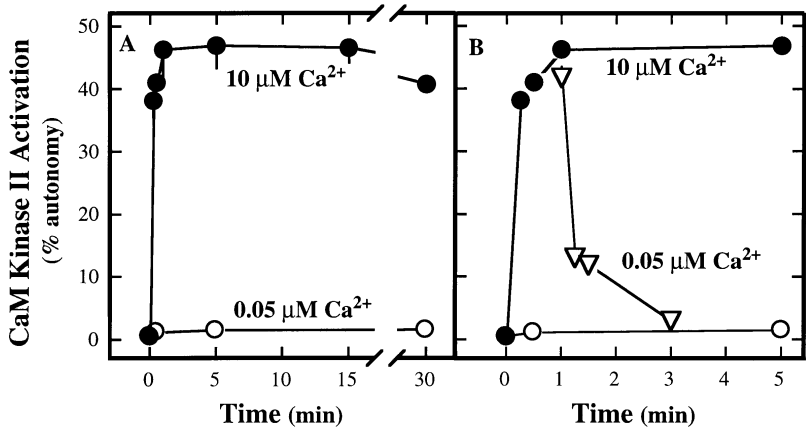

Figure 2 Rapid activation and deactivation of CaM kinase II in permeabilized βTC3 cells

βTC3 cells were permeabilized by α-toxin and incubated at 37 °C in buffers containing 0.05 μM Ca^{2+} (○, ▽) or 10 μM Ca^{2+} (●) for the times indicated. In panel (**B**), cells were incubated in 10 μM Ca^{2+} for 1 min and then 0.05 μM Ca^{2+} for the times indicated (▽). CaM kinase II activation was determined as in Figure 1.

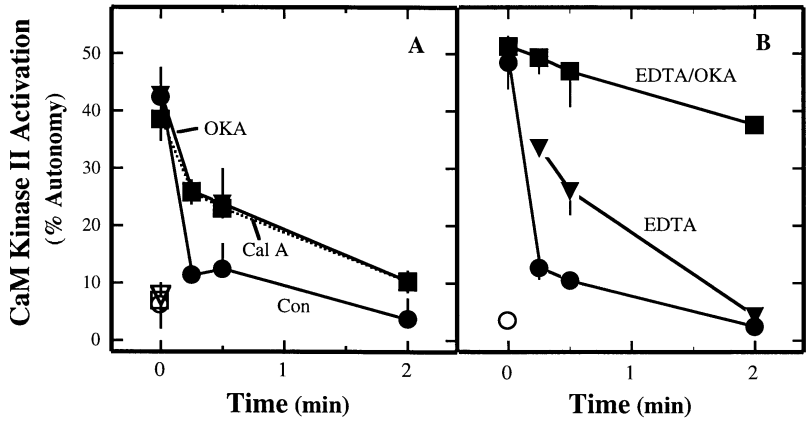

Figure 3 Modulation of CaM kinase II deactivation in permeabilized βTC3 cells by PP-1, PP-2A and PP-2C

Zero time indicates CaM kinase II activation state of permeabilized βTC3 cells incubated in 0.05 μM Ca^{2+} (open symbols) or 10 μM Ca^{2+} (filled symbols) for 1 min. Panel (**A**): stimulated cells were then incubated in 0.05 μM Ca^{2+} in the presence of vehicle alone (Con, ●), 1 μM calyculin A (Cal A, ■) or 1 μM okadaic acid (OKA, ▼) for the times indicated and then assayed for CaM kinase II activation. Panel (**B**): stimulated cells were then incubated in 0.05 μM Ca^{2+} in the presence of vehicle alone (●), 10 mM EDTA (▼) or 10 mM EDTA in the presence of 1 μM okadaic acid (EDTA/OKA, ■) for the times indicated, and then assayed for CaM kinase II activation.

phosphatase inhibitors. The deactivation of CaM kinase II was then followed by the assay of autonomous kinase activity at 0–2 min. The use of a permeabilized cell model provided an advantage in that the cell cytosol was accessible to ion chelators and organic inhibitors that otherwise would be cell-impermeant or subject to partial sequestration within the plasma membrane.

Involvement of an okadaic-acid-sensitive protein phosphatase(s) in the deactivation of β-cell CaM kinase II

Previous studies have documented the ability of the okadaic-acid-sensitive phosphatases PP-1 [17] and PP-2A [5,18] to dephosphorylate CaM kinase II *in vitro* or in cell extracts. Sensitivity to okadaic acid was similarly used in the current study to test for the involvement of these phosphatases in the observed deactivation of CaM kinase II in permeabilized β-cells. Incubation with okadaic acid (1 μM) [19,20] had no significant effect on either the basal level of CaM kinase II autonomy or enzyme activation by Ca^{2+} (10 μM) (Figure 3A). However, the presence of okadaic acid markedly slowed the rate of CaM kinase II deactivation after the removal of Ca^{2+}, such that only approximately 43 % and 46 % reversal was achieved at 15 s and 30 s respectively, compared with 80–90 % observed in control cells in the presence of vehicle alone (DMSO). Enzyme deactivation was approximately 90 % completed by 2 min in the presence of okadaic acid, such that residual activation was not significantly greater than basal levels in unstimulated cells. This datum was validated by an identical effect of a second inhibitor of PP-1 and PP-2A, calyculin A [19,20], to slow enzyme deactivation, suggesting the involvement of one or both of these phosphatases in the dephosphorylation of CaM kinase II in βTC3-cells (Figure 3A).

Evidence for the involvement of a Mg^{2+}-dependent protein phosphatase in the deactivation of CaM kinase II

The inability of okadaic acid or calyculin A to completely prevent CaM kinase II deactivation in 2 min suggested the involvement of a further phosphatase activity insensitive to these

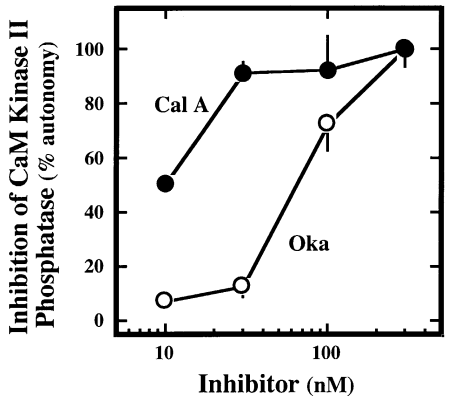

Figure 4 Preferential inhibition of CaM kinase II phosphatase by calyculin A relative to okadaic acid in permeabilized βTC3 cells

Permeabilized βTC3 cells stimulated by 10 μM Ca^{2+} were returned to a Mg^{2+}-free buffer containing 0.05 μM Ca^{2+}, 10 mM EDTA and varying concentrations (10–300 nM) of calyculin A (●) or okadaic acid (○). The incubation was continued at 37 °C for 2 min and the CaM kinase II activation state determined. Data are represented as means ± S.E.M. from 3–4 independent determinations.

compounds. Within the broad classification of phosphatases initially proposed by Ingebritsen and Cohen [21], enzymes remaining include PP-2B (calcineurin) or PP-2C. PP-2B is a Ca^{2+}-dependent phosphatase that is potently inhibited by cyclosporin A or FK-506 through the interaction of specific immunophilins [22]. PP-2C is absolutely dependent on Mg^{2+} for activity [23]. The inclusion of FK-506 at a concentration previously demonstrated to effectively inhibit PP-2B (20 nM) [24] failed to influence the deactivation of CaM kinase II. At 15 and 30 s, CaM kinase II activation had decreased to 15.6 % and 10.18 % autonomy in control cells and 15.53 % and 10.27 % autonomy in the presence of FK-506, respectively. These data suggest that PP-2B is not involved in the deactivation of CaM kinase II in the pancreatic β-cell; in fact there is, to the authors' knowledge, no evidence that CaM kinase II is a substrate for PP-2B in any cell type. In contrast, the rate of CaM kinase II deactivation was significantly lowered, relative to control cells, when activated cells were placed in a buffer in which Mg^{2+} had been chelated by the inclusion of 10 mM EDTA (Figure 3B). Compared with control cells in which 77 % and 83 % deactivation was evident at 15 s and 30 s respectively after the removal of Ca^{2+}, 32 % and 49 % deactivation was observed at equivalent time points in Mg^{2+}-free buffer. Again, CaM kinase II deactivation was only retarded and not prevented, since by 2 min virtually complete deactivation (> 95 %) of CaM kinase II was observed, independent of the presence or absence of EDTA. These latter data suggest therefore the partial involvement of a Mg^{2+}-dependent protein phosphatase, such as PP-2C, in the dephosphorylation of activated CaM kinase II.

Strikingly, the addition of okadaic acid (1 μM) facilitated markedly the ability of Mg^{2+}-free buffer to slow the deactivation of CaM kinase II (Figure 3B). Under these conditions, the fraction of CaM kinase II in the autonomous state was determined to be 51.28 ± 5.39 % (means ± S.E.M., $n = 3$–4) and 37.57 ± 0.96 % at 0 and 2 min, respectively. Only under these conditions therefore is the deactivation of CaM kinase II prevented to any great extent at 2 min. The 28 % decrease in enzyme activation could be the result of incomplete inhibition of phosphatase activity, or due to thermal instability of the autophosphorylated enzyme [5,25]. Predictably, the combined presence of EDTA and okadaic acid induced the marked activation of CaM kinase II in basal incubation buffer containing 0.05 μM Ca^{2+} (23.1 ± 8.00 % autonomy relative to 2.95 ± 0.35 % for control), that was greater than either agent alone (6.36 ± 1.60 % and 5.89 ± 0.85 % for EDTA and okadaic acid, respectively). These data suggest: (i) that both okadaic-acid-sensitive and Mg^{2+}-dependent protein phosphatase activities govern the deactivation of CaM kinase II in permeabilized β-cells; and (ii) that both phosphatases are required for the maintenance of basal activation levels of this enzyme.

Identification of okadaic-acid-sensitive CaM kinase II phosphatase

It has been previously demonstrated that the comparison of the effectiveness of okadaic acid and calyculin A to inhibit a dephosphorylation event can be used to differentiate between the involvement of PP-1 and PP-2A [26]. Thus whereas calyculin A has a similar affinity towards PP-1 and PP-2A (IC_{50} of 0.5–2 nM and 0.1–1 nM respectively), okadaic acid is a more potent inhibitor of PP-2A (IC_{50} < 0.1 nM) than PP-1 (IC_{50} 100 nM) [26]. In the absence of EDTA, calyculin A and okadaic acid, both at a concentration of 1 μM, had identical effects to slow the rate of CaM kinase II deactivation (Figure 3A). However, using the optimal conditions established in Figure 3(B) in which CaM kinase II deactivation was markedly inhibited by the combined presence of EDTA and okadaic acid, it was established that enzyme dephosphorylation was differentially sensitive to low concentrations of okadaic acid and calyculin A (Figure 4). Whereas these compounds at 300 nM were equally effective at inhibiting CaM kinase II phosphatase, lower concentrations revealed a greater sensitivity of this phosphatase to calyculin A. IC_{50} values for the CaM kinase II phosphatase were estimated to be 60–70 nM and < 10 nM for okadaic acid and calyculin A respectively, closely reflecting the published sensitivity profile of PP-1 [26].

DISCUSSION

Diverse phosphatases, including purified preparations of PP-2C [25] and the catalytic subunits of PP-1 [3] and PP-2A [18], effectively dephosphorylate the primary autophosphorylation site (Thr-286) responsible for the generation of Ca^{2+}-independent activity of CaM kinase IIα *in vitro*. This may not, however, reflect the regulation of enzyme deactivation in intact cells, where the action of catalytic phosphatase subunits towards phosphosubstrates is likely to be governed by their association with regulatory and/or targeting subunits anchored to specific regions in the cell [27,28]. Within this scheme, the cellular location of CaM kinase II could determine the identity of the phosphatase required for its deactivation. Evidence for such regulation is provided from studies in the brain, which have demonstrated that soluble CaM kinase II is dephosphorylated primarily by PP-2A [17,29], whereas the high concentration of particulate enzyme in the synaptic junction is dephosphorylated principally by PP-1 [17,30].

The principal observation from this study was that the dephosphorylation and deactivation of CaM kinase II in the permeabilized βTC3 cell was mediated by the combined action of okadaic acid-/calyculin A-sensitive and Mg^{2+}-dependent protein phosphatase activities. The characteristics of this response were remarkably similar to those observed previously in cerebellar granular cells [25,31]. For example, in intact cerebellar cells the reversal of CaM kinase II activation following stimulation with depolarizing concentrations of potassium was not completely inhibited by high concentrations of okadaic acid [31]. Fur-

thermore, prevention of CaM kinase II deactivation by extracts of cerebellar cells *in vitro* was only achieved in the combined presence of okadaic acid and concentrations of EDTA sufficient to chelate free Mg^{2+} [25].

The preferential sensitivity of CaM kinase II phosphatase to calyculin A over okadaic acid closely reflected the profile reported for PP-1 *in vitro* [19,26]. The suggestion that PP-1 is a prominent CaM kinase II phosphatase in the pancreatic β-cell would be consistent with two previously reported regulatory schemes in other cells. First, PP-1 appears to be a major phosphatase that targets CaM kinase II following autophosphorylation-dependent translocation to the postsynaptic density of rat forebrain subcellular fractions [17,32]. Secondly, PP-1 was reported as a major CaM kinase II phosphatase, on the basis of a preferential ability of calyculin A over okadaic acid to potentiate cholecystokinin-induced activation of this enzyme in rat pancreatic acinar cells [33]. However, the possibility that novel okadaic-acid-sensitive phosphatases other than PP-1 recently described [34,35] are involved in this process cannot be eliminated.

The partial inhibition of CaM kinase II phosphatase by EDTA immediately implicates the involvement of the Mg^{2+}-dependent phosphatase, PP-2C, in the deactivation of this enzyme in the β-cell. This suggestion is supported by the proven ability of recombinant PP-2C to reverse autophosphorylation and activation of CaM kinase II α/β through the dephosphorylation of Thr-286/Thr-287, as analysed by phosphopeptide-mapping analysis [25]. However, in the absence of specific inhibitors of PP-2C, it is difficult to assess the extent to which the effect of EDTA on CaM kinase II activation in the permeabilized β-cell reflects the inhibition of PP-2C, and not the perturbation of other cellular responses. The evaluation of the potential involvement of this phosphatase in this process awaits more elaborate and definitive experimental approaches that are beyond the scope of this initial study.

Although similar to studies *in vitro*, the interaction of PP-1 and Mg^{2+}-dependent phosphatases in the deactivation of CaM kinase II in the β-cells is not clear. For example, although the effects of okadaic acid and EDTA to prevent enzyme deactivation at 15 s appear additive, a more complex interaction appears to exist after longer periods of time (i.e. 2 min) where the effects appear to be synergistic. These data do not support a simple model of a limited action of distinct phosphatases on the same phosphorylation site; indeed, such a mechanism would not be expected physiologically. Rather, these data may suggest the existence of an important regulatory interaction between PP-1 and the Mg^{2+}-dependent phosphatase component which could, for example, be mediated through the dephosphorylation of interacting sites on CaM kinase II. CaM kinase II is known to be phosphorylated at multiple sites in either basal [36] or stimulated conditions [32,37] but the significance of this to enzyme regulation is not fully understood. An alternative explanation, however, is that this interaction reflects the involvement of a PP-1 species whose regulation incorporates a dependence on Mg^{2+}. Whereas a Mg^{2+}/ATP-dependent PP-1 has been characterized [38] whose activation is regulated by both the phosphatase modulator, inhibitor-2, and a Mg^{2+}-dependent auto-dephosphorylation step (by PP-1, reviewed in [39]), no relevant physiological substrate has been ascribed to this phosphatase.

The process of insulin secretion appears to be critically dependent on endogenous β-cell protein phosphorylation/dephosphorylation [40] and has stimulated numerous studies to examine the effect of phosphatase inhibitors on this process. Okadaic acid [41,42] has been shown to inhibit secretagogue-induced insulin secretion, although the specificity of this effect is questioned, on the basis of a similar inhibitory effect elicited by the non-active analogue, 1-nor-okadone [43]. Calyculin A has an effect similar to that of okadaic acid, albeit more potent, in the inhibition of glucose- (and glyceraldehyde-)induced insulin secretion from isolated rat islets [41]. It is noteworthy, however, that in electrically permeabilized islets, high concentrations of okadaic acid, but not of 1-nor-okadone, had the opposite effect to enhance basal (i.e. in 50 nM Ca^{2+}) and cAMP-induced insulin secretion [43]. The relationship of these observations to both the current study and others [44] demonstrating tendency of okadaic acid to activate CaM kinase II is, however, not clear. Whereas a functional role of CaM kinase II in insulin secretion is still to be substantiated, this study identifies this enzyme as a target of an okadaic acid-/calyculin A-sensitive protein phosphatase. The further regulation of this process by Mg^{2+} may be related to the ability of this cation to modulate insulin secretion [45,46].

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