Targeted Phosphorylation of Inositol 1,4,5-Trisphosphate Receptors Selectively Inhibits Localized Ca\(^{2+}\) Release and Shapes Oscillatory Ca\(^{2+}\) Signals*

David R. Giovannucci‡§, Guy E. Groblewski‡, James Sneyd, and David I. Yule‡

From the 1Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, New York 14642, the 1Department of Nutritional Science, University of Wisconsin, Madison, Wisconsin 53706, and the Institute of Informational and Mathematical Sciences, Massey University, Auckland 1012, New Zealand

The current study provides biochemical and functional evidence that the targeting of protein kinase A (PKA) to sites of localized Ca\(^{2+}\) release confers rapid, specific phosphoregulation of Ca\(^{2+}\) signaling in pancreatic acinar cells. Regulatory control of Ca\(^{2+}\) release by PKA-dependent phosphorylation of inositol 1,4,5-trisphosphate (InsP\(_3\)) receptors was investigated by monitoring Ca\(^{2+}\) dynamics in pancreatic acinar cells evoked by the flash photolysis of caged InsP\(_3\) prior to and following PKA activation. Ca\(^{2+}\) dynamics were imaged with high temporal resolution by digital imaging and electrophysiological methods. The whole cell patch clamp technique was used to introduce caged compounds and to record the activity of a Ca\(^{2+}\)-activated Cl\(^{-}\) current. Photolysis of low concentrations of caged InsP\(_3\) evoked Cl\(^{-}\) currents that were inhibited by treatment with dibutyryl-cAMP or forskolin. In contrast, PKA activators had no significant inhibitory effect on the activation of Cl\(^{-}\) current evoked by uncaging Ca\(^{2+}\) or by the photolytic release of higher concentrations of InsP\(_3\). Treatment with Rp-adenosine-3′,5′-cyclic monophosphorothioate, a selective inhibitor of PKA, or with H\(_8\)31, a peptide known to disrupt the targeting of PKA, largely abolished forskolin-induced inhibition of Ca\(^{2+}\) release. Further evidence for the targeting of PKA to the sites of Ca\(^{2+}\) mobilization was revealed using immunocytochemical methods demonstrating that the R\(_{116}\) subunit of PKA was localized to the apical regions of acinar cells and co-immunoprecipitated with the type III but not the type I or type II InsP\(_3\) receptors. Finally, we demonstrate that the pattern of signaling evoked by acetylcholine can be converted to one that is more "CCK-like" by raising cAMP levels. Our data provide a simple mechanism by which distinct oscillatory Ca\(^{2+}\) patterns can be shaped.

Hormone-, neurotransmitter-, or growth factor-evoked increases in intracellular calcium exert control over a vast array of cellular functions, including secretion and gene expression (1–3). The regulatory control governing the fidelity and specificity of these processes can be encoded by the amplitude, frequency, or localization of cytosolic calcium signals (Δ[Ca\(^{2+}\)]\(_{i}\)) (4–6). It is well established that Δ[Ca\(^{2+}\)]\(_{i}\) in nonexcitable cells is generally induced by InsP\(_3\) production, following activation of G\(_q\) proteins coupled to phospholipase C\(_{\beta}\) (7, 8). Many cell types express multiple distinct receptors coupled to this general transduction pathway. It is, however, not understood how the selective activation of distinct receptors that utilize the same general intracellular signaling system, such as the G\(_q\)-coupled formation of InsP\(_3\), can generate agonist-specific Δ[Ca\(^{2+}\)]\(_{i}\). (9–12). Pancreatic acinar cells represent an ideal cell model for investigating this phenomenon because despite the activation of a common Ca\(^{2+}\) release pathway by agonists, very different patterns of Δ[Ca\(^{2+}\)]\(_{i}\) emerge. Stimulation of cells with acetylcholine (ACh) or cholecystokinin (CCK) results in Δ[Ca\(^{2+}\)]\(_{i}\) that differ in spike frequency, level of baseline spiking, and local sites of initiation (11–14).

In a previous study, we showed that the acinar cell type III InsP\(_3\)-R is rapidly and selectively phosphorylated by cAMP-dependent kinase A (PKA) following stimulation with physiological levels of CCK but not by ACh (15). This observation is consistent with data in the literature suggesting that CCK but not muscarinic stimulation results in coupling to G\(_q\), in addition to G\(_q\) (16). Thus, in this study we have investigated the possibility that selective phosphoryregulation of the InsP\(_3\)-R by PKA contributes to the shaping of cytosolic Ca\(^{2+}\) signals.

**EXPERIMENTAL PROCEDURES**

Isolation of Mouse Pancreatic Acinar Cells—Single acinar cells or small two- to three-cell clusters were prepared by a standard collagenase-digestion of pancreata from wild-type C57Bl/6 mice. Briefly, 25-g mice were sacrificed in accordance with National Institutes of Health policy and established protocol with the Division of Laboratory Animal Medicine, University of Rochester following CO\(_2\) gas asphyxiation and then cervical dislocation. The pancreas was removed, and the capsule was injected with Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 30 μg/ml collagenase (Sigma), 1000 units Purified collagenase (Warington), and 1 mg/ml soybean trypsin inhibitor. Following 20–30 min of digestion at 37 °C, the pancreas was triturated through a 10-ml Falcon pipette tip, passed through a 100-μm nylon mesh, and washed with Dulbecco’s modified Eagle’s medium containing 1% bovine serum albumin (Sigma), and isolated cells and small acini were collected by centrifugation at 100 × g for 3 min. Cell were plated onto poly-l-lysine-coated glass coverslips and allowed to adhere for 5 min prior to application of recording solution perifusate.

Electrophysiology—Ionic currents were recorded at a sampling rate of 1 kHz using an Axopatch 200a patch clamp amplifier, Instrutech

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‡ To whom correspondence should be addressed. E-mail: david_giovannucci@urmc.rochester.edu.

The abbreviations used are: [Ca\(^{2+}\)]\(_{i}\), cytosolic calcium concentration; NPE-caged InsP\(_3\), D-mylo-inositol 1,4,5-trisphosphate; P\(_{4,5,6,7}\)(2-nitropheryl)-ethyl ester; CCh, carbamylcholine, carbachol; CCK, cholecystokinin; NP-BGTA, ß-nitropheryl ethylenediaminetetraacetic; dbcAMP, dibutyryl cyclic adenosine monophosphate; InsP\(_3\), inositol 1,4,5-trisphosphate; InsP\(_3\)-R, InsP\(_3\) receptor(s); PKA, protein kinase A; Rp-cAMPS, Rp-adenosine-3′,5′-cyclic monophosphorothioate; ACh, acetylcholine.

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Digital interface, and IGOR PRO/Pulse Control XOP software. The standard intracellular recording solution contained 140 mM KCl, 10 mM HEPES-KOH, 1.13 mM MgCl₂, 2 mM sodium ATP, 1 mM n-hydroxymethylene-diaminetetracetic acid, and 0.001–0.1 mM myo-inositol, 1,4,5-trisphosphate, P₃NO₃, 1/2-nitrophenyl-ethyl ester (NPE-caged InsP₃), pH 7.2. The intracellular recording solution for the photolytic release of caged Ca²⁺ contained 130 mM KCl, 10 mM HEPES-KOH, 10 mM n-nitrophenyl EGTA, 5 mM CaCl₂, 2 mM magnesium ATP, 1.2 mM MgCl₂, pH 7.3. Under whole cell conditions, resting [Ca²⁺], and free [Mg²⁺] were estimated at 175 nM and 1 mM, respectively. Intervals of 4 min were maintained following patch rupture prior to and between stimuli to allow for sufficient equilibration with the patch pipette solution. The extracellular solution contained 140 mM NaCl, 10 mM HEPES-NaOH, 10 mM d-glucose, 4.7 mM KCl, 1.13 mM MgCl₂, 1 mM CaCl₂, pH 7.3.

Flash Photolysis and Digital Imaging—Photolytic release was performed using a pulsed Xenon arc lamp (T.I.L.L. Photonics) and fiber optic guide fed to a dual port epifluorescence condenser attached to a Nikon DM400 dichroic mirror and Nikon 40×/1.3 oil immersion objective. Images were acquired at 79-ms intervals and displayed using the ECL detection system exposed on ECL Hyperfilm (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

Threshold level InsP₃-evoked Ca²⁺ Release Is Inhibited by PKA Activation—To date, no consensus exists in the literature as to the physiological consequence of phosphorylation of individual InsP₃R subtypes (19–23). Thus, we investigated the PKA-dependent regulation of Ca²⁺ release in isolated mouse pancreatic acinar cells using the controlled release of photoactivatable (caged) InsP₃ and selective pharmacological activators and inhibitors of PKA function. Whole cell patch clamp methods were used to measure Ca²⁺-activated ionic current evoked by the photolysis of caged compounds. This current has been previously established to faithfully report [Ca²⁺]ᵢ as to the physiological consequence of phosphorylation of individual InsP₃R subtypes.

As shown in Fig. 1 (A and C), high intensity UV light flash photolysis of a reproducible portion of 1–100 μM NPE-caged InsP₃ introduced via diffusional equilibration with the patch pipette solution, could repetitively evoke a Ca²⁺-activated current in a concentration-dependent manner. This indicated that prior exposure to Ca²⁺ had little effect on amplitude or kinetics at 10,000 × g. The supernatant was assayed for protein concentration. Samples of equal protein concentration were then incubated with anti-serum overnight at 4 °C. Immunoprecipitation of individual InsP₃R subtypes was performed with excess antiserum (17). (No InsP₃R was detectable in the lysate after addition of protein A.) Immobilized protein A beads (Pierce) were added to each sample. As a control, samples were processed with no cellular lysate or with no immunoprecipitating anti-serum to ascertain specific bands on the immunoblot. After rotating the samples for 2 h, the samples were microcentrifuged, and the supernatant was discarded. The beads were washed four times with lysis buffer, suspended in SDS-polyacrylamide gel electrophoresis sample buffer, and boiled. The proteins were then separated by SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose prior to immunoblotting (Schleicher & Schuell). Immuneoactivity was visualized using peroxidase-conjugated secondary antibodies followed by detection using the ECL detection system exposed on ECL Hyperfilm (Amersham Pharmacia Biotech).

Immunocytochemistry—Subcellular localization was performed using standard immunohistochemical methods in isolated acini fixed with 2% paraformaldehyde as described previously (18). A Noran/Oz laser scanning confocal microscope was used to visualize the distribution of immunofluorescence.

**FIG. 1.** PKA activation inhibits Ca²⁺ release. A, application of 60 μM dbcAMP reversibly inhibited the Ca²⁺-activated ionic current evoked by low doses of NPE-caged InsP₃ in whole cell patch clamped mouse acinar cells. The traces represent recordings from single or two- or three-cell clusters that were voltage clamped at a holding potential (Hₒ) of −30 mV at 25 °C. No significant changes in current were activated by flash photolysis when Hₒ was 0 mV (the reversal potential of both Ca²⁺-activated currents) or when caged compounds were omitted from the internal solution. B, the averaged data comparing the percentage of inhibition by dbcAMP of the ionic currents evoked by flash photolysis in cells loaded with different amounts of NPE-caged InsP₃. The percentage of inhibition was determined by comparing peak current amplitude prior to and following dbcAMP treatment. The number of cells for each data set and statistical significance (p < 0.01) is indicated (*asterisk). C, repetitive photolytic release of InsP₃ had no effect on peak amplitude or kinetics of current transients induced prior to and following treatment with dbcAMP.
els of Ca$^{2+}$ are generally within a physiological range evoked apically by InsP$_3$ (1–10 μM) (29) and at which the Cl$^-$ current is not likely to be saturated (29–31). It is generally believed that, unlike CFTR or voltage-activated Cl$^-$ channels, cAMP does not directly modulate the Ca$^{2+}$-activated Cl$^-$ channel (32–34). Furthermore, we observed that PKA activation was found to directly inhibit InsP$_3$-evoked Δ[Ca$^{2+}$]$_i$ in a permeabilized mouse pancreatic acinar cell preparation, supporting our contention that the reduction in the Ca$^{2+}$ signal largely resulted from a decreased Ca$^{2+}$ release rather than an effect on the Ca$^{2+}$-activated Cl$^-$ channel or on Ca$^{2+}$ clearance (data not shown).

A reduction of the current activated by low levels of stimulation indicated that a subset of InsP$_3$R that exhibit the highest sensitivity for InsP$_3$ is preferentially modulated by dbcAMP. Because the pancreatic acinar cell expresses all isoforms of receptor together with heterotetrameric forms (35), it is difficult to categorically identify this high sensitivity receptor. The relative contribution of an InsP$_3$R type to the initial signal is presumably determined by several factors including the relative abundance of the particular receptor, the affinity of the receptor for InsP$_3$ and susceptibility to phosphoregulation by PKA. Because the type II receptor is a poor substrate for PKA phosphorylation (23), it would seem unlikely that this receptor contributes markedly to threshold level Ca$^{2+}$ release and its modulation by PKA. In contrast, the type I receptor is a good substrate for phosphorylation by PKA and has been reported to have the highest affinity for InsP$_3$ but is expressed at very low levels in pancreatic acinar cells (17). The type III receptor has been shown to be an efficient substrate for PKA-mediated phosphorylation (15). In addition, the only quantitative study of receptor number in the exocrine pancreas has shown that the type III receptor is the most abundant receptor form (17) and, thus, by mass action would presumably contribute significantly to the initial Ca$^{2+}$ release. In support of this contention, the type III receptor has also been proposed to serve as an initial trigger for Ca$^{2+}$ release (36). Furthermore, the open probability of the receptor is modulated very steeply by near resting [Ca$^{2+}$] i (37). It should, however, be noted that the type III receptor has been reported, when studied in isolation, to have the lowest affinity for InsP$_3$ (23). These data together with the body of work in the literature indicate that the type III is a good candidate for the initial, PKA-modulated Ca$^{2+}$ signal. A contributing role for the type I receptor alone or in heterologous association with the type III receptor, however, cannot be excluded.

Because Ca$^{2+}$ spikes induced by low levels of ACh have been shown to initiate at specialized, InsP$_3$R-rich sites (trigger zones) tightly associated with the luminal borders and are often confined to the luminal pole (18, 29, 38, 39), we postulated that the effect of dbcAMP on Ca$^{2+}$ release would also be manifested in this region. To test this hypothesis, we sought to selectively activate Ca$^{2+}$ release at the acinar cell trigger zone. Thus, rather than using a high intensity flash discharge, we applied a continuous, low level photolytic strobe stimulus (indicated as I in the Fig. 3) to cells loaded with caged InsP$_3$ to achieve threshold activation levels of InsP$_3$. In most cases it was necessary to first determine empirically the concentration of caged InsP$_3$ that was needed to evoke threshold activation. As shown in Fig. 3, fluorescence digital imaging methods confirmed that this stimulation paradigm preferentially induced Ca$^{2+}$ rises that were initiated and largely maintained in the luminal pole, characteristic of threshold Ca$^{2+}$ release. Simultaneous patch clamp measurements revealed the activation of irregular current spikes that mirrored the evoked apical Δ[Ca$^{2+}$]$_i$. As expected, a subsequent, high intensity UV flash
discharge (indicate as II in the Fig. 3) evoked a global Ca\textsuperscript{2+} rise that initiated in and remain confined to the luminal pole. A, simultaneous measurements of the Ca\textsuperscript{2+}-activated current spikes (lower trace) and the luminal (black) or basal (red) Δ[Ca\textsuperscript{2+}], in a patch clamped acinar cell loaded with 75 μM OGB-2. Ca\textsuperscript{2+} release was activated by low intensity, strobe-induced photolysis of threshold amounts of InsP\textsubscript{3} (dashed line) or by high intensity flash discharge (arrow). B, a corresponding series of fluorescence images from the same cell as in A show the spatial distribution of the Ca\textsuperscript{2+} signal at various time points. Images were obtained at 79-ms intervals and are displayed sequentially as every second (first row), fifth (second row), or 25th image frame (third row) during low intensity photolysis (I). The last row shows the Δ[Ca\textsuperscript{2+}] evoked by high intensity flash photolysis (II) at sequential 79-ms intervals. The inset shows the transmitted light image of the cell doublet and placement of the regions of interest for measurement of luminal (black box) or basal (red box) Δ[Ca\textsuperscript{2+}].

To investigate the inhibitory effect of cAMP on the high sensitivity InsP\textsubscript{3}R subset, cAMP levels were increased by treatment with 10 μM forskolin, and the Ca\textsuperscript{2+}-activated current spike activity was evoked by 15–30 s of a continuous UV strobe. To accurately quantify the complex nature of the Ca\textsuperscript{2+} release events evoked by continuous strobe prior to and following forskolin application for 3–5 min, the time integral of the current produced was determined. As shown in Fig. 4A, forskolin treatment reversibly inhibited Ca\textsuperscript{2+} release at the trigger zone. Following wash-off of forskolin, current responses returned to control values within 5–20 min. Forskolin treatment induced nearly 83% inhibition of threshold Ca\textsuperscript{2+} release (Fig. 4B). Current integrals were, on average, reduced to 16.4 ± 9% of control values by forskolin treatment (p = 0.0003, n = 10). Removal of forskolin restored the current integrals over 5–20 min to a level not significantly different from control values (150.7 ± 23%). Next, we tested whether PKA activation was required for the inhibitory effect of cAMP. As shown in Fig. 4 (C and D), inclusion of a specific, competitive PKA inhibitor, Rp-adenosine-3',5'-cyclic monophosphorothioate (Rp-cAMPS) (334 μM), in the patch pipette solution largely abrogated the forskolin-induced inhibition of spike activity, and the current integrals following forskolin treatment were not significantly different from control values (107 ± 7 and 124 ± 27%, respectively, n = 5). Taken together, these data strongly indicate that intracellular rises in cAMP negatively modulate InsP\textsubscript{3} induced Ca\textsuperscript{2+} release via PKA-dependent phosphoregulation of a specialized subset of InsP\textsubscript{3}R localized at the acinar cell apical pole.

**PKA Is Functionally Targeted to the Type III InsP\textsubscript{3} Receptor**—Because forskolin treatment likely induces a spatially uniform rise in cAMP, we suggest that signaling specificity is
achieved not in the production of cAMP but in the compartmentalization of its effector, PKA. This co-localization has been shown by others to lead to rapid and efficient phosphorylation of a specific substrate, even on a uniform background of cAMP levels (40–47). Given the high degree of functional and structural organization exhibited by these polarized cells, we hypothesized that PKA might be targeted to the trigger zone to provide spatiotemporal control over the phosphoregulation of Ca\textsuperscript{2+} release. PKA targeting is often achieved by the association of the regulatory R subunit dimer with an protein kinase A-anchoring protein bearing a specific subcellular localization signal (46, 48). Immunohistochemical methods were performed to visualize the subcellular distribution of PKA in acinar cells using RII\textsubscript{a} and RII\textsubscript{b} subunit-specific monoclonal antibodies. The RII\textsubscript{a} subunit exhibited diffuse cytosolic distribution (not shown). In contrast, the RII\textsubscript{b} subunit displayed a pattern of labeling that was confined to the apical and granular region of the cells (Fig. 5). This pattern of staining overlaps and is consistent with localization of PKA to the sites of InsP\textsubscript{3}-induced Ca\textsuperscript{2+} release. It is within this region that the majority of InsP\textsubscript{3}R co-localize with a highly structured sublumenal actin web (18, 39, 49).

To determine whether RII subunits form molecular associations with type III InsP\textsubscript{3}R, we immunoprecipitated proteins from acinar cell extracts using a monoclonal antibody to the type III InsP\textsubscript{3}R. The enriched proteins were separated, and the Western blots were probed with actin, RII\textsubscript{a} and RII\textsubscript{b} subunit-specific monoclonal antibodies. As shown in Fig. 6A, actin and both RII subunits immunoprecipitated with the type III InsP\textsubscript{3}R, suggesting that PKA is targeted to its potential kinase substrate, either directly or by a common tethering mechanism, possibly through actin or actin-binding proteins. To demonstrate specificity for the immunoprecipitation of PKA, ly.

**Fig. 4. Ca\textsuperscript{2+}-activated current spikes are inhibited by PKA activation.** A, application of 10 \mu M forskolin for 3–5 min (solid line) induces the reversible inhibition of current spikes evoked by continuous low intensity UV strobe discharge (arrow and dashed line) in a single acinar cell. Breaks in the record indicate 3–5-min intervals between stimuli. Because current profiles evoked by 15–30 s of continuous strobe discharge exhibited complex kinetics, current integrals were used to quantify the changes in whole cell current. B, a comparison of the of the time-integrated current spikes induced prior to and following treatment with forskolin in cells loaded 1–10 \mu M NPE-caged InsP\textsubscript{3}. C, inclusion of 334 \mu M Rp-cAMPS in the patch pipette solution effectively blocked the forskolin-induced inhibition of current spikes evoked by low level photolytic release of InsP\textsubscript{3}. D, average current integrals measured during treatment with and following removal of 10 \mu M forskolin in the presence of Rp-cAMPS were not significantly different from control values. Number of cells for each data set and significance (asterisk) are indicated.

**Fig. 5. The type III InsP\textsubscript{3}R and PKA co-localize.** A confocal micrograph of small acinar cell clusters showing that the RII\textsubscript{b} subunit of PKA is localized to the luminal secretory poles of the cells (arrowheads) but not to the basal or perinuclear regions (large arrows). No staining was evident when primary antibody was omitted.
lanes 2 and 3). To investigate whether PKA was associated selectively with any particular InsP3R type, individual receptors were immunoprecipitated from pancreatic lysates using subtype-specific antiserum (17). Probing of separated proteins with the antiserum used to immunoprecipitate confirmed that individual InsP3R types had been immunoprecipitated (Fig. 6C, upper panel). In lane A, specific polyclonal antiserum raised against the C terminus of type I receptor were used (17, 18). In lane B, a polyclonal antiserum versus the type II receptor (17, 18), and in lane C, a monoclonal antibodies specific for type III receptor were utilized. Each antiserum was utilized in excess and is known to effectively and selectively immunoprecipitate its respective receptor (17, 18). In the lower panel, the same immune complexes were probed with antiserum raised against PKA-RIIβ. Only samples immunoprecipitated with type III InsP3R contained PKA-RIIβ. These data strongly suggest that PKA is targeted specifically to type III receptors and, thus, is ideally placed to mediate rapid, specific phosphorylation of this receptor.

To test the functional significance of type III InsP3R-PKA co-localization, we disrupted PKA targeting in patch clamped acinar cells using a synthetic peptide containing the functional RII binding motif of the thyroid protein kinase A-anchoring protein, Ht31. The Ht31 peptide has been shown to be an efficient and specific inhibitor of RII protein kinase A-anchoring protein interactions (46, 48). Fig. 7A shows that inclusion of 30 μM Ht31 in the pipette solution could block the forskolin-induced inhibition of the apical Ca2+ release. As shown in Fig. 7B, no statistically significant inhibition was observed following continuous treatment with forskolin for 5 or 10 min or following removal of forskolin, compared with control responses (76.8 ± 15, 49.5 ± 27, and 139 ± 30% of control, respectively; n = 9).

Functional Relevance of PKA Activation to the Shaping of Oscillatory Ca2+ Signatures—Although our experiments provide evidence that the targeted phosphorylation of a subset of InsP3R exerts negative regulatory control over local Ca2+ release events, we wondered whether this modulation could account for the distinctive patterns of Ca2+ oscillations induced by CCK or carbachol (CCh). Application of physiological doses of ACh or CCh to pancreatic acinar cells generates sinusoidal [Ca2+] oscillations with frequencies of 4–6/min that are superimposed on an elevated base line (11, 12). In contrast, CCK application induces oscillations consisting of base-line spikes of much longer period (1–2/min) (12, 50). To address this relationship, we tested whether PKA-dependent phosphorylation could convert CCh-induced oscillations into a pattern that resembled CCK-induced oscillations. Intact acinar cells were treated with low doses of either CCh or CCK to induce [Ca2+] oscillations and the Δ[Ca2+] monitored with the Ca2+-sensitive dye, fura-2, prior to and following application of 60 μM dbcAMP. As shown in the representative trace in Fig. 8A, introduction of dbcAMP reduced both spike amplitude and the plateau component (defined as the average intra-spike level) and slowed the frequency of [Ca2+] oscillations, suggesting that activation of PKA was sufficient to shift the CCh-induced pattern of oscillations to one that was more “CCK-like”. As expected, dbcAMP...
had little effect on CCK-induced $[\text{Ca}^{2+}]_c$ oscillations (Fig. 8B), where presumably the InsP$_3$R is already in a phosphorylated state. Analysis of the oscillation frequencies and plateau levels of $\Delta[\text{Ca}^{2+}]$, induced by these agonists are shown in Fig. 8 (C and D).

Numerous factors have been proposed to provide a mechanism underlying agonist-specific $[\text{Ca}^{2+}]_c$ oscillations observed in pancreatic acinar cells (51, 52). These include the utilization by CCK receptor signaling of alternative second messengers such as cyclic adenosine diphosphate ribose and nicotinic acid adenine dinucleotide phosphate. Although no data are available regarding the ability of agonists to stimulate levels of these putative messengers, significant modulation of $[\text{Ca}^{2+}]_c$ signaling has been reported (25, 53). It is clear that if the levels of these molecules are modulated by agonist stimulation, it must be through $\text{G}_{\text{q}}$ activation, because both CCK and muscarinic signaling are abolished by maneuvers antagonizing $\text{G}_{\text{q}}$ function (54, 55). Recent reports have also suggested that pancreatic secretagogue receptors interact with differing RGS proteins to play a role in modulating signaling events (56, 57).

$\text{G}_{\text{q}}$ modulation of $[\text{Ca}^{2+}]_c$ production at the level of the cell surface receptors would be expected to have significant effects on the generation of $[\text{Ca}^{2+}]_c$ signals. Although the data presented in this study do not preclude any of the aforementioned proposals, selective modulation of the initial $\Delta[\text{Ca}^{2+}]$ by PKA appears to contribute significantly to shaping the $[\text{Ca}^{2+}]_c$ signal.

We conclude that targeted, PKA-mediated phosphorylation specifically controls the functioning of a discrete subset of intracellular $[\text{Ca}^{2+}]$ release channels that act as the initial trigger for InsP$_3$-induced $[\text{Ca}^{2+}]$ release. Our data suggest that the type III InsP$_3$R, abundant in the apical trigger zone, fulfills this role. We contend that the negative allosteric modulation of the type III InsP$_3$R contributes significantly to the characteristic $[\text{Ca}^{2+}]_c$ oscillations induced by CCK. Given that most cells have surface receptors that can couple to multiple G protein families (58, 59), cross-talk between divergent signaling pathways to modulate $[\text{Ca}^{2+}]_c$ signaling at the level of the Ca$^{2+}$ release channel is likely to be a widespread and important mechanism by which information can be encoded for the selective activation of distinct physiological end points.

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Fig. 8. Treatment with 60 µM dbcAMP alters carbachol- but not CCK-induced $[\text{Ca}^{2+}]_c$ oscillations in intact acinar cells. Cells were loaded with 2 µM fura-2 AM and monitored by digital imaging. Representative fluorescence ratio changes evoked by bath application of low doses of carbachol (A) or CCK (B) prior to and following application of dbcAMP. C, the frequency of carbachol-induced $[\text{Ca}^{2+}]_c$ oscillations was significantly reduced from 4.33 ± 0.36 oscillation/min to 2.47 ± 0.48 oscillation/min (p ≤ 0.003, n = 6) by dbcAMP, whereas the frequency of CCK-induced oscillations remained unchanged (0.99 ± 0.18 oscillation/min versus 1.0 ± 0.05 oscillation/min, n = 3). D, similarly, the elevated $[\text{Ca}^{2+}]_c$ plateau observed during carbachol treatment was diminished by application dbcAMP (0.836 ± 0.064 ratio units versus 0.652 ± 0.037 ratio units; p ≤ 0.003, n = 6), whereas there was no significant effect on CCK-induced responses (0.566 ± 0.017 ratio units versus 0.577 ± 0.035 ratio units, n = 3). The dashed line indicates averaged basal (resting) ratio values (0.517 ± 0.055) determined betwixt $[\text{Ca}^{2+}]_c$ oscillations during the treatment periods.
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