J Korean Med Sci 2000; 15(Suppl): S55-6
ISSN 1011-8934

Modulation of $InsP_3$ Receptor Properties by Phosphorylation: Targeting of PKA to $InsP_3$ Receptors Shapes Oscillatory Calcium Signals in Pancreatic Acinar Cells

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The major role of pancreatic acinar cells is to synthesize, store in granules and secrete by regulated exocytosis digestive enzymes. Secretion is stimulated physiologically, by the hormone cholecystokinin (CCK) and the neurotransmitter acetylcholine (ACh). A key event in acinar stimulus-secretion coupling is the elevation of intracellular calcium resulting from the action of the second-messenger inositol 1,4,5-trisphosphate ($InsP_3$), formed by the Gαq- activated hydrolysis of phosphatidylinositol 4,5,bisphosphate by phospholipase Cβ (1).

Stimulation by physiological concentrations of agonists results in characteristic oscillatory calcium signals, which originate in the apical region of the acinus and spread as a wave towards the basal pole. At the lowest concentration of agonists these signals often are confined to the initiation sites of release or "trigger-zones" (2, 3). Recent work has demonstrated that structural polarity of signaling elements underlie the characteristic calcium release in the apical region of the cell (4-6). Of paramount importance to this phenomenon is the expression of the vast majority of receptors for $InsP_3$ ($InsP_3R$) directly apposed to the apical plasma-membrane. Of note, however, despite activation of this common release pathway, stimulation with ACh or CCK results in distinct calcium signals, differing in oscillatory frequency, the baseline Ca^{2+} level between oscillatory cycles and in local sites of initiation (7, 8). Since it has been reported that CCK but not ACh- stimulation will activate Gαs (9) in addition to Gαq, we investigated the possibility that phosphoregulation of the $InsP_3R$ by PKA can account for these agonist-specific Ca^{2+} signatures and thus underlie the specificity and fidelity of agonist-stimulated calcium signaling observed in pancreatic acinar cells.

To demonstrate that the $InsP_3$ receptor undergoes phosphorylation, the type-III $InsP_3$ receptor was immunoprecipitated from ^{32}P metabolically labeled acinar cells, which had been stimulated with either CCK (10 pM-10 nM) or the muscarinic agonist carbachol (CCh) (100 nM-10 μM). After separation of immunoprecipitated proteins on 5% PAGE gels, the degree of phosphorylation of $InsP_3$ receptor was revealed by autoradiography and quantified by densitometry. Stimulation with CCK resulted in a rapid phosphorylation (<1 min to peak) of the type-III receptor, which could be detected at 10 pM and reached a peak at 100 pM CCK. It should be noted that the onset and maximum phosphorylation of the receptor achieved coincides with concentrations of CCK which can be demonstrated to induce calcium oscillations. In contrast to stimulation by CCK no significant phosphorylation of the receptor was observed at concentrations of CCh which induce oscillatory calcium signals (<1 μM). The CCK-induced phosphorylation could be mimicked by agents known to activate PKA and could be blocked by antagonists of this pathway such as H89 and rp-cAMP. The effect of phosphorylation of the $InsP_3$ receptor by PKA-activators was investigated using the controlled release of photoactivatable $InsP_3$ and monitoring the effects on calcium release. Activation of PKA with either cell permeable cAMP analogues or by forskolin rapidly and reversibly reduced or abolished $InsP_3$-induced Ca^{2+} release. Surprisingly, this effect was most pronounced at the lowest concentrations of $InsP_3$, which could be demonstrated by digital imaging to evoke increases in Ca^{2+} which were initiated and primarily restricted to the apical "trigger-zone" of the acinus. Thus phosphoregulation of $InsP_3$ receptors appears to occur to a subset of the most sensitive $InsP_3$ receptors in this region.

Given the high degree of functional and structural polarization of these cells we next investigated the pos-

Key Words: *Pancreatic Acinar Cells; Ca^{2+} Oscillations; Protein Kinase A; AKAP*

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sibility that PKA was localized to the trigger-zone to 'target' phosphorylation of the $InsP_3R$. PKA-localization is often achieved by association of the regulatory subunit dimer with a family of anchoring proteins termed A-kinase Anchoring Proteins, or AKAPs. These proteins contain specific sub-cellular localization sequences allowing the targeting of PKA to diverse sites such as the actin cytoskeleton, peroxisomes and the nucleus (10). This family of proteins has been demonstrated to be physiologically important in a wide variety of systems, including the modulation of voltage gated calcium channels in neurons (10). In support of this hypothesis, regulatory subunits of PKA could be shown to co-localize and co-immunoprecipitate with $InsP_3$ receptors. In addition, a peptide, known to disrupt the association of AKAP with PKA regulatory subunits, thus disrupting its localization, significantly attenuated the inhibition of apical Ca^{2+} release induced by $InsP_3$.

A mathematical model of the above data suggests that phosphorylation of the $InsP_3$ receptor could largely account for the differences in oscillatory signatures observed (11). In support of this model activation of PKA during ACh-induced Ca^{2+} oscillations transformed oscillations in to signals markedly more like those stimulated by CCK. No effect was seen on CCK-induced signaling, since presumably the $InsP_3$ receptor is already phosphorylated in this case.

In conclusion, upon stimulation with CCK, targeted, PKA-mediated phosphorylation specifically controls the kinetics of Ca^{2+} release from a distinct subpopulation of Ca^{2+} release channels. This data provides a simple mechanism by which agonist-specific oscillatory Ca^{2+} signals are largely shaped in pancreatic acinar cells.

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