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Activation of PKCδ by Tyrosine Phosphorylation in Rat Parotid Acinar Cells

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Receptor- and nonreceptor-mediated stimuli produce increases in both PKCδ tyrosine phosphorylation and activity in rat parotid acinar cells and other cells. In vivo and in vitro increases and decreases in tyrosine phosphorylation resulted in increases and decreases, respectively, of PKCδ activity. These studies demonstrated that increases in PKCδ activity by G protein-coupled receptors and other stimuli were controlled by alterations in tyrosine phosphorylation.

Introduction

Salivary gland epithelial cells rely on multiple signal transduction events to perform their physiological functions, including ordinary cellular housekeeping chores that are common to all cells as well as those functions that are more unique to their differentiated role as initiators and modifiers of fluid secretion and saliva formation. Among the various signaling proteins that play important roles are members of the PKC family of proteins (1). These proteins are phospholipid-dependent serine-threonine kinases. PKCs have different substrate specificities, and this suggests that they each may have unique substrates and play specific roles in modulating cellular functions. Some PKCs can be modulated by phorbol esters as well as by G protein-coupled receptors that hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (1).

During the last several years we have focused on the activation of PKCδ in rat parotid acinar cells. In previous studies we noted that agonists of muscarinic and substance P receptors produce rapid increases in the tyrosine phosphorylation of PKCδ (2). Since these stimuli also initiate fluid secretion by the acinar cells, PKCδ may contribute to processes relating to fluid secretion. The contribution of tyrosine phosphorylation to PKCδ activity has a conflicting literature (3). Investigators have found that tyrosine phosphorylation of PKCδ increases, decreases, or produces no change in its activity. In addition to changes in the enzyme activity of this PKC, changes in its localization also affect its biological activity. In recent studies we examined the changes in PKCδ activity in rat parotid acinar cells exposed to both receptor agonists (carbachol, which activates the M3 muscarinic receptor in parotid acinar cells) and nonreceptor-mediated agents. All of these agents increased both the tyrosine phosphorylation of PKCδ as well as its enzymatic activity, and the activity was dependent on the tyrosine phosphorylation.

Key Words: *PKC; Parotid; Tyrosine Phosphorylation; Carbachol*

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Methods

Parotid acinar cells were prepared from male Sprague Dawley rats. The resulting cell suspension was suspended in a physiological salt solution. Cells were exposed to all agents at 37℃. The cells were lysed, and PKCδ was immunoprecipitated using a PKCδ-specific antibody. A PKC activity assay was conducted on the immunoprecipitated protein at 30℃ using a peptide based on the pseudosubstrate domain of PKCδ. Some immunoprecipitates were subjected to SDS-PAGE and immunoblotted using anti-phosphotyrosine antibodies and anti-PKCδ antibodies.

Results

Increases in PKCδ activity correlate with increases in tyrosine phosphorylation

Carbachol, a muscarinic agonist, produced rapid increases in the tyrosine phosphorylation of PKCδ. Even larger increases in PKCδ tyrosine phosphorylation were produced by PMA, a phorbol ester that binds to PKCδ, and pervanadate, an inhibitor of protein tyrosine phosphatases. Carbachol, PMA, and pervanadate also produced increases in PKCδ activity compared to the basal

activity that was found in PKCδ immunoprecipitates from unstimulated cells. The stimulated activities ranged up to 5 times the basal activity, and were ranked in the order of the degree of alterations in tyrosine phosphorylation produced by the agents (pervanadate >PMA >carbachol). Thus, there was a positive correlation between alterations in tyrosine phosphorylation and activity.

Modulations of PKCδ tyrosine phosphorylation change PKCδ activity

Although our studies suggested that increases in PKCδ tyrosine phosphorylation promoted increases in PKCδ activity, we wanted to test this correlation in a more experimental manner. Consequently, PKCδ tyrosine phosphorylation was modulated in vivo and in vitro. We exposed cells to inhibitors of tyrosine kinases, which blocked increases in PKCδ tyrosine phosphorylation by carbachol and other agents. The PKCδ activities in immunoprecipitates from inhibitor-treated cells were reduced in a comparable manner to the reduction in their tyrosine phosphorylation. A second approach was to treat PKCδ immunoprecipitates in vitro with a protein tyrosine phosphatase in order to dephosphorylate the protein. Phosphatase treatment dephosphorylated tyrosine residues in immunoprecipitates from stimulated cells. Phosphatase treatment had no effect on the PKCδ activity in unstimulated cells, but greatly reduced the activity in immunoprecipitates from stimulated cells in a comparable manner to the reduction in tyrosine phosphorylation of PKCδ. Thus, these in vivo and in vitro treatments indicate that increases in the tyrosine phosphorylation of PKCδ increase the enzyme activity of this signaling protein.

Discussion and Analysis

Although the literature regarding the contribution of tyrosine phosphorylation to PKCδ activity is conflicting (3), our results indicate that there is a positive correlation between phosphorylation and activity in rat parotid acinar cells. We also tested this in other cell types and found a similar positive correlation. Since stimuli that promote fluid secretion also increase PKCδ tyrosine phosphorylation and activity, the physiological role of PKCδ may involve modulation of fluid secretion. However, this remains speculation at this time. The results also indicate that the activation of phospholipase C-linked G protein-coupled receptors (GPCRs) in parotid cells results in alterations in tyrosine phosphorylation. This is not particularly surprising, since a large number of GPCRs utilize tyrosine phosphorylation to modulate intracellular signaling events in a manner that previously was considered to be a characteristic only of growth factor receptors. Current studies are focused on identifying the tyrosine kinase that phosphorylates PKCδ.

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