PROCEEDING

Role of protein kinase C-δ in isoproterenol-induced amylase release in rat parotid acinar cells

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Abstract : In parotid acinar cells, β-adrenergic receptor activation results in accumulation of intracellular cAMP. Subsequently, cAMP-dependent protein kinase (PKA) is activated and consequently amylase release is provoked. In this paper, we investigated involvement of protein kinase C-δ (PKCδ), a novel isoform of PKC, in amylase release induced by β-adrenergic receptor stimulation. Amylase release stimulated with the β-agonsit isoproterenol (IPR) was inhibited by rottlerin, an inhibitor of PKCδ. IPR activated PKCδ and the effect of IPR were inhibited by a PKA inhibitor, H89. Myristoylated alanine-rich C kinase substrate (MARCKS), a major cellular substrate for PKC, was detected in rat parotid acinar cells, and a MARCKS inhibitor, MARCKS-related peptide, inhibited the IPR-induced amylase release. IPR stimulated MARCKS phosphorylation, which was found to be inhibited by H89 and rottlerin. These observations suggest that PKCδ activation is a downstream pathway of PKA activation and is involved in amylase release *via* MARCKS phosphorylation in rat parotid acinar cells stimulated with β-adrenergic agonist. J. Med. Invest. 56 Suppl. : 368-370, December, 2009

Keywords **:** *PKCδ, amylase release, MARCKS, parotid acinar cells*

INTRODUCTION

In parotid acinar cells, stimulation of β-adrenergic receptors provokes amylase release (1). The amylase release is depended on the increase in intracellular cAMP levels stimulated with β-adrenergic receptors but occurs independent of elevation of intracellular calcium ion (Ca^{2+}) levels. Activation of cAMP-dependent protein kinase (PKA) followed by the increase in intracellular cAMP levels is thought to be an essential step in this process (1). On the other hand, it has also been thought that protein kinase C (PKC) activation is involved in amylase release in parotid acinar cells (2-4). We here investigated the role of PKC on amylase release induced by the β-adrenergic agonist isoproterenol (IPR) in rat parotid acinar cells.

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INVOLVEMENT OF PKCδ IN IPR-INDUCED AMYLASE RELEASE

The PKC family consists of at least 10 members, which are classified into three subgroups based on their structural characteristics and cofactor requirement. They include the conventional cPKC (α, βI, βII and γ), stimulated by Ca^{2+} and diacylglycerol (DAG) or phorbol esters ; the novel nPKC (δ, ε, η and θ), activated by DAG and phorbol esters but Ca^{2+} independent ; and the atypical aPKC (ζ and ι/λ),

unresponsive to Ca^{2+}, DAG and phorbol esters (5, 6). Of those, the expression of PKCα, δ, ε and ζ has been reported in rat parotid acinar cells (7, 8). In the presence of calphostin C, an inhibitor of DAG-sensitive PKC, IPR-induced amylase release was reduced. Since β-adrenergic receptor activation does not elicit Ca^{2+} mobilization, this observation suggests that a Ca^{2+}-independent and DAG-sensitive PKC, i.e. a novel PKC such as PKCδ, is involved in IPR-induced amylase release. Then, the effect of the PKCδ inhibitor rottlerin was examined. The inhibitor mimicked the effect of calphostin C. Although rottlerin has been used widely as a PKC-δ inhibitor, this polyphenolic compound has also been reported to cause a general inhibition of the mitochondrial metabolism which correlated with a depletion of the cellular ATP concentration (9, 10). Then we examined the effect of rottlerin on cellular ATP level in rat parotid acinar cells. However, the cellular ATP level was scarecely reduced by rottlerin. Therefore, it is unlikely that the inhibition of IRP-induced amylase release by rottlerin is caused by a toxic effect.

ACTIVATION OF PKCδ BY IPR

It is known that activated isoforms of PKC, including PKCδ, are phosphorylated and translocated from the cytosol to the cell membrane (11). When parotid acinar cells were stimulated with IPR, the increase in the amount of phosphorylated-PKCδ in the membrane fraction was observed by Western blotting using anti-phosphorylated-PKCδ antibody. This observation suggests that stimulation of β-adrenergic receptor induces PKCδ activation in parotid acinar cells. Further, we directly examined kinase activity of PKCδ using PKCδ-specific immunoprecipitates. When the kinase activity was measured with PKCδ purified from cell lysate by immunoprecipitation, the activity was shown to be increased in the acinar cells by treatment with IPR. Next we examined the effect of the PKA inhibitor H89 on IPR-induced PKCδ activation by detection of phosphorylated-PKCδ in the membrane fraction. In the presence of H89, IPR failed to increase the amount of phosphorylated-PKCδ in the membrane fraction, indicating that this PKA inhibitor inhibits IPR-induced PKCδ activation. These results suggest that PKCδ is activated via PKA activation in the IPR-stimulated rat parotid acinar cells.

MARCKS PHOSPHORYLATION INDUCED BY IPR

Myristoylated alanine-rich C kinase substrate (MARCKS) is a major cellular substrate for PKC (12). The involvement of MARCKS in the IPR-induced amylase release was examined in parotid acinar cells. The N-terminal sequence peptide of MARCKS (MANS) has been shown to be a useful inhibitor of MARCKS function because this peptide suppresses the secretory function *in vivo* (13, 14) and in cultured cells (15). In parotid acinar cells pretreated with the MANS peptide, IPR-induced amylase release was reduced, suggesting the involvement of MARCKS in IPR-induced amylase release.

Involvement of phosphorylation of MARCKS by PKC in secretory function has been demonstrated in chromaffin cells (16, 17), insulin-producing INS-1 cells (18), bovine luteal cells (19), and SH-SY5Y human neuroblastoma cells (20). When we examined the effect of IPR on MARCKS phosphorylation by Western blotting using anti-phosphorylated MARCKS antibody, this β-agonist induced MARCKS phosphorylation in parotid acinar cells. The IPR-induced MARCKS phosphorylation was inhibited in the presence of a PKA inhibitor, H89, implying that MARCKS phosphorylation stimulated by IPR is evoked via PKA activation in parotid acinar cells. In the presence of the PKCδ-specific inhibitor, rottlerin, the MARCKS phosphorylation was also inhibited. These results suggest that PKCδ activated by IPR stimulation via PKA activation provokes MARCKS phosphorylation.

Taken together, it is most likely that PKA-PKCδ-MARCKS pathway is involved in IPR-induced amylase release in rat parotid acinar cells.

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