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MINI REVIEW

# Role of protein kinase C in cAMP-dependent exocytosis in parotid acinar cells

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**Summary** It is well known that β-adrenergic receptor activation in parotid acinar cells results in increased intracellular cAMP levels, and consequently induces exocytotic amylase release. However, protein kinase C (PKC) has also been considered to contribute to amylase release. In this paper, we review the role of PKC in cAMP signaling and amylase release. PKCδ, a so-called 'novel PKC', has been shown to be activated by β-adrenergic receptor stimulation. Myristoylated alanine-rich C kinase substrate (MARCKS), a major cellular substrate for PKC, is detected in parotid acinar cells, and MARCKS phosphorylation and translocation from the membrane to the cytosol are induced by β-adrenergic receptor stimulation. cAMP-dependent protein kinase (PKA) inhibitor inhibits β-agonist-induced PKC activation and MARCKS phosphorylation. The β-agonist-induced amylase release is inhibited by inhibitors of PKCδ or by a MARCKS-related peptide. These findings suggest that MARCKS phosphorylation via PKCδ activation, which is downstream of PKA activation, is involved in the cAMP-dependent amylase release in parotid acinar cells.


## 1. Introduction

In parotid acinar cells, stimulation of β-adrenergic receptors induces the exocytotic amylase release [1]. The amylase release is regulated by intracellular cAMP levels, since adenylate cyclase activators (such as forskolin), cAMP-phosphodiesterase inhibitors (such as 3-isobutyl-1-methylxanthine) and cell-permeable forms of cAMP (such as dibutylyl cAMP), mimic the effects of β-agonist on amylase release [2—4]. The amylase release occurs without elevation of intracellular calcium ion ($Ca^{2+}$) levels, because β-agonists provoke increases in intracellular cAMP levels, but have no effect on $Ca^{2+}$ levels [5,6].

In the cAMP-dependent amylase release, activation of cAMP-dependent protein kinase (PKA) is thought to be an essential step in the process. During amylase secretion from parotid acinar cells, the activity of PKA is enhanced [1]. PKA inhibitors inhibit the cAMP-dependent amylase release [7,8]. Insertion of PKA induces amylase release in permeabilized parotid acinar cells [9]. Therefore, it seems likely that PKA phosphorylates proteins involved in amylase exocytosis. Several proteins have been reported to be phosphorylated following β-adrenergic stimulation in parotid glands [10—12]. However, it is not clear which protein is phosphorylated that is crucial for triggering the cAMP-dependent exocytosis.

On the other hand, it has also been thought that protein kinase C (PKC) activation is involved in amylase release in parotid acinar cells based on studies using PKC activators [13—15]. In this paper, we review the role and importance of PKC on the cAMP-dependent amylase release in parotid acinar cells.

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## 2. PKC in parotid acinar cells

PKCs comprise a family of phospholipid-dependent enzymes that catalyze the covalent transfer of phosphate from ATP to serine and threonine residues of proteins. PKCs are activated by a variety of extracellular signals and, in turn, modify the functions of many cellular proteins, including receptors, enzymes, cytoskeletal proteins and transcription factors. Phosphorylation of the substrate proteins induces conformational changes that modify their functional properties. Therefore, PKCs are involved in a large variety of cell functions and signal transduction pathways that regulate cell migration and polarity, proliferation, differentiation, ion fluxes, secretion and cell death [16].

The PKC family consists of at least 10 members, which are divided into three subgroups based on their structural characteristics and cofactor requirements. They include the classical cPKC (α, βI, βII and γ), the novel nPKC (δ, ε, η and θ), and the atypical aPKC (ζ and ι/λ) isoforms [17,18]. All isoforms require phosphatidylserine, a component of the phospholipid bilayer, for their activation. The classical cPKCs are $Ca^{2+}$-sensitive and also require diacylglycerol (DAG) or tumor-promoting phorbol esters for their activation. The novel nPKCs are $Ca^{2+}$-independent but still require DAG or phorbol esters [19]. The atypical aPKCs are also $Ca^{2+}$-independent, and phosphatidylserine is sufficient for their maximal activity [20]. Thus, various stimuli can lead to different responses via isoform-specific PKC signaling regulated by their expression, localization and phosphorylation status in particular biological settings.

PKC activity was initially detected in rat parotid gland [21]. Subsequently, PKCα, δ, ε and ζ were found to be expressed in parotid acinar cells [22,23], and the involvement of PKC in amylase secretion in parotid acinar cells has been demonstrated [13—15,21,23]. The PKC activator phorbol esters, such as phorbol 12,13-dibutyrate and phorbol 12-myristate 13-acetate (PMA), induce protein secretion in parotid gland slices [13], and elicit amylase release in parotid acinar cells [14,15]. It has been shown that phorbol esters have no effect on adenylate cyclase activity or $Ca^{2+}$ mobilization [13]. Those observations suggest that PKC contributes to amylase release in parotid acinar cells. However, the effects of PKC inhibitors vary; H7 inhibited amylase release induced by β-adrenergic receptor stimulation [21], whereas Ro 31-8220 and CGP 41-251 enhances amylase release provoked by norephinephrine [23].

Our current study [24] demonstrated that calphostin C, an inhibitor of DAG-sensitive PKC [25], partially inhibits amylase release induced by β-adrenergic receptor stimulation. 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. an nPKC such as PKCδ, is involved in amylase release following β-adrenergic receptor stimulation. When the effect of the PKCδ-specific inhibitor rottlerin was examined, that inhibitor mimicked the effect of calphostin C. It is known that activated isoforms of PKC, including PKCδ, are phosphorylated and translocated from the cytosol to the cell membrane [26]. In parotid acinar cells stimulated with a β-agonist (isoproterenol), the amount of phosphorylated-PKCδ in the membrane increased (Fig. 1), which strongly suggests that β-adrenergic receptor activation induces PKCδ activation in parotid acinar cells. Taken together, it is most likely that PKCδ activated by β-adrenergic receptor stimulation contributes to amylase release.

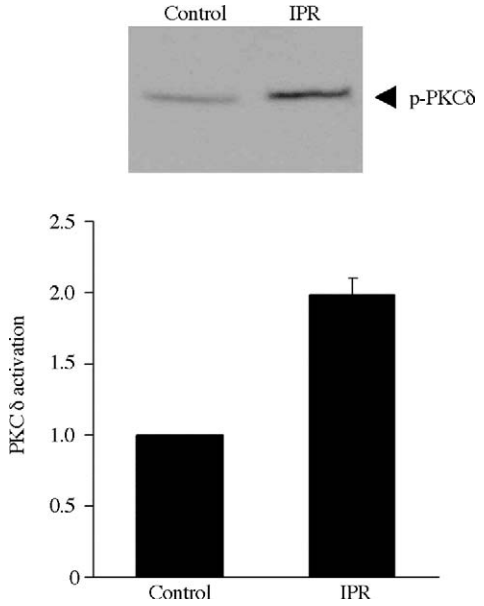


**Figure 1** PKCδ activation induced by β-adrenergic receptor stimulation in parotid acinar cells. Cells were stimulated by the β-agonist isoproterenol (IPR) for 10 min. p-PKCδ in the membrane fraction was detected by Western blotting using an anti-p-PKCδ antibody (upper). PKCδ activation is calculated as a ratio and is normalized against the control at 0 min (lower).

## 3. MARCKS in parotid acinar cells

Dozens of proteins that contain predicted phosphorylation sites for PKC have been identified. Myristoylated alanine-rich C kinase substrate (MARCKS) is a major cellular substrate for PKC [27]. MARCKS binds to the plasma membrane via the dual actions of a hydrophobically myristoylated N-terminus and a polybasic stretch within the so-called effecter domain [28] which is a phosphorylation site [29]. It is well known that phosphorylated MARCKS (p-MARCKS) translocates from the membrane to the cytosol, because phosphorylation of the effecter domain significantly decreases the binding force of MARCKS to the membrane [30].

MARCKS has been implicated in various cellular functions [31,32]. The involvement of MARCKS has been demonstrated in $Ca^{2+}$-dependent secretory function. In chromaffin cells, PMA stimulates noradrenaline release and MARCKS phosphorylation, and a synthetic peptide of the phosphorylation site domain sequence of MARCKS inhibits the PMA-induced noradrenaline release in permeabilized cells [33,34]. In insulin-producing INS-1 cells, glucagon-like peptide-1 (GLP-1) stimulates insulin secretion, PKCα and PKCε activation and MARCKS phosphorylation [35]. A PKC inhibitor inhibits MARCKS phosphorylation and insulin secretion coupled to GLP-1-stimulated signaling [35]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) stimulates MARCKS phosphorylation and oxytocin secretion in bovine luteal cells [36]. However, $PGF_{2\alpha}$ failed to stimulate oxytocin exocytosis in cells expressing a mutant MARCKS which cannot be phosphorylated by PKC [36]. In SH-SY5Y human neuroblastoma cells, PMA elicits noradrenaline