〔MINI REVIEW〕

The role of protein kinase C in protein secretion from salivary glands

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Protein secretion from salivary glands has been studied extensively using parotid acinar cells, and stimulation of β–adrenergic receptors (β–AR) expressed on these cells induces exocytotic secretion of amylase. This process is induced by the Gs protein–mediated activation of adenylyl cyclase (AC), and by the subsequent production of cAMP and the activation of cAMP–dependent protein kinase (PKA) (Baum, 1987 ; Shimomura et al., 2004 ; Takuma & Ichida, 1994). Protein kinase C (PKC) is also involved in amylase secretion from parotid acinar cells via stimulation of muscarinic receptors (Satoh et al., 2009 ; Shimomura et al., 1988 ; Terzian et al., 1996).

PKA– and PKC–dependent amylase secretion was originally thought to occur via separate pathways activated by sympathetic and parasympathetic stimulation, respectively. However, accumulating evidence indicates that PKC functions downstream of PKA and promotes protein secretion via phosphorylation of myristoylated alanine–rich C kinase substrate (MARCKS) (Fig. 1).

MARCKS is a major cellular substrate for PKC. Phosphorylated MARCKS (MARCKSp) translocates from the plasma membrane to the cytosol, and this process facilitates exocytosis (Aderem, 1992). MARCKS phosphorylation is involved in protein secretion from exocrine glands, such as parotid glands and pancreas (Satoh et al., 2009 ; Satoh et al., 2016 ; Satoh et al., 2019).

It has been found that β–AR–mediated amylase secretion from parotid acinar cells is reduced to 50%–70% by MANS peptide (a MARCKS inhibitor), calphostin C (CalC ; a non–selective PKC inhibitor), and rottlerin (a PKCδ–specific inhibitor). In addition, β–AR–mediated MARCKS phosphorylation is inhibited by H89 (a PKA inhibitor), CalC, and rottlerin. H89 also inhibits β–AR–mediated activation of PKCδ (Satoh et al., 2009). These findings indicate that MARCKS is phosphorylated by PKCδ through the activation of PKA, and this phosphorylation induces amylase secretion from parotid acinar cells (Fig. 1).

PKC has several isoforms that are classified into three types : 1) diacylglycerol (DAG) and Ca^{2+}–dependent conventional PKCs (cPKC), which contain the isoforms PKCα, PKCβ, and PKCγ ; 2) DAG–dependent and Ca^{2+}–independent novel PKC (nPKC), which contain the isoforms PKCδ, PKCε, PKCη, and PKCθ ; and 3) DAG and Ca^{2+}–independent atypical PKC (aPKC), which contain the isoforms PKCι, PKCκ, and PKCλ (Jaken, 1996 ; Newton, 1995). Rat parotid acinar cells express PKCα, PKCδ, PKCε, and PKCζ (Terzian et al., 1996), whereas rat sublingual acinar cells express PKCα, $PKC\beta_I$, PKCδ, PKCε, and PKCη (Culp et al., 2011).

PKCδ is a nPKC, which is activated by DAG in a Ca^{2+}–independent manner ; thus PKCδ is expected to be involved in $G_{q/11}$ protein–mediated protein secretion. Indeed, the role of PKCδ–MARKCS in cholecystokinin (CCK)–induced amylase release was demonstrated in rat pancreatic acinar cells (Satoh et al., 2016). CCK is a peptide hormone in gastrointestinal system and stimulates exocytotic release of digestive enzymes including amylase from pancreatic acinar cells. The CCK receptor is a $G_{q/11}$ protein–coupled receptor and activates phospholipase Cβ (PLCβ) to catalyze the cleavage of the membrane lipid phosphatidylinositol 4,5–biphosphate (PIP_2) and to generate the second messengers inositol 1,4,5–triphosphate (IP_3) and DAG. The PLCβ–mediated production of DAG could induce amylase secretion through the activation of PKCδ and subsequent phosphorylation of MARKS.

In parotid acinar cells, however, β–AR agonists induce amylase secretion via the activation of PKA. How does

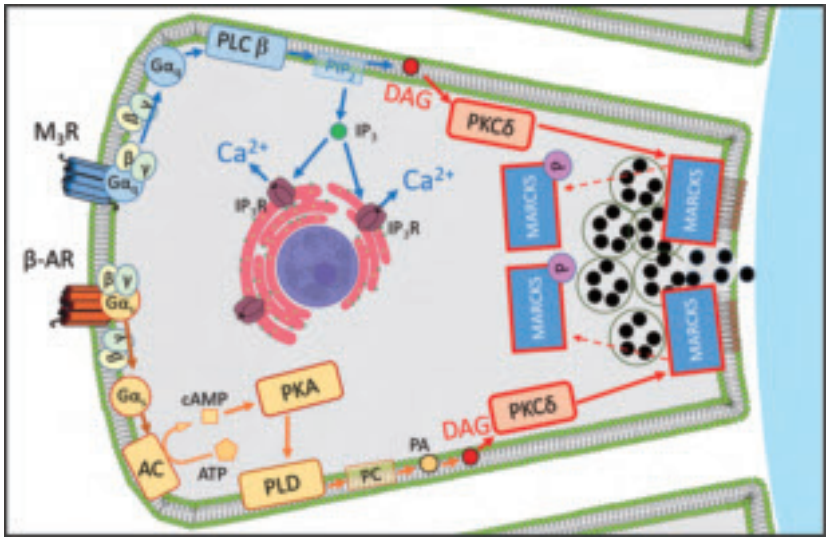

Figure 1. A schematic illustration of intracellular signaling pathways involved in protein secretion from parotid acinar cells.
Stimulation of M_3 muscarinic receptors (M_3R) results in the dissociation of $G_{q/11}$ proteins and activation of phospholipase C−β(PLCβ), which hydrolyzes the membrane lipid phosphatidylinositol 4,5−biphosphate (PIP_2) to generate inositol 1,4,5−triphosphate (IP_3) and DAG (blue line). PKCδ is activated by DAG and phosphorylates MARCKS protein. Phosphorylated MARCKS translocates from the membrane to the cytosol to facilitate exocytosis in parotid acinar cells (red line). On the other hand, stimulation of β−adrenergic receptors (β−AR) results in the dissociation of G_s proteins and activates adenylyl cyclase (AC), which converts ATP to cAMP, followed by the activation of PKA and then PLD. The activation of PLD causes hydrolysis of phosphatidylcholine (PC) to generate phosphatidic acid (PA), which is then involved in the formation of diacylglycerol (DAG) (orange line). PKCδ is activated by DAG and phosphorylates MARCKS protein (red lines).
AC : adenylate cyclase, β−AR : β−adrenergic receptor, DAG : diacylglycerol, IP_3 : inositol 1,4,5−triphosphate, M_3R : M_3 muscarinic receptor, PA : phosphatidic acid, PC : phosphatidylcholine, PIP_2 : phosphatidylinositol 4,5−biphosphate, PLCβ : phospholipase C−β, PLD : phospholipase D.

PKA activate PKCδ? There are two possible mechanisms for the PKA−mediated activation of PKCδ. One possibility is that PKA directly phosphorylates PKCδ, although there is no reported evidence to support this notion. Another possible mechanism is that PKCδ is activated by phospholipase D (PLD) through a pathway that involves DAG production mediated by the PLD–phosphatidic acid (PA) phosphatase pathway (Fig. 1).

Phospholipase D is a membrane−bound enzyme that catalyzes the hydrolysis of phospholipid substrates, including phosphatidylcholine (PC), to generate PA and water−soluble bases. In addition, PA phosphatase converts PA to form DAG (Su & Frohman, 2010).

The role of PKA in the activation of PLD was suggested by studies showing that PKA inhibitors suppress PLD activity in the rat mast cell line RBL−2H3 (Choi et al., 2002). Moreover, a PLD inhibitor suppresses GLP−1−induced amylase release in rat pancreatic acinar cells (Satoh & Kashimata, 2018). In rat parotid acinar cells, activation of PLD occurs through a Ca^{2+}−independent pathway (Guillemain & Rossignol, 1994), and PLD contributes to β−AR−mediated amylase release (Dohke et al., 2002). Taken together, these findings indicate that PKCδ is likely activated by DAG through the PKA−mediated activation of PLD (Fig. 1).

The studies described above show that PKCδ is a key enzyme in protein exocytosis and is involved in both G_s− and $G_{q/11}$−protein−mediated protein secretion from parotid acinar cells. Namely, PKCδ is activated in a DAG−dependent manner by PKA−induced activation of PLD−PA phosphatase, which mediates G_s protein−mediated protein secretion, and is also directly activated by PLC, which mediates $G_{q/11}$ protein−mediated protein secretion (Fig. 1).

Unlike amylase secretion from parotid acinar cells which is controlled primarily by sympathetic nerve, mucin secretion from sublingual glands is predominantly regulated by parasympathetic nerve. Interestingly, mucous secretion from rat sublingual glands is regulated by muscarinic receptors through the activation of PLCβ and subsequent activation of PKCα, without the involvement of PKCδ (Fig. 2) (Culp et al., 2011 ; Culp et al., 2020). In this signaling pathway, PLCβ produces DAG and IP_3, and then IP_3 triggers an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) via IP_3

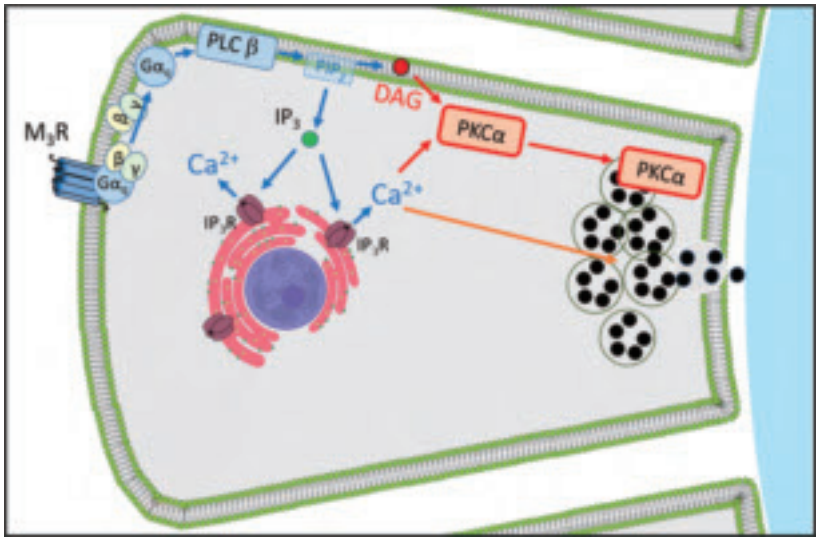

Figure 2. A schematic illustration of the intracellular signaling pathways involved in protein secretion from sublingual acinar cells.
Stimulation of the M_3 muscarinic receptor (M_3R) results in the dissociation of $G_{q/11}$ proteins and the activation of PLCβ, which hydrolyzes the membrane lipid phosphatidylinositol 4,5−biphosphate (PIP_2) to form inositol 1,4,5−triphosphate (IP_3) and diacylglycerol (DAG). IP_3 activates IP_3 receptors (IP_3Rs) on the endoplasmic reticulum and releases stored Ca^{2+} into the cytosol (blue line). PKCα is activated by DAG together with Ca^{2+}, translocates to the apical membrane of the acinar cell, and promotes protein exocytosis from sublingual acinar cells (red line).
DAG : diacylglycerol, IP_3 : inositol 1,4,5−triphosphate, IP_3R : IP_3 receptor, M_3R : muscarinic receptor, PIP_2 : phosphatidylinositol 4,5−biphosphate, PLCβ : phospholipase C−β.

receptor−mediated Ca^{2+} release from intracellular stores and subsequent Ca^{2+} entry into the cell via store−operated Ca^{2+} entry (Putney, 2010).

Protein exocytosis from the sublingual gland occurs in two phases, during which DAG and $[Ca^{2+}]_i$ act together to mediate mucin secretion via PKCα. In the resting state, PKCα is detected in the lateral and basal/perinuclear regions. After 1 minute of CCh stimulation, PKCα is detected in apical regions, where it persists for at least 30 min. This finding suggests that the translocation of PKCα to the apical membrane is responsible for the priming or docking of mucin granules during exocytosis (Culp et al., 2011).

Together, these studies reveal that PKC isoforms induce protein secretion through different mechanisms in parotid (Fig. 1) and sublingual acinar cells (Fig. 2). The studies also show that PKC isoforms have different cell−specific functions that are dependent upon their expression patterns and the expression of other regulatory molecules.

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