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Antigen-induced Ca^{2+} mobilization in RBL-2H3 cells: Role of I(1,4,5)P_3 and S1P and necessity of I(1,4,5)P_3 production

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Abstract

Inositol 1,4,5-trisphosphate (IP_3) has long been recognized as a second messenger for intracellular Ca^{2+} mobilization. Recently, sphingosine 1-phosphate (S1P) has been shown to be involved in Ca^{2+} release from the endoplasmic reticulum (ER). Here, we investigated the role of S1P and IP_3 in antigen (Ag)-induced intracellular Ca^{2+} mobilization in RBL-2H3 mast cells. Antigen-induced intracellular Ca^{2+} mobilization was only partially inhibited by the sphingosine kinase inhibitor DL-*threo*-dihydrosphingosine (DHS) or the IP_3 receptor inhibitor 2-aminoethoxydiphenyl borate (2-APB), whereas preincubation with both inhibitors led to complete inhibition. In contrast, stimulation of A_3 adenosine receptors with N^5-ethylcarboxamidoadenosine (NECA) caused intracellular Ca^{2+} mobilization that was completely abolished by 2-APB but not by DHS, suggesting that NECA required only the IP_3 pathway, while antigen used both the IP_3 and S1P pathways. Interestingly, however, inhibition of IP_3 production with the phospholipase C inhibitor U73122 completely abolished Ca^{2+} release from the ER induced by either stimulant. This suggested that S1P alone, without concomitant production of IP_3, would not cause intracellular Ca^{2+} mobilization. This was further demonstrated in some clones of RBL-2H3 cells excessively overexpressing a β isoform of Class II phosphatidylinositol 3-kinase (PI3KC2β). In such clones including clone 5A4C, PI3KC2β was overexpressed throughout the cell, although endogenous PI3KC2β was normally expressed only in the ER. Overexpression of PI3KC2β in the cytosol and the PM led to depletion of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), resulting in a marked reduction in IP_3 production. This could explain the abolishment of intracellular Ca^{2+} mobilization in clone 5A4C. Supporting this hypothesis, the Ca^{2+} mobilization was reconstituted by the addition of exogenous $PI(4,5)P_2$ in these cells. Our results suggest that both IP_3 and S1P contribute to FcεRI-induced Ca^{2+} release from the ER and production of IP_3 is necessary for S1P to cause Ca^{2+} mobilization from the ER.

Keywords: FcεRI; Calcium mobilization; Lipid mediators; IP_3; Sphingosine 1-phosphate; Signal transduction

1. Introduction

Cross-linking of the high affinity receptor for IgE (FcεRI) with antigen (Ag) in the RBL-2H3 mast cell line leads to tyrosine phosphorylation of receptor subunits by the activation of the Src family tyrosine kinase Lyn [1,2]. The tyrosine phosphorylation of γ subunits of FcεRI is thought to recruit a non-receptor tyrosine kinase, Syk. Syk, in turn, activates various signaling molecules including phospholipase Cγ, which hydrolyzes phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol [3]. IP_3 then binds to the IP_3 receptor to mobilize stored Ca^{2+} and to promote an influx of extracellular Ca^{2+} [3,4]. In addition to IP_3, we have previously shown that

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sphingosine 1-phosphate (S1P) might be also involved in FcεRI-mediated mobilization of Ca^{2+} in RBL-m1 cells [5].

S1P is a phosphorylated metabolite of sphingosine produced by sphingosine kinase. S1P can work intracellularly as a second messenger or extracellularly by binding to cell surface S1P receptors [6]. S1P was implicated in various signaling events [7–14], including Ca^{2+} homeostasis [5,15,16]. Particularly, S1P has been shown to release Ca^{2+} from intracellular Ca^{2+} stores in an IP_3-independent manner [17]. Ca^{2+} release from endoplasmic reticulum (ER)-rich microsomal preparations has been observed to be dependent on the production of S1P [18]. In addition, S1P-gated Ca^{2+} channel has been demonstrated [19]. In addition to findings that S1P mediates Ca^{2+} mobilization by FcεRI [5,15], more recent studies have demonstrated mobilization of Ca^{2+} via the sphingosine kinase pathway by various surface receptors, such as muscarinic receptors expressed in HEK293 cells [20,21], FcγR1 in U937 cells [22] and formyl peptide receptors and P2Y receptors in HL60 cells [23,24]. Exogenous sphingosine may be converted to S1P, which causes Ca^{2+} mobilization from the ER or sphingosine itself may modulate Ca^{2+} influx [25,26]. Extracellular S1P has also been shown to release Ca^{2+} from the ER by the S1P surface receptors and phospholipase C pathway [27].

While working with a β isoform (PI3KC2β) of Class II phosphatidylinositol 3-kinase (PI3K), we discovered that S1P production in PI3KC2β-overexpressing RBL-2H3 cells was increased. PI3KC2β belongs to the family of Class II PI3Ks, which are characterized by the presence of a C2 domain at the carboxyl terminus. Three mammalian Class II PI3Ks (PI3KC2) have been identified and cloned [28–31]: Drosophila 68D enzyme [32], murine m-cpk [33] and p170 [34] belong to this class of PI3K. Unlike the Class I PI3Ks, these enzymes preferentially utilize phosphatidylinositol and phosphatidylinositol 4-monophosphate as lipid substrates measured in vitro [35]. Little is understood about the physiological role of the class II PI3Ks or how their enzymatic activity is regulated in vivo. PI3KC2β is sensitive to the PI3K inhibitor wortmannin, but PI3KC2α is not [29]. Recently, it has been shown that PI3KC2α and PI3KC2β are recruited to the activated epidermal growth factor (EGF) receptor complex upon stimulation of human A431 cells with EGF [36]. The N-terminal region of PI3KC2β was shown to selectively interact with the EGF receptor [36] by binding to receptor-associated Grb2 [37].

When PI3KC2β is overexpressed at low levels (<10 folds), PI3KC2β is expressed mostly in the ER, similar to the endogenous enzyme [35]. In these cells, antigen-stimulated S1P production and Ca^{2+} mobilization are markedly enhanced (data not shown). However, when PI3KC2β is expressed at high levels (>100 folds), antigen-induced Ca^{2+} release from the ER was completely abolished. We thought characterization of RBL-2H3 clones that overexpress PI3KC2β at high levels would provide us with a useful tool to study antigen-induced Ca^{2+} signaling. Using pharmacological reagents as well as our RBL-PI3KC2β transfectants, the present study investigated the mechanism by which Ca^{2+} is mobilized from the ER by FcεRI via the S1P and IP_3 pathways.

2. Methods

2.1. Reagents

Materials were obtained from the following sources: radiolabeled compounds from NEN (Boston, MA); fura-2 AM from Molecular Probes Inc. (Eugene, OR); dinitrophenyl-human serum albumin (DNP-HSA) from Sigma (St. Louis, MO); *sn*-2-*O*-palmiotyl PI(4,5)P_2-7-nitrobenz-2-oxa-1,3-diazole (NBD) from Echelon Research Laboratory Inc. (Salt Lake City, UT); mouse anti-PI(4,5)P_2 IgG_{2b} from Assay Design, Inc. (Ann Arbor, MI); monoclonal rhodamine-X-conjugated anti-mouse IgG from Jackson Immuno Laboratory Inc. (West Grove, PA); rabbit polyclonal affinity purified antibody against green fluorescence protein (GFP) was obtained from Clontech (Palo Alto, CA); DL-*threo*-dihydrosphingosine (DHS), U73122 and U73343 from Biomol (Plymouth Meeting, PA); and 2-APB from Calbiochem (San Diego, CA). Materials for cell culture were obtained from GIBCO-BRL (Gaithersberg, MD). Silica gel G TLC plates were obtained from Bodman (Aston, PA).

2.2. Isolation of cDNA clones encoding PI3KC2β and construction of expression vector

Approximately 1×10^6 plaques from a Jurkat T-cell cDNA library (Stratagene) were screened with a 353 bp [^{32}P]-labelled DNA fragment obtained by PCR using two PCR primers (sense, 5′-GGIGACGACTGITGTCAIGACATG-3′; antisense, 5′-CATIATGTTGICGTIGTGICIGTC-3′). A total of 34 overlapping clones were sequenced. The longest cDNA fragment obtained was a 4.5-kb, which covers the 3′-end of the full open reading frame for PI3KC2β. The DNA fragment was subcloned into the EcoRI site of pBluscript KSII(+), yielding pBluscript KSII(+)-3′PI3KC2β. We isolated the 5′-end of the cDNA by reverse transcription-PCR using selected primers [sense (5′-ATGTCTTCGACTCAGGACAATGGGGA-3′) and antisense (5′-GGCGAGCACCAGGGGG-AGCGAGCT-3′)] based on the sequence in the GenBank Database (Accession No. Y11312) for full-length PI3KC2β [28]. The amplified DNA fragment was subcloned into pCR2.1, yielding pCR2.1-5′PI3KC2β. A full-length cDNA was constructed by ligation of inserts from pCR2.1-5′PI3KC2β and pBluscript KSII(+)-3′PI3KC2β. Subsequently, the 4.9 kb EcoRI/XbaI fragment containing the full-length coding region was cloned into the pEGFP-C3 mammalian expression vector (Clontech) to allow the expression of GFP-PI3KC2β.