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L-type Ca^{2+} channels in mast cells: Activation by membrane depolarization and distinct roles in regulating mediator release from store-operated Ca^{2+} channels

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ABSTRACT

Store-operated Ca^{2+} channels (SOCs) are considered to be the principal route of Ca^{2+} influx in non-excitable cells. We have previously shown that in mast cells IgE + antigen (Ag) induces a dihydropyridine (DHP)-sensitive Ca^{2+} influx independently of Ca^{2+} store depletion. Since the DHP receptor is the α subunit of L-type Ca^{2+} channels (LTCCs), we examined the possible role of LTCCs in mast cell activation. Mast cells exhibited substantial expression of the α_{1C} ($Ca_V1.2$) subunit mRNA and protein on their cell surface. IgE + Ag-induced Ca^{2+} influx was substantially reduced by the LTCC inhibitor nifedipine, and enhanced by the LTCC activator (S)-BayK8644, whereas these agents had minimal effects on thapsigargin (TG)-induced Ca^{2+} influx. These LTCC-modulating agents regulated IgE + Ag-induced cell activation but not TG-induced cell activation. Inhibition of SOCs by 2-aminoethoxydiphenyl borate reduced both degranulation and production of cytokines, including interleukin-13 and tumor necrosis factor-α, whereas LTCC modulation reciprocally regulated degranulation and cytokine production. IgE + Ag, but not TG, induced substantial plasma membrane depolarization, which stimulated a DHP-sensitive Ca^{2+} response. Moreover, IgE + Ag-, but not TG-induced mitochondrial Ca^{2+} increase was regulated by LTCC modulators. Finally, gene silencing analyses using small interfering RNA revealed that the α_{1C} ($Ca_V1.2$) LTCC mediated the pharmacological effects of the LTCC-modulating agents. These results demonstrate that mast cells express LTCCs, which becomes activated by membrane depolarization to regulate cytosolic and mitochondrial Ca^{2+}, thereby controlling mast cell activation in a distinct manner from SOCs.

1. Introduction

Ca^{2+} is a highly versatile intracellular second messenger that regulates many complicated cellular processes, including cell functions, cell proliferation and apoptosis. Agonists such as hormones and growth factors induce changes in the intracellular Ca^{2+} dynamics by activating C-type phospholipases (Putney, 1987; Berridge and Irvine, 1989; Berridge, 1993). In hematopoietic cells, agonist-induced cross-linking of antigen (Ag) receptors activates the release of Ca^{2+} from intracellular stores, which is followed by Ca^{2+} entry across the plasma membrane. In T cells, the former process is mediated by both inositol-1,4,5-triphosphate (IP_3)-dependent and -independent intracellular Ca^{2+} channels, while the latter process depends on not only on plasma membrane Ca^{2+} channels, but also on at least two different types of intracellular channels (Grafton and Thwaite, 2001). The pathway of Ca^{2+} entry from the extracellular space still remains unclear, although it contributes most of the elevated intracellular Ca^{2+} during T cell activation (Haverstick et al., 1991). The roles of Ca^{2+} entry through store-operated Ca^{2+} channels (SOCs) have been the subject of extensive research, since store-operated Ca^{2+} entry (SOCE) or capacitative Ca^{2+} entry is considered to be the principal route of Ca^{2+} influx in non-excitable cells (Parekh and Penner, 1997; Parekh and Putney, 2005). SOCs are activated by emptying intracellular stores through the action of IP_3 produced after activation of Ag receptors by mechanisms that remain to be elucidated (Barritt, 1999; Elliott, 2001; Putney et al., 2001). SOCs are also activated by a pharmacological emptying of intracellular stores via pharmacological agents, such as thapsigargin (TG) (Montero et al., 1990, 1991; Alvarez et al., 1994). Despite extensive efforts to determine the molecular entities that are responsible for SOCE, they are still only defined electrophysiologically in terms of the current passing through the channels. The best characterized SOC current in hematopoietic cells is the current passing through Ca^{2+} release-activated Ca^{2+} (CRAC) channels I_{CRAC}, which was firstly identified in rat basophilic leukemia (RBL) cells (Hoth and Penner, 1992) and subsequently in Jurkat T cells (Zweifach and Lewis, 1993). Recent evidence indicates that the mammalian proteins STIM1

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and Orai1 are required for the activation of SOCs and CRAC channels in a variety of mammalian cells (Smyth et al., 2006; Feske, 2007).

In electrically excitable cells, voltage-gated channels, including long-lasting L-type Ca^{2+} channels (LTCCs) are activated by membrane depolarization, and serve as the principal route of Ca^{2+} entry, thereby controlling a variety of crucial physiological processes. It has long been thought that voltage-operated channels are a characteristic feature of excitable cells. However, a growing body of evidence suggests the existence of functional voltage-gated or -independent dihydropyridine (DHP)-sensitive channels/LTCCs in a variety of non-excitable cells, including various hematopoietic cells such as B cells (Grafton et al., 2003; Akha et al., 1996), dendritic cells (Poggi et al., 1998), NK cells (Zocchi et al., 1998), neutrophils (Rosales and Brown, 1992), and T cells (Kotturi et al., 2003; Stokes et al., 2004; Savignac et al., 2001, 2004; Badou et al., 1997). The DHP receptor is well known originally as the α_1 subunit of LTCCs in excitable cells (Bodi et al., 2005). The LTCCs are heterotetrameric polypeptide complexes consisting of three transmembrane subunits (α_1, γ and α_2/δ complex) and one cytoplasmic chain (the β1 chain) that allow depolarization-induced Ca^{2+} influx into the cytosol (Bodi et al., 2005). A spectrum of DHP derivatives, which specifically bind with high affinities to the α_1 subunit of LTCCs and regulates their functional state from closing to opening or vice versa, allows both the identification and the functional analyses of this class of molecule. Human and rodent T cells express transcripts and/or proteins of the α_{1C} ($Ca_v1.2$), α_{1D} ($Ca_v1.3$) and/or α_{1F} ($Ca_v1.4$) subunits of LTCCs (Kotturi et al., 2003; Stokes et al., 2004). Anti-Ig-induced Ca^{2+} influx in B cells has been shown to occur through a DHP-sensitive channel with similarities to a subtype of LTCCs (Akha et al., 1996). These studies suggest that LTCCs exist in immune cells and are activated upon Ag receptor stimulation.

Mast cells play key roles in allergic and inflammatory reactions and are involved in innate and acquired immunity (Galli et al., 1999). Mast cells and RBL-2H3 cells, an experimental model of mucosal-type mast cells, express the high-affinity receptor for IgE (FcεRI) on their cell surface. Cross-linking of IgE-occupied FcεRI by an agonist (*e.g.*, Ags, allergens and anti-IgE antibodies) initiates a cascade of biochemical events leading to degranulation as well as new synthesis and release of arachidonic acid metabolites, such as leukotrienes (LTs) and cytokines (Galli et al., 1999). Several signal transduction pathways are involved in this process, including the activation of protein and lipid kinases, such as protein kinase C, mitogen-activated protein kinases, and phosphatidylinositol-3-kinase. Elevation in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) is thought to be one of the earliest and requisite events in the signal transduction pathways. In mast cell biology, however, little attention has been paid to the roles of Ca^{2+} channels other than SOCs, probably because SOC agonists like TG, only mimic FcεRI-mediated biological effects such as degranulation and LTC_4 release (Huber et al., 2000). However, we recently reported that IgE + Ag, but not TG, induced a substantial Ca^{2+} influx that was independent of Ca^{2+} store emptying (Suzuki et al., 2008). Moreover, the store-independent Ca^{2+} influx was highly sensitive to DHP derivatives. In addition, despite their comparable abilities to stimulate degranulation, TG stimulation was far less effective than IgE + Ag in inducing cytokine production, suggesting that this type of Ca^{2+} entry pathway may play a role in cytokine production. Since DHP-sensitive channels are well known as the α_1 subunit of LTCCs, we became interested in the possible involvement of LTCCs in mast cell activation. Here we demonstrate that mast cells express the α_{1C} ($Ca_v1.2$) subunit of LTCCs on their cell surface. We further demonstrate that FcεRI cross-linking activates plasma membrane depolarization, which results in activation of LTCCs and mitochondrial Ca^{2+} uptake. Our data suggest that the α_{1C} ($Ca_v1.2$) LTCC may regulate mast cell effector responses in a distinct manner from SOCs.

2. Materials and methods

2.1. Materials

Nifedipine was obtained from Calbiochem (San Diego, CA, USA). (S)-BayK8644, 2-aminoethoxydiphenyl borate (2-APB), and TG were obtained from Sigma (St. Louis, MO). TG was dissolved in dimethyl sulfoxide (DMSO) and diluted with Hank's balanced salt solution (HBSS) to a final concentration of <0.1% before use. At a concentration of 0.1%, DMSO had no effects on its own throughout the present study. Anti-TNP IgE monoclonal antibody (clone IgE-3) was purchased from BD PharMingen Japan (Tokyo, Japan). TNP-conjugated bovine serum albumin (TNP-BSA; TNP:BSA conjugation ratio of 25:1) was purchased from Cosmo Bio (Tokyo, Japan). An anti-α_{1C} antibody (antigen peptide TTKINMDDLQPSENEDKS corresponding to residues 848-865 of rat $Ca_v1.2$) was purchased from Alomone Laboratories (Jerusalem, Israel). Fluo3-acetoxyl-methyl ester (Fluo3/AM) and rhod-2-acetoxylmethyl ester (rhod-2/AM) were purchased from Dojindo Laboratories (Kumamoto, Japan) and Invitrogen Corporation (Carlsbad, CA, USA), respectively. All other chemicals were of analytical grade.

2.2. RBL-2H3 cells

RBL-2H3 cells were obtained from the National Institute of Health Sciences (Japanese Collection of Research Bioresources (JCRB), Tokyo Japan; Cell number, JCRB0023) and grown in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 10% fetal bovine serum (FBS; SAFC Biosciences, Tokyo, Japan) in a 5% CO_2-containing atmosphere. The cells were harvested by incubation in HBSS containing 1 mM ethylenediamine tetraacetate and 0.25% trypsin for 5 min at 37 °C. Cells suspended in supplemented DMEM were plated on 100-mm culture dishes (1×10^7 cells/10 ml) or in 6-well plates (3×10^6 cells/well) and cultured at 37 °C.

2.3. Bone marrow-derived mast cells (BMMCs)

BMMCs were prepared from the femurs of 4–8-week-old C57BL/6J mice as previously described (Suzuki et al., 2002). All animal experiments were performed according to Nihon University guidelines. The cells were cultured in RPMI 1640 medium (Sigma) supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen), β-mercaptoethanol (5×10^{-5} M; Wako Pure Chemicals, Osaka, Japan), 100 μg/ml sodium pyruvate (Invitrogen), 1% minimal essential medium (MEM) non-essential amino acid solution (Invitrogen) and 5 ng/ml of recombinant interleukin-3 (IL-3) (PeproTech Inc., Rocky Hill, NJ) in a 5% CO_2-containing atmosphere at 37 °C. After 4–6 weeks of culture, the cells were stained for their cell surface expression of FcεRI. BMMCs were used for experiments after 4–8 weeks of culture (>95% mast cells).

2.4. RT-PCR

Total RNA was isolated from 1×10^6 cells using ISOGEN (Nippon Gene Corporation, Tokyo, Japan) and reverse transcribed to cDNA using SuperScript™ II reverse transcriptase (invitrogen), oligo dT primer (Invitrogen) and 0.5 mM dNTP Mixture (Invitrogen). α_{1C} subunit, α_{1D} subunit and GAPDH mRNA were measured by RT-PCR using a Model PC-802 thermal cycler (Astec Corporation, Fukuoka, Japan). Primers were for rat α_{1C}, 5′-GACAACCTGGCTGATGCGGAGAGCCTGAC-3′ and 5′-ATGCGGTGGCACTGCAGGCGGAACCTG-3′; for mouse α_{1C}, 5′-GACAACCTGGCTGATGCGGAGAGCCTGAC-3′ and 5′-ATACGGTGGCACTGCAGGCGGAACCTG-3′; for rat α_{1D}, 5′-GGTCACTTTGCTCCGACGTATCCAGCC-3′ and 5′-GTTTGGAGTCTTCTGGTTCGTCATCTT-3′; for mouse α_{1D}, 5′-GGTCACTCTGCTCCGGCGGATCCAGCC-3′ and 5′-GTTTGGAGTCTTCTGGCTCGTC-