Biochemical and Biophysical Research Communications 391 (2010) 1714–1720

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Fyn kinase controls FcεRI receptor-operated calcium entry necessary for full degranulation in mast cells

Elizabeth Sanchez-Miranda, Alfredo Ibarra-Sanchez, Claudia Gonzalez-Espinosa *

Departamento de Farmacobiología, Centro de Investigación y de Estudios Avanzados (Cinvestav), Sede Sur, Calzada de los Tenorios 235, Col. Granjas Coapa, CP 14330 Mexico City, Mexico

ARTICLE INFO

Article history:
Received 9 December 2009
Available online 30 December 2009

Keywords:
Mast cells
Allergy
IgE receptor
Fyn kinase
Calcium
Degranulation
IL-2

ABSTRACT

IgE–antigen-dependent crosslinking of the high affinity IgE receptor (FcεRI) on mast cells leads to degranulation, leukotriene synthesis and cytokine production. Calcium (Ca^{2+}) mobilization is a *sine qua non* requisite for degranulation, allowing the rapid secretion of stored pro-inflammatory mediators responsible for allergy symptoms. Fyn is a Src-family kinase that positively controls FcεRI-induced mast cell degranulation. However, our understanding of the mechanism connecting Fyn activation to secretion of pre-synthesized mediators is very limited. We analyzed FcεRI-dependent Ca^{2+} mobilization in bone marrow-derived mast cells (BMMCs) differentiated from WT and Fyn −/− knock out mice. Fyn −/− BMMCs showed a marked defect in extracellular Ca^{2+} influx after FcεRI crosslinking but not after thapsigargin addition. High concentrations of Gadolinium (Gd^{3+}) partially blocked FcεRI-induced Ca^{2+} influx in WT cells but, in contrast, completely inhibited Ca^{2+} mobilization in Fyn −/− cells. Low concentrations of an inhibitor of the canonical transient receptor potential (TRPC) Ca^{2+} channels (2-aminoethoxyphenyl-borane, 2-APB) blocked FcεRI-induced maximal Ca^{2+} rise in WT but not in Fyn −/− cells. Ca^{2+} entry through Fyn-controlled, 2-APB sensitive channels was found to be important for full degranulation and IL-2 mRNA accumulation in WT cells. Immunoprecipitation assays showed that Fyn kinase interacts with TRPC 3/6/7 channels after IgE–antigen stimulation, but its association is not related to protein tyrosine phosphorylation. Results indicate Fyn kinase mediates the receptor-dependent activation of TRPC channels that contribute to degranulation in FcεRI-stimulated mast cells.

Introduction

Mast cells are central players in adaptive and innate immune responses. After IgE–antigen stimulation of the high affinity immunoglobulin E receptor (FcεRI), are able to rapidly secrete different granule-stored mediators responsible for allergies and other inflammatory diseases [1]. This degranulation process depends on the increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and the activation of protein kinase C (PKC), particularly, PKCδ [2]. Calcium needed for degranulation includes ions from intracellular stores and from the extracellular medium, which enter to the cell through plasma membrane channels [3]. Intracellular Ca^{2+} is released from inner stores responding to the generation of inositol 1,4,5-trisphosphate (IP3) by phospholipase C (PLC) [4], whereas extracellular Ca^{2+} comes from channels dependent on internal store depletion, event recognized as capacitative calcium entry (CCE) [5]. Evidence also has been obtained indicating that an initial, non-store controlled Ca^{2+} influx is necessary for full mast cell activation [6,7]. Canonical transient potential receptor (TRPC) Ca^{2+} channels have been involved in capacitative and non-capacitative Ca^{2+} entry in mast cells [8].

FcεRI receptor is coupled to the activation of two early Src-family kinases, named Lyn and Fyn. Lyn phosphorylates the immunotyrosine activation motifs (ITAMs) of the FcεRI β and γ chains [7,9]. ITAM phosphorylation allows Syk translocation and activation, leading to tyrosine phosphorylation of the adapter molecule LAT (linker for activation of T-cells). LAT, in turn, coordinates the formation of a macromolecular complex composed by different proteins, such as the PLCγ [9]. Production of IP3 and diacylglycerol (DAG) by the Lyn-LAT-PLCγ pathway has been involved in mediator-release after FcεRI receptor in mast cells [10], although the generation of negative signals for mast cell degranulation have been also attributed to Lyn [11]. On the other hand, Fyn kinase has been recently found to be an essential element for the positive control of FcεRI-induced degranulation, since bone marrow-derived mast cells (BMMCs) produced from Fyn −/− mice are unable to fully degranulate after FcεRI crosslinking and no PKCδ activation after IgE–antigen stimulation is observed either [12]. However, full

Abbreviations: BMMCs, bone marrow-derived mast cells; CCE, capacitative calcium entry; PKC, protein kinase C; TRPC, canonical transient receptor potential channel; 2-APB, 2-aminoethoxydiphenyl-borane.

* Corresponding author.
E-mail address: cgonzal@cinvestav.mx (C. Gonzalez-Espinosa).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.12.139

degranulation of Fyn −/− BMMCs can be obtained with a combination of a calcium ionophore (A23187) and an activator of PKC (Phorbol Myristate Acetate, PMA) [12]. Fyn kinase phosphorylates Gab2 adapter and controls PI3 kinase activity and AKT phosphorylation after IgE–antigen stimulation of mast cells [12]. Fyn −/− BMMCs are also defective in the activation of NFκB transcription factor and in the synthesis of different pro-inflammatory cytokines such as IL-2 [13]. Attention on Fyn kinase role on mast cell degranulation has been increased during the past years, since inhibition of mast cell secretion of pro-inflammatory mediators is an important therapeutic target to control allergic diseases, but our understanding of the mechanism that couples Fyn kinase with degranulation is far from being complete.

In the present work, we analyzed Ca^{2+} mobilization after FcεRI receptor crosslinking in BMMCs derived from Fyn −/− mice. We found an important role of Fyn kinase in the activation of membrane TRPC Ca^{2+} channels responsible for full degranulation and IL-2 mRNA synthesis after IgE/antigen stimulation of mast cells.

Materials and methods

Cells. BMMCs were differentiated from bone marrow cultures of 129S1/SvImJ WT and Fyn −/− mice from Jackson Laboratories (Maine, USA) using reported methods [13]. All procedures involving mice were approved by the Committee for the Experimental Use and Animal Care in Cinvestav (CICUAL, Protocol # 383-07). After 4–6 weeks of culture, 97–99% of the cells were positive to FcεRI staining as measured by a standard flow cytometry assay.

IgE-dependent sensitization. WT and Fyn −/− BMMCs were diluted to 2 million cells per mL of culture media and were sensitized with 300 ng/mL of a monoclonal anti-DNP IgE (Clone SPE-7, Sigma) for one hour at 37 °C. Unbound IgE was removed by washing the

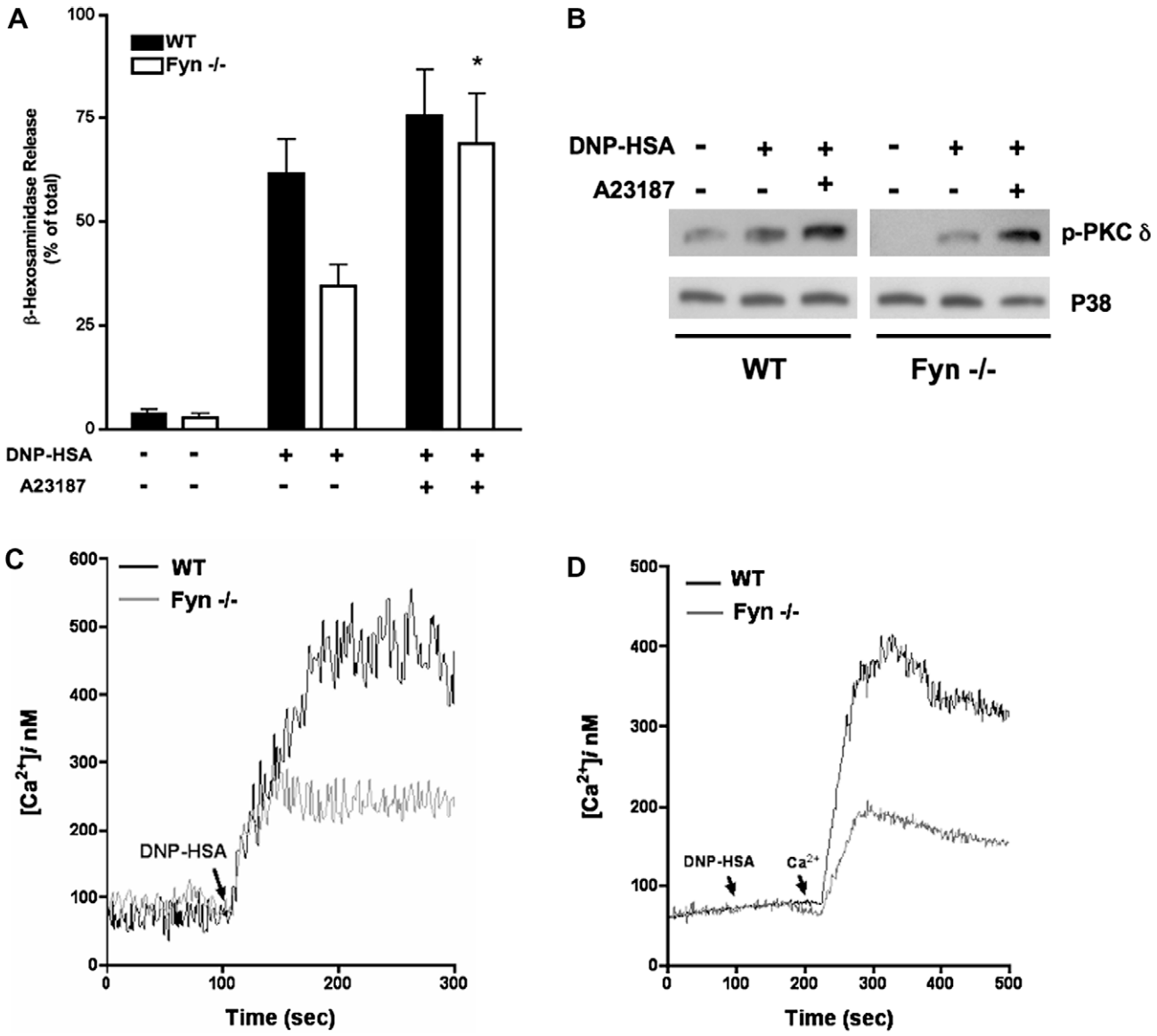

Fig. 1. Restoration of FcεRI-dependent degranulation and PKCδ phosphorylation in Fyn −/− BMMCs by Ca^{2+} ionophore. (A) WT and Fyn −/− BMMCs were IgE-sensitized and stimulated with 9 ng/mL of DNP–HSA or with DNP–HSA + 10 nM A23187. After 30 min at 37 °C, β-hexosaminidase activity was determined as described. * $p < 0.01$, respect to Fyn −/− cells stimulated with DNP–HSA alone, ANOVA. (B) WT and Fyn −/− BMMCs were IgE-sensitized and stimulated with 9 ng/mL DNP–HSA or DNP–HSA + 10 nM A23187 for 10 min at 37 °C. A representative Western blot against p-PKCδ and p38 as loading control is shown. (C) WT or Fyn −/− BMMCs were sensitized and loaded with 5 mM Fura 2-AM for 30 min. After basal fluorescence registration, DNP–HSA (9 ng/mL) was added. (D) Fura-2-loaded WT and Fyn −/− BMMCs were suspended in Ca^{2+}-free Tyrode's/BSA buffer. Antigen and Ca^{2+} to 1.8 mM were added at the indicated times.