Activation of CD47 receptors causes histamine secretion from mast cells

E. Sick*, N. Niederhoffer, K. Takeda, Y. Landry and J.-P. Gies

Université de Strasbourg, CNRS UMR 7213, Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie, 74 route du Rhin, BP 60024, 67401, Illkirch (France)

Received 8 December 2008; received after revision 12 January 2009; accepted 29 January 2009

Abstract. Mast cells play pivotal roles in allergic and inflammatory processes via distinct activation pathways. Mucosal and serosal mast cells are activated by the IgE/FcεRI pathway, while only serosal mast cells are activated by basic secretagogues. We show that CD47 receptors are expressed on rat peritoneal mast cells. 4N1K, a peptide agonist of CD47, rapidly caused exocytosis. Such exocytosis required increased intracellular calcium and was inhibited by pertussis toxin and an antibody against the βγ dimer of a G protein. Cooperation with integrins and glycosylphosphatidylinositol-anchored proteins was necessary, since anti-integrin antibodies and pretreatment with phosphatidylinositol-phospholipase C reduced exocytosis. Depletion of membrane cholesterol inhibited exocytosis and decreased CD47 in lipid rafts, consistent with a CD47/integrin/Gi protein complex being located in rafts. An anti-CD47 antibody inhibited exocytosis induced by 4N1K and by mastoparan and spermine, suggesting that basic secretagogues might target CD47. We propose that 4N1K-stimulated mast cell exocytosis involves a CD47/integrin/Gi protein complex.

Keywords. CD47, integrin-associated protein, mast cell exocytosis, inflammation, basic secretagogues.

Introduction

Mast cells are tissue dwelling cells that play a pivotal role in allergic reactions and take part in other pathophysiological conditions such as innate and acquired immunity, autoimmune diseases, inflammation, wound healing, fibrosis and tumors [1, 2]. Activated mast cells release stored and de novo-synthesized mediators including histamine, cytokines, leukotrienes, prostaglandins and proteases [3]. Both mucosal and serosal (or connective tissue) mast cells are activated by interaction of antigen to IgE bound to the high-affinity IgE receptor FcεRI [4]. In addition, serosal mast cells, such as human skin and rat peritoneal mast cells, are also activated by a variety of compounds generically known as basic secretagogues [5], via an IgE-independent pathway. These compounds include endogenous and venom peptides (e.g., substance P, bradykinin, anaphylatoxin C3a and mastoparan), drugs (e.g., neomycin and cannabinoids, defensins), and natural or synthetic polyamines (e.g., spermine and compound 48/80) [5–7]. Although many attempts to identify candidate receptors for these compounds have been made, none have been described until recently. It was thus proposed that after penetration into mast cells in an apparently receptor-independent fashion, basic secretagogues interact directly with G proteins to induce exocytosis [5–9]. The inhibitory effects of pertussis toxin pretreatment and specific blocking antibodies against G protein subunits, together with stimulation of

* Corresponding author. E-mail: esick@pharma.u-strasbg.fr
purified G proteins by mastoparan and other basic peptides, support this hypothesis [7, 9, 10]. Nevertheless, the possible involvement of membrane receptors accounting for basic secretagogue-induced mast cell activation has never been formally excluded [5]. Indeed, newly discovered G protein-coupled receptors (GPCR) called Mas-related genes (Mrg) have been recently suggested as candidate receptors for basic secretagogues [11], based on the correlation between cell expression levels and induced responses. Furthermore, trimeric G proteins may also transduce the activation of some non-heptahelical receptors [12]. This has been extensively demonstrated for CD47 (or integrin-associated protein, IAP) [13–15]. CD47, a 50-kDa transmembrane glycoprotein, is a member of the immunoglobulin superfamily that was also termed IAP because it co-purifies and interacts with integrins [16, 17]. CD47 has a single Ig-V like domain heavily glycosylated at its N terminus, five transmembrane domains and an alternatively spliced cytoplasmic C terminus. CD47 is ubiquitously expressed and, based on its modulatory interactions with several types of integrins [18–20], it has been proposed that CD47 and integrin form a complex having seven transmembrane helices that mimics GPCR [18]. CD47 also mediates cell-cell interactions via SIRP (signal regulatory protein) family receptors [21] and cell-extracellular matrix interactions via thrombospondin family members [22–24]. Thrombospondin is an endogenous ligand for CD47 [20, 23], and peptide 4N1K, widely used as a specific CD47 agonist, corresponds to the C-terminal binding motif of thrombospondin [23–25].

CD47 is implicated in many different physiological or pathophysiological processes [18], including infection [26], inflammation [27, 28], cell spreading, proliferation and apoptosis [20, 27, 29]. Given that CD47 is functionally coupled to trimeric G proteins [13–15], has pro-inflammatory effects [27] and is expressed in a variety of mast cells [30–32], we tested whether exocytosis of mast cells is triggered by activating CD47. Here, we show for the first time that stimulation of CD47 by peptide 4N1K rapidly induces secretion of histamine from rat peritoneal mast cells. Pretreatment with a specific anti-CD47 monoclonal antibody (mAb) or pertussis toxin blocked secretion induced by 4N1K. The interaction of activated CD47 with G proteins was confirmed by the inhibition of histamine release upon pretreatment with mAb directed against the Gαβγ dimer of G proteins. CD47-mediated secretion required cooperation with integrins, as mAb directed against β integrin subunits decreased histamine release. Interestingly, pretreatment with an anti-CD47 mAb also caused inhibition of secretion induced by two reference basic secretagogues, masto-

Materials and methods

Antibodies and reagents. Peptides 4N1K, K-4NGG and 4N1-2 were from Bachem (Bubendorf, Switzerland). 4N1K and 4NGG were also synthesized in the lab by Dr. H. de Rocquigny, and were verified by mass spectroscopy. Anti-CD47 (clone OX101), anti-CD48 (clone OX45), anti-CD18/β2 integrin (clone 6G2) and anti-CD61/β3 integrin (clone F11) mAbs were from Serotec (Oxford, UK). Anti-CD29/β1 integrin mAb (clone Ha2/5) was from BD Pharmingen Biosciences (San Diego, CA, USA). Anti-GpIb mAb (directed against the C-terminal 20 amino acids of mouse G protein β1 subunit, with broad specificity to mouse, rat and human GpIbβ1–4 subunits) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Fura-2/AM and 2- aminoethoxydiphenyl borate (2-APB) were from Calbiochem (San Diego, CA, USA). Alexa Fluor® 568 goat anti-mouse IgG was from Invitrogen (Paisley, UK). All other reagents were from Sigma (St. Louis, MO, USA).

Isolation and purification of mast cells. Male Wistar rats (300–500 g) were anesthetized before bleeding. Rats were injected intraperitoneally with 10 ml HEPES buffer (137 mM NaCl, 2.7 mM KCl, 0.3 mM CaCl₂, 1.0 mM MgCl₂, 0.4 mM NaH₂PO₄, 5.6 mM glucose, 10 mM HEPES and NaOH to pH 7.4) supplemented with 0.1% BSA. Peritoneal fluid was collected after gentle abdominal massage and centrifuged for 3 min at 180 g. The pellet of mixed peritoneal cells was resuspended in the same buffer and mast cells were purified by centrifugation for 5 min at 220 g on a discontinuous BSA gradient (30% and 40%, w/v). The pellet was then resuspended and mast cells were examined under a light microscope for viability (>95%) and purity (>97%) using Trypan blue and toluidine blue, respectively. Rats were raised in the animal house facilities in the Faculty of Pharmacy and used in accordance with Institutional policies (No. D-67-218-26, Direction D/Direction des Services Vétérinaires du Bas-Rhin).

Quantification of mast cell exocytosis. Purified mast cells (2×10⁵ cells/assay) were pre-incubated for 5 min at 37°C before challenge with different secretagogues.