Biochemical and Biophysical Research Communications 469 (2016) 559–564

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Intestinal and peritoneal mast cells differ in kinetics of quantal release

Santiago Balseiro-Gomez, M. Pilar Ramirez-Ponce, Jorge Acosta, Eva Ales**, Juan A. Flores*

Departamento de Fisiología Médica y Biofísica, Facultad de Medicina, Universidad de Sevilla, 41009 Seville, Spain

ARTICLE INFO

Article history:
Received 25 November 2015
Accepted 9 December 2015
Available online 12 December 2015

Keywords:
Mast cells
Exocytosis
Amperometry
Proteoglycans and serotonin

ABSTRACT

5-hydroxytriptamine (5-HT, serotonin) storage and release in mast cell (MC) secretory granules (SG) are dependent on serglycin proteoglycans (PG). This notion is based on the studies of MC of the connective tissue subtype that predominantly contain PG of the heparin type, whereas intestinal mucosal MC, which contain predominantly chondroitin sulfate, have been poorly explored. In the present study, we addressed the possibility that PG contents may differently affect the storage and release of preformed mediators in these two MC subclasses and explain in part their different functional properties. Rat peritoneal (PMC) and intestinal mast cells (IMC) were isolated and purified using a percoll gradient, and the efflux of 5-HT from each SG was measured by amperometric detection. IMC exhibited a ~34% reduction in the release of 5-HT compared with PMC because of a lower number of exocytotic events, rather than a lower secretion per single exocytotic event. Amperometric spikes from IMC exhibited a slower decay phase and increased half-width but a similar ascending phase and foot parameters, indicating that the fusion pore kinetics are comparable in both MC subclasses. We conclude that both PG subtypes are equally efficient systems, directly involved in serotonin accumulation, and play a crucial role in regulating the kinetics of exocytosis from SG, providing specific secretory properties for the two cellular subtypes.

1. Introduction

Mast cells (MC) derive from the bone marrow and migrate into connective and mucosal tissues [1], where, due to their localization close to the host/environment surface, they play a role in the first line of defense against invaders [2]. MC are specialized secretory cells that respond to inflammatory signals with the massive secretion of a wide range of inflammatory products. Some products, such as histamine, serotonin (5-HT), proteases and proteoglycans, are stored in cytoplasmic secretory granules (SG). SG in secretory cells perform two different functions: they act as a storage depot for small molecules and they release these molecules during exocytosis. Storage in dense core vesicles requires stable packaging for an extended period of time. This is accomplished by association of the vesicular contents. On the other hand, release requires dissociation of this storage matrix on a relatively fast time scale so the contents can be extruded [3,4]. In MC, histamine and 5-HT are associated with a negatively charged serglycin proteoglycan (PG) matrix in the granules through a cation exchange type of interaction [5]. The absence of serglycin has a dramatic effect on the ability of MC to store a number of granule compounds, including several MC-specific proteases and biogenic amines [6].

Two major subclasses of MC can be distinguished: mucosal MC (MMC) and connective tissue MC (CTMC) [7]. CTMC typically reside in the skin and the peritoneal cavity. In contrast, MMC are predominant in the mucosal layer of the intestine, where their numbers expand dramatically during, for example, parasitic infection [7]. The different functional properties and tissue localizations of CTMC and MMC are reflected by clearly distinct contents of SG components, in particular, with respect to the specific composition of PG. Sulfated proteoglycans constitute the major constituents of MC granules. Of these, heparin is mostly found in CTMC, whereas chondroitin sulfate is found in MMC [8,9].

During exocytosis, secretory products are released through a pore that connects the lumen of the SG to the extracellular medium. Release from single granules can be monitored by

Abbreviations: 5-HT, serotonin; MC, mast cells; PMC, peritoneal mast cells; IMC, intestinal mast cells; MMC, mucosal mast cells; CTMC, connective tissue mast cells; PG, proteoglycans; SG, secretory granule; I_{max}, maximum oxidation current; $t_{1/2}$, spike width at half-height; Q, spike net charge; $Rise_m$, ascending slope of spike; I_{foot}, foot maximum current; t_{foot}, foot life-time; Q_{foot}, foot charge.

* Corresponding author.
** Corresponding author.
E-mail addresses: sanbalgom@alum.us.es (S. Balseiro-Gomez), pponce@us.es (M.P. Ramirez-Ponce), jorgealo@us.es (J. Acosta), eales@us.es (E. Ales), jaflores@us.es (J.A. Flores).

http://dx.doi.org/10.1016/j.bbrc.2015.12.033
0006-291X/

amperometry with a carbon fiber electrode [10–13], which ensures that each exocytotic event, i.e., the release of the vesicular content, can be observed as an amperometric spike whose main features give information about the nature (kinetics, charge) of the whole process [14]. The dilation of the fusion pore and the release of the granule contents are associated with a rapid swelling of the granule matrix [15], which has been attributed to the displacement of associated cations in the granule with hydrated cations, such as Na^+ from the external solution [16]. Thus, the PG matrix functions as an ion exchanger, and the storage and release of secretory products occur by ion exchange with the counter ions that neutralize the charge groups that are fixed in the matrix. In chromaffin cells, the presence of a granule matrix (chromogranin) that binds catecholamines slowed the discharge of these transmitters by several orders of magnitude [3]. This role was also proposed for the peritoneal mast cell (PMC) granule matrix, where changes in the ionic composition of the surrounding media, pH, or electric potential produced changes in the association of soluble monoamines with the heparin sulfate matrix [17,18]. Less is known about the mechanisms that govern the storage and release of 5-HT in MMC because mature MC isolation from mucosal sites is complicated. In addition, despite the strong implication of 5-HT in immune regulation, the contribution of the sulfated glycosaminoglycans (heparin or chondroitin sulfate) attached to the serglycin protein core in the control of storage and efflux of 5-HT has been poorly investigated. In the present study, we addressed the latter issue by comparing single exocytotic events obtained by amperometry from peritoneal and intestinal MC.

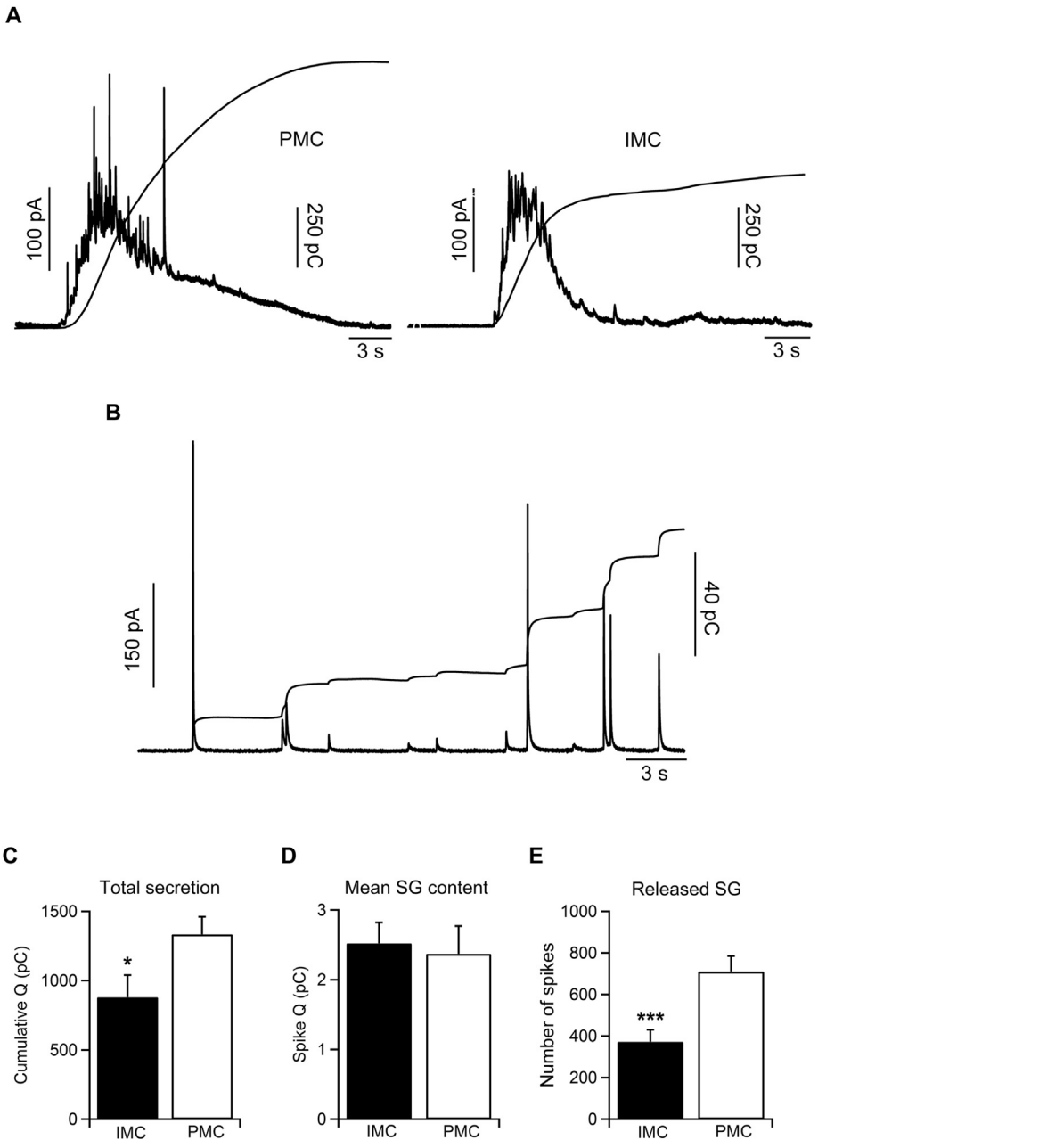

Fig. 1. Secretion of serotonin from cultured mast cells. Typical amperometric traces obtained from PMC (panel A) and IMC (panel A and B) after the application of C48/80; the left calibration bar is for the amperometry trace and the right bar is for the integrated signal. Histogram plotting the quantification of the cumulated release (Panel C) ($^*p = 0.038$; Mann–Whitney *U* test) and spike charge measured per individual amperometric spike (Panel D). The average number of events for each cell illustrates the number of exocytotic events that is reduced in IMC (panel E) ($^{***}p = 0.0010$; Mann–Whitney *U* test).