# Upregulation of Mast Cells Degranulation and Anaphylaxis Biomarkers via Substance P and Calcium Ionophore: Possible implication in Extracellular Allergic Disease

**Naglaa K Idriss[a,b,d], Andrew F Walls[a], Laurie C Lau[a], Mogib E Khedr[a], Rana S Abadalkareem[a], Reham I El-Mahdy[b,d], Amr Ashry[c], Marwa A. Gaber[b, e*]**

[a]Immunopharmacology Group, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD, UK.

[b]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[c]Department of Cardiothoracic Surgery, Assiut University Hospital, Faculty of Medicine, Assiut University, Assiut, Egypt

[d]Department of Basic Medical Science, Badr University, New Naser City, West of Assiut, Assiut, Egypt.

[e]Biochemistry Department, Sphinx University, New Assiut, Egypt

**Abstract**

**Background:** Mast cells play crucial roles in the pathogenesis of allergies through the secretion of powerful mediators, prominent among which is a group of proteases that are stored and function within the intracellular granules of mast cells.

**Objectives:** We aimed to investigate the secretion of these proteases from mast cells during degranulation and to explore the factors that can influence the secretion and gene expression of mast cell mediators.

**Materials and methods:** Mast cell line (LAD2) were stimulated to degranulate with calcium ionophore A23187 (CaI) or substance P (SP). Gene expressions of dipeptidyl peptidase I (DPPI) and carboxypeptidase A3 (CPA3), chymase (CHY), tryptase (TRY) were determined by quantitative RT-PCR. Alterations in gene expression were investigated at time points ranging from 0.5 to 6 hours following cell stimulation. Beta-hexosaminidase activity and DPPI levels were measured using ELISA.

**Results**: Experimental stimulation of sensitized mast cells by CaI or SP, revealed by the release of the activation marker β-hexosaminidase in a time dependent manner (net release=63% ± 2.4 at 6 h), this was associated with significant increases in gene expression for DPPI and also for CPA3, CHY and TRY. Cal sensitization produced a significant increase in DPPI, CPA3 and CHY in the first 30m of stimulation with gradual decrease in levels with time but still significantly higher than non-stimulated cells ($p \leq 0.05$ for all). The reverse for TRY, as the most significant increase in its expression was at 2H. SP produced a gradual increase in gene expression for all studied parameters ($p \leq 0.05$ for all) with the most significant increase was for TRY gene at 2h ($p < 0.01$ vs cal).

**Conclusion:** DPPI can be released from mast cells on degranulation, and the subsequent increases in gene expression would be consistent with the later replenishment of secretory granules. This protease may participate in processes of protease activation and other activities outside the cell in inflammatory disease. Thus, DPPI could be represented as an innovative marker for mast cell sensitization which may accurately diagnose the allergic responses specially when measured as panel with CPA3, CHY and TRY.

**Keywords**: Degranulation; DPPI; Inflammatory disease; Mast cells; Proteases

**DOI: 10.21608/SVUIJM.2025.373499.2159**

***Correspondence**: marwagaber@aun.edu.eg

**Received:** 12 March,2025. **Revised:** 12 April, 2025.

**Accepted:** 17 April, 2025. **Published**: 17 April, 2025

## Introduction

Allergic reactions denote an augmented sensitivity to various stimuli, spanning from drugs and food to insect bites, as well as environmental triggers like pollens, animal dander, and dust mites **(Basu and Banik, 2018).** Anaphylaxis can be demarcated as systemic allergic response which affects multiple systems, that is rapid in onset and can be fatal, and requires immediate medical intervention **(Kim and Fischer, 2011; Muraro et al., 2014).** Over last years, the prevalence of allergic reactions has been increased significantly, sparking numerous endeavors aimed at elucidating the underlying mechanisms and predisposing factors. While demographic correlations have been identified, the molecular and cellular processes remain less comprehensively understood. Moreover, the diagnosis of allergic reactions poses challenges due to their often rapid onset and unpredictable trajectory which may range from restricted skin reactions to potentially fatal respiratory and circulatory complications **(Guibas et al., 2017).** Besides, routine clinical laboratory tests used to confirm allergic reactions face substantial limitations.

There has been ongoing research into the role of mast cells in health and illness meanwhile their detection. They considered as multifunctional tissue-dwelling cells that traditionally implicated with allergic conditions, although their participation in defense machines and inflammatory conditions is becoming increasingly appreciated **(Pejler et al., 2010; Caughey, 2011; Wernersson and Pejler., 2014)**. Mast cell stimulation leads to its degradation and excretion of numerous intermediates nevertheless those with specific probability as allergic molecules are proteases: dipeptidyl peptidase I (DPPI), carboxypeptidase A3 (CPA3), chymase (CHY) and tryptase (TRY). Because they are unique to mast cells, determining the serum levels of such mediators in allergic patients with would approve the presence of an immune reaction **(NICE, 2011; Xiao et al., 2021)**.

Both immunological and non-immunological mechanisms are implicated in allergic reactions precipitation. The immunological one could be IgE-dependent or IgE-independent responses **(Khan and Kemp, 2011).** In the IgE-mediated reactions, the exposure of the sensitized person to the offending allergen results in its binding with IgE antibody, which in turn binds to the highly specified IgE receptor (FcεRI) on mast cells.

As a sequel, mast cells exhibit a cascade of reactions subsequently ended by allergy signs. This includes the release of various inflammatory compounds especially proteases, that induces augmented vascular permeability, bronchospasm, increased mucous production and eosinophil enrollment **(Sampson et al., 2006; Kalesnikoff and Galli 2010)**.

==========

**Cite this article as** Naglaa K Idriss, Andrew F Walls, Laurie C Lau, Mogib E Khedr, Rana S Abadalkareem, Reham I El-Mahdy , Amr Ashry, Marwa A. Gaber. (2025). Upregulation of Mast Cells Degranulation and Anaphylaxis Biomarkers via Substance P and Calcium Ionophore: Possible implication in Extracellular Allergic Disease. *SVU-International Journal of Medical Sciences*. Vol.8, Issue 1, pp: 852-865.

DPPI (or cathepsin C) is a lysosomal cysteine protease belonging to the papain-like cysteine peptidases family found mainly in mast cells and other inflammatory cells **(Chitsamankhun et al., 2024).** Upon maturation, the human recombinant DPPI adopts a compact tetramer structure with each monomer housing an exposed active site cleft to allow its catalytic action **(Chitsamankhun et al., 2024).** DPPI acts intracellularly to activate a variety of proteases by cleaving their amino-terminal dipeptides. It has been reported that there were a 90% decrease in tryptase activity of a mastocytoma cell line with DPPI inhibition. On the other hand, DPPI management of recombinant pro-chymase resulted in its transformation into the enzymatically active chymase **(McEuen et al., 1998).** Moreover, DPPI is thought to play a crucial role in regulating levels of CPA3 **(Henningsson et al., 2003; NICE, 2014).**

Consequently, our study aimed to investigate the secretion of DPPI from mast cells upon degranulation and determine whether this protease may play a role in processes of other protease activation and extracellular activities in inflammatory diseases in a time dependent manor. Additionally, the present study evaluated the impact of calcium ionophore A23187 and SP on mast cell stimulation and degranulation, which can control the expression and secretion of mast cell molecules, aiming to establish a theoretical foundation for the treatment of allergic diseases.

## Materials and methods

### *Culturing of LAD2 cells*

LAD2 cell line is considered the most appreciated one for studying the human mast cell biology as it expresses the highly specified FcεRI and contain abundant granules thus degranulate easily in response to antigen. In addition, it can be used for the studying of signaling procedures that regulate these reactions. However, the main disadvantage of LAD2 cells is their comparatively slow development rate, which doubles every two weeks on average. LAD2 cells were purchased from Brooke Laboratory, Southampton University. StemPro-34 serum free medium was used for the culture of LAD2. It contains 2% (v/v) StemPro-34 supplement, 1% (v/v) penicillin-streptomycin-glutamine, and 100ng/ml stem cell factor. When the cells reached $1x10^6$ cells/ml, they were collected and centrifuged for 10 minutes at 270 g. Cells were washed and resuspended in PBS and recentrifuged again as previously mentioned. The cell pellet collected, counted and the number was attuned according to the subsequent procedure **(ePrints Soton, 2018; Kirshenbaum et al., 2019).**

### *Degranulation assay*

LAD2 Cells and IgE Sensitization: A cell pellet containing 20 x 10^6 LAD2 cells was suspended in 10 mL of serum-free media alone (StemPro-34®, ThermoFisher Scientific, UK). Nearly, 3.0 x 10^6 cells (or 1.5 mL) from that 10 mL were then added to designated wells in a sterile 6-well culture plate. In one set, only LAD2 cells (0%) and 3.5 mL of serum-free media were added (non-sensitized, negative control), while in the second set, 1.5 mL of cells, along with 15 µL of human myeloma IgE and 1.485 mL of serum-free media, were added (sensitized, positive control). The plate was subsequently incubated overnight at 37°C in 5% CO2 under humidified conditions (**Adnan et al., 2023**). The next day, the cells were harvested and collected into sterile tubes, centrifuged and the supernatants were discarded. The cell pellets were resuspended in 3.5 mL of Tyrode's buffer and 180 µL of the cells was added to the wells of a sterile v-bottom plate according to the design template.

**1- Stimulation with calcium ionophore A23187 (CaI) and substance P (SP):**

An incubation step in dehydroleucodine, xanthatin or 3-benzyloxymethyl-5H-furan-2-one was done before the LAD2 cells were cultured in calcium ionophore A23187 (CaI) and substance P (SP) (both final concentration 10 µg/mL) for 20 min at 37°C (5% CO2 and humidified atmosphere) (Schulman et al., 2023).

Twenty µL each of 10 µM CaI and 3% anti-IgE (resulting in a final concentration of 1 µM) or 10 µg/mL SP and 3% anti-IgE were prepared and added to all appropriate wells. A control of 20 µl/well 3% anti-IgE (including spontaneous and total positive stimuli) was prepared for each group. The plate was then incubated at 37°C for 1 hour then centrifuged. The supernatants were carefully removed without disturbing the cell pellets, and the plate was kept on ice **(Adnan et al., 2023).**

2- **β-Hexosaminidase assay:** The final step of the assay involved transferring 30 µl of the previously prepared supernatant into a 96-well plate in triplicate. These included tubes containing supernatant from negative control cell lysates, positive spontaneous release (The release of molecules upon addition of Tyrode's buffer (20 µl/well) only without any stimulus), positive total release (upon treating with 1% Triton X-100, 20 µl/well) and supernatant was induced by CaI and substance P. Subsequently, 50 µl of the β-hexosaminidase substrate (Sigma N-9376) was added and the plate was incubated for 1 hour at 37°C. The reactions were halted by 0.2 M glycine (100 µL/well) (**Rujitharanawong et al., 2022**). Optical density readings were taken at 410 nm using a microplate reader, and the results were expressed as % net release of β-hexosaminidase calculated by the subsequent equation:

Net release (%) = (cell lysate sample Abs – spontaneous release Abs) X 100/ total release Abs.

***DPPI assay by ELISA method***

The reagents used consisted of monoclonal anti-DPPI antibody (supplied by St. John's laboratories, London, UK) that can combined with both recombinant DPPI (capture antibody) and rabbit polyclonal anti-DPPI (detecting one). Standards (up to 1000 ng/ml) were prepared from human recombinant DPPI. The reaction was blocked by foetal bovine serum (FBS) in PBS in concentration of 20% and the 3% FBS was used as the diluting agent.

***Quantitative real-time polymerase chain reaction (qRT-PCR) for genes***

The effect of CaI and SP on the degranulation-induced changes in the gene expression of the DPPI, CPA3, CHY and TRY in the supernatant of cell line was detected using quantitative RT-PCR at time points ranging from 0.5 to 6 hours following cell stimulation, with GPDH as the reference gene. RNA Isolation and purification from LAD2 cells were performed with Qiagen's RNA Mini Kit (RNeasy Mini Kit, Qiagen #74106) according to the manufacturer's guidelines. The extracted total RNA was assayed spectrophotometrically at 260 and 280 nm by mean of Nanodrop® (Epoch Microplate Spectrophotometer, Biotek, USA). The genes expression was assessed by real time PCR (ABI Prism SDS 7000). For cDNA synthesis, reverse transcription was done by the Super Script First-Strand Synthesis System for RT-PCR using 1000 ng of the total RNA from each sample. Next, PCR amplification cycles were done as the thermal cycling profile was 50 °C for 2 min followed by 95 °C for 10 min. Subsequently, 40 cycles of PCR amplification were done, as 15s DNA denaturation at 95 °C, 30s primers annealing at 60 °C and 30s at 72 °C followed by one cycle of 10 min at 72 °C. Normalization of each target gene was done

relative to the mean critical threshold (CT) values of GAPDH as housekeeping gene by the ΔΔCt method. (**Table.1)** listed the primer sequences of every tested gene.

**Table 1. Primers sequences used for the qRT-PCR reaction**

| Target gene | Primers sequences 5'-3' |
|---|---|
| CPA3 | F: CCATTCCTACTCCCAGATGC<br>R: TTGATAGAACATCAGTGCCAATC |
| TRY | F: GCGATGTGGACAATGATGAG<br>R: TCCATTATGGGGACCTTCAC |
| CHY | F: ACGGAACTTTGTGCTGACG<br>R: GGCTCCAAGGGTGACTGTTA |
| DPP1 | F: TTGTAGCCAGCATGCTCAAG<br>R: GCCTGTGTAGGGGAAGCA |
| GAPDH | F: ATGACCCCTTCATTGACC<br>R: GAAGATGGTGATGGGATTTC |

**Statistical analysis**

All data analysis was done with IBM_SPSS version 24 and GraphPad Prism software version 8.0.1 (GraphPad Software, USA). All values were presented as mean ± standard error (SEM). Normality was tested for the main parameters using Shapiro Wilk test. Student t-test/Mann Whitney U test was used to test the mean/median differences of the data according to normality. The results achieved were considered significant at the $p < 0.05$ level.

**Results**

***Characterization of cultured LAD2 cells***

Cultured LAD2 were recognized by their morphology as displayed in **(Fig.1).**

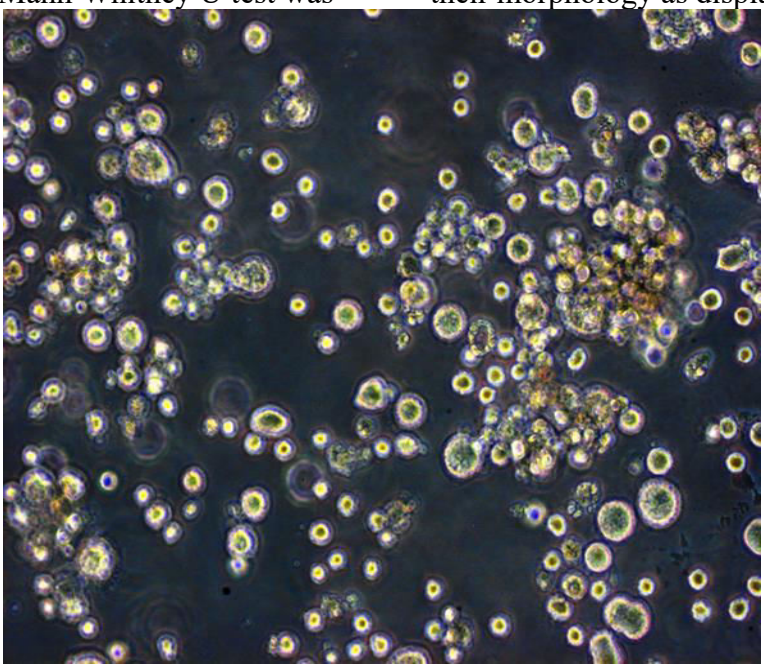

**Fig. 1. The microscopic morphology of mature LAD2 cells**.Cells are rounded, oval or irregularly mononuclear cells with dense granular cytoplasm that frequently obscuring the nucleus

***Effect of incubation with calcium ionophore and SP for 30m on activation and degranulation of LAD2 cells***

To examine the ability of calcium ionophore and SP to degranulate and activate mast cell, LAD2 cells were incubated with calcium ionophore (1.00 µM) and SP (1.00 µM), and the genes expression profile of CPA3, TRY, CHY, and DPP1 were evaluated after 30 min. of treatment using quantitative real-time PCR technique. As shown in (**Fig.2),** calcium ionophore significantly increased CPA3, TRY, CHY, and DPP1 expression levels whereas SP treatment caused a significant increase in TRY, CHY, and DPP1 expression levels compared to LAD2 cells without stimulation. In addition, a significant increase in TRY, and DPP1 expression levels with SP vs calcium ionophore was observed **(Fig.2).**

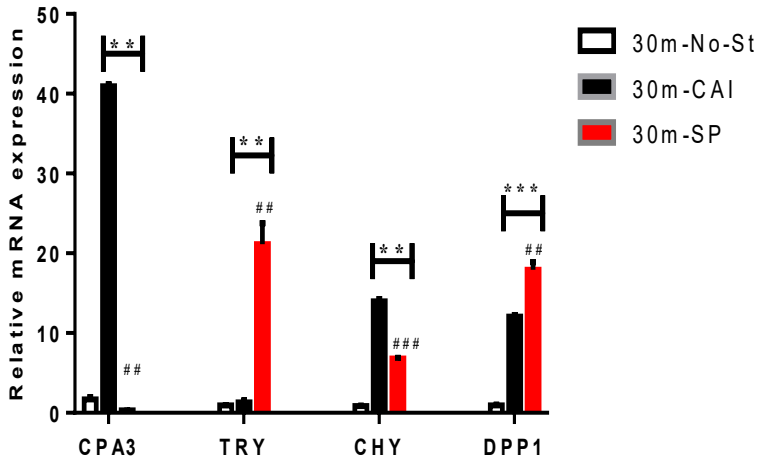


**Fig. 2.Relative quantitative expression of mRNA levels of CPA3, TRY, CHY, and DPP1 in LAD2 cells** .Without stimulation, after incubation of calcium ionophore (1.00 µM) for 30m, and after incubation of SP (1.00 µM) for 30m. Results were normalized to GAPDH and expressed as means ± SEM.

** P< 0.01, *** P<0.001 vs no stimulation, ## P < 0.01, ###P < 0.001 vs calcium ionophore.

***Effect of incubation with calcium ionophore and SP for 1 hour on activation and degranulation of LAD2 cells***

Treatment of LAD2 cells with calcium ionophore (1.00 µM) for 1hr. resulted in a significant amplified relative expression of CPA3, TRY, and CHY in comparison to the native LAD2 cells. Moreover, supplementation of the LAD2 cells with SP (1.00 µM) for 1hr. caused a significant upregulation of CPA3,and TRY expression levels as compared to native LAD2 cells and in TRY expression in contrast to the calcium ionophore treated LAD2 cells ($p<0.01$) **(Fig.3).**

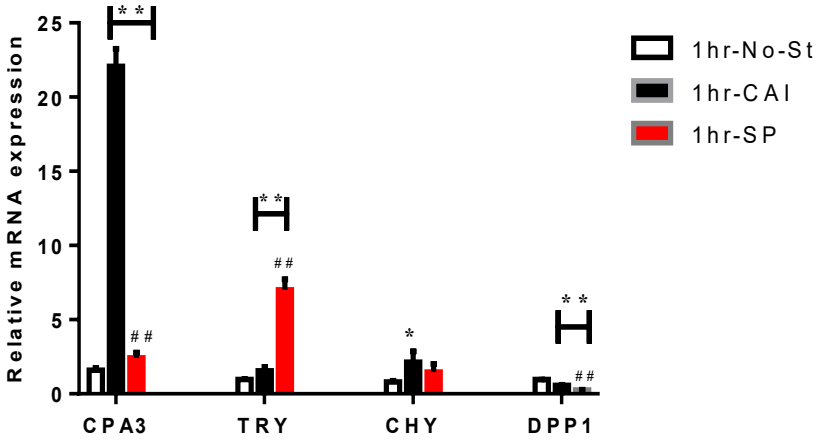


**Fig. 3. Relative quantitative expression of mRNA levels of CPA3, TRY, CHY, and DPP1 in LAD2 cells**. Without stimulation, after incubation of calcium ionophore (1.00 µM) for 1hr, and after incubation of SP (1.00 µM) for 1hr. Results were normalized to GAPDH and expressed as means ± SEM.

* P< 0.05, **P<0.01 vs no stimulation, ## P < 0.01 vs calcium ionophore.

***Effect of incubation with calcium ionophore and SP for 2 hrs on activation and degranulation of LAD2 cells***

CPA3, TRY, and DPP1 expression levels were augmented significantly in the mast cell line treated with calcium ionophore (1.00 µM) for 2 hr. compared to non-treated cells. The SP treated cells (1.00 µM) for 2 hr. showed a significant increase in the expression levels of all studied genes compared to non-treated cells and significant upregulation in TRY, CHY and DPP1 expression levels compared to the calcium ionophore treated LAD2 cells **(Fig.4).**

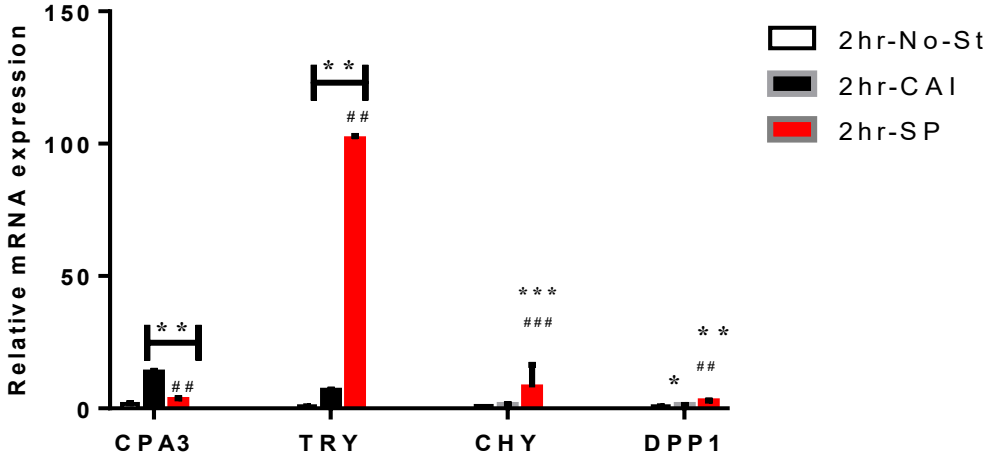


**Fig. 4. Relative quantitative expression of mRNA levels of CPA3, TRY, CHY, and DPP1 in LAD2 cells**. Without stimulation, after incubation of calcium ionophore (1.00 µM) for 2hr, and after incubation of SP (1.00 µM) for 2hr. Results were normalized to GAPDH and expressed as means ± SEM.

* P< 0.05, ** P< 0.01, *** P<0.001 vs no stimulation, ## P < 0.01, ###P < 0.001 vs calcium ionophore.

***Effect of incubation with calcium ionophore and SP for 6 hrs on activation and degranulation of LAD2 cells***

Regarding CPA3, TRY, CHY, and DPP1, relative quantitative expression of mRNA levels after incubation of calcium ionophore and SP for 6 hr were shown in Fig. 5. Calcium ionophore (1.00 µM) treatment for 6 hr. associated with a significant upregulation in the expression of mast cell mediators (CPA3, TRY, CHY, and DPP1) in comparison with the untreated LAD2 cells. Moreover, LAD2 cells treated with SP (1.00 µM) verified a time-dependent upregulation in CPA3, TRY, and DPP1 expression levels compared to the native LAD2 cells ($p<0.01$) and significant upregulation in TRY, and DPP1 expression levels compared to the calcium ionophore treated LAD2 cells ($p<0.01$) **(Fig.5).**

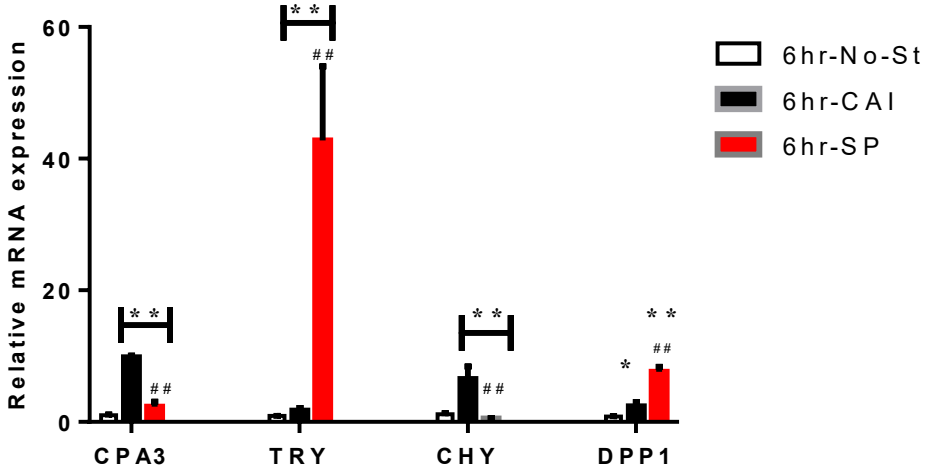


**Fig. 5. Relative quantitative expression of mRNA levels of CPA3, TRY, CHY, and DPP1 in LAD2 cells**. Without stimulation, after incubation of calcium ionophore (1.00 µM) for 6hr, and after incubation of SP (1.00 µM) for 6hr. Results were normalized to GAPDH and expressed as means ± SEM.

* P< 0.05, ** P< 0.01 vs no stimulation, ## P < 0.01 vs calcium ionophore.

**The release of B-hexoamindase from LAD2 cells**

As a biomarker of mast cell activation and degran ulation β-hexosaminidase was assesed. LAD2 cells were treated with calcium ionophore (1.00 µM) and the release was evaluated together with DPP1 after 1hr., 2hr., 3hr., 4hr., 5hr., and 6 hr. of treatment using the ELISA assay. As shown in (**Fig.6)**, release of B-hexoamindase and DPP1 significantly increased in mast cell line after treatment with calcium ionophore (1.00 µM). This suggests that calcium ionophore (1.00 µM) have an activation effect on LAD2 cells as a function of time.

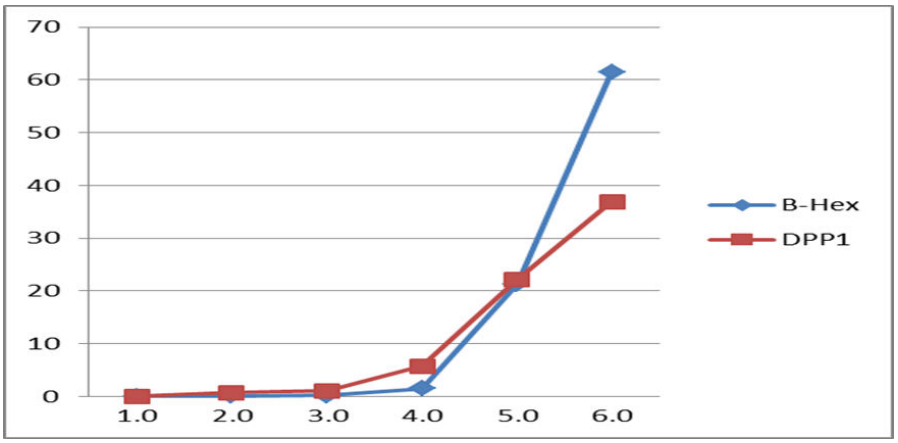


**Fig 6. The release of B-hexoamindase and DPP1 from LAD2 cells challenge with Ca Inophore**

## Discussion

In recent years, there has been a notable rise in the prevalence of allergy-related conditions and asthma, impacting approximately 20% of the global population, though with considerable variations among countries **(Burchett et al., 2022).** Understanding the pathophysiology of allergy and discovering innovative treatment protocols for anaphylaxis and allergic reactions have remained focal areas of research activity. A variety of cells, including eosinophils, neutrophils, macrophages, and mast cells, exert significant influence on the development and progression of allergy. Among these, mast cells emerge as pivotal actors in the asthmatic response, as they release various mediators that provoke pro-inflammatory cytokine release and induce airway constriction **(Burchett et al., 2022).** Consequently, the primary emphasis in developing effective allergy treatments is on identifying the factors triggering mast cell activation and understanding the associated molecular processes **(Burchett et al., 2022).**

The current study on LAD2 mast cell line revealed a significant increases in gene expression for DPPI and also for CPA3, CHY and TRY in a time dependent manor after stimulation of mast cells by CaI or SP (1.00 µM, for both). In addition, our investigations revealed that DPPI is present in association with mast cell proteases (CPA3, TRY, CHY) and in parallel with the activation marker β-hexosaminidase, suggesting that DPPI and other mast cell proteases may be released during mast cell sensitization and degranulation.

Substance P (SP), a neuropeptide belonging to the tachykinin 1 (TAC 1) polypeptide family, is distributed throughout the nervous systems, the digestive system and various immune cells. SP typically attaches to its primary high-affinity neurokinin-1 (NK 1) receptor when it is released ,initiating cellular signaling pathways associated with peripheral neurogenic inflammation, digestive motility, and several functions within the CNS, such as pain perception, itch response, sleep regulation, cognition, memory, and mood **(Garcia and Gascon, 2015).** Thus, the effects of SP on mast cell activation that we report in our study might occur through activation of the NK-1 receptor which earlier demonstrated to be expressed by human mast cells **(Taracanova et al., 2017).** Various concentrations of SP were employed to elicit a mast cell response, but we ultimately settled on using 1µM for optimal efficacy. The rationale behind the utilization of this higher concentration of SP in our assay is

that, as previously noted, SP might undergo decomposition throughout the assay process due to chymase released from human mast cells **(Taracanova et al., 2018).**

Mast cells are renowned for their pivotal involvement in IgE-dependent allergic inflammatory conditions and are frequently situated adjacent to sensory neurons. But IgE-independent methods, such through the Mas-related G protein coupled receptor X2 (MRGPRX2), can also activate human mast cells **(Roy et al., 2021).** MRGPRX2 responds to various typically polycationic ligands, including certain antimicrobial peptides, peptidergic and non-peptidergic medications, and SP. The tachykinin neuropeptide SP, when bound to MRGPRX2, induces mast cell degranulation, leading to the release of inflammatory molecules like histamine and proteases, thereby triggering allergic inflammatory reactions. There is increasing evidence that this route has a role in chronic urticaria, asthma, and SP-mediated pain and itching signals. **(Green et al., 2019).**

In the current study, the measured mast cell mediators revealed high levels of TRY and DPP1 relative gene expression following incubation with SP (1.00 μM) for 30 minutes, 2 hours, and 6 hours compared to LAD2 cells without stimulation. This finding is consistent with the study of **Taracanova and his colleagues (2017)** as they considered the effects of the cytokine IL-33 alone and in combination with SP on the synthesis and expression of IL-1β, pro-IL-1β, and caspase-1 **(Taracanova et al., 2017).** Additionally, **Petrosino et al. (2019)** stated that Palmitoylethanolamide inhibits the response to SP stimulation in a dose-dependent manner, leading to the suppression of SP-induced mast cell degranulation and the secretion of β-hexosaminidase and histamine **(Petrosino et al., 2019).**

Furthermore, this study revealed that the LAD2 mast cell line releases CPA3, TRY, CHY, and DPP1 in response to calcium ionophore A23187 at a concentration of 1.00 μM. Following treatment with the calcium ionophore, protein levels of β-hexosaminidase and DPP1 significantly increased in the mast cell line. This activation effect on LAD2 cells occurred as a function of time. The response is primarily mediated by the extracellular influx of $Ca^{2+}$ into the mast cells, moving against the concentration gradient, which elevates the $Ca^{2+}$ concentration within the mast cells and ultimately triggers degranulation of the mast cell granules **(Moon et al., 2014).**

It has been proposed that an increase in intracellular $Ca^{2+}$ causes microtubule and actin assemblies to reorganize, which is req uired for the translocation and membrane fus ion of mast cell secretory granules.

Similarly, a previous study demonstrated that usnic acid, acting as a calcium ionophore, modulates intracellular calcium levels and secretory granule dynamics, leading to the degranulation of basophilic leukemia cell culture (RBL-2H3) and the release of β-hexosaminidase into the extracellular media after a 30-minute incubation **(Chelombitko et al., 2020)**.

Stimulation of FcεRI on mast cells enhances rapid upsurges in intracellular free calcium concentrations and activation of numerous transcription factors as NF-κB, JNK and CREB **(Holowaka et al., 2012).** In a variety of immune system cells, including T cells, B cells, and mast cells, $Ca^{2+}$ acts as a universal second messenger. Many cellular processes in mast cells, including migration, adhesion, exocytosis, gene expression, and proliferation, depend on $Ca^{2+}$ signaling. Mobilization of calcium in lymphocytes and mast cells occurs in two stages **(Oh-hora, 2009).** Phospholipase Cγ (PLCγ) is activated in the first stage by tyrosine kinases linked

to FcεRI. Phosphatidylinositol bisphosphate is hydrolyzed by PLCγ to produce inositol triphosphate (IP3), which triggers the quick nevertheless transitory mobilization of Ca2+ from endoplasmic reticulum reserves. A prolonged extracellular Ca2+ influx through the plasma membrane characterizes the second stage. Specialized plasma membrane Ca2+ channels, also known as store-operated Ca2+ channels or calcium release-activated Ca2+ channels, are responsible for this so-called "store-operated" mechanism **(Hosokawa et al., 2013).** Our results are in line with **Park and his colleagues (2023)** who demonstrated that the anti-allergic effect of caffeic acid methyl ester (CAME) is due to the concentration-dependent suppression of the CaI 23187-induced TNF-α secretion, and β-hexosaminidase and histamine release **(Park et al., 2023).** In addition, it has been reported that Adenophorae Radix (AR) successfully diminished the PMACI-induced production of TNF-α, IL-6, IL-1β and IL-8 in stimulated HMC-1 **(Myung, 2020).**

Mast cell-specific proteases, such as CPA3, TRY, CHY, and DPP1, maintain their activity within secretory granules. Among these proteases, tryptase is the primary one in human mast cells, and the ratio of tryptase to chymase is used to define specific mast cell types. Chymase is also found in notable concentrations within particular mast cell subsets. These proteases create complexes with proteoglycans inside the granules, which stabilize them and regulate their function. When mast cells are activated and release their granules, the proteases gradually separate from the proteoglycans and are swiftly discharged into the extracellular matrix as active monomers. These mediators profoundly affect nearby tissues, triggering diverse cellular responses, including the initiation of immunological and inflammatory reactions, as well as cellular hyperplasia **(Méndez-Enríquez and Hallgren, 2019)**

**Limitations of the study:** Our study involved the stimulatory effect of cal and sp only IgE dependant responses, further studies on IgE-independent responses are mandatory.

**Conclusion**

Our study demonstrated that mast cells express group of proteases including DPPI, CPA3, tryptase, chymase, and B-hexoamindase upon degranulation with calcium ionophore (1.00 μM) and SP (1.00 μM). These findings highlight the significant involvement of calcium ionophore and SP in mast cell secretion. Therefore, in allergic inflammation, calcium ionophore and SP may represent a potential target in modulation/regulation of mast cell responses . Given the potential therapeutic value of inhibiting calcium ionophore and SP function to prevent inflammation, further study of these substances in the in vivo mast cell models is required.

**Conflict of interest:** The authors report no conflict of interest.

**List of abbreviation: CaI**: calcium ionophore A23187; **CHY:** chymase; **CPA3:** carboxypeptidase A3; **DPPI**: dipeptidyl peptidase I; **FcεRI:** Fc-epsilon receptor I; **LAD2:** laboratory of allergic diseases 2**; SP**: substance P; **TRY:** tryptase;

**References**


- **Basu S, Banik B. (2018).** Hypersensitivity: an overview. Immunol Curr Res, 102:105.
- **Kim H, Fischer D. (2011).** Anaphylaxis. Allergy Asthma Clin Immunol, 7 Suppl 1:S6.
- **Muraro A, Roberts G, Worm M, Bilo MB, Brockow K, Fernandez Rivas M, et al. (2014).** Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy, 69:1026-1045.

- **Guibas GV, Mathioudakis AG, Tsoumani M, Tsabouri S. (2017)**. Relationship of allergy with asthma: there are more than the allergy "eggs" in the asthma "basket". Frontiers in pediatrics, 5:92.
- **Pejler G, Ronnberg E, Waern I, Wernersson S. (2010).** Mast cell proteases: multifaceted regulators of inflammatory disease. Blood, 115:4981-4990.
- **Caughey GH. (2011).** Mast Cell Proteases as Protective and Inflammatory Mediators. Mast Cell Biology: Contemporary and Emerging Topics, 716:212-234.
- **Wernersson S, Pejler G. (2014).** Mast cell secretory granules: armed for battle. Nature Reviews Immunology , 14:478-494.
- **NICE. (2011).** Anaphylaxis: assessment and referral after emergency treatment. In NICE Guidlines: https://www.nice.org.uk/guidance/cg134/chapter/1-Recommendations;.
- **Xiao Y, Cong M, Li J, He D, Wu Q, Tian P, et al. (2021).** Cathepsin C promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. Cancer Cell, 39:423-437. e427.
- **Khan BQ, Kemp SF. (2011).** Pathophysiology of anaphylaxis. Curr Opin Allergy Clin Immunol, 11:319-325.
- **Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Bock SA, Branum A, et al. (2006).** Second symposium on the definition and management of anaphylaxis: summary report-Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol, 117:391-397.
- **Kalesnikoff J, Galli SJ. (2010).** Anaphylaxis: mechanisms of mast cell activation. Chem Immunol Allergy, 95:45-66.
- **Chitsamankhun C, Siritongtaworn N, Fournier BPJ, Sriwattanapong K, Theerapanon T, Samaranayake L, et al. (2024).** Cathepsin C in health and disease: from structural insights to therapeutic prospects. J Transl Med. 20;22(1):777.
- **McEuen AR, Ashworth DM, Walls AF. (1998).** The conversion of recombinant human mast cell prochymase to enzymatically active chymase by dipeptidyl peptidase I is inhibited by heparin and histamine. Eur J Biochem, 253:300-308.
- **Henningsson F, Wolters P, Chapman HA, Caughey GH, Pejler G. (2003).** Mast cell cathepsins C and S control levels of carboxypeptidase A and the chymase, mouse mast cell protease 5. Biological Chemistry, 384:1527-1531.
- **NICE. (2014).** Drug Allergy: Diagnosis and Management of Drug Allergy in Adults, Children and Young People. In NICE Guidlines: https://www.nice.org.uk/guidance/cg183/chapter/1-Recommendations.
- **ePrints Soton - University of Southampton. (2018).** Mast cell mediators: their subcellular localization, bacterial influence on their release and expression and their potential value as markers for predicting the severity of allergic reactions Rana Abadalkareem PhD thesis Final pdf.
- **Kirshenbaum A.S., Yin Y., Sundstrom J.B., Bandara G., Metcalfe D.D. (2019).** Description and characterization of a novel human mast cell line for scientific study. Int. J. Mol. Sci., 20:E5520.
- **Adnan A, Acharya S, Alenazy LA, Vecilla L, Bianchi P, Picard M, et al.**

**(2023).** Multistep IgE Mast Cell Desensitization Is a Dose- and Time-Dependent Process Partially Regulated by SHIP-1, The Jour J Immunol, 210 (6): 709–720.

- **Schulman ES, Schulman ES, Nishi HN, Pelleg AP. (2023).** Degranulation of human mast cells: modulation by P2 receptors' agonists. Front. Immunol. 14.
- **Rujitharanawong C, Yoodee S, Sueksakit K, Peerapen P, Tuchinda P, Kulthanan K, et al., (2022).** Systematic comparisons of various markers for mast cell activation in RBL-2H3 cells. Cell Tissue Res. 390(3):413-428.
- **Burchett JR, Dailey JM, Kee SA, Pryor DT, Kotha A, Kankaria RA, et al. (2022).** Targeting Mast Cells in Allergic Disease: Current Therapies and Drug Repurposing. Cells, 11:3031.
- **Garcia-Recio S, Gascón P. (2015).** Biological and pharmacological aspects of the NK1-receptor. BioMed research international; 2015:495704.
- **Taracanova A, Alevizos M, Karagkouni A, Weng Z, Norwitz E, Conti P, et al. (2017).** SP and IL-33 together markedly enhance TNF synthesis and secretion from human mast cells mediated by the interaction of their receptors. Proceedings of the National Academy of Sciences, 114:E4002-E4009.
- **Taracanova A, Tsilioni I, Conti P, Norwitz ER, Leeman SE, Theoharides TC. (2018).** Substance P and IL-33 administered together stimulate a marked secretion of IL-1β from human mast cells, inhibited by methoxyluteolin. Proceedings of the National Academy of Sciences, 115:E9381-E9390.
- **Roy S, Ayudhya CCN, Thapaliya M, Deepak V, Ali H. (2021).** Multifaceted MRGPRX2: New insight into the role of mast cells in health and disease. Journal of Allergy and Clinical Immunology, 148:293-308.
- **Green DP, Limjunyawong N, Gour N, Pundir P, Dong X. (2019).** A mast-cell-specific receptor mediates neurogenic inflammation and pain. Neuron, 101:412-420. e413.
- **Petrosino S, Schiano Moriello A, Verde R, Allarà M, Imperatore R, Ligresti A, et al. (2019).** Palmitoylethanolamide counteracts substance P-induced mast cell activation in vitro by stimulating diacylglycerol lipase activity. Journal of Neuroinflammation, 16:1-16.
- **Moon TC, Befus AD, Kulka M. (2014).** Mast cell mediators: their differential release and the secretory pathways involved. Frontiers in immunology, 565:569.
- **Chelombitko MA, Firsov AM, Kotova EA, Rokitskaya TI, Khailova LS, Popova LB, et al. (2020).** Usnic acid as calcium ionophore and mast cells stimulator. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1862:183303.
- **Holowka D, Calloway N, Cohen R, Gadi D, Lee J, Smith NL, et al. (2012).** Roles for Ca2+ mobilization and its regulation in mast cell functions. Frontiers in immunology, 3:104.
- **Oh-hora M. (2009).** Calcium signaling in the development and function of T-lineage cells. Immunological reviews, 231:210-224.
- **Hosokawa J, Suzuki K, Nakagomi D, Tamachi T, Takatori H, Suto A, et al. (2013).** Role of calcium ionophore A23187-induced activation of IkappaB kinase 2 in mast cells. International Archives of Allergy and Immunology, 161:37-43.
- **Park JY, Lee HJ, Han ET, Han JH, Park WS, Kwon YS, et al. (2023).**

Caffeic acid methyl ester inhibits mast cell activation through the suppresion of MAPKs and NF-κB signaling in RBL-2H3 cells. Heliyon, 9(6):e16529.

- **Myung NY. (2020).** Adenophorae Radix Attenuates Mast Cell-mediated Allergic Inflammation through Down-regulation of NF-κB/Caspase-1 Activation. Korean Journal of Plant Resources. 33(6):659-665.
- **Méndez-Enríquez E, Hallgren J. (2019).** Mast cells and their progenitors in allergic asthma. Frontiers in immunology, 810:821.