# Sodium butyrate potentiates mast cell responses to MRGPRX2 by increasing glycosaminoglycan

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## Abstract

Sodium butyrate (NaBu), a histone deacetylase inhibitor, regulates immune responses and mast cell-mediated diseases. We investigated how NaBu affects human mast cells and found out that it inhibits mast cell proliferation. NaBu also enhanced mast cell responsiveness to mas-related G protein-coupled receptor X2 (MRGPRX2) agonist compound 48/80 (C48/80), leading to increased degranulation. Additionally, flow cytometry analysis using Berberine (BBR), which specifically targets glycosaminoglycan-rich granules in mast cells, revealed that NaBu potentially enhances glycosaminoglycan storage within mast cells. This research expands our knowledge of NaBu's effects on mast cells, offering potential therapeutic applications for mast cell-related diseases. Further studies are required to understand specific molecular events for precise therapeutic development, promising advancements in immunomodulatory medicine.

## Introduction

**C48/80:** A ligand that attaches to the MRGPRX2 receptor, possibly activating ERK1/2, pPKC and pPLC pathways that lead to mast cell degranulation.

**BBR:** A benzylisoquinoline alkaloid, that binds to heparin, and upon activation with ultraviolet light, it emits a vivid yellow fluorescence while targeting glycosaminoglycan-rich granules in mast cells

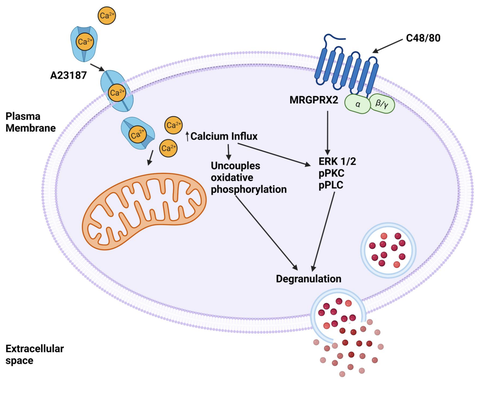


Figure 1: Mast cell activation by MRGPRX2 and Calcium Ionophore (A23187) leads to pathway activation that results in mast cell degranulation.

## Methodology

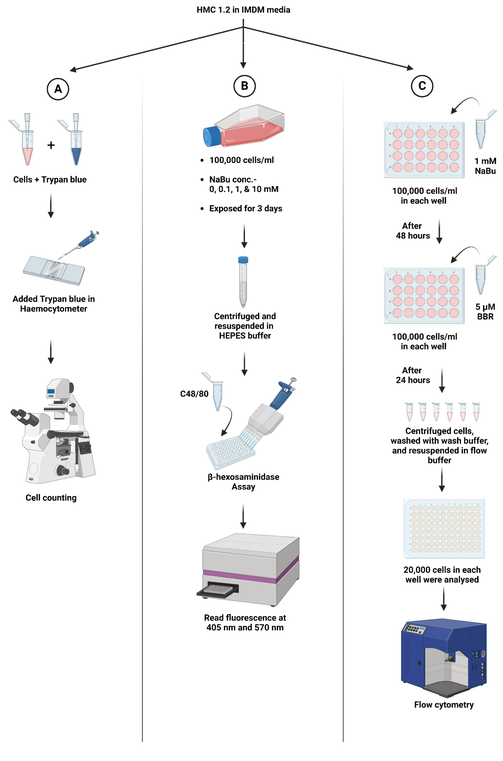


Figure 2: Representation of the Methodology used: (A) HMC 1.2 cell proliferation and viability determined by cell counting using Trypan Blue in Haemocytometer; (B) β-hexosaminidase assay to identify cell degranulation; (C) Flow cytometry with BBR and NaBu treatments.

## Results

### NaBu inhibits cell proliferation without affecting the viability

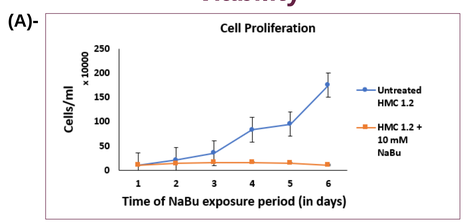


Figure 3: NaBu inhibits HMC-1.2 cell proliferation. HMC-1.2 cells were cultured with NaBu (10 mM) for a 5-day time course, and (A)- cell number and (B)- cell viability were measured (n = 7 for days 1 to 3 and n=3 for days 4 and 5). *p < 0.05, Student's t-test.

### NaBu enhances the responsiveness to degranulation of HMC 1.2. after 3 days of exposure

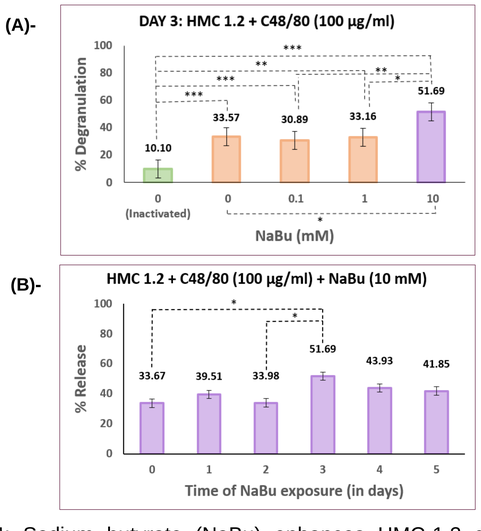


Figure 4: Sodium butyrate (NaBu) enhances HMC-1.2 degranulation. (A)- HMC-1.2 cells were exposed to different concentrations of NaBu (0, 0.1, 1, and 10 mM) for 3 days and then activated with compound 48/80 (100 µg/ml). (B)- HMC-1.2 cells were treated with NaBu (10 mM) for 5 days. β-hexosaminidase release was measured to evaluate degranulation (n = 7 for days 1 to 3 and n=3 for days 4 and 5). *p < 0.05, **p < 0.01, ***p < 0.001, Student's t-test.

## Results

### NaBu increases BBR internalization in HMC-1.2

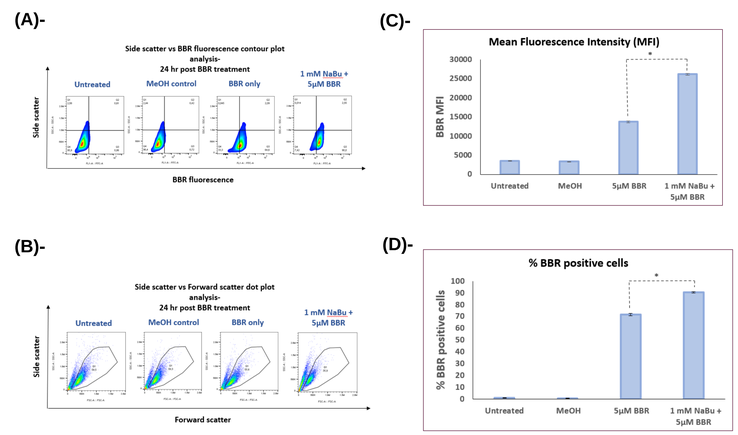


Figure 5: Pre-treatment with NaBu increases BBR internalization. 100,000/mL HMC-1.2 were treated with 1 mM NaBu for 48 hr followed by treatment with 5 µM BBR or Methanol control (MeOH). At 24 hr cells were processed for flow cytometry to determine (A)- Side scatter (Y axis) versus BBR fluorescence (X-axis), (B)- Side scatter (Y axis) versus Forward scatter (X-axis), (C) BBR Mean fluorescence intensity (MFI) and (D) % BBR positive cells. n = 3, *p < 0.05, Student's t-test.

## Conclusion/Future Directions

-NaBu activates mast cell degranulation potentially via MRGPRX2 activation.
-NaBu increases BBR internalization in mast cells and potentially enhances glycosaminoglycan storage.
-Further research is needed to elucidate specific phosphorylation events and gene expression for NaBu effects for precise therapeutic development.

## Acknowledgment

DEPARTMENT OF Chemical and Materials Engineering

## References

MacDonald CA, Qian H, Pundir P, Kulka M. Sodium butyrate suppresses malignant human mast cell proliferation, downregulates expression of KIT and promotes differentiation. Frontiers in Allergy. 2023 Mar 10;4:1109717.