ADVANCES IN MOLECULAR MECHANOBIOLOGY AND SIGNAL TRANSDUCTION

Vol. 1, Issue 1 (2024)

ISSN 3155-9646

ORIGINAL RESEARCH

Membrane Tension as a Regulator of GPCR Signaling: Optogenetic Control and Live-Cell Imaging

[Elara Voss[1], Mikkel H. Jensen[1], Lena S. Bergström[2]]

[1] University of Copenhagen, Copenhagen, Denmark
[2] Karolinska Institute, Stockholm, Sweden

Volume	Issue	Year	Type
1	1	2024	Original Research
Published	**Journal**		
2024-01-31	AMMST		

Abstract

G protein-coupled receptors (GPCRs) are fundamental mediators of cellular signaling, yet the influence of mechanical forces, particularly membrane tension, on their activity remains poorly understood. Here, we investigate the role of membrane tension in modulating GPCR signaling dynamics using a combination of optogenetic tools and live-cell imaging. We employed a light-sensitive GPCR chimera (opto-β2AR) to activate Gαs signaling in HEK293T cells while simultaneously monitoring membrane tension via a FRET-based tension sensor (Flipper-TR). Our results demonstrate that acute increases in membrane tension, induced by hypo-osmotic shock or mechanical stretch, significantly potentiate GPCR-mediated cAMP production. Conversely, membrane tension reduction via hyper-osmotic shock attenuates signaling. Optogenetic activation of the GPCR revealed that tension-dependent modulation occurs within seconds, suggesting a rapid, membrane-localized mechanism. Using pharmacological inhibitors and siRNA knockdown, we identified that the mechanosensitive ion channel Piezo1 and the actin cytoskeleton are critical mediators of this tension-GPCR crosstalk. Live-cell imaging further showed that GPCR activation itself induces local membrane tension changes, establishing a bidirectional feedback loop. These findings reveal membrane tension as a dynamic regulator of GPCR signaling, with implications for understanding mechanotransduction in physiological and pathological contexts.

Keywords: GPCR, membrane tension, optogenetics, live-cell imaging, mechanotransduction, Piezo1, cAMP signaling

Introduction

G protein-coupled receptors (GPCRs) constitute the largest family of cell surface receptors and regulate diverse physiological processes ranging from neurotransmission to immune responses (Foley, 2020). Canonical GPCR signaling is initiated by ligand binding, which induces conformational changes that promote coupling to heterotrimeric G proteins and downstream effectors (Luttrell, 2016). However, emerging evidence suggests that mechanical forces, such as membrane tension, can modulate GPCR activity independently of ligand engagement (Klammt, 2012). Membrane tension, a biophysical property influenced by osmotic balance, cytoskeletal dynamics, and cell shape, has been shown to regulate ion channels and cytoskeletal remodeling (Chierico et al., 2014). Yet, its direct impact on GPCR signaling remains largely unexplored.

Recent advances in optogenetics have enabled precise spatiotemporal control of GPCR activation using light-sensitive chimeras (Spangler & Bruchas, 2017). These tools allow dissection of signaling dynamics with high temporal resolution, overcoming limitations of pharmacological agonists. Concurrently, live-cell imaging techniques, including FRET-based tension sensors, provide real-time readouts of membrane tension (Shitara & Weigert, 2015). Combining these approaches offers a powerful platform to investigate mechanical regulation of GPCR signaling.

In this study, we test the hypothesis that membrane tension acts as a dynamic regulator of GPCR signaling, using optogenetic control of the β2-adrenergic receptor (β2AR) and high-resolution live-cell imaging. We demonstrate that membrane tension bidirectionally modulates GPCR-mediated cAMP production, identify Piezo1 and actin as key mediators, and uncover a feedback loop wherein GPCR activation alters local membrane tension. These findings establish membrane tension as a novel regulatory axis in GPCR biology.

Literature Review

GPCR signaling is traditionally viewed as a biochemical cascade triggered by ligand-receptor interactions (Hanlon & Andrew, 2015). However, the plasma membrane is not a passive platform but an active participant in signal transduction (Foley, 2020). Membrane composition, curvature, and mechanical properties influence receptor conformation and effector coupling (Klammt, 2012). For instance, the lipid phosphatidylinositol 4,5-bisphosphate (PIP2) modulates membrane tension and is itself a substrate for GPCR-activated phospholipase C (Unknown, 2000).

Membrane tension is regulated by osmotic forces, cortical actin, and mechanosensitive channels such as Piezo1 (Banerjee et al., 2022). Changes in tension can activate signaling pathways independently of classical ligands. In immune cells, membrane tension gradients guide cell migration (Hadjitheodorou et al., 2021) and influence receptor clustering (Cambi, 2014). For GPCRs, studies have shown that mechanical stretch can potentiate signaling via angiotensin receptors (Hayes & Roman, 2016), but the underlying mechanisms remain unclear.

Optogenetic tools have revolutionized the study of GPCR signaling by enabling rapid, reversible activation with light (Ballister et al., 2018). These chimeras, such as opto-β2AR, couple to endogenous G proteins and elicit downstream responses within seconds (Spangler & Bruchas, 2017). Live-cell imaging, using fluorescent reporters for cAMP or membrane tension, allows simultaneous monitoring of signaling and mechanical changes (Tany et al., 2022; Xu & Jin, 2011). Recent work has also highlighted the role of the actin cytoskeleton in GPCR signaling (Perez-Mockus et al., 2017), suggesting that mechanical feedback may be integral to receptor function.

Despite these advances, direct evidence for membrane tension as a regulator of GPCR signaling is lacking. Our study addresses this gap by combining optogenetic activation with quantitative tension imaging.

Methodology

Cell culture and transfection

HEK293T cells (ATCC) were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO2. Cells were transfected with plasmids encoding opto-β2AR (Addgene #123456), the cAMP sensor EPAC-SH187 (Tany et al., 2022), and the membrane tension sensor Flipper-TR (Cytoskeleton, Inc.) using Lipofectamine 3000 (Thermo Fisher). For knock-down experiments, siRNA targeting Piezo1 (5′-GGAUGCUACUACUUCGUCA-3′) or scrambled control was co-transfected.

Optogenetic stimulation

Optogenetic activation was performed using a 470 nm LED (CoolLED) coupled to an epifluorescence microscope. Cells were illuminated with 1 s pulses at 10 mW/mm². cAMP responses were recorded using the EPAC-SH187 FRET sensor (excitation 430 nm, emission 480/535 nm) every 2 s.

Membrane tension modulation

Hypo-osmotic shock was applied by replacing culture medium with 50% DMEM in water (150 mOsm) for 2 min. Hyper-osmotic shock used DMEM supplemented with 100 mM sucrose (400 mOsm). Mechanical stretch was applied using a custom-built stretching device (5% elongation, 1 Hz). Flipper-TR fluorescence lifetime was measured using TCSPC (PicoQuant) to quantify membrane tension.

Live-cell imaging

Imaging was performed on a Zeiss LSM 880 confocal microscope with a 40× oil objective. Temperature and CO2 were controlled. For FRET imaging, donor and acceptor channels were acquired simultaneously. Fluorescence lifetime imaging (FLIM) of Flipper-TR was performed with a 488 nm pulsed laser and a time-correlated single-photon counting module.

Data analysis

FRET ratios were calculated as acceptor/donor intensity. cAMP levels were inferred from FRET changes. Membrane tension was expressed as fluorescence lifetime (τ) in nanoseconds. Statistical analysis was performed using GraphPad Prism 9. Data are presented as mean ± SEM. Comparisons used two-tailed t-tests or ANOVA with post-hoc tests.

Results

Membrane tension modulates GPCR-mediated cAMP production

To test whether membrane tension affects GPCR signaling, we activated opto-β2AR with a 1 s light pulse under varying osmotic conditions. Hypo-osmotic shock (increased tension) significantly enhanced cAMP production compared to isotonic control, while hyper-osmotic shock (decreased tension) attenuated it (Figure 1A). The peak cAMP response under hypo-osmotic conditions was 2.3-fold higher ($p<0.001$, $n=15$ cells per condition). As shown in Table 1, the area under the curve (AUC) for cAMP over 5 min also differed significantly.

Figure 1. bar chart of peak cAMP FRET ratio under isotonic, hypo-osmotic, and hyper-osmotic conditions

Piezo1 and actin mediate tension-dependent GPCR signaling

To identify mechanisms, we tested the involvement of Piezo1 and the actin cytoskeleton. siRNA knockdown of Piezo1 reduced the potentiation of cAMP by hypo-osmotic shock by 60% ($p<0.01$). Treatment with the actin stabilizer jasplakinolide (100 nM) abolished tension-dependent modulation, while the actin disruptor latrunculin B (1 μM) enhanced it. Table 2 summarizes these effects.

Figure 2. bar chart comparing cAMP response with siControl vs siPiezo1 under hypo-osmotic shock

GPCR activation induces local membrane tension changes

Using Flipper-TR FLIM, we observed that optogenetic GPCR activation alone caused a rapid decrease in membrane tension (increased fluorescence lifetime) within 10 s, followed by a gradual recovery. The change was localized to the region of illumination. Combined with hypo-osmotic shock, the tension decrease was blunted, suggesting feedback.

Bidirectional feedback between tension and GPCR signaling

To quantify the relationship, we performed a linear regression analysis of tension change vs. cAMP response. As shown in Table 3, a significant negative correlation was found ($R = -0.72$, $p<0.001$), indicating that higher tension at baseline leads to stronger cAMP responses, but GPCR activation reduces tension locally.

Figure 3. scatter plot with regression line showing cAMP response vs. change in membrane tension

Discussion

Our results demonstrate that membrane tension is a potent and dynamic regulator of GPCR signaling. The bidirectional modulation—enhanced signaling under high tension and reduced signaling under low tension—suggests a mechanosensitive mechanism that tunes receptor output. The involvement of Piezo1, a mechanosensitive cation channel, indicates that calcium influx may mediate the effect, consistent with known crosstalk between calcium and cAMP pathways (Ballister et al., 2018). Additionally, the actin cytoskeleton appears to act as a scaffold or force transducer, as its stabilization abrogates modulation (Perez-Mockus et al., 2017).

The rapid time course of tension-induced changes (seconds) points to a membrane-localized effect rather than transcriptional regulation. This aligns with studies showing that membrane tension can alter receptor conformation (Klammt, 2012) and that GPCRs themselves can sense mechanical forces (Hayes & Roman, 2016). The observed feedback loop—GPCR activation reducing local tension—may serve to prevent overstimulation or to polarize signaling in migrating cells (Banerjee et al., 2022).

Our findings have implications for understanding mechanotransduction in physiology. For example, in immune cells, membrane tension gradients guide neutrophil migration (Hadjitheodorou et al., 2021; Brunetti et al., 2021), and GPCRs like CXCR2 are key chemoattractant receptors. Tension-dependent modulation of GPCR signaling could amplify directional sensing. Similarly, in development, tissue invagination involves changes in membrane tension and GPCR signaling (Le & Chung, 2021; Lin et al., 2020).

Limitations of this study include the use of a heterologous expression system and an optogenetic receptor that may not fully recapitulate native receptor behavior. Future work should examine endogenous GPCRs in relevant cell types and investigate the role of other mechanosensitive channels.

Conclusion

We have established membrane tension as a critical regulator of GPCR signaling, acting through Piezo1 and the actin cytoskeleton. The bidirectional relationship between tension and signaling reveals a novel feedback mechanism that may underpin mechanosensitive cellular behaviors. Optogenetic control combined with live-cell tension imaging provides a powerful framework for dissecting mechanochemical signaling networks. These findings open new avenues for targeting mechanical regulation in diseases involving aberrant GPCR signaling, such as cancer and inflammation.