Abstract

G protein-coupled receptor signaling is mediated by distinct families of G alpha proteins, each associated with specific intracellular responses. Among these, receptors coupled to the G alpha q/11 protein family play a key role in regulating intracellular signaling pathways involved in diverse physiological processes. Traditionally, G protein-coupled receptor signaling was viewed as a transient event occurring exclusively at the plasma membrane. However, advances in molecular pharmacology and imaging technologies have expanded this view, revealing that G protein-coupled receptor signaling is spatially and temporally regulated within cells, with increasing emphasis on endosomal signaling. This expanded understanding has important implications for pharmacological efficacy and signaling specificity, including improved drug selectivity, reduced off-target effects, enhanced signal duration, and the potential development of compartment-specific therapies that more precisely modulate disease-relevant pathways. This study investigated whether phosphatidylinositol 4-phosphate serves as a substrate for phospholipase C beta-mediated diacylglycerol production at early endosomes following activation of G alpha q/11-coupled receptors. Using NanoBiT-based diacylglycerol biosensors targeted to the plasma membrane and early endosomes, real-time diacylglycerol production was monitored in human embryonic kidney 293 cells expressing angiotensin II type 1 receptor, oxytocin receptor, and muscarinic acetylcholine receptor 3, in the presence or absence of PI-273, a selective inhibitor of phosphatidylinositol 4-kinase type II alpha, the enzyme responsible for phosphatidylinositol 4-phosphate synthesis at early endosomes. PI-273 treatment reduced endosomal diacylglycerol production in angiotensin II type 1 receptor-expressing cells and oxytocin receptor-expressing cells, while plasma membrane diacylglycerol production remained largely unaffected, consistent with the reported selectivity of PI-273 for endosomal phosphatidylinositol 4-kinase type II alpha. Preliminary data from muscarinic acetylcholine receptor 3-expressing cells suggested a similar trend. Confocal microscopy provided qualitative support for receptor internalization into early endosomes upon angiotensin II stimulation. Collectively, these findings provide evidence that phosphatidylinositol 4-phosphate is a substrate for phospholipase C beta-mediated diacylglycerol production at early endosomes, contributing to the growing understanding of spatially compartmentalized G alpha q/11 signaling and its pharmacological implications.

Keywords: G protein-coupled receptor; G alpha q/11 signaling; phosphatidylinositol 4-phosphate; diacylglycerol; early endosomes