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Characterization of Agonist-Induced Ca^{2+} Signals in Human Airway Smooth Muscle Cells Using Excitation Scanning Hyperspectral Imaging and Image Analysis Approaches

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Calcium signals plays critical roles in the physiology and pathophysiology of the respiratory system. Thus, proteins that regulate Ca^{2+} signaling pathways are often potential therapeutic targets. However, there are significant gaps in our understanding Ca^{2+} signaling pathways complicating the use of agents that alter Ca^{2+} signals as potential pharmacologic therapies. Thus, there is a need to better understand signal specificity and information content within Ca^{2+} signaling pathways. We propose the use of spectral imaging and dynamic region of interest (ROI) tracking software to better understand the impacts of G_qPCR agonists on Ca^{2+} signals in human airway smooth muscle cells (HASMCs). Unlike traditional approaches in which investigators analyze static ROIs, we identify and track the location of ROIs as a function of time. Characteristics of Ca^{2+} signals in specific ROIs can then be quantified. This approach allows for a more nuanced and precise representation of agonist-induced Ca^{2+} signals in HASMCs. We have focused on the response of HASMCs to three distinct G_qPCR agonists: two agonists that trigger contraction of HASMCs, carbachol and histamine; and one agonist that triggers relaxation of HASMCs, chloroquine. Utilizing excitation scan-based hyperspectral imaging, we capture and visualize the Ca^{2+} signals induced by these agonists. This advanced imaging method is complemented by a comprehensive analytical approach that integrates with dynamic ROI tracking software. ROI features (Ca^{2+} signal characteristics) were then analyzed using principal component analysis (PCA) and subsequent k-means clustering or K nearest neighbor methods. This approach enables us to dynamically track Ca^{2+} signals and categorize them based on parameters including area, duration, and amplitude. This approach has revealed unique patterns and characteristics of Ca^{2+} signals distinct to each agonist, offering potential insight into signal specificity. These observations may shed light on the distinct effects of carbachol, histamine, and chloroquine in modulating Ca^{2+} signals and subsequent regulation of HASMC contractile state. In addition, this overall approach may be applicable to the scientific interrogation of a wide range of intracellular signals.

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