Modeling and Modulating the Functional Desensitization of β2AR in Real-Time

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Desensitization of the beta-2 adrenoceptor (β2AR) is a phenomenon associated with prolonged or repeated exposure to beta-agonists, contributing to increased morbidity and decreased quality of life for asthma patients. Stimulation of the β2AR by Gαs-agonists triggers Gαs-mediated activation of adenylyl cyclase, which converts ATP to cAMP, ultimately triggering smooth muscle relaxation. Betaarrestin binding is a homeostatic mechanism to desensitize and internalize β2AR via endocytosis. The purpose of this study was to develop a new experimental paradigm to detect, in real-time, functional desensitization of β2AR expressed on human airway smooth muscle cells. We used an RGD-coated ferrimagnetic microbead functionalized to the cytoskeleton through cell surface integrin receptors and measured the dynamic changes in cytoskeletal stiffness of human airway smooth muscle cells in response to the β-agonist isoproterenol via magnetic twisting cytometry (MTC). We observed a 32% loss of cell relaxation as soon as 20 minutes after stimulation with isoproterenol, which we believe to be the observable impact of β2AR desensitization. We verified this assumption through experimentation with isobutylmethylxanthine (IBMX), a wide-ranging phosphodiesterase inhibitor. Observing a loss of relaxation from cells pretreated with IBMX, we concluded that loss of cell relaxation was not solely due to the backdoor mechanism of cAMP breakdown by phosphodiesterases. In one IBMX-pretreated cell line, we observed 100% loss of isoproterenolinduced relaxation within 30 minutes, thus suggesting functional β2AR desensitization. Using MTC, we observed that the desensitization-driven wane of cell relaxation occurs rapidly and determined that the breakdown of cAMP by phosphodiesterases was not the sole cause of the loss of relaxation. Supported by R25ES020721.