Demystifying Bitter Taste Receptor Relaxation of Airway Smooth Muscle

β-adrenoceptor agonists are the first-line medications used to manage bronchoconstriction, a key characteristic of asthma, which affects approximately 300 million patients worldwide. Unfortunately, β-agonists have safety issues (1) and limited clinical effectiveness (2, 3). Therefore, the development of new bronchodilators could provide novel approaches to control asthma symptoms.

In 2010, Dr. Liggett and colleagues identified six bitter taste receptors (TAS2Rs) in human airway smooth muscle (HASM) cells (4). TAS2Rs are G protein-coupled receptors that were thought to solely express on taste cells in the tongue and to protect against ingestion of toxins from plants (5). The finding of TAS2Rs on HASM cells has attracted attention within the smooth muscle research community. Subsequently, TAS2Rs were found in other tissues, including the pancreas, gastrointestinal tract, thyroid, and uterus (6). In HASM, TAS2R agonists induce significant relaxation, which is associated with increases in localized Ca^{2+} at the membrane along with membrane hyperpolarization (4). This localized Ca^{2+} increase triggered by TAS2Rs seems inconsistent with the established paradigm that increased $[Ca^{2+}]_i$ (at least by the G_q-coupled receptors in HASM) leads to contraction rather than relaxation. Thus, more in-depth studies are required to understand how TAS2R agonists cause HASM relaxation.

LINK (LIM kinase) has been shown to catalyze the phosphorylation of cofilin, which inhibits the ability of cofilin to sever F-actin and thus increases F-actin in cells (7). However, its role in TAS2Rs is largely unknown. In this issue of the *Journal*, Dr. Woo and colleagues (pp. 417–429) provide evidence to suggest that agonist activation of TAS2R14 (the most abundant TAS2R in HASM) affects the polarity protein Par3, which inhibits the LIMK-cofilin pathway and induces F-actin severing and HASM relaxation (8). Smooth muscle contraction/relaxation is mainly regulated by two pathways: Myosin activation and actin cytoskeletal reorganization (9–13). Myosin activation initiates contractile filament sliding, whereas remodeling of the actin cytoskeleton facilitates force transmission between the contractile filaments and the extracellular matrix (9–15). The authors show that treatment with the TAS2R14 agonists DPD and AA did not affect myosin light chain phosphorylation induced by contractile stimuli. In contrast, treatment with DPD and AA induced dephosphorylation of LIMK and cofilin and decreased the F-actin/G-actin ratio. Furthermore, the TAS2R14 agonists enhanced the colocalization of cofilin and F-actin. The authors also discovered that Par3 knockdown reduced cofilin phosphorylation. These new and important findings demonstrate that agonist activation of TAS2R14 regulates the Par3-LIMK-cofilin-actin filament pathway rather than myosin activation.

The authors performed elegant experiments to show that TAS2R14 activation regulates cofilin via G_i but not β-arrestin1/2 in HASM cells. Consistent with their previous findings (4), treatment with the TAS2R14 agonist increased $[Ca^{2+}]_i$, whereas $[Ca^{2+}]_i$ chelation reduced AA-induced cofilin dephosphorylation.

To assess the role of Par3 and cofilin in HASM relaxation, the authors used two models: Single-cell mechanics using magnetic twisting cytometry and precision-cut lung slices with airway luminal diameter measurements. They provide convincing evidence that the knockdown of Par3 or cofilin attenuates the inhibitory effects of the TAS2R14 agonist in single-cell experiments. Moreover, the knockdown of Par3 or cofilin reduced the TAS2R14 agonist-mediated bronchodilation. These functional studies demonstrate the role of Par3 and cofilin in regulating HASM relaxation as well as contractility.

This study has several strengths. First, they propose a new concept that agonist activation of TAS2R induces HASM relaxation by inhibiting the Par3-LIMK-cofilin-actin filament pathway. Second, they propose that TAS2R activates G_i and PLCβ, which complexes with the polarity protein Par3 and inhibits the LIMK cascade. Third, they propose that TAS2R-activated PLCβ increases compartmentalized Ca^{2+}, which facilitates inhibition of the Par3-LIMK pathway. Thus, these studies unveil a new mechanism by which TAS2R activation can increase localized Ca^{2+} but also induce HASM relaxation or inhibit HASM contraction. Fourth, they employed advanced and translational technologies such as HASM cells, RNA interference, the fractionation assay, phospho-specific antibodies, magnetic twisting cytometry, cell imaging, and precision-cut lung slices.

Like many other papers, this article has some limitations. For instance, we do not know whether TAS2R activation increases the association of the PLCβ with Par3 in HASM cells as assessed by imaging and biochemical analysis. We also do not know whether the knockdown of Par3 or cofilin induces the relaxation of asthmatic HASM in animal asthma models. However, these minor weaknesses should not diminish our appreciation for the authors who present strong evidence elucidating a new mechanism by which TAS2R activation induces HASM relaxation and attenuates contraction. ■

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