Olfactory Receptor Interactions with Other Receptors

Randy A. Hall

Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia, USA

Olfactory receptors expressed in heterologous cells often suffer from retention in the endoplasmic reticulum and poor trafficking to the plasma membrane. Interactions of the olfactory receptor M71 with several subtypes of adrenergic and purinergic receptors can alleviate M71 trafficking deficits in heterologous cells and allow for robust M71 plasma membrane expression. Furthermore, the signaling pathway to which M71 couples upon agonist stimulation can be strongly influenced by the partner receptor's driving M71 trafficking to the plasma membrane. These studies provide evidence that olfactory receptors can interact with other G protein–coupled receptors and that these interactions can promote olfactory receptor surface expression and functionality in heterologous cells.

Key words: **odorant; heterodimerization; homodimerization; dimerization; oligomerization; hetero-oligomerization; association; olfaction; sensory; neuron**

A major hindrance in studying the pharmacology and signaling properties of mammalian olfactory receptors (ORs) has been that these receptors typically exhibit poor trafficking to the plasma membrane because of retention in the endoplasmic reticulum when expressed in most types of heterologous cells.[1,2] ORs are by no means unique among G protein–coupled receptors (GPCRs) in suffering from such localization problems upon heterologous expression. For a variety of other GPCRs that exhibit trafficking and signaling deficits in heterologous cells, including $GABA_BR1$, the taste receptor T1R3, and the α_{1D}-adrenergic receptor, coexpression with specific partner receptors can form heteromeric receptor complexes with greatly improved plasma membrane trafficking and functionality.[3]

To examine whether OR trafficking in heterologous cells might be enhanced through interactions with other receptors, my colleagues and I coexpressed a Flag-tagged version of the OR M71 with more than 40 other GPCRs and quantified M71 trafficking to the plasma membrane in HEK-293 cells.[4,5] Nearly all coexpressed receptors had no effect on M71 trafficking and exhibited no physical interactions with M71. However, a few receptors did significantly improve M71 surface trafficking upon coexpression: the β_2-adrenergic receptor, $P2Y_1$ and $P2Y_2$ purinergic receptors, and A_{2A} adenosine receptor. These four receptors were furthermore found in coimmunoprecipitation studies to robustly form physical complexes with M71.[4,5]

After observing these enhancements in M71 surface trafficking upon coexpression with certain adrenergic and purinergic receptors, we wondered whether M71 in these heterologous cells exhibited increased functionality concomitant with its increased localization in the plasma membrane. Interestingly, we found that the signaling activity of M71 could be differentially influenced by the different partner receptors. When M71 surface expression was achieved via coexpression with the G_s-coupled β_2-adrenergic receptor, stimulation with the M71

Address for correspondence: Randy A. Hall, Rollins Research Center, 1510 Clifton Rd., Rm. 5113, Emory University School of Medicine, Atlanta, GA 30322. Voice: 404-727-3699; fax: 404-727-0365. rhall@pharm.emory.edu

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agonist acetophenone (ACP) resulted in significant cyclic AMP (cAMP) generation, a classical downstream signaling output for many ORs. In control studies, ACP-stimulated increases in cAMP were not observed for mock-transfected HEK-293 cells or cells transfected with either M71 or the β_2-adrenergic receptor alone.[4] Similarly, in cells cotransfected with M71 and either P2Y1 or P2Y2, no ACP-stimulated cAMP generation could be detected, even though M71 exhibited strong plasma membrane localization in these doubly transfected cells. However, ACP-induced increases in the phosphorylation state of extracellular signal–regulated kinases 1 and 2 (ERK1/2) could be detected in M71/P2Y1- and M71/P2Y2-expressing cells, especially when $G_{\alpha o}$ was cotransfected.[5] Odorant stimulation of olfactory sensory neurons (OSNs) can robustly increase phospho-ERK,[6] and moreover, odorant stimulation of some native ORs in OSNs can activate $G_{\alpha o}$.[7] The data from our transfection experiments suggest that an individual OR, such as M71, can couple to both cAMP generation through $G_{\alpha s}/G_{\alpha olf}$ and phospho-ERK increases through $G_{\alpha o}$, as well as that the G protein–coupling preferences of the OR can be influenced by differential assembly with coexpressed partner receptors.

All the preceding studies concerning M71 interactions with other receptors were performed in heterologous cells in which the receptors involved were overexpressed. What is the relevance of this work to ORs *in vivo*? We think it unlikely that OR surface expression in native OSNs is widely dependent on interactions with adrenergic or purinergic receptors. First, although we have shown that M71 and a closely related OR subtype exhibit robust interactions with the adrenergic and purinergic receptor subtypes mentioned earlier, more distantly related ORs (and probably most ORs) do not exhibit detectable interactions with the adrenergic and purinergic receptors.[5] Second, for at least a few of the adrenergic and purinergic receptor subtypes that we found to be capable of interacting with M71 family members in transfected cells, it is not clear whether these subtypes are expressed at physiologically significant levels in OSNs. Thus, we propose that our studies represent proof of concept that ORs, such as M71, can interact with other receptors and that these interactions can strongly promote OR surface expression. Interestingly, several non-OR GPCRs are expressed at high levels in OSNs,[8] some of them orphan receptors of unknown function, and perhaps one or several of these receptors represent authentic *in vivo* heterodimer partners for ORs. Furthermore, we have recent data that several ORs, including M71 and rat I7, are capable of robust homodimerization, as shown in coimmunoprecipitation studies using heterologous cells expressing two differentially tagged versions of the same receptor. It has been speculated that homodimerization is an important step in the biosynthetic processing and trafficking of other GPCRs,[9] but this topic has not yet been explored for ORs. We foresee that studies on the physiological significance of OR homodimerization, as well as studies on potential OR interactions with certain non-OR GPCRs that are enriched in OSNs, will be interesting areas for future investigation.

Conflicts of Interest

The author declares no conflicts of interest.

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