Abstract

β-Arrestin belongs to the protein family which has a huge impact not only on GPCR signaling, but its role exceeds the function of the membrane channel, its own signaling cascade, or as a scaffold protein, etc. Here we aimed to study β-arrestin roles on the MOR behaviour in the plasma membrane or μ-opioid receptor (MOR) signaling and effect on adenylyl cyclase (AC) function using the siRNA to decrease the expression of β-arrestin isoforms. Furthermore, we focused on investigating the role of β-arrestin on the crosstalk between MOR and TRPV1 channels, which are important parts of pain transduction. For this purpose, we used HEK293 cells that stably expressed MOR-YFP or transiently transfected with TRPV1-CFP.

We observed that both β-arrestin isoforms have an effect on the lateral mobility of MOR in the plasma membrane and the silencing of one or another β-arrestin isoforms abolishes the effect of MOR agonists to affect its diffusion in the plasma membrane. Interestingly, silencing of β-arrestin1 diminish the internalization of MOR induced by the endogenous agonist endomorphin-2. On the other hand, silencing of β-arrestin2 did not abolish the endomorphin-2 induced MOR internalization. Moreover, both isoforms exhibit a distinct impact on the inhibition of AC induced by the agonists of MOR. Forskolin-induced AC activity was enhanced in cells lacking β-arrestin2 and suppressed by silencing the β-arrestin1. Furthermore, we observed an important role of $G_{\alpha s}$ in forskolin-induced cAMP accumulation in cells lacking β-arrestins. For the first time, we showed a possible interaction of β-arrestin1 with AC activated by isoprenaline.

The next part of our investigation was to focus on the role of β-arrestin2 in the MOR-TRPV1 crosstalk. We observed that the elimination of β-arrestin2 abolished the effect of MOR or TRPV1 agonists to induce changes in the lateral mobility of one receptor or the other. Furthermore, the level of β-arrestin2 within the plasma membrane was decreased after activation of MOR or TRPV1 in cells expressing both receptors and that β-arrestin2 plays an important role in MOR-induced ERK1/2 phosphorylation in cells expressing TRPV1.

In the last part of our study, we examined the possible cooperation between TRPV1 and TLR4 in the plasma membrane and observe potential crosstalk between TLR4 and TRPV1 after TRPV1 activation.

Together, our study demonstrates the differences between the β-arrestin isoforms in MOR signaling and in modulation of AC activity and that β-arrestin2 is an important mediator in the crosstalk between MOR and TRPV1.

Key words: β-arrestin, μ-opioid receptor, TRPV1 receptor, TLR4 receptor, signaling, adenylyl cyclase, receptor lateral mobility.