ABSTRACT - EN

The muscarinic acetylcholine M_2 receptor that was originally identified as the predominant muscarinic receptor subtype in the heart is also widely distributed in the central nervous system. Its signal transduction is effected by both the βγ dimer of heterotrimeric G-protein that activates potassium or inhibits calcium conductance and the α_i subunit that preferentially inhibits cAMP synthesis. However, M_2 muscarinic receptors expressed in CHO cells (CHO-M_2) directly activate signalling pathways of all three major subclasses of G-proteins, i.e. preferred $G_{i/o}$ subclass and at concentrations higher than needed for standard inhibition of forskolin-stimulated cAMP synthesis also G_s and $G_{q/11}$ subclasses to cause stimulation of cAMP synthesis and accumulation of inositolphosphates (IP), respectively . In the present experiments we investigated influence of membrane cholesterol content on activation of signalling pathways of these three G-protein subclasses in CHO-M_2 cells by carbachol, a non-hydrolysable acetylcholine analogue. Treatment of cells with methyl-β-cyclodextrin decreased cell and membrane cholesterol content by 74% and 39%, respectively, and incubation in the presence of cholesterol-saturated methyl-β-cyclodextrin increased cholesterol content by 169% and 137%, respectively . Cholesterol depletion significantly decreased the affinity of M_2 receptors for the tritiated non-permeable antagonist [^{3}H]-N-methylscopolamine binding and increased of plasma membrane receptor density in intact cells and membranes whereas the increase in cholesterol had no significant effect. Membranes displayed two-affinity agonist binding sites for carbachol and cholesterol depletion doubled the fraction of high-affinity binding sites. In intact cells the decrease of membrane cholesterol strongly decelerated and reduced the extent of receptor internalization induced by carbachol whereas cholesterol enrichment had no effect. The increase of membrane cholesterol suppressed efficacy of carbachol on cAMP synthesis inhibition (G_i), cAMP synthesis stimulation (G_s), and inositolphosphates accumulation ($G_{q/11}$). On the other hand, the decrease of membrane cholesterol increased efficacy, without change of potency, of carbachol on cAMP synthesis stimulation and inhibition while efficacy of stimulation of inositolphosphates accumulation was reduced and potency augmeted. Noteworthy, modifications of membrane cholesterol had no effect on membrane permeability, oxidative activity, protein content, or relative expression of G_s, $G_{i/o}$, and $G_{q/11}$ alpha subunits. These results demonstrate the important role of membrane cholesterol content that may underlie development of various pathological processes on signal transduction through muscarinic receptors.