ABSTRACT

Study of HEK293 cells stably expressing fusion protein between delta opioid receptor (δ-OR) and pertussis toxin-insensitive mutant of $G_i1\alpha$ protein, δ-OR-$G_i1\alpha$ (Cys^{351}-Ile^{351}), provided the following results. Decrease of plasma membrane cholesterol content (cholesterol depletion) induced by cyclic oligosaccharide β-cyclodextrin did not affect binding of specific δ-OR agonist, [^{3}H]DADLE. Neither the maximum number of binding sites nor the affinity of [^{3}H]DADLE binding was changed by cholesterol depletion. However, the ability of δ-OR to activate cognate trimeric G proteins was impaired. EC_{50} value of agonist-stimulated [^{35}S]GTPγS binding was an order of magnitude higher. This effect was observed in case of both control and pertussis toxin-treated cells. It means that cholesterol depletion markedly reduced the efficiency of functional coupling of δ-OR to endogenously expressed pertussis toxin-sensitive G proteins of G_i/G_o family as well as covalently bound $G_i1\alpha$ (Cys^{351}-Ile^{351}) protein. Unchanged plasma membrane cholesterol content is therefore important requirement for proper δ-OR function.

Detection of the effect of cholesterol depletion on the functional activity of δ-OR was supported by the analysis of changes in biophysical state of plasma membrane using fluorescent membrane probes, hydrophobic probe 1,6-diphenyl-1,3,5-hexatriene (DPH) and polar probe Laurdan. Cholesterol depletion induced an increase of the overall plasma membrane fluidity that resulted from an increased degree of disorder and mobility of hydrophobic membrane interior constituents. Polarity of the hydrophilic region of membrane bilayer was increased by cholesterol depletion. This can be ascribed to an increase in number of water molecules – hydration.

Studies of HEK293T cells transiently expressing N-terminally epitope-tagged form of δ-OR (FLAG-δ-OR) indicated that cholesterol depletion reduce the efficiency of δ-OR endocytosis (internalization) which occurs in response to binding of an agonist. This form of internalization occurs through the clathrin-coated vesicles. Furthermore, cholesterol depletion induced internalization of agonist-unbound FLAG-δ-OR. The portion of receptors internalized in this way corresponded to ~40 % of agonist-induced internalized receptors. The analysis of colocalization of endocytosed forms of receptor with intracellular vesicle markers (Rab4, Rab5, Rab7 and Rab11 proteins) demonstrated that cholesterol depletion-induced and agonist-induced FLAG-δ-OR internalization proceed in a different manner.