

Membrane domains are an important structure in plasamatic membrane. They concentrate various signaling molecules. Their main structural component is cholesterol and by its removal the membrane domains are disrupted. The aim of our work was to examine the effect of cholesterol depeletion on signaling initiated thyreothropin releasing hormone (TRH). Although its signaling cascade is located within membrane domains the receptor itself is not.

We showed that cholesterol depletion by -cyclodextrin caused release of Gq/11 proteins and caveolin 2 from membrane domains. We also discovered that cholesterol depletion decreases potency of TRH to activate G proteins as well as induction of release of intracellular Ca²⁺

In the last part we investigated the effect of disruption of the cell membrane integrity by cholesterol depletion on thyrotropin-releasing hormone receptor (TRH-R) surface mobility and internalization in HEK293 cells stably expressing TRH-R-eGFP fusion protein. CLSM studies indicated that the internalization of receptor molecules initiated by TRH stimulation was significantly attenuated. The detailed analysis of recovery of TRH-R-eGFP fluorecence in bleached spots of different sizes indicated that cholesterol depletion results in an increase of overall receptor mobility. We suggest that migration of receptor molecules in intact cell membrane proceeds as a diffusion plus exchange between “binding sites” represented by membrane domains. In -cyclodextrin-treated cells, migration of receptor molecules from the surrounding (unbleached) areas of cell membrane proceeds faster than the exchange of the newly arrived (unbleached) receptors between “binding sites” existing in the bleached spot. In control cells, migration of receptors into the bleached spot proceeds much slower; the rate of exchange between the “binding sites” is faster than the influx of new receptors.

The unchanged, intact state of the cell membrane represents an obligatory condition for an optimum functioning of TRH-initiated signaling cascade.