Conclusions

Our purpose was to separate plasma membrane fraction characterised as lipid rafts (or membrane domains) and find the extent of G protein coupled receptors (GPCRs) raft association. Three different techniques were applied – detergent extraction, alkaline treatment with sonication, and homogenisation. We found that only Triton X-100 treatment led to lipid rafts separation from bulk membrane phase. Many marker molecules helped us to characterise low (sucrose 15-25%) and high-density (sucrose 40%) membrane fractions separated on sucrose density gradients after cells homogenisation, alkaline or detergent extraction. Low density, detergent resistant membrane fragments, were characterised by presence of membrane domains proteins caveolin, flotillin, and GPI-bound alkaline phosphatase, CD55, CD58, and CD59, whereas high-density sucrose gradient fraction were enriched in non-raft proteins NA/K-ATPase, MHC I, CD147, calnexin and transferrine receptor. Using alkaline extraction and homogenisation (detergent-free methods) did not provide membrane domains separated from non-raft phase of plasma membrane.

In addition to various marker proteins, gradient distributions of GPCRs and trimeric G protein Gsα, Gqα/G11α, Gi1,2α and Gβ were analysed as well. In non-detergent preparations, all monitored proteins co-localised in gradient fractions representing 35-40% sucrose. On the other hand, application of Triton X-100 enabled us to analyse the extent of trimeric G proteins raft association. The fraction of trimeric G proteins localised in membrane domains represented 20-50% of total cellular amount, and the amount of Gsα protein in membrane domains decreased specifically after long-term agonist (iloprost) treatment in HEK293 cells stably expressing Flag-tagged form of human IP prosthacycline receptor (FhIPR) and fusion protein between Gsα and FhIPR (FhIPR-Gsα). On the other hand, gradient distribution of receptor molecules did not change, and nearly all receptor remained in detergent soluble, non-raft phase of plasma membrane. Similar results were obtained for receptor for thyreotropin-releasing hormone (thyreoliberin, TRH) expressed in HEK293 cells as fusion protein between VSV (vesicular stomatitis virus), GFP (green fluorescent protein) and thyreoliberin (TRH) receptor.

Further analysis of Triton X-100 extracted domains from raft brain cortex showed only low β-adrenergic receptors association, similarly as was observed for IP prosthacycline and TRH receptors in HEK293 cells. On the other hand, these domains were enriched in $GABA_B$

receptor. Hence, we may conclude that after Triton X-100 extraction, lipid rafts are separated from plasma membrane. These rafts are rich in caveolin, flotillin, and other domains markers, and contain a significant portion of cellular trimeric G proteins. However, with exception of $GABA_B$ receptor, only very low amount of monitored GPCRs were present in them.