Abstract

The first part of this thesis is preoccupied with the identification of protein alterations in the membrane fraction of HEK293-E2M11 cells after prolonged TRH treatment. The isolated membrane fraction enriched in plasma membranes contained high amounts of Na^+,K^+-ATPase, TRH receptor and G-proteins compared to the postnuclear supernatant. By using 2D electrophoresis and mass spectrometry, the levels of 42 proteins were identified to be altered in samples of PM-enriched fractions from TRH-treated (16 h; 10 μM) cells. Out of these proteins only ezrin and stomatin-like 2 are known to be localized in the plasma membrane. Five proteins (mitofilin, MTHSP75, prohibitin, stomatin like-2, peroxiredoxin III) whose levels were increased after the prolonged TRH treatment represent proteins localized in mitochondria. All of them are important for proper structure and function of mitochondria. The ratio of anti-apoptotic Bcl-2 to pro-apoptotic Bax was markedly higher in cells treated with TRH compared to control cells. Hence, it can be concluded that prolonged TRH treatment may significantly affect mitochondrial membrane and function of mitochondria.

The second part of this thesis deals with the identification of molecular protein complexes of TRH-R and/or $G_{q/11}$ protein. The presumed effects of TRH on the stability of these complexes have also been investigated. By native electrophoresis, three complexes of TRH-R and four complexes of $G_{q/11}\alpha$ protein were identified. The TRH-R complex found in the 80 kDa region corresponds to TRH-R dimer, which was proved by experiments analysing the effect of solubilisation at different temperature. The molecular complex detected in the 140 kDa region represents a pre-associated TRH-R–$G_{q/11}$ complex, which was verified by co-immunoprecipitation and experiments on cells with decreased levels of the Gα and Gβ subunits of $G_{q/11}$ proteins. Short-term (10–30 min) treatment with TRH led to dissociation of the pre-associated TRH-R–$G_{q/11}$ complex with concomitant increase in the level of TRH-R dimer while long-term TRH treatment resulted in partial re-association of TRH-R–$G_{q/11}$ complex apparently due to up-regulation of TRH-R. The immunoblot signal of $G_{q/11}$ protein in the 140 kDa region apparently corresponds not only to the pre-associated TRH-R–$G_{q/11}$ complex but also to molecular complexes of $G_{q/11}$ protein with other $G_{q/11}$-coupled receptors.

The $G_{q/11}$ complexes found in the 140 kDa and 300 kDa regions were observed to be membrane-bound while $G_{q/11}\alpha$ complexes in the 70 kDa region were cytosolic. The cytosolic $G_{q/11}\alpha$ complexes were observed to associate after prolonged TRH treatment which was connected with dissociation of membrane-bound $G_{q/11}$ complexes in the 140 kDa and 300 kDa regions and translocation of $G_{q/11}\alpha$ from the plasma membrane into the cytosol. The second reason for a decrease in the level of membrane-bound $G_{q/11}$ complexes is down-regulation of $G_{q/11}\alpha$ proteins after prolonged TRH treatment.

High-molecular-weight complexes of TRH-R or $G_{q/11}\alpha$ were observed in the 500 kDa or 700 kDa regions, respectively. The levels of both these complexes were found to increase after short-term TRH treatment suggesting that TRH-R and $G_{q/11}\alpha$ can be translocated from the pre-associated TRH-R–$G_{q/11}$ complex. In case of $G_{q/11}\alpha$, this hypothesis was confirmed by [^{35}S]GTPγS binding assay followed by autoradiography. The signal of bound [^{35}S]GTPγS in the 700 kDa $G_{q/11}\alpha$ complex was markedly enhanced after TRH treatment. GRK2 and PLCβ were assessed as potential components of these high-molecular-weight complexes.

Altogether, our present studies have demonstrated that native electrophoresis can serve as a highly suitable method for identification of molecular complexes. By using this approach we were able to show that the TRH receptor may form a pre-associated complex with its cognate G-protein and therefore it can be included among other GPCRs that constitute such complexes. The stability of this TRH-R–$G_{q/11}$ complex as well as other resolved receptor or $G_{q/11}$ protein complexes were markedly influenced by TRH treatment, which indicates that hormones can modulate the interactions between proteins and re-arrange proteins within complexes in the plasma membrane.