Summary

The vasopressin-V_2-receptor (V_2R) belongs to the large protein family of G protein-coupled receptors and is expressed mainly in the basolateral membrane of the principal cells of the renal collecting duct (Nonoguchi et al., 1995; Schülein et al., 1998). Activation of the receptor by its ligand 8-Arginine-vasopressine (AVP, also known as antidiuretic hormone) leads to the stimulation of the Gs/adenylyl cyclase system and consequently to a regulated water reabsorption from the urine. Mutations in the gene encoding the V_2R cause nephrogenic diabetes insipidus (NDI), a disease characterized by the inability of the kidney to concentrate urine (Oksche et al., 1998; Wüller et al., 2004). The majority of the NDI mutations lead to folding-defective proteins which are recognized by the quality control system (QCS) of the early secretory pathway (Oksche et al., 1998). These misfolded forms are usually retained intracellularly and finally subjected to proteolysis. It has recently been shown that misfolded receptors are not only retained in the endoplasmic reticulum of the early secretory pathway but also in the ER/Golgi intermediate compartment (ERGIC; Hermosilla et al., 2004; Oueslati et al., 2007). The mechanisms underlying this different retention behaviour of the mutant receptors are unknown.

During this work, the coherence between the localisation of the mutation within the receptor molecule and the retention mechanism was determined with the help of numerous artificial V_2R-mutants designed by molecular modelling. Moreover, the involvement of the different components of the quality control system in the retention process was tested. In the second part, a novel high throughput assay using automated microscopy was established to find small molecules that influence misfolded V_2Rs. These compounds may bind specifically to the mutant proteins and improve receptor folding (and consequently transport) as so called pharmacological chaperones. Alternatively, they may improve only trafficking by the inhibition of quality control components.

The data presented in this study showed a significant coherence between the localisation of the mutation within the receptor molecule and the retention mechanism. Mutations within the α-helical domains of the receptor more frequently led to stronger folding defects causing an exclusive ER-retention. In contrast, mutants within the extra- and intracellular loops more frequently reached post-ER compartments such as the ERGIC (transversal theory of mutation

position and retention mechanism). The interactions between the components of the quality control system and the V_2R-mutants were analysed with co-immunoprecipitations. It could be demonstrated that the interaction of Hsp70, Calnexin, Derlin1 and the individual receptor mutants was variable. However, a significant decrease in chaperone interactions was observed in more C-terminally located mutations (longitudinal theory of mutation position and chaperone interaction).

The novel high throughput assay to identify small molecules influencing receptor folding and/or transport by automated microscopy was successfully established. Using plasma membrane Trypan blue stains and nuclear Hoechst 33258 stains, it was for the first time possible to localize and quantify receptor GFP fluorescence signals in the plasma membrane and intracellular compartments of live HEK293 cells automatically. This methodology should lead to the identification of novel pharmacological chaperones for the V_2R and/or inhibitors of quality control components in the future. Moreover, the assay may be useful for other membrane proteins where folding defects play a role as disease-causing mechanisms.