5. Summary

The accessory β-subunits of Shaker-related voltage-dependent potassium channels (Kv1) form tetramers arranged with 4-fold rotational symmetry, like the membrane-integral and pore-forming alpha subunits (Gulbis et al., 1999). The crystal structure of the conserved Kvβ2 core domain shows that Kvβ subunits are potentially oxidoreductase enzymes containing an active site composed of conserved catalytic residues, a nicotinamide cofactor ($NADP^+$) and a substrate-binding site. At the other hand Kvβ subunits with an N-terminal inactivating domain like Kvβ1.1 (Rettig et al., 1994) and Kvβ3.1 (Heinemann et al., 1996) confer a rapid N-type inactivation to otherwise non-inactivating Kv1 channels. By a combination of structural modelling and electrophysiological characterization of structure-based mutations, as well as by direct measurement of nucleotide coenzyme binding, it is shown, that changes in Kvβ oxidoreductase activity may markedly influence the gating mode of Kv1 channels: Kvβ1.1 subunit reversibly binds different nucleotide coenzymes. Mutating the residues, that make direct contact with the $NADP^+$ molecule, can attenuate the nucleotide coenzymes binding of Kvβ1.1. These mutants of Kvβ1.1 are no more able to confer rapid inactivation to Kv1 upon their co-expression in Xenopus oocytes. Similarly, amino acid substitutions at the putative catalytic residues in the Kvβ1.1 attenuate the inactivating activity of Kvβ1.1. I propose that Kvβ oxidoreductase activity couples Kv channel inactivation to cellular redox regulation. The sensitivity of this kind of regulation is dependent on cell type. In CHO and HEK 293 expression systems, the effect of oxidoreductase features of Kvβ subunits on Kvβ-mediated rapid inactivation is less pronounced.

The molecular aspects of N-type inactivation have been recently elucidated in unprecedented details (Zhou et al. 2001). A small domain composed of several amino acids at the N-terminus of Kvβ1.1 blocks the channel by binding to its receptor site in the pore. By co-expression of

Kvβ1.1 with chimeric channels, made between Kv1.2 and Kv2.1 it is shown, that, as yet unknown structural elements in the transmembrane region of Kv2.1 can prevent the Kvβ1.1-mediated rapid inactivation. We conclude that the presence of N-terminal inactivating domain and an appropriate receptor site in the pore of the channel are necessary but not sufficient for rapid N-type inactivation of the channel.