SUMMARY

It is essential for the activation and clonal proliferation of T cells that the membrane potential required for signal transduction be provided by the K^+ conductance of the cell membrane, in which the Kv1.3 channel has a major role. The operation of the channel depends on the biophysical parameters characterizing the gating of the channel and on molecules specifically blocking the channel. Of these factors we studied the molecular mechanism of the dependence of inactivation kinetics on the extracellular pH and K^+ concentration and the Kv1.3 blocking ability and selectivity of toxins purified from the venom of the scorpion Centruroides elegans.

The Kv1.3 channel inactivates exclusively by the slow, so-called C-type mechanism, the rate of which is influenced by, among other factors, the properties of the amino acid residue at position 399. Based on our results the reversible protonation of H399 affects inactivation via the following molecular mechanism: Through an electrostatic interaction the protonated histidines regulate the occupancy of the K^+ binding site in the selectivity filter that determines the rate of inactivation. The occupancy of the K^+ binding site is influenced by filling from both the intra- and extracellular sides. The protonation of H399 has opposite effects on these two processes: it enhances filling from the intracellular side (slowing of inactivation) while it inhibits filling from the extracellular side (acceleration of inactivation). In order to verify the validity of our model we examined the dependence of the rate of inactivation on the extracellular pH and K^+ concentration, the effect of pH_o on inactivation in solutions of various ionic strengths, as well as the kinetics of the filling of the K^+ binding site by measuring the association and dissociation rates of Ba^{2+} ions. We modeled the properties of the wild type channel containing deprotonated or protonated H399 residues with H399L and H399K mutants, respectively. Our findings about the pH_o and K^+ dependence of inactivation have significance in the understanding of the inactivation mechanism of *Shaker* K^+ channels. This mechanism may also help the adaptation of T cells to conditions in which the pH_o and K^+ concentration differ from normal conditions (e.g. in areas of inflammation).

Toxins Ce1, Ce2 and Ce4 of the five (Ce1-5) toxins purified from the venom of the scorpion Centruroides elegans that belong in the Noxiustoxin subfamily blocked Kv1.3 with high affinity without blocking IKCa1. None of the effective toxins blocked *Shaker* channels or Kv2.1 belonging to the *Shab* family. Peptides selectively blocking Kv1.3 over IKCa1 may have great significance in the therapeutical inhibition of T cell subtypes whose proliferation is dependent on Kv1.3 channels.