Abstract

While inhibiting most neurons of the central vertebrate nervous system (CNS), Cl^--currents activate somatosensory neurons and olfactory sensory neurons (OSN). Both cell types show Cl^--currents coupled to intracellular Ca^{2+}-signalling. The receptor current of OSN is mostly carried by a Ca^{2+}-acitvated Cl^--current. While Ca^{2+}-activated Cl^--currents have already been detected by electrophysiological means in a variety of cells, no coding gene has been identified yet. The products of the clca gene family seem to have properties of Ca^{2+}-activated Cl^--channels. Until now, *clca* genes have been cloned from non-neuronal epithelia only. We have now cloned the first neuronal *clca*-gene from olfactory epithelium.

This thesis deals with the hypothesis that *rclca1* codes for the Ca^{2+}-activeted Cl^--channel of the olfactory signal transduction cascade. rCLCA1-specific antibodies have been generated to characterize and compare the rCLCA1-protein with other CLCA-proteins. rCLCA1 is a glycosylated 125 kDa membrane protein with four transmembrane domains. It is proteolytically cleaved into two 35 kDa and 97 kDa proteins. Both rCLCA1-fragments are slightly enriched in olfactory cilia in comparison with whole olfactory epithelium. This has been shown for all olfactory signalling cascade-proteins. However, on slices of olfactory epithelium rCLCA1-antibodies do not localize the protein in cilia but detect tight-junction structures. Functional expression of rCLCA1 shows, that it generates an enhanced Cl^--conductance in *rclca1*-transfected cells which has completely different properties than the native Cl^--current of OSN. By examining rCLCA1 in Odora cells, an OSN-cell lineage, it could be proved that *rclca1* can not code for the Ca^{2+}-activated Cl^--channel in OSN: Although Odora cells showed large Ca^{2+}-acitvated Cl^--currents with properties of the native current, the *rclca1*-gene and its protein could not be detected in these cells.

Ca^{2+}-activated Cl^--currents do depolarize somatosensory neurons and OSN, because these neurons have an outstandingly high $[Cl^-]_i$ compared with most CNS-neurons. In this thesis the $[Cl^-]_i$ of freshly dissociated somatosensory neurons has been measured by fluorescence-lifetime imaging (FLIM) for the first time. The $[Cl^-]_i$was 30 mM, thus Cl^--currents can indeed activate somatosensory neurons. The $[Cl^-]_i$ is determined by the expression of different chloride-transport molecules, like the cation/Cl^--cotransporter (CCC) proteins. This thesis shows that both OSN and somatosensory neurons do not express KCC2, the CCC-

molecule that leads Cl^- out of most neurons. In addition to this both types of neurons do not express NKCC1. The Cl^--accumulation process could not be elucidated by this thesis but an active Cl^--accumulation process will be discussed.