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Sommario

This PHD thesis is composed on 100 pages and is recorded in PDF format Ca2+- activated Cl- channels (CaCCs) play important roles in many physiological processes, including olfactory transduction, modulation of light photoreceptor responses, epithelial secretion. The molecular identity of these channels has been elusive for a long time, but recently it was shown that two members of the TMEM16/anoctamin family, TMEM16A/anoctamin1 and TMEM16B/anoctamin2, are responsible for CaCCs in several cells. At present, little is known about the structure-function relations for TMEM16A and TMEM16B. In the first part of this Thesis, we performed for the first time a site-directed mutagenesis study on TMEM16B to understand the molecular mechanisms of voltage and Ca2+ dependence. We mutated aminoacids in the first putative intracellular loop and measured the properties of the wild-type and mutant TMEM16B channels expressed in HEK 293T cells using the whole cell patch-clamp technique. In particular, we obtained three different mutants of TMEM16B: E367Q, ?E5 (deletion of the five consecutive glutamates 386EEEEE390), and ?EYE (deletion of three aminoacids 399EYE401) in the first intracellular loop. Ca2+ and voltage dependence of channel activation were not significantly modified in the ?EYE mutant compared to the wild-type channel. E367Q and deletion of five glutamates modified the voltage dependence of the TMEM16B, shifting the conductance-voltage

	relation toward more positive voltages. None of these mutants affected the apparent Ca2+ affinity. These results show that glutamates E367 and 386EEEEE390 in the first putative intracellular loop contribute to the voltage-dependent regulation of the TMEM16B channel. In the second part of this Thesis, we studied the relation between permeation and gating of TMEM16B, using both the whole cell and the inside-out patch clamp techniques. The permeability ratio sequence substituting Cl- with other anions at the extracellular or intracellular side was: SCN-> I-> NO3 -> Br-> Cl-> F-> gluconate. Activation and deactivation kinetics at 0.5 ?M Ca2+ were affected by the presence of various anions. The time constants of deactivation became slower in the presence of anions more permeant than Cl-, and faster with anions less permeant than Cl-. The voltage-dependence of channel activation was also modified by anions. When external Cl- was reduced by partial replacement with the less permeant gluconate, or 5 with sucrose, the conductance-voltage relation was shifted toward more positive voltages. Conversely, the substitution of Cl- with more permeant anions shifted the conductance-voltage relation to more negative voltages. Moreover, in the presence of different extracellular anions the apparent affinity for Ca2+ increased with increasing the permeability ratios. We also investigated the effect of replacing Cl? with SCN? at the intracellular side of the channel and found similar gating modifications as from the extracellular side. These results provide the first evidence that gating of TMEM16B is modulated by permeant anions.
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