1. Record Nr. TD18041658

Autore	Ozhathil, Lijo Cherian
Titolo	A structural and functional insight into TMEM16B, a calcium-activated chloride channel. [Tesi di dottorato]
Editore	SISSA, 2014-10-22
Lingua di pubblicazione	Inglese
Formato	Tesi di dottorato
Livello bibliografico	Monografia
Note	diritti: info:eu-repo/semantics/openAccess In relazione con info:eu-repo/semantics/altIdentifier/hdl/20.500.11767/3921
Sommario	TMEM16B, also known as anoctamin 2 has been recently identified as a calcium-activated chloride channel. It is expressed at the synaptic terminals of photoreceptors, in hippocampal cells, in the cilia of olfactory sensory neurons and in the microvilli of vomeronasal sensory neurons. TMEM16B, as its most known cousin TMEM16A, is activated both by calcium and voltage. When this thesis was started there was no available data correlating the gating function and protein structure in TMEM16B. In our first manuscript, we show a coupling between calcium and voltage in TMEM16B activation. Primary sequence analysis did not show any canonical calcium binding sites nor S4-like dedicated voltage sensors. However, the first intracellular loop contains several negatively charged amino acids. We performed site directed mutagenesis at 367E, and 386EEEEE390 in the first intracellular loop and investigated their role in calcium or voltage dependence of TMEM16B. Either neutralizing or deleting these acidic residues strongly shifted the conductance-voltage relation towards more positive voltages without a significant effect on the apparent calcium sensitivity. Our findings indicate involvement of glutamic acids from the first intracellular loop in voltage dependent activation of TMEM16B, and provides an initial structure-function study for this channel. In our second manuscript,

	we focused on understanding the effect of permeant anions on TMEM16B activation. Our results show TMEM16B is poorly selective among anions and has a permeability sequence of SCN- > I- > NO3- > Br- > Cl- > F- > gluconate. The channel kinetics also shows dependence on the permeant anion, with more permeable anions, such as SCN-, causing a much slower activation and deactivation kinetics than Cl-. Moreover, SCN- facilitated the channel activation by lowering the half-maximal concentration of calcium required for opening the channel and shifting the conductance-voltage relation towards less positive voltages. From this work we report the existence of a crosstalk between calcium, voltage and permeant anion in TMEM16B activation. Furthermore, we looked for a compound that could modulate the function of TMEM16B. We found that anthracene-9-carboyxlic acid, one of the traditional calcium-activated chloride channel blockers is very interesting since it had multiple effects on TMEM16B. In our third manuscript we report the block by A9C as voltage and concentration dependent, with maximal inhibition at positive voltages. Surprisingly, A9C also potentiated the current at intermediate concentrations and negative voltages. However, anthracene-9-methanol (A9M), a non-charged analog of A9C, completely abolished the voltage dependent inhibition and the potentiation effect seen with A9C. Both A9C and A9M had much slower current kinetics. This indicates the requirement of negative charge of A9C for its voltage dependent block of outward currents and potentiation of inward currents. In summary, the studies included in this thesis reveal a complex coupling between calcium, voltage, and permeant anion in TMEM16B activation. The identification of a compound have contrasting effects on the channel activation, provides a new tool for future structure-function studies on this channel.
Localizzazioni e accesso	http://memoria.depositolegale.it/*/http://hdl.handle.net/20.500.11767/3921