Amiloride-Sensitive Epithelial Na^+ Channel Is Made of Three Homologous Subunits

Research by C.M. Canessa, L. Schild, G. Buell, B. Thorens, I. Gautschi, J.-D. Horisberger, and B.C. Rossier, Nature ***1994,*** 367, *463*

Condensation and commentary by ***Thomas Dandekar*** *and* ***Patrick Argos,*** *European Molecular Biology Laboratory, Heidelberg*

CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To identify the two missing subunits complementing the α-subunit of an important epithelial ion channel

RESEARCHERS' APPROACH

Having already identified the α-subunit of the rat epithelial sodium channel (α-ErNaC),[1] the research group identified the two missing subunits by expression cloning in *Xenopus* oocytes and functional complementation.

OBSERVATIONS

Pools of roughly 20,000 were derived from a total of 250,000 cDNA clones made from 2.5–3.5-kb mRNA purified from the distal colon of rats maintained on a low salt diet. In vitro transcribed cRNA from these pools yielded no amiloride-sensitive current measured by patch clamp techniques in oocytes. However, in the presence of the α-subunit, one pool gave larger currents than the α-subunit alone. Further permutations of the positive pool isolated two independent clones, β and γ. The amiloride-sensitive current, in the presence of all three subunits together was more than 100-fold higher than that from the α-subunit alone. Removing the α-subunit led to a negligible signal. The inhibitory concentrations of amiloride (104 nM) and the analog benzamil (11 nM) did not differ from those obtained for the intact, complete channel. Unitary channel currents showed the long time periods of opening and closing which are characteristic of the epithelial sodium channel. Further channel characteristics like conductance also compared well. Moreover, the α-, β-, and γ-subunits are coexpressed in colon, kidney, and lung tissue. The amino acid sequences of all three subunits show around 35% residue identity between pairs; the subunits are also similar to MEC4, DEG1, and MEC10, a remarkable conservation between mammals and these *C. elegans* gene family members involved in sensory touch transduction and, when mutated, neuronal degener-

CHEMTRACTS—BIOCHEMISTRY AND MOLECULAR BIOLOGY 5:82–84 (1994)
 CCC 1045-2680/94/010082-03$04.00

ation. None of the subunits express a leader sequence, and thus the N terminus is predicted to be on the cytoplasmatic side of the plasma membrane. The authors see in each of the three subunits two transmembrane domains, one near the N-terminal region and the other close to the C terminus. β-sheet-like structures are predicted to follow the N-terminal helix and precede the C-terminal transmembrane segments. The latter are connected by a large (over 400 residues) hydrophilic and putative extracellular loop with conserved cysteine boxes. Glycosylation of the loops is supported by four potential *N*-glycosylation sites.

COMMENTARY ON THE RESEARCH

The first voltage gated channel was cloned by the group of Numa about 10 years ago,[2] but the first identification of the epithelial sodium channel proved to be difficult[3] and was only achieved independently last year.[1,4] The authors show that the basic function of the channel can be provided just by the three subunits, leading to a trimer model for the channel $\alpha\beta\gamma$. As multimeric structures like $\alpha_2\beta\gamma$ (voltage-gated potassium channel[5]) or $\alpha_3\beta\gamma$ (nicotine receptor[6]) are also conceivable, a future task will be to determine the exact stoichiometry of the complex. Furthermore, the authors pointed out that associated proteins (e.g., G protein[7] or cytoskeleton proteins[8]) may be quite important to modulate the channel.

Further data strengthen the generality and detail of this new channel model. In accompanying papers Hong and Driscoll[9] show data for the homologous protein MEC4 to be a subunit of an ion channel and that the second transmembrane domain of DEG1 or α-ErNaC may specifically replace the equivalent segment of MEC4 without destroying function. Moreover, Huang and Chalfie[10] demonstrate coexpression of MEC4 and MEC10 in the same neuron and the requirement of MEC6 for mechanosensation. Their several mutants resulting in loss of function suggest that the putative α-helix in the second transmembrane domain may line the pore. Characterization[11] of MEC7 hints that tubulins have to be included to form touch receptors in *C. elegans*.

A new family of cation channels may now be proposed; their original function involved in the control of cellular and extracellular volume may now be extended to sophisticated mechano-sensitive channels such as those in cochlear hair cells.[12] Potential medical implications concern cystic fibrosis through treatment of the increased sodium reabsorption and human neurodegenaratory diseases.

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