Commentary

Epithelial Na Channels

Why All the Subunits?

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Epithelial Na channels mediate Na reabsorption in the distal segments of the kidney, gut, and other organs (Garty and Palmer, 1997). They are vital to the control of blood volume and arterial blood pressure, as evidenced by various forms of hypertension involving defects in the channels themselves or the renin–angiotensin–aldosterone axis regulating them (Lifton, 1996). Several years ago the molecules comprising these channels were cloned and sequenced (Canessa et al., 1993; Lingueglia et al., 1993; Canessa et al., 1994). The first clone, called αrENaC, was sufficient to produce amiloride-sensitive Na currents when expressed in *Xenopus* oocytes. The physiological and pharmacological properties of these channels resembled those in the kidney and other native epithelia, but the magnitude of the currents was small. Much larger currents were obtained when αrENaC was coexpressed with two additional subunits termed βrENaC and γrENaC. The β and γ subunits themselves did not produce measurable currents. A molecular basis for this synergism was suggested by measurements of the surface expression of the subunits (Firsov et al., 1996). Coexpression of all three subunits was essential to have a significant number of any of the subunits in the plasma membrane of the oocyte.

The question of how the subunits interacted with each other was unresolved in the earlier studies. On the one hand, many properties of the holochannels were similar to those of the α subunit expressed by itself. These included ion selectivity (Li > Na >> K), current-voltage relationship, and the affinity for the canonical blocker amiloride ($K_I \sim 100$ nM). This suggests that the α subunit might form the pore by itself, while the other subunits could serve to help transport α to or stabilize it in the membrane. On the other hand, the three subunits are very similar in structure. They are all about the same size, they have two predicted membrane-spanning regions (M1 and M2) separated by a large extracellular domain, and share ~30% overall homology. This situation is more reminiscent of the nicotinic ACh receptor, in which 5 subunits arrange themselves pseudo-symmetrically around a central pore (Brisson and Unwin, 1985). A similar structure was proposed early on for ENaC (Jentsch, 1994), but until recently there was little or no direct support for this model.

In the January issue of *The Journal of General Physiology* Schild et al. reported results which argue strongly in favor of the three subunits making similar contributions to the formation of the pore (Schild et al., 1997). The basic finding involved the identification of a location in the presumed extracellular domain of all three subunits which affects channel conduction and channel block in qualitatively similar ways. The specific amino acids are S583 in the α subunit, G525 in β, and G537 in γ. These residues are located just before M2, presumably in contact with the extracellular fluid. Substitution of a cysteine at this position of any one of the subunits reduced both the conductance and the sensitivity to amiloride, although these effects were much larger for the β and γ subunits than for the α. These results suggest that the residues could form part of the pore itself; amiloride is thought to bind within the lumen of the channel (Garty and Palmer, 1997). More strikingly, introduction of these mutations created blocking sites for Zn^{2+} ions, presumably the result of a direct interaction with the sulfhydryl group of the cysteine. Zn^{2+} had little effect on the wild-type channel but blocked the α S583C, β G525C, and γ G537C mutants. In the case of α S583C the block was voltage dependent, consistent with the idea that the blocking site resides within the pore. These results greatly strengthen the notion that the three subunits contribute in similar ways to the formation of the channel. In particular, it is difficult to imagine how the mutations could produce such effects if the β and γ subunits were acting just as chaperones or stabilizing agents.

In an article appearing in this month's issue of the *Journal*, McNicholas and Canessa add support to this general idea (McNicholas and Canessa, 1997). They report experiments defining the properties of channels formed from only α + β or only α + γ subunits. Whereas the α + γ channels had properties rather similar to the wild-type holochannel, the α + β channels were much less sensitive to amiloride and had a very different concentration-conductance relationship with a larger apparent K_m for Na. Construction of chimeric subunits suggested that the key regions involved in these differences were once again in the extracellular domain. The region affecting amiloride block was near the M2 do-

main, in a region including the residue studied by Schild et al. The region affecting Na affinity was closer to the M1 domain. The general conclusion is that the β and γ subunits can substitute for each other in the formation of the holochannel, but that these substitutions affect channel properties. Thus the subunits must have similar but distinct roles within the channel structure. McNicholas and Canessa (1997) raise the intriguing possibility that channels with different subunit composition might exist in nature. This kind of mixing and matching of subunits could account for some of the variability in the properties of amiloride-sensitive channels found in different tissues (Smith and Benos, 1991; Palmer, 1992).

Which parts of the subunits form the pore itself? Previous studies had identified serine residues in the M2 domain of the α subunit which when mutated altered the single-channel conductance, Na:Li selectivity and amiloride affinity (Waldmann et al., 1995). M2 is therefore a good candidate for a pore-lining structure. The results of Schild et al. on the effects of Zn^{2+} block strongly implicate the pre-M2 domain of all three subunits as another possible contributor. The results of McNicholas and Canessa on the Na affinity of the channels suggest that a third region, just outside M1, may also play a role in the conduction system. However, a caveat to this conclusion is that older experiments on frog skin implied the existence of allosteric binding sites for Na that might modify channel activity and contribute to the apparent K_m for Na transport (Lindemann and Van-Driessche, 1978).

The subunit stoichiometry of the channel is a major question yet to be answered. McNicholas and Canessa found that optimal expression of α + β and α + γ channels occurred with the injection of equivalent doses of cRNA. This provides indirect evidence that roughly equal numbers of each subunit might be required. However, neither the exact proportions nor the absolute number of molecules needed to form a functioning channel has been determined. This information will be required in order to advance more detailed models of how the various subunits might interact with each other as well as with Na ions moving through the pore.

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