Commentary

Mechanosensitivity of the Epithelial Sodium Channel (ENaC): Controversy or Pseudocontroversy?

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Amiloride-sensitive epithelial sodium channels constitute the rate-limiting step for sodium reabsorption in the distal part of the renal tubule, in the distal colon, in the ducts of several exocrine glands, and in the airways (Rossier et al., 1994). Until recently, the classification of the epithelial sodium channels was exclusively based on biophysical and pharmacological properties: sodium and potassium selectivity, single channel conductance, kinetics of gating and amiloride sensitivity. As recently pointed out by Garty and Palmer (1997), the molecular cloning of the three homologous channel subunits denoted α, β, and γ epithelial sodium channels (ENaC) has provided a molecular definition of at least one class of amiloride-blockable channels.

ENaC is the major sodium-conducting pathway in the distal nephron, participating in the fine control of sodium balance, blood volume, and blood pressure. ENaC also has a unique role in controlling lung fluid clearance, especially at birth (Hummler et al., 1996). The ENaC genes share striking homologies with a set of genes that has been identified in *Caenorhabditis elegans*, using a genetic screening for loss of mechanosensation (Tavernarakis and Driscoll, 1997). The high degree of homology in the predicted transmembrane domains and in the structure of the ectodomain of the ENaC family suggested early on that ENaC may itself be involved in mechanosensitivity in mammalian cells.

Awayda et al. (1995) have followed up on this idea. They have reported that they were able to in vitro translate an α ENaC subunit from bovine kidney in a rabbit reticulocyte lysate and reconstitute the polypeptide product into a liposome (Awayda et al., 1995). Incorporation into planar lipid bilayers leads to sodium channel activity with a single-channel conductance of 40 pS. The channel was inhibited by a low concentration of amiloride (K_i 150 nM) and was moderately sodium selective. Mechanosensitivity of the channel was demonstrated by channel activation in response to small hydrostatic pressure differences across the bilayer. Surprisingly, selectivity and amiloride affinity were drastically altered by stretch activation. In a subsequent report (Ismailov et al., 1996), the study was extended to the reconstitution of α, β, γ ENaC subunits, either from in vitro translation in the presence of dog pancreas microsomes or from a crude microsomal membrane fraction of *Xenopus* oocytes in which the subunits were heterologously expressed. On incorporation into planar lipid bilayers, sodium channel activity was observed that was (*a*) sodium selective (P_{Na}/P_K = 10), (*b*) amiloride sensitive (K_i 170 nM), (*c*) comprised of three different open-state conductance levels (13, 26, and 40 pS), and (*d*) mechanosensitive. A hydrostatic pressure difference as low as 0.26 mmHg induced a fourfold increase in open probability (P_o).

Should we conclude from these studies that the epithelial sodium channel is mechanosensitive? Probably not. In the present issue of *The Journal of General Physiology*, one of the authors of the previous papers has now used the *Xenopus* oocyte expression system to examine in a living cell the effects of changes of membrane tension on α, β, and γ ENaC. In this setting, it is clear that ENaC is not mechanosensitive at either the whole-cell or single-channel levels (Awayda and Subramanyam, 1998). Are these results really contradictory? or should they trigger one of these long lasting controversies that we see (and sometimes enjoy) in science? I do not believe so. However, they do serve to emphasize the advantages and limitations of the experimental systems that were used.

In the oocyte model, the advantage is that one can express well defined and purified molecules (i.e., mRNA coding for the gene of interest) and measure their function in the membrane of a living cell. Within one experiment, hundreds of oocytes from the same female can be injected, insuring a well-controlled statistical analysis of the data. The main disadvantages of the system are twofold. First, there is a large biological variability from one set of oocytes coming from an animal to another, independent of the traditional factors invoked, such as season, water quality, or other ill-defined and somewhat mythic components that make the life of the oocyte fan sometimes so miserable. This intrinsic variability requires one to perform a large number of independent experiments. The second pitfall is that the oocyte can lack or express endogenous components that may be important physiologically to regulate the activity of the channel under study.

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The reconstitution of channel activity in planar lipid bilayers also presents distinct experimental advantages. It is a very well controlled experimental system, especially when the channel protein is biochemically pure and its reconstitution into proteoliposomes at less than one molecule per vesicle is achieved. This was, for instance, accomplished by Bear et al. (1992) for the cystic fibrosis transmembrane conductance regulator (CFTR). Upon incorporation, purified CFTR exhibited the basic biophysical and regulatory properties of the type of chloride channel found in native cells and believed to underlie cAMP-evoked secretion in epithelial cells (Bear et al., 1992). Reconstitution of channel activity in planar lipid bilayer suffers from different technical difficulties, however, when the channel protein studied is not biochemically pure and fully characterized as it was for CFTR. When using nonpurified membrane proteins, as in the papers cited above (Awayda et al., 1995; Ismailov et al., 1996), one has to take great precautions to insure that the protein translated in vitro is normally folded, properly oligomerized, and assembled in a physiologically active form. The technical problem is difficult enough for a homomeric channel (Awayda et al., 1995). The problem becomes even more severe when one wants to fold, oligomerize, and assemble an heterotetrameric protein (Firsov et al., 1998) such as ENaC, which is made of three homologous subunits (α, β, and γ). Experimental evidence was not provided that this was achieved (Ismailov et al., 1996). When crude microsomal membranes from oocytes expressing ENaC subunits are used as the source of protein for reconstitution into planar bilayers, one also has to realize that these membranes will contain monomeric, dimeric, and heteromultimeric channel proteins in ill defined proportions with different degrees of oligomerization and maturation. Nor should one overlook the problem that microsomal membranes (dog pancreas microsomes as well as oocyte microsomes) are likely to express various endogenous channel activities that will have to be characterized separately in control experiments.

Assuming that these parameters can be experimentally controlled, two possible observations can be made. Either reconstituted channels in the planar bilayer recapitulate most, if not all, the biophysical properties of the native channel measured in a cell expressing the ionic transport of interest, or they do not. In the present case, the properties of ENaC in apical membrane of native epithelial cells have been established by Palmer and Frindt (1986) and Hamilton (1985). Thus far, these basic biophysical properties have not been fully reproduced in the planar lipid bilayer. Conversely, no consistent and significant effects of pressure (10–60 mmHg applied to the patch pipette) could be documented in apical membranes of cortical collecting duct cells (Palmer and Frindt, 1996).

Some other basic properties, such as the consistent appearance of multiple conductance states and sensitivity to stretch activation are observed in planar lipid bilayers, suggesting a triple barrel structure of the channel (Ismailov et al., 1996). These observations have never been made in native cells or in oocytes expressing ENaC.

At the present time, considering the respective limitations of each of the two experimental systems, I am inclined to believe that expression systems that reproduce the properties of the native channels are more likely to be more physiologically relevant. In my view, the more "reductionist" approach of reconstituting ENaC in lipid bilayers should be well suited to address questions about detailed ion channel behavior. I am convinced that the planar lipid bilayer system will be useful in the future once the experimental conditions for reconstituting the physiological activity of ENaC are fully defined. In this respect, it will be of special interest to look whether the properties of a biochemically purified ENaC channel protein can reproduce the biophysical properties of ENaC observed in the apical membrane of renal cells. The answer to this question should come sooner or later.

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