New and Notable

Pore Models for Transporters?

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This issue of the *Biophysical Journal* contains an article by Harold Lecar and his colleagues H.P. Larsson, S.A. Picaud, and F.S. Werblin, who use noise analysis to measure the elementary events underlying glutamate-activated Cl currents in salamander photoreceptors. The 0.7 pS conductance uncovered by noise analysis describes an amino acid transporter and is the distinguishing feature of this paper. Whereas 0.7 pS is small for an ion channel, it is enormously large for a carrier. Standard models of electrogenic glutamate transporters (Nicholls and Attwell, 1990) predict a much tinier elementary event. The expected transporter current is in fact so small that the concept of unitary conductance rarely enters into traditional models. Instead, the literature suggests that a set number of charged or uncharged species (the substrate plus cotransported ions) cross the membrane at a fixed rate. For glutamate transporters the stoichiometry is one glutamate anion plus two or three Na ions (and possibly one retrograde K). A net charge of 1e per cycle at rate of 1000 cycles per second (high for most transporters) would by a naive calculation generate an elementary current of only 0.16 fA. The relatively large macroscopic current that many have observed therefore requires rapid cycling or an enormous number of transporters. Larsson et al. (1996) have proposed a different model; they explain the whole-cell currents in terms of a colossal (30 fA) microscopic current over 100 times greater than predicted from fixed stoichiometry models. This result raises questions; for example, if the underlying currents are so large, why have we not encountered them before? What are these currents for, and how are they related to transport?

Before discussing these fundamental issues, let us consider other explanations that may account for the inconsistency between the predicted single-transporter event and the measured event. Rods and cones contain ligand-activated receptors as well as transporters; thus, glutamate-induced currents (which are carried by Cl) may derive from glutamate receptors that gate conventional ion channels. Against this explanation, blockers that inhibit glutamate-receptors do not eliminate the glutamate-induced Cl current, whereas antagonists of glutamate-transporters do. Furthermore, the glutamate-activated Cl current has a very specific requirement for Na (even Li cannot substitute). This feature is a well-known characteristic of the class of proteins to which glutamate transporters belong. Indeed, the necessity for Na is so strong that it has lead to the concept of a "Na/glutamate-gate." In contrast, glutamate receptors do not have a selective requirement for Na. Such observations first led researchers to speculate that the large glutamate-induced current in rods and cones flows through transporters. Larsson et al., in this issue of the *Journal*, contribute new data that support this interpretation, and they explain the size of the whole-cell current in terms of the microscopic events.

To briefly summarize, at -50 mV 1 mM glutamate induces -350 pA in salamander photoreceptors with a variance-to-mean ratio of 0.034 pA. A Lorentzian fit to the spectral density gives a corner frequency of 215 Hz. The conductance of the unitary event does not depend on the concentration of glutamate or Na. However, the corner frequency (which partly reflects the opening rate of the channel) is proportional to glutamate concentration. Larsson et al. combine these data with the requirement for uptake in a model that joins transporters and channels in the same molecular scheme. In this model, glutamate binding increases the open-channel probability; however, once the channel is open neither Na nor glutamate affects the passage of Cl ions. An empty transporter can act as a Cl channel, it can bind two Na ions and transport glutamate, or it can do both. The model explains why THA, an aspartate derivative that can substitute for glutamate, induces a smaller current than glutamate. An analysis of the THA-induced fluctuations—by analogy to classical work on surrogate transmitters at the neuromuscular junction—reveals that THA decreases open-channel probability, not single-channel conductance. Thus the model can explain macroscopic properties associated with transporters in molecular terms. An exciting advance in this regard would be to analyze the corresponding rate of THA transport, thereby relating channel events to transporter efficiency.

The noise data themselves merely indicate the presence of a standard ligand-gated ion channel. As noted above, however, previous work (see Picaud et al., 1995 for additional references) indicates that glutamate transport and glutamate-gated Cl current reside in the same molecule. Although the possibility remains that the Cl conductance derives from an unrelated glutamate-gated ion channel, this becomes less and less plausible. Recently, the likelihood that glutamate transporters and Cl channels occupy the same integral membrane protein has received dramatic support. Fairman et al. (1995) demonstrate that a cloned human glutamate transporter expressed in *Xenopus* oocytes confers the properties of both glutamate uptake and glutamate-induced Cl conductance. The problem is complex and potentially rich because not all clones in this family have transporter and channel properties in equal measure (Wadiche et al., 1995). These amino

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acid transporters have another interesting feature. Unlike Na/Cl-dependent neurotransmitter cotransporters, glutamate uptake does not require Cl. Thus the ions required for the transport of glutamate (Na) and the ions that carry the current through the channel (Cl) are distinct in glutamate transporters and can be separately manipulated by ion substitution. Perhaps one reason we have not discovered pores in transporters before now is that in many cases the ion required for transport and the ion that carries the current are the same. Comparing glutamate transporters that conduct Cl with transporters that carry Na will undoubtedly provide insights into the mechanisms of transporter versus channel modes of operation. Indeed, the channel-like activity of glutamate transporters is no isolated phenomenon. Heterologously expressed GABA transporters, serotonin transporters, and norepinephrine transporters (Cammack et al., 1994; Mager et al., 1994; Lester et al., 1994; Risso et al., 1995; Galli et al., 1995) have transmitter-activated currents that in many respects resemble ligand-gated ion channels. Interest in the topic has increased enough to warrant a Symposium at the 1996 meeting of the Biophysical Society on *Transporters and Channels: Functional Similarities* organized by Henry Lester.

If transporters have fundamental similarities to ion channels, as Lecar and his colleagues propose, questions arise concerning the contribution of the pore to uptake or to other hallmarks of coupled transport, such as transmitter flux against concentration gradients and substrate-induced efflux of transmitter. In addition, we may ask whether the pore in the transporter contributes significantly to the resting potential or to membrane excitability. When an action potential initiates transmitter release, does the current generated by the reuptake mechanism add to the voltage of the presynaptic terminal? The answer to this question requires information we do not yet have, namely the number of transporters in the membrane relative to other pores. However, if the present data are any indication, even modest numbers of transmitter-binding transporters could have significant electrical effects. In this sense, neurotransmitter transporters differ minimally from traditional ligand-gated receptors that act as ion channels, except here the agonists move across the membrane. As this concept evolves, the borders between ion channels, ligand-gated receptors, and neurotransmitter transporters become obscure. This may force us to rethink many of the traditional concepts associated with cotransport, such as "electrogenic uptake." Theoretical explanations of the dual nature of transporters are underway. Su et al. (1996) in this issue of the *Journal* suggest a single-file model for cotransport; however, the high rate of charge transfer inferred from the data in Larsson et al. (1996) suggests an open pore. It will be interesting to see how this controversy turns out, and whether one model will suffice for all transporters. One thing is certain—the existence of pores in neurotransmitter transporters forces us to reexamine the kinds of schemes that we will have to use to relate transport phenomena to molecular mechanisms.

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