Commentary

No Easy Way Out (or In)

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In retrospect, when Jardetzky (1966) published a "simple allosteric model for membrane pumps," he initiated a conceptual movement away from the (then still) prevailing anthropomorphic view of transporters and pumps as "carriers" in the literal sense, membrane-bound entities that somehow made their way across the membrane, loaded with cargo. The idea of an "alternating access" model (as it is now called) as a way to explain transmembrane transport had already been proposed by Patlak (1957) and by Vidaver (1966), but Jardetzky (1966) and especially Läuger (1979) popularized it.

A trio of papers in this volume (Lu and Hilgemann, 1999a,b; Hilgemann and Lu, 1999) presents an alternating-access model for a GABA transporter (GAT1, the first to be cloned, by Guastella et al., 1990). The experimental kinetic database already accumulated on this transporter expressed at high levels in *Xenopus* oocytes, obtained both by two-electrode voltage clamp (Mager et al., 1993, 1996) and by the giant excised patch (Hilgemann, 1989) technique, is one of the most extensive on any carrier (certainly in a single expression system). The proposed model (Hilgemann and Lu, 1999) is probably the most complete "fitted" model extant for any transporter, active or passive. The term "fitted" is in opposition to those exercises where an entire kinetic model is presented, and "plausible" or literature values are assigned to its numerous rate coefficients to generate model behavior that mimics a variety of known experimental results. In the present case, no more rate coefficients, charge movements, Eyring barriers, stoichiometries, etc. are extracted than the data are capable of yielding. (The authors here clearly identify two inaccessible rate coefficients, and "fix" them arbitrarily.) The results make for a sobering read, even by those without specific interest in GABA transport, because they illustrate the magnitude of the modeling enterprise. There is simply no easy way. The database must be vast, with multiple approaches and extensive coverage of both steady state and pre–steady state experiments, before an attempt at global modeling can realistically be undertaken. "True" kinetic effects of the substrates must be distinguished from possible modulating ones. The starting model itself must then be ruthlessly pruned of all experimentally inaccessible parameters to yield the minimal model, and the minimal set of adjustable parameters, that will account for all accumulated observations. Several kinetic steps will need to be lumped mathematically because experiments to date are unable to resolve them. Thermodynamic constraints, such as reversal potentials for electrogenic carriers and microscopic reversibility rules, must be obeyed. The sum of all charges translocated in the various steps must match the known stoichiometry. Such "honest" minimal models will constitute a strong incentive for focusing future experiments on missing or ambiguous steps in the cycle.

The model proposed by the authors for the GAT1 cotransporter, which has a $1GABA:2Na^+:1Cl^-$ stoichiometry and therefore translocates one net charge, is an archetypal alternating-access model with two stable states E_{in} and E_{out}, designated after the side from which they offer access to the substrates, and two states with such rapid exit rates that their being lumped into a single pseudo-step does not materially affect kinetic predictions. Ion and GABA binding reactions are assumed instantaneous. A surprising feature of the model is that the major charge translocating event (carrying $\sim$1.1 charge inward) takes place when the empty E_{in} state reverts to the E_{out} state, a process during which an external Na^+ becomes occluded. The feature explains how cytoplasmic Cl^- binding, by preventing return of the empty carrier to the outside (note that the anthropomorphic terminology is still with us), competes with external Na^+ binding. Three additional (small) charge-carrying events are also identified. The predictive powers of the proposed model are impressive. It accounts, among others, for all known current–voltage relations (steady state and pre–steady state), including the kinetic effects of substrates thereon; transient charge movements in the absence of net current or in the absence of substrates; GABA–GABA exchange, including in the absence of extracellular chloride; and cis–cis and cis–trans substrate interactions (a tough test on any model).

The carrier modeling bar has been reset, and it is very high.

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references

Guastella, J., N. Nelson, H. Nelson, L. Czyzyk, S. Keynan, M.C. Miedel, N. Davidson, H.A. Lester, and B.I. Kanner, 1990. Cloning and expression of a rat brain GABA transporter. *Science*. 249: 1303–1306.

Hilgemann, D.W. 1989. Giant excised cardiac sarcolemmal membrane patches: sodium and sodium–calcium exchange currents. *Pflügers Arch.* 415:247–249.

Hilgemann, D.W., and C.-C. Lu. 1999. GAT1 (GABA:Na^+:Cl^-) cotransport function: database reconstruction with an alternating access model. *J. Gen. Physiol.* 114:459–475.

Jardetzky, O. 1966. Simple allosteric model for membrane pumps. *Nature*. 211:969–970.

Läuger, P. 1979. A channel mechanism for electrogenic pumps. *Biochim. Biophys. Acta*. 552:143–161.

Lu, C.-C., and D.W. Hilgemann. 1999a. GAT1 (GABA:Na^+:Cl^-) cotransport function: steady state studies in giant *Xenopus* oocyte membrane patches. *J. Gen. Physiol.* 114:429–444.

Lu, C.-C., and D.W. Hilgemann. 1999b. GAT1 (GABA:Na^+:Cl^-) cotransport function: kinetic studies in giant *Xenopus* oocyte membrane patches. *J. Gen. Physiol.* 114:445–457.

Mager, S., N. Kleinberger-Doron, G.I. Keshet, N. Davidson, B.I. Kanner, and H.A. Lester. 1996. Ion binding and permeation of the GABA transporter GAT1. *J. Neurosci.* 16:5405–5414.

Mager, S., J. Naeve, M. Quick, C. Labarca, N. Davidson, and H.A. Lester. 1993. Steady states, charge movements, and rates for a cloned GABA transporter expressed in *Xenopus* oocytes. *Neuron*. 10:177–188.

Patlak, C.S. 1957. Contributions to the theory of active transport: II. The gate type non-carrier mechanism and generalizations concerning tracer flow, efficiency, and measurement of energy expenditure. *Bull. Math. Biophys.* 19:209–235.

Vidaver, G.A. 1966. Inhibition of parallel flux and augmentation of counter flux shown by transport models not involving a mobile carrier. *J. Theoret. Biol.* 10:301–306.