

Glutamate Transporters: Redefining the View of a Transporter

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Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system and activates a wide range of ionotropic and metabotropic receptors to mediate a complex array of functions. The extracellular glutamate concentration is tightly controlled by a family of glutamate transporters (also termed excitatory amino acid transporters, EAATs), which serve to maintain a dynamic signalling system between neurones. The failure or down-regulation of EAAT function will lead to elevations in extracellular glutamate concentrations. This in turn leads to excessive stimulation of glutamate receptors which, if prolonged, will result in excitotoxicity and cell death. These processes are thought to underlie the pathogenesis of ischaemic brain damage following a stroke (1) and also various neurodegenerative disorders, such as amyotrophic lateral sclerosis (2) and Alzheimer's disease (3).

Glutamate transporters and chloride channels

Glutamate transporters are secondary active transporters. These transporters utilise a secondary energy source (in this case Na^+ , K^+ and H^+ ion gradients) to drive the movement of a solute (in this case glutamate) against its concentration gradient with the number of co- and counter-transported ions determining the concentrating capacity of the transporter. Glutamate transport is coupled to the co-transport of 3 Na^+ ions, 1 H^+ and the counter-transport of 1 K^+ ion, which will maintain a glutamate concentration gradient across the cell membrane of approximately 10^6 (4). Glutamate transporters can also function as ligand-gated chloride channels (6). This has raised many important issues from a range of

perspectives and also challenges many of the concepts of how transporters function.

From a physiological point of view these questions include the following. To what extent does the chloride channel function influence cell excitability and ion homeostasis, and can the chloride channel activity be manipulated under physiological or pathological conditions? From the biochemical point of view they include another issue, as follows. What is the molecular basis for the dual functions of the transporter, and can pharmacological or endogenous agents differentially modulate the dual functions? A number of studies have addressed aspects of these questions (e.g. refs 7-9). As yet, however, there is no clear understanding of how glutamate transporters allow chloride ions to permeate the transporter or what is the physical relationship between the transport process and the chloride channel. In this article, we shall briefly review the progress in understanding how glutamate transporters bind glutamate and translocate it through the membrane, and how chloride ions permeate glutamate transporters. We then present some plausible models for the structural relationship for the dual functions of this intriguing class of membrane proteins.

The glutamate translocation domain

Five different glutamate transporters have been identified and the human cDNAs are termed EAAT1-5 (5, 10, 11), with rat homologues of EAAT1-3 also termed GLAST1, GLT1 and EAAC1 respectively (12-14). Expression of individual recombinant EAAT clones in

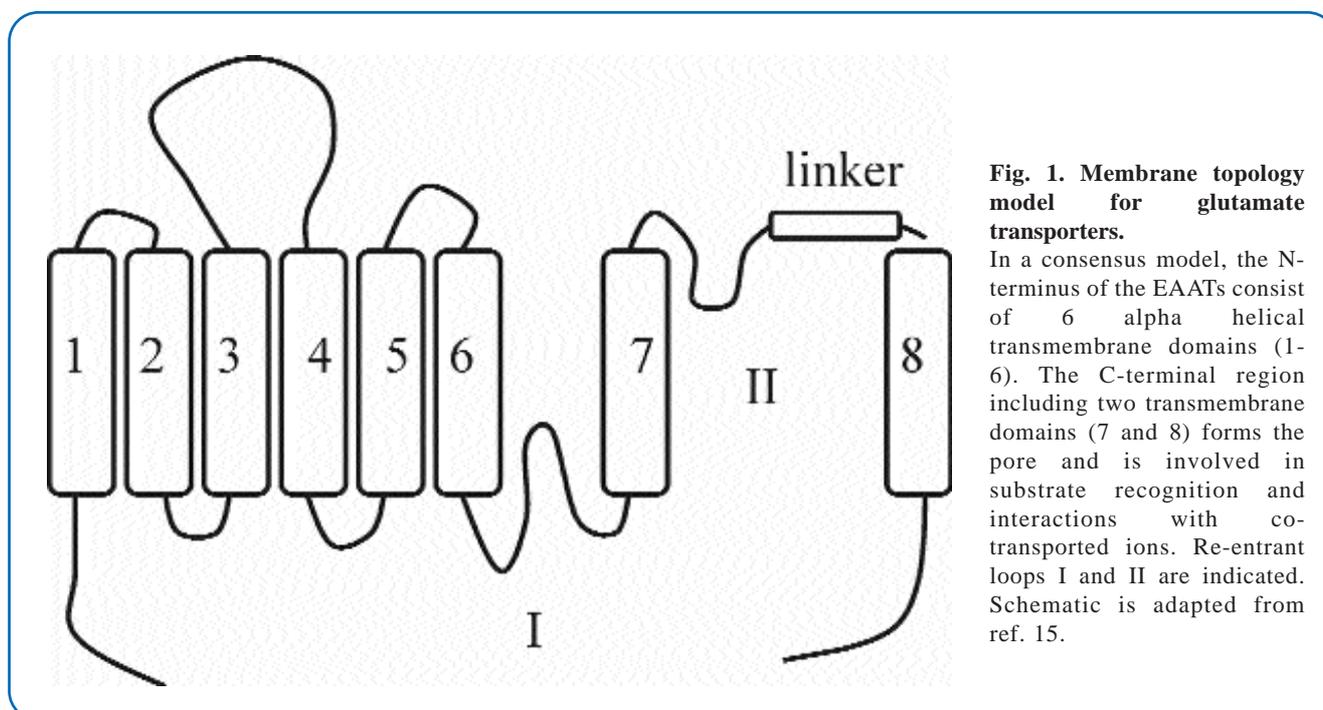


Fig. 1. Membrane topology model for glutamate transporters.

In a consensus model, the N-terminus of the EAATs consist of 6 alpha helical transmembrane domains (1-6). The C-terminal region including two transmembrane domains (7 and 8) forms the pore and is involved in substrate recognition and interactions with co-transported ions. Re-entrant loops I and II are indicated. Schematic is adapted from ref. 15.

Xenopus oocytes or HEK-293 cells generates glutamate transporters that can mimic, to a large extent, the pharmacological and electrophysiological properties of various native glutamate transporters (10). This suggests that the basic functional units of the transporter are formed from a single protein species. The amino acid sequences of the five glutamate transporters are closely related, with 40-60% of residues identical between the various subtypes (11). Hydropathy plots of the amino acid sequences of the five glutamate transporters together with scanning cysteine mutagenesis and chemical modification studies using methanethiosulphonate derivatives (12-16), have been used to predict the transmembrane topology of the transporters shown in **Fig. 1**. The general consensus is that EAATs consist of 6 α -helical transmembrane domains (TMD) in the N-terminal half of the sequence. An alternate model has also been proposed with a different assignment of TMD7 and re-entrant loop I as well as re-entrant loop II being assigned as a linker region (16).

Despite the differences in assignment of transmembrane domains, there is consensus that the C-terminal region forms a pore region accessible to an aqueous environment and that this region is involved in substrate recognition and co-transported ion interactions. Point mutations in the C-terminal half of the transporter have identified a number of residues that are involved with substrate and coupled ion recognition and/or translocation. The first re-entrant loop (I in **Fig. 1**), located between TMD6 and 7, contains a conserved series of serine and threonine residues. Two of the serine residues are accessible from both the extracellular and cytoplasmic sides while the threonine residues are accessible only from the cytoplasm (18). Accessibility from both sides of the membrane implies an important role in the transport cycle, in which those

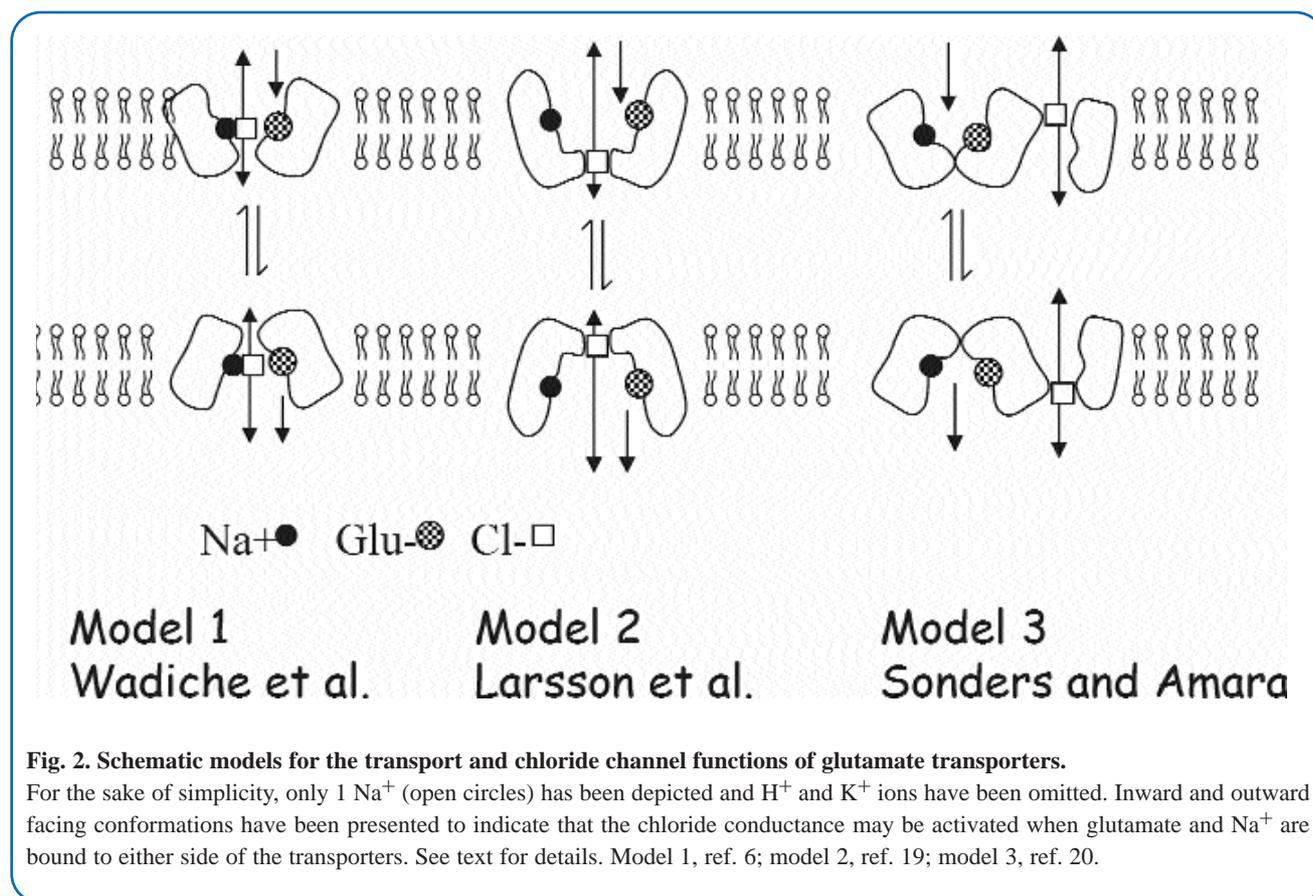
residues with substrate and cation bound are exposed alternatively to the extra and intracellular milieu to catalyse the translocation process. Two serine residues S440 and S443 (GLT1 numbering) within the second re-entrant loop (II in **Fig. 2**), are also important to both substrate specificity and selectivity for co-transported cations (18).

Furthermore the two re-entrant loops are in close proximity to one another, as demonstrated by formation of disulfide bonds between cysteine mutants at positions 364 in re-entrant loop I and 440 in re-entrant loop II (18). TMD7 contains residues that bind the potassium ion, sodium ions and protons (18) and that also appear to be accessible from both sides of the membrane. This implies that these residues form part of the translocation machinery.

Finally, TMD8 is an amphipathic domain that contains residues that are crucial for substrate discrimination. R447 (EAAC1) is involved sequentially in both the recognition of the γ -carboxyl group of substrates and in binding of potassium, enabling the coupling of their fluxes (18). Thus, TMD7 and 8 together with the two re-entrant loops are likely to be in close proximity to one another to form the principal binding sites for glutamate, Na^+ , K^+ and H^+ and catalyse their coordinated co- and counter-transport. Despite the steady progress being made in understanding the molecular details of how substrates are transported across the membrane, these studies also serve to highlight a major shortcoming of our understanding of the function of glutamate transporters, namely, how these transporters could also function as chloride channels.

The chloride channel of glutamate transporters

A number of recent studies suggest that it is possible to selectively manipulate the two functions by either mutations or



through the actions of the endogenous modulator zinc. This suggests that a separate structural domain may form the chloride channel. Three residues in the hydrophobic linker region between the second re-entrant loop and TMD8, namely, V452, V449 (EAAT1) and I421 (GLT1) were mutated to cysteine and modified by sulfhydryl reagents. These modified residues allow glutamate to bind to the transporter but do not allow transport to occur. However, the chloride channel function is unaffected (9). This suggests that the two functions are generated by distinct conformational states of the transporter. Conversely, addition of zinc to the glutamate transporter EAAT4 selectively inhibits the chloride channel function with little or no effect on transport. This also suggests that there are likely to be independent molecular determinants for the two functions. Our laboratory has located the zinc-binding site on EAAT4 and demonstrated that it is located at the extracellular edge of TMD3, which is distinct from the glutamate translocation domain. We are currently investigating the roles of transmembrane domains in the N-terminal half of the transporter to see if they are involved in formation of the chloride channel. We have obtained preliminary data that suggests that TMD2 and 3 may form part of the chloride channel.

A single pore or a dual pore model for EAAT function?

A number of kinetic schemes for glutamate transport have been proposed, which differ in the order of substrate and ion binding steps and the rates of the various steps in the process (see e.g. refs 6, 17). A common theme that has emerged from these models is that activation of the uncoupled chloride conductance may occur at a limited number of stages in the process. The uncoupled chloride conductance may be activated by 3 Na⁺ ions bound to either the external or internal surface of the transporter and the extent of activation is further facilitated by subsequent binding of glutamate at either surface. A number of schematic models has also been developed to better understand the physical basis of the dual functions of glutamate transporters. We shall consider three of these models, which are schematically presented in Fig. 2.

The first model, proposed by Wadiche *et al.* (6), postulates that sodium ions, bound within the pore of the channel and required for transport, form a chloride-binding site. In the presence of sodium and glutamate, the transporter may adopt a transient conformation that allows chloride ions to pass through the transporter. In this single pore model, glutamate and sodium bound to the transporter serve as part of a chloride selectivity filter and also gate for the chloride channel of the transporter and the two processes are tightly linked (see Fig. 2, model 1). Larsson *et al.* (19) have proposed a similar model containing a single pore that may adopt various conformations. In contrast to the first model, the molecular determinants of chloride permeation are separate to that of the transport process. In a third model, postulated by Sonders and Amara (20), glutamate and sodium bind to the transporter and may induce conformational changes in the transmembrane domains, thus resulting in the opening of a second pore and allowing chloride ions to pass. At this stage it is not possible to definitively rule out any of these models, but there are some experimental observations that make model 1 less likely than the others. As mentioned above, if the chloride selectivity filter and gate for the channel were formed by the sodium bound within the pore of the transporter, then the two processes should be tightly linked, yet this is clearly not the case.

It is interesting to note that a dual pore model has recently been proposed for the facilitative glucose transporter. It has been suggested that there is a main pore that is responsible for glucose transport and a secondary pore, partially overlapping with the primary pore, which allows the passive flux of water molecules through the transporter (21). In terms of glutamate transporters, we need to map the proximity of the molecular determinants for the two functions, before we can begin to address this issue of single or dual pore for the two functions of glutamate transporters.

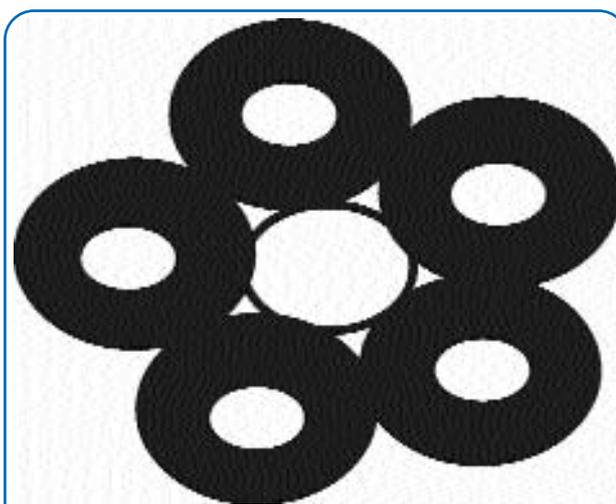


Fig. 3. A schematic model for pentameric glutamate transporters in the cell membrane.

See text for discussions of the various functional interpretations of the model.

Glutamate transporters are pentamers in the cell membrane

At this stage, glutamate transporters have not been crystallised for structure determination but a crude picture of the transporters has been obtained from freeze-fracture electron microscopy. The EAAT3 subtype of transporters appears to form homopentameric oligomers in the cell membrane (22). How then might such a structure fit in with the functional models for glutamate transporters? If a single pore model holds true, then two possibilities exist. First, the pentamer may form a single pore at the interface of the five subunits in which both glutamate and chloride pass. Second, each subunit within the pentamer contains a single pore in which both glutamate and chloride pass and each subunit would function independently of each other (Fig. 3). However, if the dual pore model turns out to be the more plausible, then the issue is whether the two pores arise from each subunit or whether they arise as a consequence of pentamer formation. In this last scenario, each of the subunits may form a glutamate/Na⁺/K⁺/H⁺ translocation pore and the interface of the five subunits may form the chloride channel. Such a structural solution would explain the large differences in chloride permeation between the five subtypes of the glutamate transporter family and suggests how the chloride pore can be modified independently of the structure of the glutamate pore.

Clearly, more experiments are required to elucidate the structural basis of the dual functions of glutamate transporters. Elucidation of the structural basis for these

properties will help resolve a number of fundamental questions about how transporters work. The chloride channel function of transporters is an intriguing property which redefines our understanding of how transporters operate. At this stage we do not know whether the channel function is a necessary by-product of forming a powerful and efficient transporter or whether it plays a truly secondary function of maintaining levels of membrane excitability and cell homeostasis. The answer to this question may well turn out to be both dependent on the transporter subtype, the expression levels and cellular location of the transporters. In general terms, transporter proteins are involved in regulating many aspects of cell biology and also are the targets for a wide variety of therapeutic agents and drugs of abuse. As such, the ideas and models developed from understanding how these proteins work will impact on our understanding of a range of current issues in biochemistry, physiology and pharmacology.

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