Glutamate and Glutamine in the Brain

Brain Uptake of Glutamate: Food for Thought1,2

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ABSTRACT Glutamate transporters in cells of the central nervous system play a key role, not only in providing glutamate for metabolic and protein synthesis purposes, but also in terminating glutamate’s synaptic actions and keeping the extracellular glutamate concentration below levels that cause neuronal death. Recent advances in our understanding of how glutamate transport is powered allow a prediction of how glutamate transport will fail in stroke, releasing excess glutamate that triggers the death of neurons, thereby causing mental and physical handicap. J. Nutr. 130: 1023S–1025S, 2000.

KEY WORDS: • glutamate • brain • uptake • stroke

The transmission of information at most excitatory synapses in the brain is mediated by the release of glutamate from the presynaptic cell. After diffusing to the postsynaptic cell, glutamate activates cation channels or G protein–linked receptors, which alters the membrane potential or intracellular biochemistry of the postsynaptic cell. For brain neurons to transmit information at a high rate, it is necessary for glutamate’s postsynaptic actions to be terminated rapidly. This occurs in part by removal of glutamate from the extracellular space by glutamate transporters. These transporters also have the role of keeping the extracellular glutamate concentration ([glu]o) below levels that are excitotoxic to neurons. That is, if [glu]o rises too high for too long, then excessive activation of postsynaptic N-methyl-D-aspartate (NMDA)3 receptors causes an excessive calcium influx into neurons, which triggers their death (Ankarcrona et al. 1995, Choi et al. 1988). In this article, I will discuss how glutamate transporters function normally, and how they malfunction in brain ischemia.

Types and locations of glutamate transporters

Five different mammalian glutamate transporters have been cloned. Their names and the cells in which they are expressed are shown in Figure 1. Apart from cells in the retina and cerebellum, which express high levels of the tissue-specific transporters listed in Figure 1, the transporters expressed most commonly throughout the brain are GLT-1/EAA1 in glial cells and EAAC1/EAA1 in neurons.

Powering glutamate transport

The extracellular concentration of glutamate is usually in the low micromolar range, whereas that inside cells is in the millimolar range. Furthermore, the potential inside cells is negative, and glutamate bears a net negative charge. Thus, energy is required to pump glutamate into cells up its electrochemical gradient. This energy does not come directly from ATP, but is derived from the cotransport of ions moving down their electrochemical gradients, as schematized in Figure 2. Figure 2 shows that net positive charge enters the cell with

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3 Abbreviations used: EAAC, excitatory amino acid carrier; EAAT, excitatory amino acid transporter; GLT, glutamate transporter; NMDA, N-methyl-D-aspartate.

FIGURE 1 The names of the glutamate transporter family members, and their location in the brain. The excitatory amino acid transporter (EAAT) names are for the human transporters; other names were given when the transporters were first cloned in nonhuman species. Abbreviations: GLAST, glutamate and aspartate transporter; GLT, glutamate transporter; EAAC, excitatory amino acid carrier.

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each glutamate, allowing glutamate transport to be monitored as a membrane current (Fig. 3; Brew and Attwell 1987).

When a nontransported blocker of glutamate transport is applied, a current change is seen that reflects the direction of glutamate transport, i.e., outward at very negative potentials where inward glutamate transport generates an inward current (before the blocker is applied), and inward at very positive potentials where outward glutamate transport generates an outward current (Zerangue and Kavanaugh 1996). At some intermediate potential (the reversal potential), there is no net transport, and applying the blocker generates no current. This reversal potential is set by the number and identity of the ions cotransported to drive glutamate transport. Measurement of the reversal potential, and how it is altered by changes of ion concentration, has revealed that for both the neuronal transporter EAAT3 and the glial transporter GLT-1, three Na$^{+}$

**Forward uptake**

**FIGURE 2** The ion movements powering uptake of glutamate into the cell. Three Na$^{+}$ and one H$^{+}$ enter, while one K$^{-}$ is transported out of the cell.

**FIGURE 3** Schematic diagram of recording of glutamate transport in a glial cell (wine glass–shaped object) as a membrane current. A whole-cell pipette (right) is attached to the cell, allowing recording of an inward current (bottom right inset) when glutamate is applied and activates the ion movements shown on the transporter (black circle).

**FIGURE 4** Schematic diagram of detection of glutamate release by reversed uptake in conditions mimicking stroke. A glial cell (wine glass–shaped object) is whole-cell clamped with an electrode containing glutamate and Na$^{+}$ (right). Depolarizing the glial cell potential ($V_M$: bottom right inset) in high [K$^{+}$]$_o$ solution activates reversed uptake, which can be measured as an outward current flowing through the right pipette because of the ion movements on the reversed transporter (black circle). At the same time a whole-cell clamped neuron (snail-shaped object on left) with glutamate-gated Na$^{+}$-permeable channels in its membrane is positioned near the glial cell. Glutamate leaving the glial cell is detected by opening of channels and inward current flow (bottom left inset) in the neuron.
ions and one H⁺ ion enter the cell with each glutamate, and one K⁺ ion is transported out of the cell (Fig. 2: Levy et al. 1998, Zerangue and Kavánagh 1996).

It is clear from Figure 2 that glutamate uptake is critically dependent on the ion gradients that make favorable the entry of Na⁺ and H⁺ into the cell. Below, I will discuss what happens when this situation is not maintained.

Anion channel opening associated with glutamate transport

Glutamate transporters generate current by virtue of the net charge entry that occurs as a result of the ion movements coupled to glutamate movement. However, in addition, recent work has shown that glutamate transport leads to the opening of an anion channel (Wadiche et al. 1995). Movement of Cl⁻ ions through this conductance generates a particularly large current for the transporter EAAT4 expressed in cerebellar Purkinje cells (Fairman et al. 1995) and for the transporter EAAT5 expressed in retinal photoreceptors and bipolar cells (Arriza et al. 1997, Eliasof et al. 1998, Grant and Dowling 1995, Sarantis et al. 1988). In the retinal cells, the EAAT5 anion conductance generates glutamate-gated voltage changes that are large enough to contribute to the cells' responses to light. For all of the other transporters, however, the current flow through the anion conductance is too small to affect the cells' membrane potential, and the functional significance of this anion conductance is uncertain.

Reversal of glutamate uptake in brain ischemia

An inevitable consequence of the dependence of glutamate uptake on transmembrane ionic gradients is that uptake will fail if those gradients run down. This happens dramatically in brain ischemia or hypoxia (Artwell et al. 1993, Szatkowski and Artwell 1994; Rossi et al. 2000). A few minutes after the onset of an occlusive stroke, which cuts off the blood supply to an area of brain, there is a dramatic alteration in the transmembrane ion gradients. The extracellular potassium concentration, [K⁺]o, is usually maintained at ~3 mmol/L by the Na⁺/K⁺ pump transporting K⁺ into cells. But after this pump's ATP supply is removed, [K⁺]o rises to 60 mmol/L, and Na⁺, falls by a similar amount. The [K⁺]o rise depolarizes cells to ~20 mV, and this anoxic depolarization terminates information processing by the affected area of brain.

The rise of [K⁺]o, fall of [Na⁺]o, and concomitant fall of [K⁺], rise of [Na⁺], and membrane depolarization, all decrease the energy available to power glutamate uptake. Consequently glutamate transporters are expected to reverse and pump glutamate out of cells into the extracellular space. Figure 4 shows a diagram of an experiment to measure this reversed uptake. Glutamate release from a glial cell, whole-cell clamped with glutamate and Na⁺ inside, is sensed by positioning a whole-cell clamped neuron nearby and using the neuron's glutamate-gated ion channels to detect the glutamate. When the glial cell is depolarized in high [K⁺]o, solution, to mimic the conditions of ischemia, glutamate release is detected by the sensing neuron (Billups and Artwell 1996, Szatkowski et al. 1990). Detailed calculations (Billups et al. 1998) predict that the

ion concentration changes occurring in ischemia will result automatically in reversed operation of glutamate transporters occurring until [glu], rises to ~60 μmol/L, a level known to trigger the death of neurons. Microdialysis experiments on intact brain confirm this idea (Wahl et al. 1994). Because this rise of [glu], is determined solely by the ionic stoichiometry of the transport process, the prospects for blocking this glutamate release therapeutically are slim. Release can be slowed, indeed it is slowed > 90% (Billups and Artwell 1996, Takahashi et al. 1997) by the brain acidification that occurs in stroke (resulting from the switch to anaerobic metabolism); eventually, however, it will still raise [glu], to a neurotoxic level. For this reason the best outlook for stroke therapy involves the blockade of glutamate's actions on its receptors, as well as of the downstream consequences of this receptor activation.

LITERATURE CITED


