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THE ROLE OF GLUTAMATE TRANSPORTERS IN GLUTAMATE HOMEOSTASIS IN THE BRAIN

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Summary

Glutamate transporters in neurones and glia, four of which have been cloned from mammals, play a crucial role in controlling the extracellular glutamate concentration in the brain. In normal conditions, they remove glutamate from the extracellular space and thereby help to terminate glutamatergic synaptic transmission and to prevent the extracellular glutamate concentration from rising to neurotoxic values. Glutamate transport on these carriers is thought to be driven by the cotransport of Na^+, the counter-transport of K^+, and either the cotransport of H^+ or the counter-transport of OH^-. Activating the transporters also activates an anion conductance in their structure, the anion flux through which is not coupled to glutamate movement and varies widely for the different transporters. During hypoxia or ischaemia, glutamate transporters can run backwards, releasing glutamate into the extracellular space, triggering the death of neurones and thus causing mental and physical handicap. The rate of glutamate release by this process is slowed by the acid pH occurring in hypoxia/ischaemia, which may help protect the brain during transient, but not sustained, ischaemia.

Key words: glutamate transporter, pH, synaptic transmission, hypoxia, ischaemia, epilepsy, anion conductance, glial cell, neurone, postsynaptic uptake, stoichiometry, cloning.

Introduction

The brain can be said to contain the seeds of its own destruction. Glutamate is released from neurones to communicate information rapidly by activating receptors in other neurones; in fact, glutamate is the most important and widespread excitatory neurotransmitter in the brain. However, if the extracellular glutamate concentration rises too high for too long, then it triggers the death of neurones (reviewed by Szatkowski and Attwell, 1994). Indeed, during brain hypoxia (such as occurs in perinatal asphyxia) and ischaemia (stroke), there is a massive release of glutamate which can cause severe brain damage.

Fundamental to the use of glutamate as a transmitter in the brain is the existence of a system to terminate its synaptic action – there is no extracellular enzyme to do this, unlike the situation for acetylcholine at the neuromuscular junction. The synaptic action of glutamate is eventually terminated by it being removed from the extracellular space by a family of uptake carriers in the plasma membrane of neurones and glial cells. These transporters acquire their energy to accumulate glutamate inside cells from the transmembrane ion gradients (for Na^+, K^+ and H^+) which are ultimately set up by the Na^+/K^+ pump.

Inherent in the use of transmembrane ion gradients to power glutamate transport into cells is the consequence that, if those gradients are ever run down, glutamate removal from the extracellular space may fail or even be transformed into pumping of glutamate from the inside of the cell to the outside.

In this review, we will describe the glutamate transporters that have been cloned to date and their localization, identify the ion gradients which drive glutamate transport, and assess the transporters' role in synaptic transmission. We will also discuss the possible functional significance of the anion channel present in the carrier structure, and describe how reversed operation of glutamate transporters releases glutamate during brain hypoxia or ischaemia.

Cloning and localization of glutamate transporters

Cloning of the plasma membrane glutamate transporters has revealed that they belong to a family of molecules separate from the transporters for other transmitters [γ-aminobutyric acid (GABA), glycine, noradrenaline, dopamine, 5-hydroxytryptamine: reviewed by Amara and Kuhar, 1993; Kanai *et al.* 1994]. Four distinct glutamate transporters have now been cloned, together with a related molecule that transports neutral amino acids (Arriza *et al.* 1993; Shafgat *et al.* 1993). *In situ* hybridization indicates that mRNA for these transporters is produced only in certain cells of the brain. Two of the first transporters to be cloned, GLAST or EAAT1 (Storck *et al.* 1992; Arriza *et al.* 1994)

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and GLT-1 or EAAT2 (Pines *et al.* 1992; Arriza *et al.* 1994), are located predominantly in glial cells. GLT-1 is expressed widely among astrocytes throughout the brain, whereas GLAST is much more localized, being concentrated in Bergmann glia of the cerebellum. One cloned carrier, EAAC1 or EAAT3, is expressed in neurones throughout the brain (Kanai and Hediger, 1992; Arriza *et al.* 1994), whereas the more recently cloned EAAT4 (Fairman *et al.* 1995) is expressed in cerebellum but its cellular location has not yet been published.

Using antibodies to the cloned transporters, their subcellular localization in the cerebellum has been examined in particular detail. GLT-1 carriers are not expressed uniformly in the membrane of astrocytes: there are more carriers in the parts of the glial cell membrane that face nerve terminals, axons and dendritic spines than in the membrane that faces other glial cells, capillaries, pia or stem dendrites (Chaudhry *et al.* 1995), consistent with a major role for glial uptake being to maintain the glutamate concentration around neurones below neurotoxic levels. Conventionally, neuronal glutamate uptake has been thought of as being in presynaptic terminals, where it would both help to terminate the synaptic action of glutamate and recycle glutamate to synaptic vesicles for re-use as a transmitter. However, Rothstein *et al.* (1994) found that EAAC1 is expressed throughout the soma and dendritic tree of Purkinje cells. These are not even glutamatergic neurones (although they may take up glutamate as a precursor for the synthesis of their transmitter, GABA), and this subcellular location suggests a role in terminating the synaptic action of glutamate released from the parallel and climbing fibres onto the Purkinje cells (Takahashi *et al.* 1996).

A different function for the glial transporters GLAST and GLT-1, and for the neuronal transporter EAAC1, has been suggested by experiments in which molecular biological methods were used to prevent these different transporters being expressed (Rothstein *et al.* 1996). Deleting the glial transporters led to a general rise of extracellular glutamate concentration, which caused cell death. By contrast, preventing EAAC1 function did not raise the extracellular glutamate concentration, but caused epileptic-like seizures. These results suggest that glial transporters may function mainly to keep the glutamate concentration low in the extracellular space, whereas neuronal transporters play a more specific role in synaptic transmission.

Ionic stoichiometry of glutamate transporters

One glutamate anion appears to be transported on each cycle of glutamate transport. This stoichiometry is based on the fact that all studies of high-affinity glutamate uptake have shown a Michaelis–Menten (first-order) dependence on external glutamate concentration.

Sodium ions are necessary outside cells to drive the uptake of radioactive glutamate (Hertz, 1970; Erecinska, 1987). Glutamate uptake by all four cloned expressed carriers was almost abolished by removal of extracellular Na^+. Furthermore, glutamate stimulates uptake of radioactive Na^+, suggesting that Na^+ is cotransported on the uptake carrier with glutamate. Direct measurements of glutamate and Na^+ fluxes suggest that 2 Na^+ are transported per glutamate (Stallcup *et al.* 1979; Baetge *et al.* 1979). A similar conclusion (2 Na^+ per glutamate) was deduced from the Na^+-dependence of the equilibrium accumulation of D-aspartate into synaptosomes (Erecinska *et al.* 1983). Consistent with this stoichiometry, the current produced by glutamate transport (which partly reflects the cotransport with glutamate of an excess of Na^+) has a sigmoid dependence on extracellular Na^+ concentration (Barbour *et al.* 1991).

Radiotracing experiments suggested that glutamate uptake requires, in addition to extracellular Na^+, intracellular K^+ (Kanner and Sharon, 1978; Burckhardt *et al.* 1980). Similarly, when uptake is studied by whole-cell clamping (Barbour *et al.* 1988), the glutamate-evoked current is activated by intracellular K^+ (controlled by the whole-cell pipette). The current depends on intracellular [K^+] in a Michaelis–Menten fashion, showing that 1 K^+ has to bind to activate the transporter. A direct demonstration that K^+ is actually transported out of the cell on the glutamate transporter, rather than just binding to an intracellular activating site, was obtained by measuring K^+ efflux using K^+-sensitive microelectrodes (Amato *et al.* 1994).

In addition to transporting 2 Na^+ and 1 glutamate anion into the cell, and transporting 1 K^+ out of the cell, glutamate transporters also generate pH changes, acidic inside and alkaline outside the cell, and these are not a secondary consequence of glutamate transport raising $[Na^+]_i$ and modulating the action of pH-regulating carriers (Erecinska *et al.* 1983; Bouvier *et al.* 1992; Nelson *et al.* 1983). The magnitude of the pH change implies that the glutamate transporter in salamander glia transports approximately one proton equivalent into the cell with each unitary charge. These pH changes may therefore be produced by the transport either of 1 H^+ into the cell or of 1 OH^- out of the cell with each glutamate anion. Bouvier *et al.* (1992), on the basis of anion substitution experiments, suggested that an OH^- was transported out of the cell rather than an H^+ into the cell, but the discovery that glutamate transporters activate an anion conductance (see below) undermines the methods used to reach this conclusion: it is currently unknown whether an H^+ or an OH^- is transported.

In summary, glutamate uptake is powered by the cotransport of 2 Na^+ into the cell, the counter-transport of 1 K^+, and either the cotransport of 1 H^+ or the counter-transport of 1 OH^-. Evidence supporting this stoichiometry is summarized in Fig. 1.

Glutamate transporters activate an anion channel present in their structure

Wadiche *et al.* (1995*a*,*b*) found that the charge movement associated with glutamate transport by cloned human glutamate transporters was not as predicted (entry of one

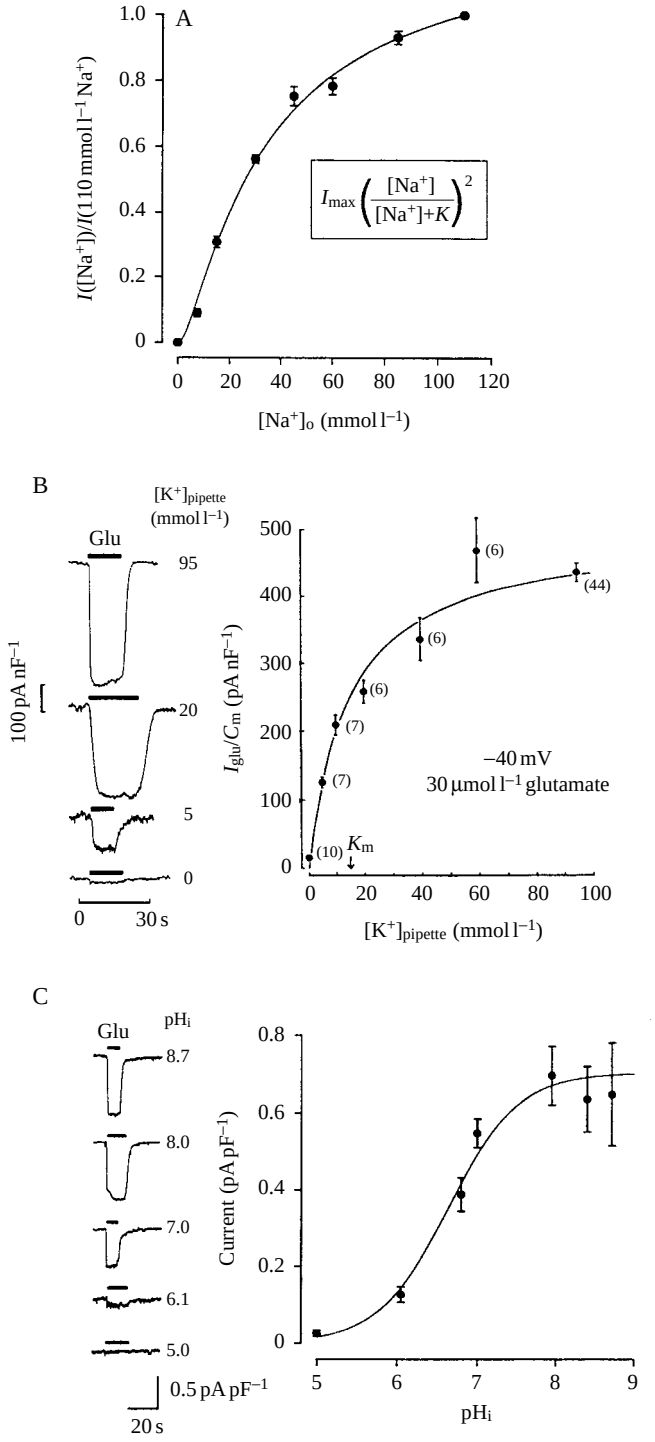

Fig. 1. Ion-dependence of glutamate uptake in retinal glial cells of the tiger salamander. (A) Dependence on $[Na^+]_o$ (abscissa) of the current (ordinate, normalized to its value with 110 mmol l^{-1} $[Na^+]_o$) produced at −40 mV by glutamate transporters in response to 200 μmol l^{-1} glutamate (from Billups and Attwell, 1996). The smooth curve is the square of a Michaelis–Menten function (inset). (B) $[K^+]_i$-dependence (abscissa) of the uptake-associated current (produced by 30 μmol l^{-1} glutamate at −40 mV, normalized by cell capacitance, C_m, to compensate for variations in cell size). Left: specimen data with different pipette (and hence internal) K^+ concentrations. Right: mean dose–response curve fitted with a Michaelis–Menten curve. From Barbour *et al.* (1988). (C) pH_i-dependence of the uptake-associated current (produced by 200 μmol l^{-1} glutamate, and normalized by cell capacitance: from Billups and Attwell, 1996). Left: specimen data; right: mean dose–response curve (the smooth curve is a Michaelis–Menten dependence on $[OH^-]_i$).

positive charge with each glutamate) from the stoichiometry just described. Instead, extra charge entry occurred, which they showed was produced by Cl^- efflux through an anion conductance activated in the transporter structure when glutamate was transported. The contribution of the Cl^- flux to the membrane current produced by the transporter is different for the different transporters, but for three of the transporters approximately 1 Cl^- leaves the cell per glutamate entering (in oocytes; Wadiche *et al.* 1995*a*,*b*). There is apparently no thermodynamic coupling of glutamate transport to the Cl^- flux, so glutamate transport is not powered by the Cl^- gradient (Billups *et al.* 1996). The cloning of EAAT4 (Fairman *et al.* 1995) showed the potential importance of the anion conductance: for this transporter, over 95 % of the current generated by the transporter is produced by Cl^- movement.

The properties of EAAT4 are very similar to those of what is now recognized as a glutamate transporter in the presynaptic terminal of salamander retinal cones (Sarantis *et al.* 1988; Eliasof and Werblin, 1993) and in fish retinal bipolar cells (Grant and Dowling, 1995). These transporters generate a current which is abolished when external Na^+ is removed, but which has all the properties of a current flowing through an anion conductance (Fig. 2).

Activation of the anion conductance during glutamate uptake into a presynaptic terminal would tend to clamp the presynaptic terminal at a relatively negative potential (since physiological values of the Nernst potential for Cl^- are close to the resting potential). In central neurones (where the presynaptic signal is an action potential, unlike the graded voltage changes occurring in cones), this negative potential would tend to reduce further exocytotic release (by making it harder for action potentials to invade the synaptic terminal and activate Ca^{2+} channels) and to potentiate the (voltage-dependent) re-uptake. As mentioned above, Rothstein *et al.* (1996) have shown that preventing the expression of neuronal EAAC1 carriers leads to epileptic behaviour of neurones. It is not yet known whether the anti-epileptic properties of EAAC1 transporters derive solely from their ability to take up glutamate, or whether their contribution to the anion conductance of neurones is also involved.

Recent work on the salamander Müller cell transporter has shown that its anion conductance is activated both by extracellular glutamate during forward uptake and by intracellular glutamate during release of glutamate by reversed operation of the uptake carrier (Billups *et al.* 1996). Thus, unlike normal glutamate-gated channels, this conductance can be activated by glutamate from either side of the membrane.

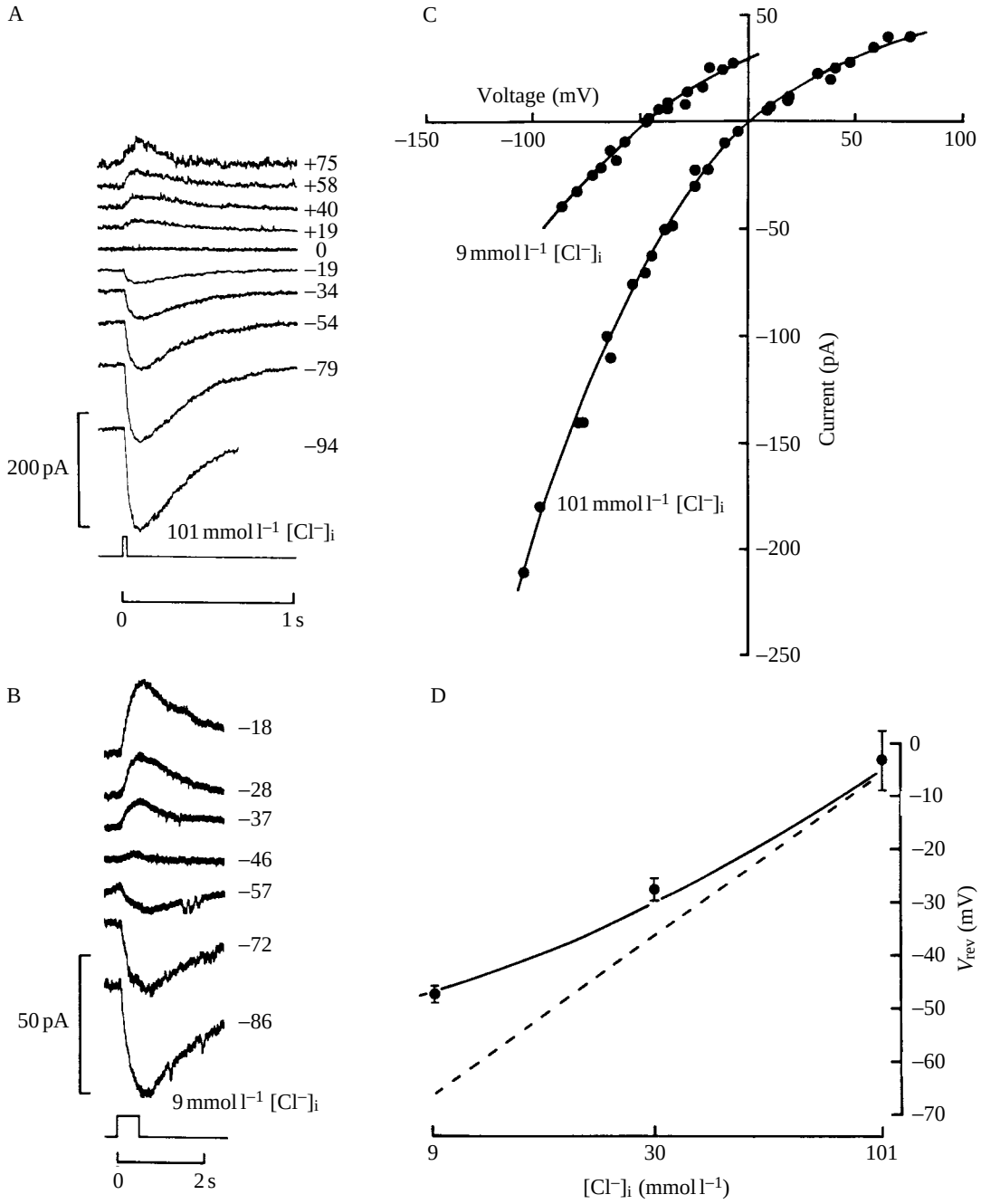

Fig. 2. Anion channel activation during glutamate uptake into cone photoreceptors of the tiger salamander (from Sarantis *et al.* 1988, with permission). (A) Currents produced by ionophoresed glutamate at different potentials (shown in mV by each trace) with 101 mmol l^{-1} intracellular $[Cl^-]$. (B) Same as A, but with 9 mmol l^{-1} internal $[Cl^-]$. (C) Current–voltage relationships from A and B. (D) Dependence of the current reversal potential, V_{rev}, on internal $[Cl^-]$ (solid line). Dashed line shows the Nernst potential for Cl^-.

Role of glutamate transport in terminating synaptic transmission

To process information at a high rate, it is necessary to terminate glutamate's synaptic action rapidly. One might expect glutamate uptake to play a key role in this termination.

It was surprising to find, therefore, that the glutamate uptake blocker PDC (L-*trans*-pyrrolidine-2,4-dicarboxylate) has essentially no effect on the decay phase of the non-NMDA (non-*N*-methyl-D-aspartate) receptor component of the synaptic current at the hippocampal Schaffer collateral to pyramidal cell synapse and at the cerebellar mossy fibre to granule cell synapse (Sarantis *et al.* 1993). There are two possible interpretations of this finding. One is simply that the particular type of glutamate transporter expressed near these synapses has too low an affinity for PDC for much block to occur. Alternatively, it is possible that, even with uptake partly

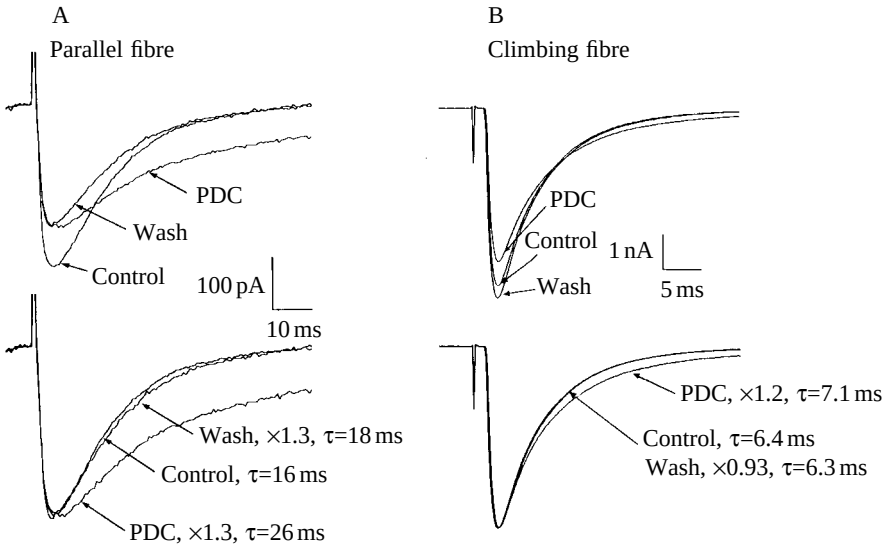

Fig. 3. Effect of blocking glutamate uptake (with 300 μmol l^{-1} PDC) on the synaptic currents at the parallel (A) and climbing (B) fibre to Purkinje cell synapses in rat cerebellar slices (from Takahashi *et al.* 1995, with permission). Top panels show excitatory postsynaptic currents (EPSCs) before (Control), during (PDC) and after (Wash) application of the uptake blocker. Bottom panels show the same data normalized to the peak current, with the time constant of the EPSC, τ, shown by each trace.

blocked, glutamate diffuses rapidly from the synaptic cleft and is taken up by carriers in nearby glial cells, so that the decay time course of the non-NMDA component of the synaptic current is determined by the kinetics of channel closing following a fall in glutamate concentration (Colquhoun *et al.* 1992; Hestrin, 1992).

Not all glutamatergic synapses behave like the Schaffer collateral and mossy fibre to granule cell synapses. For the cerebellar climbing fibre and parallel fibre to Purkinje cell synapses, PDC prolongs the synaptic current (Barbour *et al.* 1994; Takahashi *et al.* 1995), as shown in Fig. 3. For these synapses, it appears that a PDC-sensitive glutamate transporter near the synapse may play a key role in terminating synaptic transmission (although desensitization of postsynaptic receptors also contributes significantly: Takahashi *et al.* 1995). A glutamatergic synapse in culture has also been shown to have its postsynaptic current duration partly determined by glutamate uptake (Mennerick and Zorumski, 1994). Similarly, at GABAergic synapses in cerebellar slices (M. Hamann and D. Rossi, unpublished observations) and cultured hippocampal slices (Thompson and Gähwiler, 1992), blocking GABA uptake prolongs the synaptic current.

Above we noted that Rothstein *et al.* (1994) found that the neuronal transporter EAAC1 is localized in the dendritic tree and soma of cerebellar Purkinje cells. A role for this postsynaptic uptake in terminating synaptic transmission was suggested by Takahashi *et al.* (1996) who found, first, that an uptake current can be detected when D-aspartate (which is transported by glutamate carriers) is applied to Purkinje cells, and second, that when D-aspartate is loaded into Purkinje cells (*via* a whole-cell patch pipette) to slow the uptake of glutamate then the duration of the climbing fibre postsynaptic current was increased.

In considering the possible role of glutamate transport in terminating glutamate's synaptic action, it is important to know how fast glutamate transporters can cycle. Early experiments with photo-induced release of glutamate (Schwartz and Tachibana, 1990) suggested that carrier cycling could be rapid (on a millisecond time scale). More recently, however, experiments measuring a 'gating charge movement' associated with Na^+ binding to a cloned transporter (Wadiche *et al.* 1995*b*) have provided a measure of the number of transporters present; comparing this with the steady current produced by glutamate application led to the conclusion that carrier cycling was rather slow, roughly once every 70 ms. If the latter result is correct, glutamate transporters near synapses could only help to terminate synaptic transmission by rapidly binding glutamate (i.e. acting as a glutamate buffer) rather than by actually transporting it into the cell – such transport could only occur on a time scale much longer than the synaptic current. Consistent with this hypothesis, Tong and Jahr (1994) have reported that, for cultured synapses, blocking the glutamate binding site on transporters can increase the synaptic current amplitude, presumably because less glutamate is buffered by the transporters.

Reversed operation of glutamate transporters when ATP levels fall

Calculations based on the stoichiometry of glutamate transport predict that, for the normal transmembrane gradients of Na^+, K^+, pH and voltage, glutamate uptake will be able to lower the extracellular glutamate concentration, $[glutamate]_o$, to around 0.2 μmol l^{-1} (Attwell *et al.* 1993).

This minimum predicted value is altered during brain hypoxia or ischaemia because of the altered ion gradients occurring (reviewed by Attwell *et al.* 1993; Szatkowski and Attwell, 1994). The first ionic concentration to change is that of H^+: because of the switch to anaerobic respiration, the pH starts to become acid, eventually reaching about 6.1 both inside and outside cells. The significance of this acidification will be discussed below. Next, the fall of brain ATP levels produced by oxygen lack leads to slowing of the Na^+/K^+ pump and a rundown of the transmembrane gradients for $[K^+]$, $[Na^+]$ and

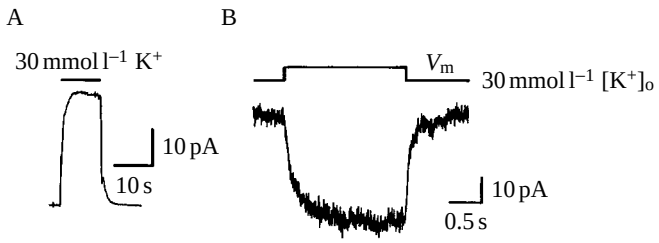

Fig. 4. Release of glutamate by reversed operation of glutamate transporters in salamander retinal glia (from Billups and Attwell, 1996, with permission). (A) Reversed uptake current evoked by raising the external K^+ concentration (from 0 to 30 mmol l^{-1}) around a cell clamped to 0 mV with a pipette containing Na^+ and glutamate. An outward current is evoked which reflects the transport of 1 glutamate anion and 2 Na^+ out of the cell and the transport of 1 K^+ and 1 OH^- into the cell. (B) Detection of glutamate release from a salamander retinal glial cell using non-NMDA channels in an adjacent rat cerebellar Purkinje cell. The glial cell was depolarized from −60 to +20 mV (membrane potential, V_m, shown as top trace) in 30 mmol l^{-1} [K^+] solution to release glutamate. This release evoked an inward current (noisy trace) in the Purkinje cell clamped to −60 mV.

voltage. Initially, the extracellular K^+ concentration, $[K^+]_o$, rises slowly, but after 2 min of oxygen lack $[K^+]_o$ rises suddenly to about 60 mmol l^{-1}, while $[Na^+]_o$ falls, and the cells are depolarized to −20 mV. This change of gradients leads to glutamate transporters running backwards (because of the reduced $[Na^+]$, $[K^+]$ and voltage gradients), releasing glutamate into the extracellular space, where it activates glutamate receptors and triggers neuronal death.

We have detected this release of glutamate by reversal of the uptake process in two ways (Fig. 4). First, raising the K^+ concentration around glial cells clamped to a depolarized potential with pipettes containing Na^+ and glutamate, to mimic the events of ischaemia, evokes an outward membrane current produced by reversed glutamate uptake (Szatkowski *et al.* 1990). Second, if a whole-cell clamped neurone is pushed up against a glial cell which is depolarized in high-$[K^+]$ solution, glutamate release from the glial cell by reversal of uptake can be detected as an opening of glutamate-gated channels in the neurone (Billups and Attwell, 1996). These experiments suggested that release of glutamate by reversed uptake does indeed occur with the same stoichiometry as forward uptake, so that 2 Na^+ and 1 glutamate anion come out of the cell while 1 K^+ is transported into the cell and either 1 OH^- is transported in or 1 H^+ is transported out.

The glutamate transporters run backwards during anoxia/ischaemia until $[glutamate]_o$ rises high enough for a new equilibrium to be reached. Predicting the lowest value of $[glutamate]_o$ that can be maintained in this situation is complicated because the necessary calculation depends on the intracellular glutamate concentration, $[glutamate]_i$, which may be different in neurones and glia. In normoxic conditions, conversion of glutamate to glutamine in glia, catalysed by glutamine synthetase, keeps the glutamate concentration low in these cells. However, because this reaction is driven by ATP, during anoxia/ischaemia the glutamate concentration rises in glia (Storm-Mathisen *et al.* 1992). Assuming an average value of 3 mmol l^{-1} for $[glutamate]_i$ during anoxia/ischaemia predicts that when $[K^+]_o$ rises during ischaemia then glutamate will be released by reversed operation of uptake carriers until $[glutamate]_o$ rises to 260 μmol l^{-1} (Attwell *et al.* 1993). This value is high enough to trigger the death of neurones if it is sustained for more than a few minutes (Choi *et al.* 1987).

Slowing of transporter-mediated glutamate release in the acidic conditions of anoxia/ischaemia

If release of glutamate by reversed operation of uptake carriers involves the transport of 2 Na^+ and 1 glutamate anion out of the cell while 1 K^+ and 1 OH^- are transported into the cell, then one would expect the acid pH shift occurring during ischaemia to slow the release of glutamate because it will deprive the carrier of external OH^- (for simplicity here we analyse the situation in terms of OH^- counter-transport, but similar considerations apply if H^+ cotransport occurs: it will be hard for the carrier to lose cotransported H^+ at the outer face of the membrane). We have detected this slowing of reversed uptake by an acid pH in salamander retinal glia by measuring the effect of an acid external pH both on the reversed uptake current and on the released glutamate detected with a sensing neurone (Billups and Attwell, 1996). When the external pH is altered from 7.3 to 6.1, glutamate release by reversed uptake was slowed by a factor of 14. This result is roughly what would be predicted if reversed uptake were proportional to the external $[OH^-]$, which decreases by a factor of 15.8 for the above pH change.

The acid pH change occurring in ischaemia is largely complete by the time (2 min after the start of the ischaemic insult) when $[K^+]_o$ rises, leading to glutamate release by reversed uptake (Mutch and Hansen, 1984; Silver and Erecinska, 1992), and so will have the effect of prolonging the time needed for the glutamate concentration to rise to a neurotoxic level. A simple model can help quantify this prolongation. If v is the volume of tissue per cell and f is the extracellular volume fraction, then the rate of rise of $[glutamate]_o$ is given by:

$$f v \mathrm{d}[\mathrm{glutamate}]_o/\mathrm{d}t = \mathrm{efflux} - \mathrm{influx}, \quad (1)$$

where efflux and influx are the unidirectional fluxes of glutamate on its transporters. Simplistically, we can postulate that at time t:

$$\mathrm{efflux} = k_e[Na^+]_i^2[K^+]_o[OH^-]_o[\mathrm{glutamate}]_i, \quad (2)$$

and

$$\mathrm{influx} = k_i[Na^+]_o^2[K^+]_i[OH^-]_i[\mathrm{glutamate}]_o, \quad (3)$$

where k_e and k_i are the rate constants for efflux and influx and the fluxes are assumed to be proportional to the fraction of transporters loaded with substrate at the membrane surface appropriate for the flux considered. The value of k_e can be obtained by equating the expression in equation 2 to I_{rev}/F,

where I_{rev} is the reversed uptake current measured experimentally with particular substrate concentrations and membrane potential, and F is the Faraday constant. The value of k_i can then be obtained by noting that, at equilibrium, the efflux and influx are equal and the equilibrium $[glutamate]_o$ is given by thermodynamics as (Attwell *et al.* 1993):

$$[glutamate]_o(equilibrium) = [glutamate]_i([Na^+]_i/[Na^+]_o)^2([K^+]_o/[K^+]_i)([OH^-]_o/[OH^-]_i) \exp(EF/\boldsymbol{R}T), \quad (4)$$

where E is the membrane potential, $\boldsymbol{R}$ is the gas constant and T is the temperature. With these substitutions, equation 1 becomes:

$$fv\mathrm{d}[glutamate]_o/\mathrm{d}t = (I_{rev}/F)\{1 - [glutamate]_o/[glutamate]_o(equilibrium)\}, \quad (5)$$

where I_{rev} is proportional to $[OH^-]_o$. In Fig. 5, we show the solution of this equation for the particular situation of the salamander retina, where all the necessary parameters are known for Müller cells (the retinal volume per cell is $v=9\times10^{-14}\,m^3$, the extracellular volume fraction is $f=0.07$, and when $[K^+]_o$ rises to 60 mmol l^{-1} and depolarizes the cells to −20 mV, I_{rev} is of the order of 20 pA at pH_o 7.3 and pH_i 7.0: Attwell *et al.* 1993). If the pH did not alter from these values, Fig. 5 shows that, after 2 min of ischaemia when the $[K^+]_o$ rises to 60 mmol l^{-1}, $[glutamate]_o$ is predicted to rise towards its equilibrium value of 260 μmol l^{-1} with a time constant of 8 s. Thus, 3.8 s after the $[K^+]_o$ rises (which in turn occurs 2 min after the start of ischaemia), $[glutamate]_o$ is predicted to reach 100 μmol l^{-1}, a value which if sustained for more than a few minutes will trigger the death of more than half the neurones (Choi *et al.* 1987). (Glutamate release from neurones, which is not considered here, would lower this value of 3.8 s further.) What is the effect of the acid pH shift on the rise of [glutamate]? Since the intra- and extracellular pH change by roughly the same amount (approximately 1 unit) during ischaemia (Silver and Erecinska, 1992; Mutch and Hansen, 1984), the equilibrium value of [glutamate] reached will not be affected (from equation 4). However, the time constant with which this equilibrium value is reached will be prolonged by an amount proportional to the decrease of $[OH^-]$: from equation 5 the time constant is:

$$\tau_{glu\ rise} = fvF[glutamate]_o(equilibrium)/I_{rev}, \quad (6)$$

and I_{rev} is proportional to $[OH^-]_o$. If the intra- and extracellular pH become more acid by 1.2 units (so pH_o goes to 6.1 as found experimentally), then the 14-fold reduction of I_{rev} found experimentally (Billups and Attwell, 1996) or the 15.8-fold reduction predicted theoretically if I_{rev} is proportional to $[OH^-]_o$ will greatly prolong the time needed for $[glutamate]_o$ to rise, as shown in Fig. 5. A 14-fold prolongation produces a time constant of 111 s for the rise of $[glutamate]_o$ and the time needed for $[glutamate]_o$ to reach 100 μmol l^{-1} is increased to 53 s.

This prolongation of the time needed for the glutamate concentration to rise may be neuroprotective in cases of transient ischaemia. If a blood vessel becomes blocked, then if the extracellular compartment did not become more acid glutamate would rise to a neurotoxic level just 2 min (the time taken for $[K^+]_o$ to rise) and 3.8 s (the time needed for $[glutamate]_o$ to reach 100 μmol l^{-1} after the $[K^+]_o$ rises) after the start of ischaemia. The slowing of release by reversed uptake produced by the acid pH shift prolongs this time to 2 min and 53 s, providing nearly an extra minute for the blood vessel to become unblocked before glutamate reaches a neurotoxic level. This neuroprotective consequence of the involvement of OH^- (or H^+) in the glutamate transporter stoichiometry may help explain why transient ischaemia (Nadeau, 1994; Lord, 1990) need not produce major damage

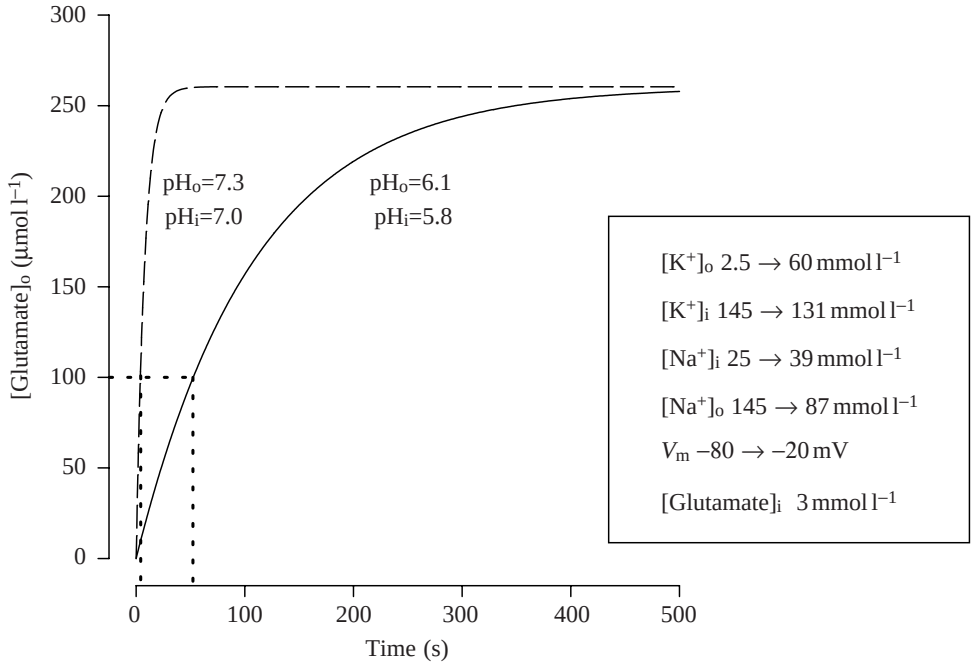

Fig. 5. Predicted rise in extracellular glutamate concentration after the rise in K^+ concentration which occurs during ischaemia (see box for assumed values of ion concentrations before and after ischaemia). The calculation is presented from equation 5 for normal values of intra- (pH_i) and extracellular (pH_o) pH (dashed curve) and for the values likely to occur during ischaemia (smooth curve). Vertical dotted lines show the time needed for the glutamate concentration to reach 100 μmol l^{-1}.

in the nervous system. [The inhibition of NMDA receptor activation by an acid pH (Traynelis and Cull-Candy, 1990) may also reduce the damage occurring in transient ischaemia.] If ischaemia is prolonged, however, the glutamate concentration will eventually reach values which trigger the death of neurones (Fig. 5).

Conclusions

The Na^+-dependent glutamate transporters play a key role in controlling the level of extracellular glutamate in the brain. An inevitable consequence of the transporters being powered by the transmembrane transport of Na^+ and K^+ is that in conditions such as ischaemia these transporters will reverse and pump glutamate into the extracellular space, leading to neuronal death.

There are a number of unanswered questions about glutamate homeostasis in the brain, some of which we list here as a stimulus to further work. First, are there more Na^+-dependent glutamate transporters which have not been cloned yet? Second, is the ionic stoichiometry of all the Na^+-dependent transporters the same as that of the salamander Müller cell transporter? Third, do Na^+-independent glutamate transporters, such as the cystine/glutamate exchanger, play a significant role in controlling the level of extracellular glutamate? Fourth, what is the role of these transporters in controlling the extracellular glutamate concentration during the development of the nervous system, before synapses have formed?

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