Currents evoked in Bergmann glial cells by parallel fibre stimulation in rat cerebellar slices

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1. Whole-cell recordings were obtained from Bergmann glial cells in rat cerebellar slices.

2. The cells had low input resistances (70 ± 38 MΩ; $n = 13$) and a mean resting potential of −82 ± 6 mV ($n = 12$) with a potassium-based internal solution. Electrical and dye coupling between Bergmann glia were confirmed.

3. Stimulation of parallel fibres induced a complex, mostly inward current which could be decomposed pharmacologically.

4. The ionotropic glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM), but not DL-2-amino-5-phosphonopentanoic acid (DL-APV; 100 μM) consistently blocked an early inward current component that may reflect synaptic activation of AMPA/kainate receptors in Bergmann glia.

5. Addition of cadmium ions (100 μM) to inhibit transmitter release blocked most of the CNQX–APV-insensitive current. This component probably reflects electrogenic uptake of the synaptically released glutamate.

6. Tetrodotoxin (TTX; 1 μM) blocked the remaining inward current: a slow component, possibly produced by the potassium ion efflux during action potential propagation in parallel fibres. An initial triphasic component of the response was also TTX sensitive and reflected passage of the parallel fibre action potential volley.

7. The putative glutamate uptake current was further characterized; it was blocked by the competitive uptake blockers D-aspartate (0·5 mM) and L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC; 0·5 mM), and by replacement of sodium with lithium. Monitoring the triphasic TTX-sensitive component showed that this inhibition did not result from changes of action potential excitation and propagation.

8. Intracellular nitrate ions increased the putative uptake current, consistent with the effect of this anion on glutamate transporters.

9. The putative uptake current was reduced by depolarization, consistent with the voltage dependence of glutamate uptake.

10. It is concluded that a large fraction of the current induced by parallel fibre stimulation reflects the uptake of synaptically released glutamate. The uptake current activated rapidly, with a 20–80% rise time of 2·3 ± 0·7 ms ($n = 10$), and decayed with a principal time constant of 25 ± 6 ms ($n = 10$).

Glutamate is the dominant excitatory neurotransmitter in the CNS. There are no extracellular enzymes which inactivate glutamate, and it is removed from the extracellular space by uptake via transporters expressed in both neurones and glia. Maintenance of a low extracellular glutamate concentration by uptake is not only necessary for synaptic function, it is also vital in preventing the neurotoxicity resulting from extended exposures to glutamate (Choi, 1990; Nicholls & Attwell, 1990). Several different forms of glutamate transporters have been cloned (Pines *et al.* 1992; Kanai & Hediger, 1992; Storck, Schulte, Hofmann & Stoffel, 1992). The original clones were named EAAC1, GLAST and GLT1. EAAC1 is found in many epithelial tissues throughout the body, and in neurones (Kanai & Hediger, 1992). GLAST and GLT-1 are found in the brain (GLT-1 exclusively so), in glial cells (Pines *et al.* 1992; Storck *et al.* 1992). A unifying nomenclature has been proposed, following cloning of the human forms of these transporters: EAAT1 = GLAST, EAAT2 = GLT-1, EAAT3 = EAAC1 and EAAT4 is a form found principally in the cerebellum (Arriza, Fairman,

Wadiche, Murdoch, Kavanaugh & Amara, 1994; Fairman, Vandenberg, Arriza, Kavanaugh & Amara, 1995; Kanai, Nussberger, Romero, Boron, Hebert & Hediger, 1995).

Glutamate transporters operate with a complex stoichiometry, harnessing several ionic gradients to drive the translocation of glutamate into cells. The uptake is electrogenic, which has permitted its study by electrophysiological methods (Brew & Attwell, 1987; Barbour, Brew & Attwell, 1991; Wyllie, Mathie, Symonds & Cull-Candy, 1991). It is currently thought that each negatively charged glutamate ion is transported into a cell with two (Stallcup, Bulloch & Baetge, 1979; Erecinska, Wantorsky & Wilson, 1983; Kanai *et al.* 1995) or three (Zerangue & Kavanaugh, 1996) sodium ions while a potassium ion is countertransported out of the cell (Kanner & Sharon, 1978; Barbour, Brew & Attwell, 1988; Amato, Barbour, Szatkowski & Attwell, 1992). During each carrier cycle a pH-changing ion is also transported – the equivalent of a proton entering the cell with glutamate (Nelson, Dean, Aronson & Rudnick, 1983; Erecinska *et al.* 1983; Bouvier, Szatkowski, Amato & Attwell, 1992). This was initially thought to be a countertransported hydroxyl ion (Bouvier *et al.* 1992), but the data supporting this proposal have now been reinterpreted in terms of an anion conductance intimately associated with the uptake process (Picaud, Larsson, Grant, Lecar & Werblin, 1995; Fairman *et al.* 1995; Wadiche, Amara & Kavanaugh, 1995; Eliasof & Jahr, 1996; Larsson, Picaud, Werblin & Lecar, 1996; Billups, Rossi & Attwell, 1996).

The role of glutamate uptake during synaptic transmission remains unclear. In a minimalist scenario, transmitter diffuses rapidly out of the synaptic cleft following release (Eccles & Jaeger, 1958), the role of uptake being simply to maintain the necessary concentration gradient by keeping the extracellular glutamate concentration low. In such a situation, block of glutamate uptake should have no immediate effect on the synaptic current – a result reported for several types of synapse (Hestrin, Sah & Nicoll, 1990; Sarantis, Ballerini, Miller, Silver, Edwards & Attwell, 1993; Isaacson & Nicoll, 1993). In other preparations, however, block of uptake prolongs compound synaptic currents (Barbour, Keller, Llano & Marty, 1994; Takahashi, Kovalchuk & Attwell, 1995; Mennerick & Zorumski, 1995), indicating that uptake is important for terminating the actions of glutamate when many presynaptic fibres are simultaneously active. In some of these studies the effects of uptake block on small compound or miniature synaptic currents were also examined (Tong & Jahr, 1994; Mennerick & Zorumski, 1995). These were not slowed, suggesting that uptake may only prevent some of the synaptic activation arising from 'spillover' and 'cross-talk', a synergistic action of glutamate released from multiple synapses during compound currents. A novel proposal for the synaptic role of uptake arose from the observation that uptake block could increase the amplitude of miniature synaptic currents (Tong & Jahr, 1994). It was suggested that only binding to transporter glutamate sites, and not intracellular sequestration, is sufficiently rapid to reduce the synaptic glutamate concentration during the synaptic current. This plausible idea is supported by the only reported measure of the turnover rate of a glutamate transporter (Wadiche, Arriza, Amara & Kavanaugh, 1995). However, it is difficult to relate the measure of steady-state turnover to the phasic nature of synaptic transmission. In the only direct measurements of uptake of synaptically released glutamate (Mennerick & Zorumski, 1994; Mennerick, Benz & Zorumski, 1996) the activation kinetics of uptake were not examined in detail.

D-[^{3}H]Aspartate, an analogue of glutamate that is also transported, is found concentrated in Bergmann glia following application to cerebellar slices (Garthwaite & Garthwaite, 1985), indicating that these cells express glutamate uptake transporters. *In situ* hybridization shows that GLAST and to a lesser extent GLT-1 are expressed in Bergmann glia (Rothstein *et al.* 1994; Chaudhry, Lehre, van Lookeren Campagne, Ottersen, Danbolt & Storm-Mathisen, 1995). At least two types of glial cell are present in the cerebellar molecular layer (Palay & Chan-Palay, 1974): the somata of Fañanas cells are found within this layer, while Bergmann glia extend radial processes through the molecular layer, ensheathing Purkinje cell dendrites and the excitatory synapses formed on them by parallel and climbing fibres. Both of these synaptic inputs are among the compound inputs affected by block of uptake (Barbour *et al.* 1994; Takahashi *et al.* 1995). It seemed probable therefore that glutamate uptake into Bergmann glia contributes to terminating the compound synaptic currents in the molecular layer. We investigated this possibility by recording from Bergmann glia during stimulation of parallel fibres. In this paper we present a characterization of the membrane currents observed in Bergmann glia following such synaptic activation. A current whose properties were consistent with electrogenic uptake of synaptically released glutamate was detected. Additional current components were observed, which could be attributed to: the extracellular field potential, the AMPA/kainate receptors expressed by Bergmann glia (Burnashev *et al.* 1992; Müller, Möller, Berger, Schnitzer & Kettenmann, 1992), and the potassium efflux following the presynaptic action potential volley.

METHODS

Coronal cerebellar slices (300 μm) were obtained from 15- to 22-day-old male Wistar rats as previously described (Llano, Marty, Armstrong & Konnerth, 1991; Barbour *et al.* 1994). Animals were killed by decapitation. Slices were maintained at 35 °C in a continuously bubbled (95% O_2, 5% CO_2) solution containing (mM): 125 NaCl, 2·5 KCl, 1·25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$ and 25 glucose. Slices in the recording chamber were superfused with the same solution at room temperature (20–26 °C) and drugs were added as described. In some experiments, a low-sodium solution was applied, the NaCl being replaced by LiCl; the solution still contained the 26 mM Na^+ from the bicarbonate salt.

Whole-cell patch-clamp recordings from Bergmann glial cells were made under visual control (using an Axioskop microscope, Carl Zeiss, Oberkocken, Gemany) with an Axopatch 200A patch-clamp amplifier (Axon Instruments). Several intracellular solutions were used. They were of the following compositions (mM). Caesium–TEA solution: 80 CsCl, 45 TEACl, 4 $MgCl_2$, 3·5 $CaCl_2$, 9 Hepes, 3·5 Na_2ATP, 0·4 Na_3GTP, 9 EGTA and 29 CsOH, pH 7·3. Potassium–Mops solution: 155 Mops, 1·5 $MgCl_2$, 3·5 $CaCl_2$, 3·5 MgATP, 0·4 Na_3GTP, 9 EGTA, 110 KOH and 9 NaOH, pH 7·3. Nitrate solution: 105 KNO_3, 17 TEACl, 4·5 $MgCl_2$, 3·5 $CaCl_2$, 9 Hepes, 3·5 Na_2ATP, 9 EGTA and 29 KOH, pH 7·3. The control solution for the experiments in which the intracellular anion was changed was the same as the nitrate solution except that KCl replaced KNO_3. For the experiments testing the effects of intracellular D-aspartate, the following solution pair was used (mM): 9 or 0 D-aspartate, 70 or 79 CsCl, 17 TEACl, 4·5 $MgCl_2$, 3·5 $CaCl_2$, 3·5 Na_2ATP, 9 Hepes, 26 NaCl, 9 EGTA and 29 CsOH, pH 7·3. The osmolarities of the pipette solutions were in the range 275–290 mosmol l^{-1}. Correction was made for the junction potential error of the potassium–Mops solution (−11 mV). Junction potentials for the other solutions were < 5 mV and were ignored. Differences of junction potential between the control and test solutions for the experiments changing internal anions were negligible (< 2 mV). For determination of the current–voltage relation, a KCl-based solution was used (mM): 130 KCl, 10 Hepes, 2 Na_2ATP, 2 MgATP, 10 sodium phosphocreatine, 1 EGTA, pH 7·3 (KOH). Parallel fibres were stimulated (< 50 V, 100–250 μs, ± biphasic pulses) using a large patch electrode filled with Hepes-buffered saline. The passive electrical properties of the cells, including the series resistance, were routinely monitored throughout the experiments by the application of 10 mV hyperpolarizing steps and the series resistance compensation parameters readjusted as necessary (Llano *et al.* 1991). Extracellular recordings were made using saline-filled patch pipettes and an Axoclamp-2B in bridge mode.

Glutamate analogues and antagonists were purchased from Tocris Cookson (Bristol, UK). Gabazine (SR 95531) was obtained from Research Biochemicals International (RBI). Most other compounds were purchased from Sigma.

For some experiments, 13 mM Neurobiotin (Vectastain, Biosys, Compeigne, France) was included in the patch electrode. Post-fixation (3% paraformaldehyde, 0·15 M phosphate-buffered saline (PBS)), endogenous peroxidases were blocked (1% H_2O_2, 10% methanol, PBS), and slices were permeabilized with Triton X-100 and treated with the ABC kit (Elite, Vectastain). Cells were visualized using diaminobenzidine (DAB) as the chromophore and slices were mounted in Mowiol (Hoescht, Frankfurt, Germany) for inspection.

Standard procedures

All traces shown in the figures, except those of Fig. 1, are averages of 4–30 sweeps. Baseline holding currents have been subtracted to facilitate comparison of current amplitudes and time courses. Stimulus artefacts were usually blanked and the time of stimulation marked in the figures by a filled triangle. Statistics are given as the means ± the standard deviation (n = number of observations). Tests of significance were made using Student's two-tailed t tests (paired when appropriate). Unless otherwise stated, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was applied at a concentration of 10 μM, DL-2-amino-5-phosphonpentanoic acid (DL-APV) at 100 μM (or alternatively 7-chlorokynurenic acid, 7-Cl-Kyn, at 50 μM), gabazine at 20 μM, $CdCl_2$ at 100 μM, and tetrodotoxin (TTX) at 1 μM.

RESULTS

Identification and electrical properties of Bergmann glial cells in slices

Experiments were performed using 300 μm coronal cerebellar slices from 15- to 22-day-old rats. This slice orientation preserves parallel fibres which run in the plane of the slice, perpendicular to the surviving Purkinje neurones (i.e. whose dendrites were not cut during slicing) and their associated Bergmann glia. Bergmann glial cells were identified by the location of their soma between the Purkinje cell and molecular layers and, once recorded, by their low input resistance, apparently linear membrane currents and the absence of spontaneous synaptic currents. When recorded with a potassium-based (potassium–Mops) internal solution, the Bergmann glia had resting membrane potentials of −82 ± 6 mV (n = 12; cf. a nominal potassium equilibrium potential (E_K) of −96 mV) and an input resistance of 70 ± 38 MΩ (n = 13). The input resistance was measured without series resistance compensation.

Some cells were also filled with Neurobiotin, which permitted the morphological confirmation of the cells' identification as Bergmann glia (Fig. 1*A*). When filled with Neurobiotin they showed a classical morphology, with two or more bushy processes extending to the pial surface where they formed end-foot specializations. This technique also revealed extensive coupling between Bergmann glia, an example of which is shown in Fig. 1*A* (in which a parasagittal section was used). For a single glial cell patched, an average of eight cells were filled with Neurobiotin over a recording period of 15 min (n = 20) in parasagittal slices, while in coronal slices an average of ten filled cells were counted (n = 3). Each glial cell extended characteristic glial fibres towards the pial surface, where they formed end-foot specializations.

The electrical correlate of the above dye coupling was observed when paired recordings of neighbouring Bergmann glia were made. Figure 1*B* shows voltage steps in one cell of such a pair resulting in currents in the neighbour. The apparent coupling conductance between these two cells was low: 3 nS (equivalent to a resistance of about 330 MΩ). Such electrical coupling was observed in 9/14 pairs of closely neighbouring (somatic centres < 15 μm apart) glial cells recorded. The apparent coupling conductances between pairs ranged from 3 nS to 100 pS (mean, 260 ± 300 pS; n = 9). These values probably underestimate the strength of coupling, since a higher true conductance would be masked by (i) poor uniformity of the voltage clamp and (ii) run-down of the coupling conductance, which was often short-lived in whole-cell recording.

The presence of the long glial processes, the electrical coupling between cells and their low input resistance indicate that uniformity of the voltage clamp is likely to be poor in these cells. For the majority of the experiments which follow, an internal solution containing TEA^+ and Cs^+ ions was used, in an attempt to improve the voltage

uniformity by reducing the potassium conductance. Somewhat counter-intuitively, the input resistance measured with this solution was 53 ± 21 MΩ ($n = 8$), compared with 70 MΩ with the potassium–Mops solution. This decreased input resistance may be accounted for by the fact that the caesium–TEA pipette solution has a lower resistivity than the potassium–Mops solution, and that series resistance compensation was used in the present experiments but not in those above. The zero-current potential was altered with the caesium–TEA solution, compared with the potassium–Mops solution, since the holding current was usually inward at −70 mV. It has been reported that the potassium conductance of Bergmann glia is insensitive to TEA^+ and Cs^+ (Müller, Fritschy, Grosche, Pratt, Möhler & Kettenmann, 1994). Intracellular caesium ions can replace potassium ions in activating glutamate uptake (Barbour *et al.* 1991).

What follows should be interpreted in the knowledge of possible voltage-clamp artefacts. An unknown fraction of charge entering the cell is lost across the membrane conductance rather than appearing as pipette current. Typically, upon entry into the whole-cell configuration, the access resistance was 7–12 MΩ (mean, 9 ± 2 MΩ; $n = 8$), 60–90% of which was compensated; the access resistance could double over the course of an experiment. The apparent absence of non-linear currents in the current–voltage relation in the negative potential range (Fig. 1*B*) suggests that the main effects of the poor voltage clamp will be loss of charge and filtering of currents rather than the activation of additional voltage-dependent currents.

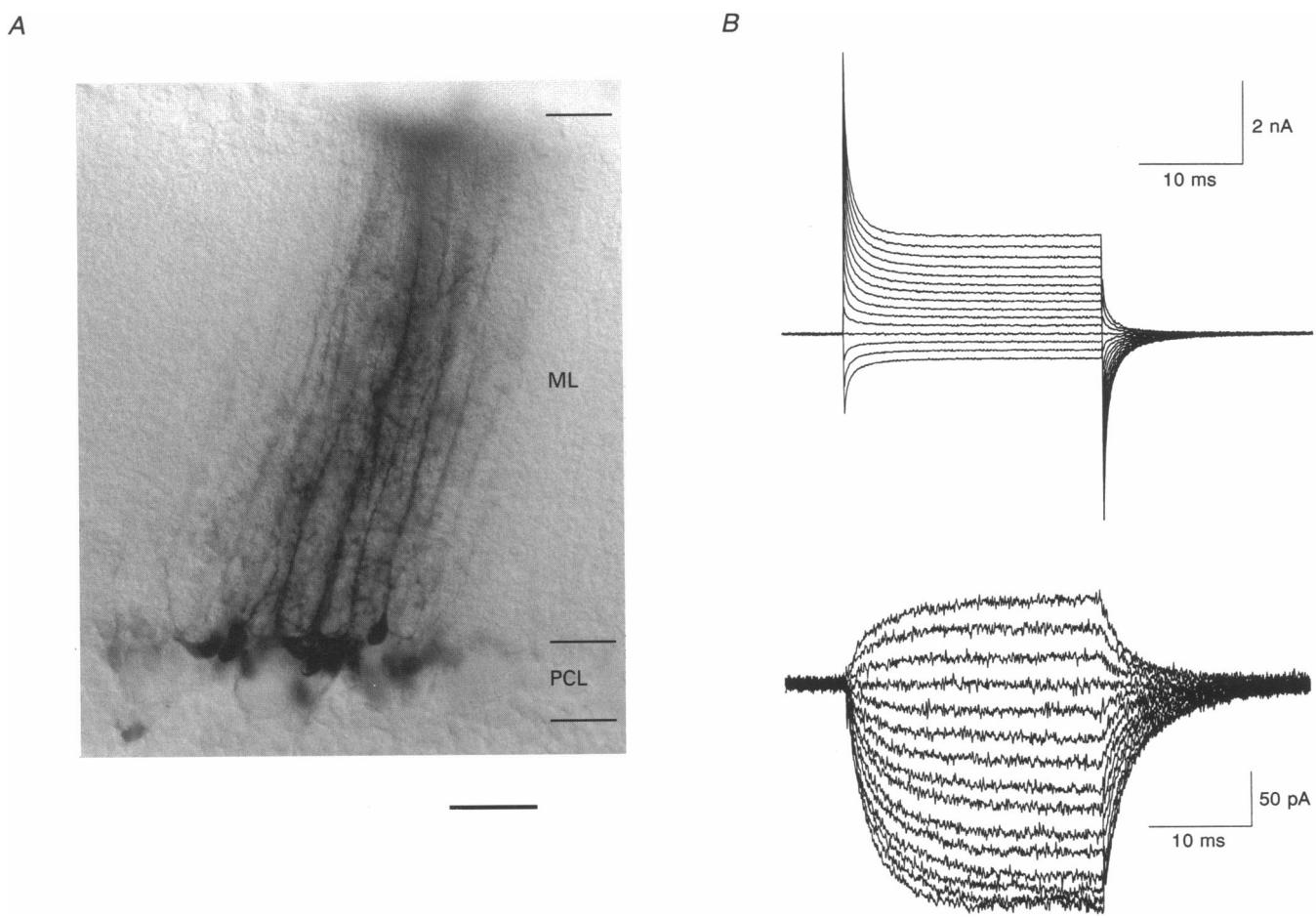

Figure 1. Bergmann glia are dye coupled and electrically coupled

A, parasagittal view of a group of Bergmann glial cells filled with Neurobiotin as a result of the whole-cell recording of one cell at the centre of the group. Glial somata are arranged near Purkinje cell somata, which are also visible under Nomarski optics. The radial glial fibres project to the surface of the folium where they form end-foot specializations (out of the focal plane). PCL, Purkinje cell layer; ML, molecular layer. Scale bar, 25 μm. *B*, simultaneous whole-cell voltage-clamp recordings from two electrically coupled Bergmann glia. The top panel shows the currents resulting from a series of voltage jumps in one of the cells ($V_h = -111$ mV to +29 mV in 10 mV steps, from an initial V_h of −81 mV), while the bottom panel shows the resulting currents recorded in a nearby coupled cell (also at a V_h of −81 mV). The recordings were made using a potassium–Mops pipette solution (see Methods). The apparent coupling conductance was 3 nS.

Initial description of currents evoked in Bergmann glia by parallel fibre stimulation

Stimulation of parallel fibres induced complex membrane currents in Bergmann glia whole-cell clamped at −70 mV (Fig. 2*A*). Shortly after the stimulus artefact (or often partly obscured by it) a rapid triphasic current with a central outward peak could be observed (it is rather compressed in time and partly obscured in Fig. 2*A*). This sequence lasted about 1–2 ms. We show below that the triphasic current reflects the passage of the presynaptic action potential volley, recorded as if the glial cell were an extracellular electrode. This brief component was followed by an inward current that developed over a few milliseconds (rising to 80% of peak 3·8 ± 0·4 ms after the outward peak of the early triphasic current; $n = 7$) and decayed more slowly, often requiring several hundreds of milliseconds to return to baseline (the mean time from current peak to 37% of the peak amplitude was 52 ± 32 ms). Its mean peak amplitude, measured in ten cells shortly after beginning whole-cell recording, was 82 ± 36 pA. The measurements were made near the beginning of recording because the current often ran down with time; the rate of decline was most marked near the beginning of the experiment. The recordings were all obtained in the presence of a $GABA_A$ antagonist, 20 μM gabazine (SR 95531). $GABA_A$ receptors therefore do not contribute to the observed currents. Figure 2*A* also shows a pharmacological decomposition of the inward current.

The application of glutamate receptor antagonists, 10 μM CNQX and 50 μM DL-APV (or 50 μM 7-Cl-Kyn), had complex and variable effects (investigated in more detail below). The most obvious in Fig. 2 is a reduction of the amplitude of the inward current, distinguishable from run-down of the response (which is only slight in this case) by the presence of a clear discontinuity of the slope of the amplitude plot (Fig. 2*B*) following application of CNQX–APV (observed in 4/10 cells). The average reduction of amplitude (without selecting cells with such discontinuities) was 35 ± 18% ($n = 10$). This value overestimates the effect of the antagonists because it includes run-down. Synaptic activation of ionotropic glutamate receptors, though not necessarily in the Bergmann glia, can thus induce currents in these glial cells. The mean amplitude of the inward

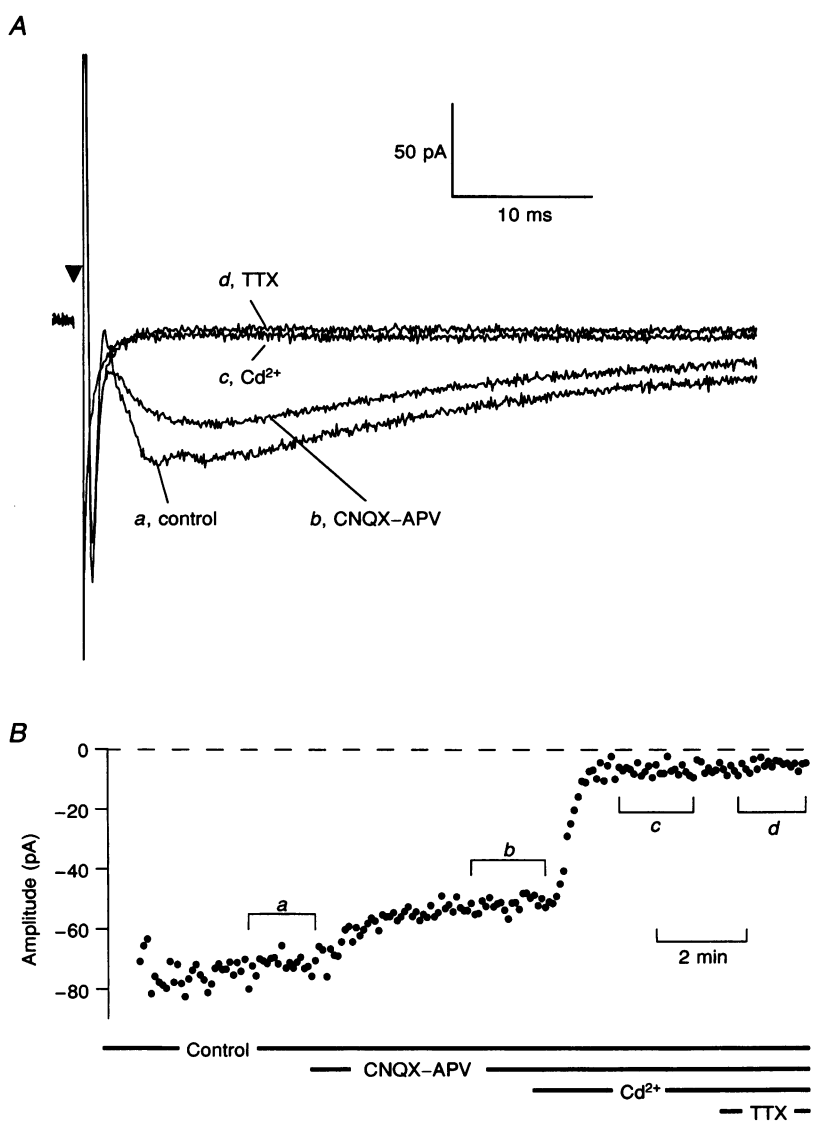

Figure 2. Pharmacological separation of the currents induced in a Bergmann glial cell by parallel fibre stimulation

A, average current responses to parallel fibre stimulation in the presence of 20 μM gabazine (*a*, control), 10 μM CNQX and 50 μM DL-APV (*b*), 100 μM Cd^{2+} (*c*) and 1 μM TTX (*d*). The letters identify the sweeps in the amplitude–time plot of *B* that were averaged. Drug applications were cumulative, i.e. trace *d* was recorded in the presence of all the above drugs. The stimulus artefact has been blanked. The time of stimulation is marked by the filled triangle. *B*, peak inward current amplitude (ignoring stimulus artefact and the current associated with the presynaptic action potential volley; see Fig. 3 and text) plotted against time. Bracketed points indicate those sweeps used to obtain the average traces shown in *A*. The times of drug applications are shown by the continuous bars below the plot. The control solution contained 20 μM gabazine.

current remaining in the presence of the glutamatergic antagonists was 53 ± 18 pA ($n = 10$).

A large part of the remaining inward current was blocked by the addition of 100 μM cadmium ions to the bathing solution. Cadmium ions block voltage-dependent calcium channels and thus the liberation of transmitter (glutamate). This was confirmed by measuring the effect of the same concentration of cadmium ions on the parallel fibre synaptic current recorded in Purkinje cells: its amplitude was reduced to 0·8 ± 0·4% ($n = 3$) of the control value. The cadmium-sensitive fraction of the inward current in the Bergmann glia thus presumably depends upon the liberation of transmitter but is not mediated by ionotropic glutamate receptors. We postulate that it reflects the electrogenic uptake of transmitter glutamate into the Bergmann glia, a hypothesis that is investigated further below.

A small, slowly decaying inward current (mean amplitude; 11 ± 8 pA; $n = 10$) remained in the presence of cadmium ions. It was blocked (as was the initial rapid triphasic component) by TTX (1 μM), indicating that it was associated with the presynaptic action potentials. We suggest that this component may reflect the effect on the glial cell of the potassium ion efflux occurring during the presynaptic action potential.

Contributions of the extracellular field

The form of the early triphasic current that immediately follows stimulation of the parallel fibres resembled an extracellular recording of a propagating action potential. To examine whether the extracellular field potential could be responsible for this (or any other) component of the currents observed in whole-cell recording of the Bergmann glia we made extracellular recordings of the field potential in the molecular layer during parallel fibre stimulation. Such a recording is shown in Fig. 3*A*, where the triphasic potential with a central negative peak caused by the passage of the presynaptic action potentials is evident. The timing and form of the intracellular triphasic current (Fig. 3*B*; data from a different experiment) are almost identical to those of the extracellular potential. The polarity of the intracellular current is consistent with its proposed link with the extracellular field. For instance, the negative peak of the extracellular field will draw current out of nearby cells (through both the membrane capacitance and resistance), generating an outward current, such as is observed in Bergmann glia. The triphasic intracellular current and extracellular field showed similar pharmacologies, both being insensitive to CNQX(–APV) and Cd^{2+} but abolished by TTX (Fig. 3*A* and *B*). We conclude that the triphasic intracellular current reflects current flow driven by the extracellular field potential changes during propagation of the presynaptic action potential volley. As such, the triphasic current served as a useful monitor of the excitation and propagation of the presynaptic action potentials.

Interestingly, if the field potential is viewed at a higher amplification and slower time base, a slower negative potential can also be observed (Fig. 3*C*). It is probably generated by the excitatory synaptic currents and subsequent action potentials activated by glutamate in Purkinje cells and interneurones, since this field component was blocked by CNQX (alone). Close examination of the intracellular records showed a possible correlate of this component of the field. In some records CNQX–APV caused an early inward current shift just after the early triphasic current (Fig. 3*D*; observed in 2/10 cells). This current shift appears much briefer than the slow component of the extracellular field. However, if a similarly slow current did flow in the glial cell, all except the initial portion would be obscured by the other current shifts induced by CNQX (see below).

Effects of glutamate receptor antagonists on the currents recorded in Bergmann glia

As shown above (Figs 2 and 3 and text), application of the CNQX–APV antagonist combination could alter both the amplitude and time course of the currents recorded in Bergmann glia. The most consistent effect of these drugs was to slow the rise of the response. The rise time of the inward current (from the central peak of the action potential waveform to the 80% point) was slowed on average by 0·5 ± 0·6 ms ($n = 10$; $P < 0·05$) to 3·5 ± 0·5 ms upon application of CNQX–APV.

Bergmann glial cells express AMPA/kainate channels (Müller *et al.* 1992; Burnashev *et al.* 1992) and it is of interest to know whether they are activated by synaptic stimulation. The slowing of the rising phase of the response to parallel fibre stimulation, i.e. block of a rapid inward current, is the effect of the glutamate receptor antagonists we consider most likely to reflect activation of these receptors (see Discussion). Although we do not prove that the changes induced by CNQX–APV result from activation of receptors in the Bergmann glia themselves, rather than indirectly following activation of neuronal receptors, separate applications of CNQX and DL-APV showed that the slowed rise was an effect of CNQX and not DL-APV.

Such an experiment is shown in Fig. 4. Average traces are shown under control conditions (in the presence of gabazine) and following the addition of DL-APV and then CNQX to the bath solution (Fig. 4*A*). The large reduction of amplitude between the control and DL-APV traces is likely to be due, at least in part, to run-down of the response, as can be seen from the gradual change of amplitude in Fig. 4*C*. The smaller amplitude decrease following CNQX application may be a genuine effect of the antagonist, since the change is rapid and well correlated with the application. When the average responses are normalized to their peak amplitudes (Fig. 4*B*), the different effects of DL-APV and CNQX on the current kinetics become apparent: CNQX slows the rising phase, while DL-APV has no effect. That this effect is correlated with the application of CNQX is confirmed by the plot of rise times in Fig. 4*D*. In twelve cells, DL-APV did not significantly affect the mean stimulus–80% rise time,

slowing it by only 0·06 ± 0·13 ms ($P = 0·14$). The subsequent addition of CNQX slowed the rise time by 0·53 ± 0·32 ms ($P < 0·0002$).

The mean amplitude of the current in the presence of DL-APV was 68 ± 36 pA, compared with 91 ± 51 pA under control conditions ($n = 13$). The mean amplitude following the subsequent addition of CNQX was 51 ± 25 pA. The run-down of the response makes it impossible to attribute these changes to the antagonists. Note that DL-APV was always applied before CNQX in this series of experiments. No significant effect of the antagonists was observed on the peak–37% decay times: control, 53 ± 39 ms; in DL-APV, 65 ± 65 ms; and in DL-APV and CNQX, 43 ± 17 ms. Comparisons of these means using Student's paired t tests gave $P > 0·15$ for all three possible combinations.

Effects of glutamate uptake blockers on the CNQX–APV-insensitive current

The inward current insensitive to glutamate receptor antagonists was largely blocked by the application of 100 μM $CdCl_2$. There are two principal hypotheses concerning the generation of this current component. One is that it represents electrogenic uptake of the synaptically released glutamate, the other that it is the result of activation of metabotropic glutamate receptors.

The first piece of experimental evidence supporting the involvement of uptake is the action on the evoked current of the glutamate uptake blockers D-aspartate (D-Asp) and L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC), both bath applied at a concentration of 0·5 mM in the continuous presence of CNQX and DL-APV. These compounds induced

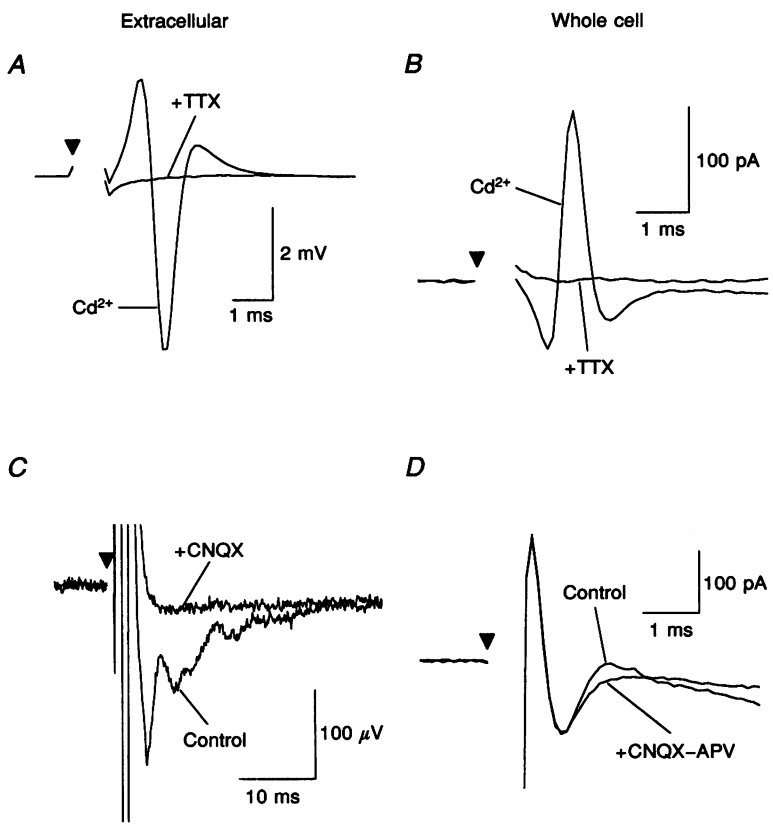

Figure 3. Extracellular field potential produced in the molecular layer by parallel fibre stimulation, and possible intracellular correlates

A, extracellular voltage recording in the molecular layer showing the characteristic triphasic waveform, with a peak negativity (negativity is plotted downwards) of the propagating action potential volley in the parallel fibres. The recording was obtained in the presence of gabazine, CNQX and Cd^{2+} (labelled Cd^{2+}). The triphasic potential was abolished by the addition of TTX (labelled +TTX). *B*, whole-cell recording from a Bergmann glial cell (from a different experiment) showing a very similar triphasic waveform in the presence of gabazine, CNQX, DL-APV, and Cd^{2+}. It was abolished by TTX. *C*, extracellular field potential in the presence of gabazine only (Control), at a higher amplification and slower time base (same trace as in *A*). A slowly decaying negativity can be observed. It is blocked by CNQX (labelled +CNQX). *D*, whole-cell record from a Bergmann glial cell from a different experiment in which the addition of CNQX (together with DL-APV) to the control solution containing gabazine (Control) blocked an early outward current that may be the correlate of the slow negativity described in *C*. In each panel, the time of parallel fibre stimulation is marked by the filled triangle.

an inward current in the Bergmann glia: 88 ± 27 pA ($n = 4$) for D-Asp and 122 ± 43 pA ($n = 8$) for PDC, consistent with the known electrogenic uptake of these drugs themselves (Barbour *et al.* 1991; Sarantis *et al.* 1993). Both D-Asp and PDC also inhibited the putative uptake current induced by parallel fibre stimulation. A typical experiment for D-Asp is shown in Fig. 5. On average, D-Asp inhibited the inward current in the presence of glutamate receptor antagonists by 78 ± 9% ($n = 4$); the mean Cd^{2+}-insensitive current in these experiments was 14 ± 7% of the same control amplitude. Similar values were obtained for PDC: a reduction of 66 ± 7% ($n = 8$) and a mean Cd^{2+}-insensitive component of 13 ± 6%. These figures probably represent an underestimate of the effect of these drugs, since, in some experiments, they were removed before a steady level of inhibition was attained. During these experiments the early triphasic current recorded in the Bergmann glia was monitored, to check whether changes in response amplitude were likely to result from variations of presynaptic excitability or conduction. Although for the cell in Fig. 5 this component remained unaffected by the application of the uptake blockers, on average ($n = 4$ cells with D-Asp) a small increase of its amplitude, 18 ± 32% ($n = 4$), was observed. This suggests that presynaptic changes of excitability do not account for the strong inhibition of the putative uptake current. The slightly increased amplitude of the early triphasic current may have been caused by the concomitant change of input resistance, a decrease of 10 ± 6%, that was also induced by the uptake blockers.

Replacement of external sodium by lithium also inhibited the putative uptake current. A mean reduction of 78 ± 10% ($n = 12$) was observed, while the early triphasic current representing the presynaptic action potential volley was reduced by only 15 ± 6%. This result is consistent with a sodium-dependent electrogenic uptake mechanism. Sodium channels, in contrast, have similar permeabilities for lithium and sodium, explaining why the presynaptic compound action potential was little affected by the ionic substitution. The input resistance was unaffected by the lithium

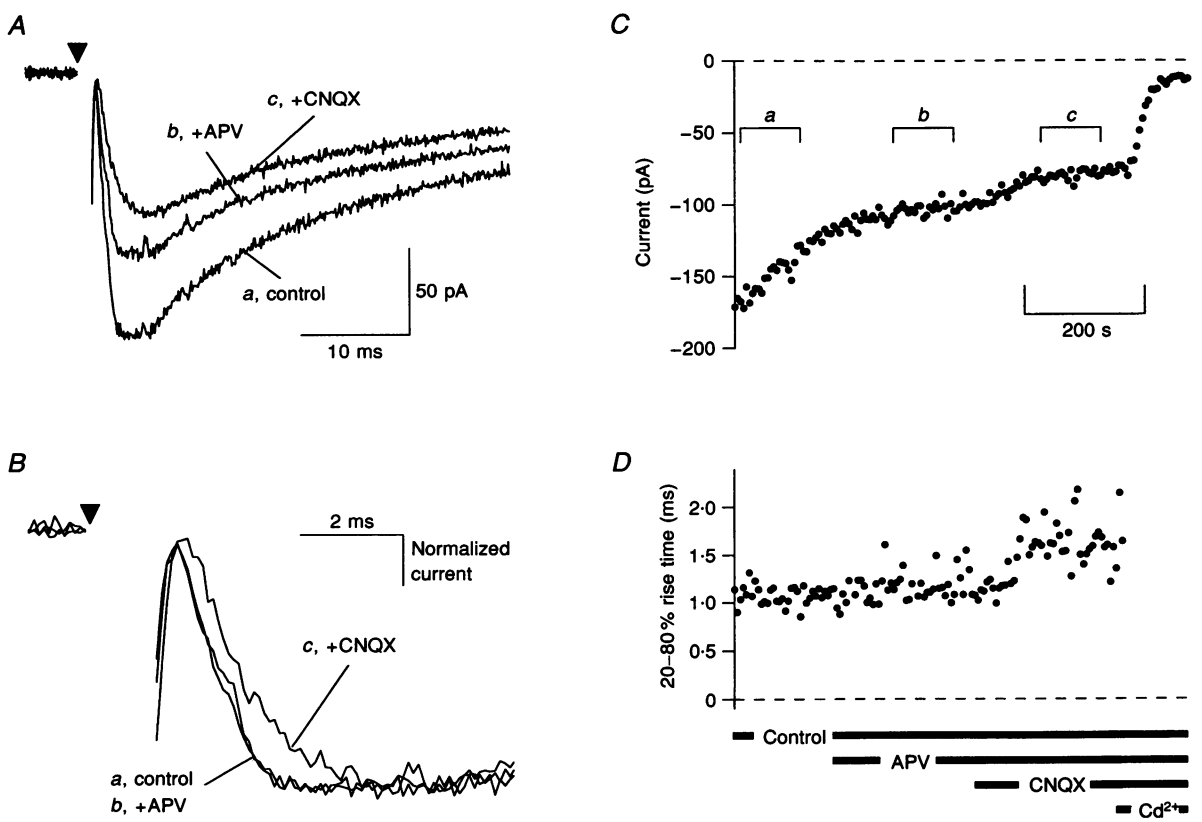

Figure 4. Effects of DL-APV and CNQX on the rising phase of the inward current following parallel fibre stimulation

A, average responses to parallel fibre stimulation in the presence of gabazine (*a*, control), after the addition of DL-APV (*b*) to the control solution and after the subsequent addition of CNQX (*c*). *B*, the same responses normalized to their peak amplitudes. *C*, the amplitudes of the individual responses throughout the experiment. An initial run-down is apparent. The bracketed points indicate the responses averaged to obtain the traces of *A* and *B*. *D*, a similar plot of the 20–80% response rise times from the same recording. The rise time increases from 1·1 to 1·5 ms upon application of CNQX. The timing of parallel fibre stimulation is marked by the filled triangles in *A* and *B*.

substitution (101 ± 7% of control), but an inward baseline current shift of 110 ± 76 pA was recorded.

Effects of changing the internal solution on the CNQX–APV-insensitive current

Manipulations of the bath solution such as described above often do not have unequivocal interpretations; many alternative explanations involving complex intercellular interactions can be imagined for the observed effects. A better-targeted approach to investigating the putative uptake current would be to try to modulate it via the intracellular solution.

Certain anions, such as nitrate, thiocyanate and perchlorate, increase the current generated by electrogenic uptake, because they are highly permeant through an anion conductance intimately associated with the glutamate uptake carrier (see Introduction). We tested the effect of replacing intracellular chloride with nitrate. A comparison was made of the mean amplitudes of the putative uptake current in two groups of cells: one recorded with a chloride-based control internal solution and the other with a nitrate-based solution (see Methods). In order to reduce intercellular variation, the experiments were carried out as follows. Cells were recorded successively, in pairs. In the first cell of the pair the amplitude of the CNQX–APV-insensitive current was measured. The second cell was always a close neighbour, and its responses to stimulation with identical, i.e. unchanged, parameters (position, intensity, duration) were recorded. The amplitude of the CNQX–APV-insensitive current and that of the residual component insensitive to 100 μM Cd^{2+} were measured. This allowed us to check whether changing the intracellular anion altered the amplitude of the Cd^{2+}-insensitive component; this is necessary because the CNQX–APV-insensitive component is the sum of both the putative uptake current and the Cd^{2+}-insensitive component. The first anion for the pairs of recorded cells was strictly alternated, to prevent any possible bias arising from the order of recording.

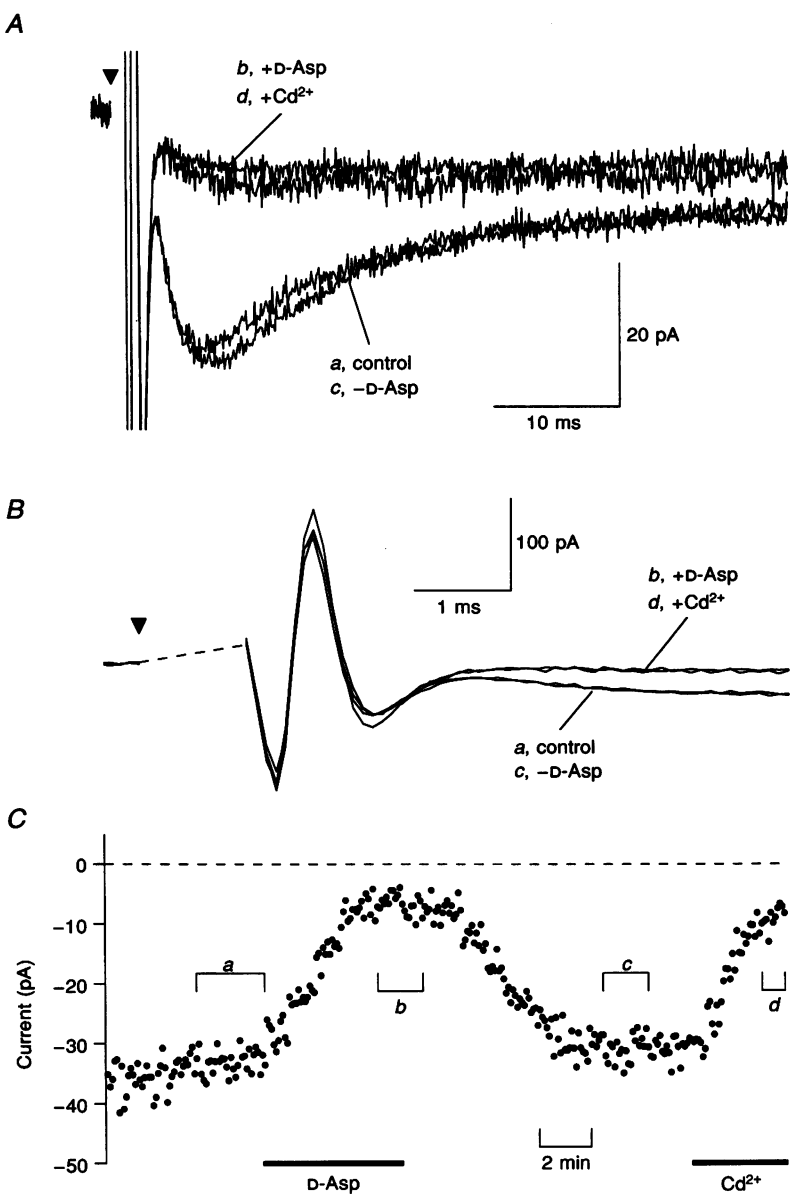

Figure 5. The glutamate uptake blocker D-aspartate inhibits the putative uptake current

A, average responses to parallel fibre stimulation obtained before (*a*, control), during (*b*), and after (*c*) application of 0·5 mM D-aspartate to the slice. Cd^{2+} was then added to the bathing solution (*d*). Gabazine, CNQX and DL-APV were present throughout. D-Aspartate induced a reversible inward current shift of 60 pA which has been subtracted in the figure to aid comparison of the current amplitudes. *B*, the same average responses shown on a faster time base (and at a lower amplification). The triphasic current reflecting the presynaptic action potential volley was little affected by the application of D-aspartate. *C*, amplitude–time plot of the responses. Bracketed points indicate the sweeps averaged to obtain the traces shown in *A* and *B*. Drug applications are shown by the filled bars. The timing of parallel fibre stimulation is marked by the filled triangles in *A* and *B*.

Comparison of the input resistances and series resistances of nitrate and control (Cl^-) cells revealed no significant differences.

The results of these experiments (35 pairs) are shown in Fig. 6. It displays specimen traces and a scatter plot of the amplitude of the CNQX–APV-insensitive current with internal nitrate as a function of that with internal chloride for each pair of cells. The mean amplitude of the CNQX–APV-insensitive current with nitrate was 91 ± 54 pA, and with chloride, 60 ± 32 pA. This difference was significant ($P < 0{\cdot}0002$; paired t test). In contrast, the mean Cd^{2+}-insensitive currents were small, irrespective of the internal anion: 14 ± 16 pA ($n = 16$) for nitrate and 9 ± 8 pA ($n = 17$) for chloride. Subtraction of the mean amplitudes of these Cd^{2+}-insensitive components from those of the CNQX–APV-insensitive currents reduced but did not abolish the significance of the effect of nitrate ($P < 0{\cdot}005$).

We also attempted to show an inhibition of the putative uptake current by internal D-aspartate. D-Aspartate competes with glutamate for the transporter, and by occupying glutamate binding sites on the cytoplasmic side it should reduce the rate of glutamate uptake into the cell. Such inhibition, by internal glutamate, has been demonstrated in another preparation (Barbour *et al.* 1991). We chose to use D-aspartate because it is not metabolized. Only a small, non-significant ($P < 0{\cdot}2$) difference was found between the amplitudes of the CNQX–APV-insensitive responses with 9 mM internal D-aspartate (43 ± 24 pA; $n = 20$) and with none (51 ± 30 pA).

Effects of metabotropic receptor antagonists

The recent advent of effective antagonists of metabotropic glutamate receptors permitted a direct test of the hypothesis that the putative uptake current was generated in part or entirely by metabotropic receptor activation, rather than by electrogenic glutamate uptake. Two antagonists were tested: (+)-α-methyl-4-carboxyphenyl-glycine (MCPG), which is most potent against group I metabotropic receptors (Bashir *et al.* 1993); and (*RS*)-α-methylserine *O*-phosphate monophenyl ester (MSOPPE), which is active against group II and III receptors (Thomas, Jane, Tse & Watkins, 1996). Neither antagonist, applied at a concentration of 1 mM, induced a clear inhibition of the current evoked in the

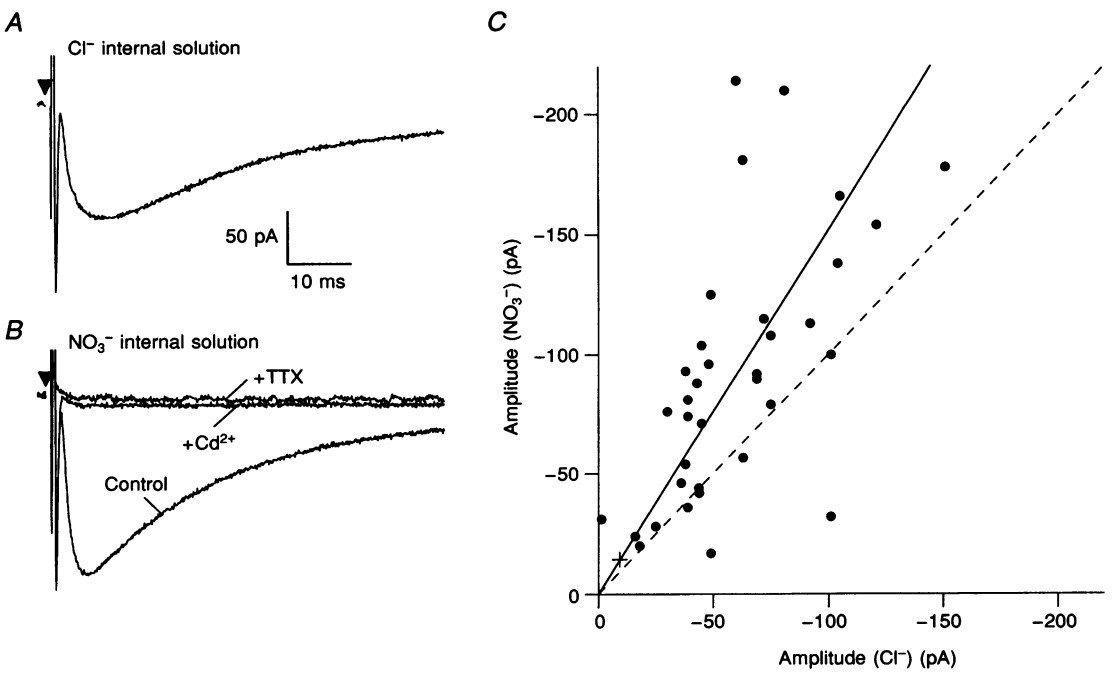

Figure 6. Intracellular nitrate ions potentiate the putative uptake current

A and *B*, specimen traces illustrating the experimental protocol with which the effect of replacing chloride by nitrate ions in the pipette solution was determined (see text). *A*, the average response to parallel fibre stimulation of a Bergmann glial cell recorded with a chloride-based pipette solution, in the presence of gabazine, CNQX and DL-APV. *B*, the response of a neighbouring cell recorded immediately afterwards with a nitrate-based pipette solution, with identical stimulation parameters. The response in the presence of gabazine, CNQX and DL-APV (Control) was recorded, then responses following addition of Cd^{2+} and then of TTX. *C*, the amplitudes of the control responses (i.e. in the presence of gabazine, CNQX and DL-APV) with the nitrate-based pipette solution as a function of those of the paired cells with the chloride-based internal solution (●). The continuous line passes through the origin and the point whose co-ordinates are the mean amplitudes for each pipette solution. The dashed line represents equality of the amplitudes for each internal solution (i.e. $y = x$). The cross marks the mean amplitudes of the residual currents insensitive to Cd^{2+}.

presence of CNQX and DL-APV (Fig. 7). The mean reductions in amplitude (after subtraction of the Cd^{2+}-insensitive current amplitudes; see Fig. 7 legend) upon antagonist application were $17 \pm 33\%$ (MCPG; $n = 5$) and $15 \pm 23\%$ (MSOPPE; $n = 12$). These reductions represent an upper limit on the effect of the antagonists on the putative uptake current, because they include run-down.

Voltage dependence of the putative uptake current

The low input resistance of the Bergmann glial cells is very likely to result in poor voltage uniformity during displacements of the holding potential from the resting potential. Nevertheless, we obtained current–voltage relations for the putative uptake current in order to determine the sign of its voltage dependence. This required subtraction of currents measured in the presence of cadmium ions from those previously recorded in the presence of CNQX and DL-APV. Subtracted currents from such an experiment are shown in Fig. 8*A*, in which jumps from −70 mV to the command voltages indicated were made (the voltage jumps themselves do not appear in the figure). The subtracted responses were plotted following offset of residual pre-stimulus baseline currents of the order of ± 100 pA. The recordings were made with a KCl-based internal solution (i.e. without caesium; see Methods) so as not to alter the Nernst potential for potassium ions. As may be seen, depolarization tended to reduce the amplitude of the current (Fig. 8*B*). For five cells, the amplitudes of the subtracted currents relative to those at −70 mV were $1{\cdot}3 \pm 0{\cdot}24$ at −100 mV and $0{\cdot}92 \pm 0{\cdot}21$ at −40 mV. The difference between the −100 and −40 mV amplitudes was significant ($P < 0{\cdot}001$). For jumps to potentials more positive than those shown, the baseline current became too noisy for the 'evoked' currents to be accurately measured.

Cd^{2+}-insensitive component

The residual inward current in the presence of cadmium ions, as well as the early triphasic current, was blocked by TTX (Fig. 9). This inward current thus probably results from the passage of the presynaptic action potentials; its generation does not require the release of transmitter. This component decayed slowly with a time constant, when fitted with a single exponential, of 480 ± 170 ms ($n = 8$).

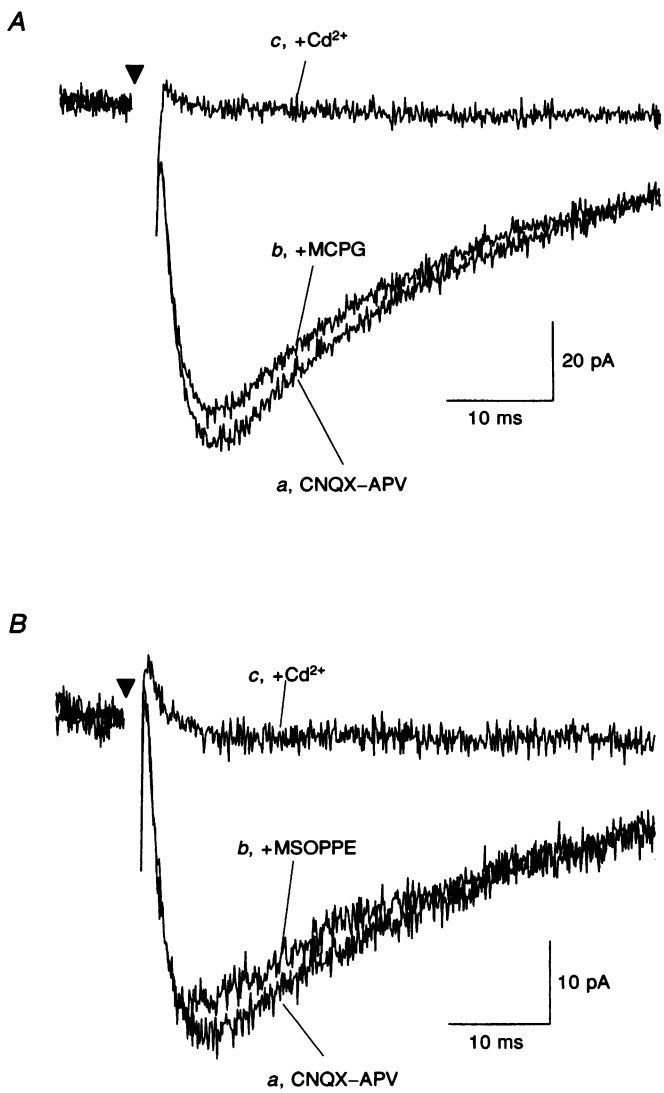

Figure 7. Effects of metabotropic glutamate receptor antagonists on the putative uptake current

A, addition of MCPG (*b*), a group I mGluR antagonist, to a bath solution containing gabazine, CNQX and DL-APV (*a*) had little effect on the putative uptake current. The subsequent addition of Cd^{2+} (*c*) blocked most of the current. *B*, experiment following an identical protocol to that of *A*, but with MSOPPE, a group II/III mGluR receptor antagonist.

Kinetics of the putative uptake current

Some care was necessary to extract the kinetics of the putative uptake current from the CNQX–APV-insensitive component, because it is the sum of both the putative uptake current and the Cd^{2+}-insensitive current. The approach taken was to select cells in which the Cd^{2+}-insensitive current was small (< 10% of the CNQX–APV-insensitive component amplitude) and then to subtract the Cd^{2+}-insensitive component from the CNQX–APV-insensitive component (Fig. 10). This selection minimizes errors arising from subtraction of currents whose time course may have changed during the experiment. The kinetics of these subtracted currents were then analysed.

The mean latency between the outward peak of the early triphasic current and the 20% point of the rising phase of the subtracted uptake current was 1·6 ± 0·3 ms ($n = 9$). The average 20–80% rise time for the uptake current was 2·3 ± 0·7 ms ($n = 10$). In order to fit the decay phase of the uptake current it was in most cases necessary to use two exponential components. The mean fit parameters were: $\tau_1 = 25{\cdot}2 \pm 5{\cdot}8$ ms ($n = 10$) and $\tau_2 = 975 \pm 807$ ms ($n = 9$), where the amplitude of the smaller, slow component was 7 ± 4% ($n = 9$) of the total (extrapolated to the time of the peak current).

DISCUSSION

Stimulation of parallel fibres in rat cerebellar slices elicits complex membrane currents in Bergmann glial cells. Pharmacological experiments show that the largest current component is likely to represent electrogenic uptake of synaptically released glutamate. These data provide new insights into the role of synaptic glutamate transporters since they are a direct measure of synaptic glutamate uptake *in situ*. Another current component was observed whose properties are consistent with synaptic activation of AMPA/kainate receptors in Bergmann glia. Our results confirm that adjacent Bergmann glia are electrically coupled and show that this gap junction coupling extends through large groups of cells to form a glial syncitium.

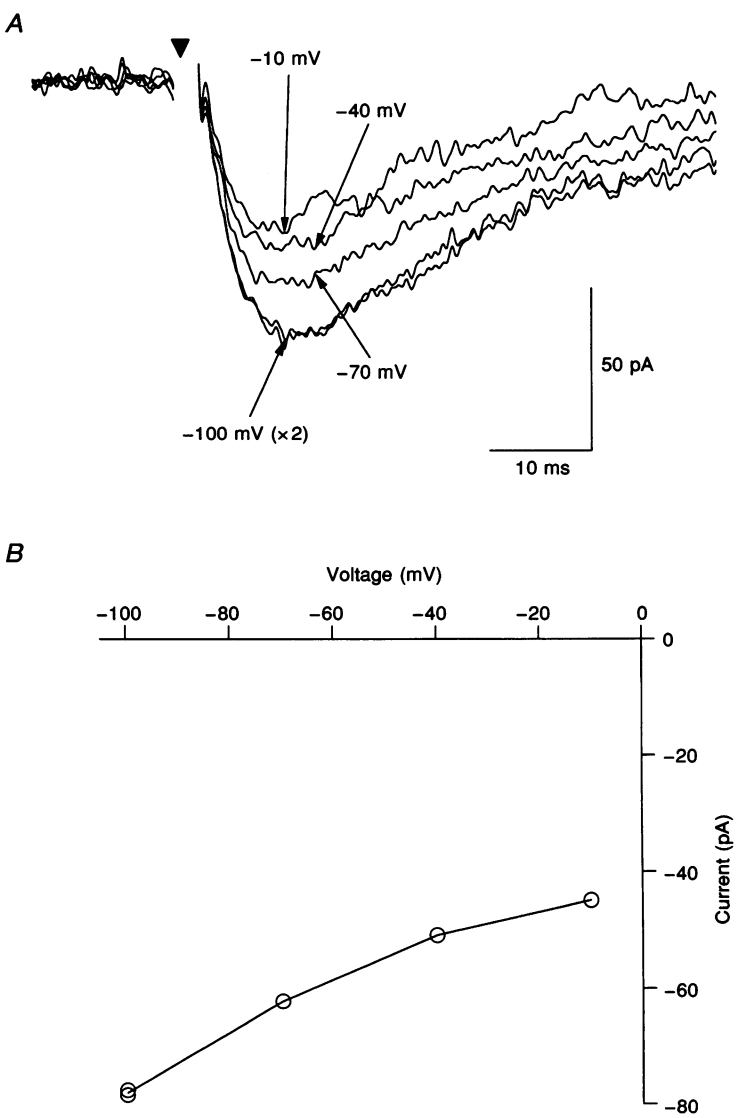

Figure 8. Voltage dependence of the putative uptake current

The putative uptake current was isolated pharmacologically by subtracting from responses recorded in the presence of CNQX and DL-APV those subsequently recorded after addition of cadmium ions. The voltage dependence of the putative uptake current was determined from such subtracted responses obtained at different holding potentials. *A*, subtracted current traces recorded in a Bergmann glial cell during voltage steps to −100, −70, −40 and −10 mV from −70 mV. Parallel fibres were stimulated (▼) 40 ms after the beginning of each voltage step (not visible), eliciting a current response which was inhibited by depolarization. The above sequence of voltage steps was repeated several times. Each trace is the average of 4 individual voltage steps. There are two traces for the −100 mV step; these averages are first and last in the sequence of voltage steps, showing that run-down was negligible during the time of the experiment. The pipette solution was KCl based and contained no Cs^+ ions. The bath solution contained gabazine. *B*, current–voltage relation for the peak inward (subtracted) current evoked by parallel fibre stimulation.

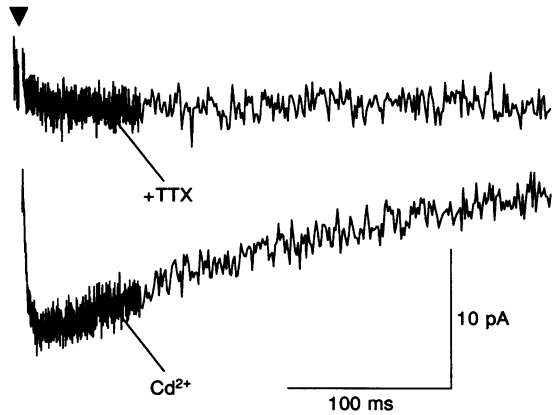

Figure 9. The Cd^{2+}-insensitive component of the response to parallel fibre stimulation
A small, slowly decaying current response remains in the presence of gabazine, CNQX, DL-APV and Cd^{2+} (labelled Cd^{2+}). It is blocked by the addition of TTX to the bath solution (labelled +TTX). The change in trace character after the initial portion of the decay reflects the switch to a slower sampling rate.

Identification of the components of the Bergmann glial response to synaptic activity

A previous study analogous to the present one investigated glial currents associated with autaptic transmission in a culture system (Mennerick & Zorumski, 1994; Mennerick *et al.* 1996). The interpretation of our results, obtained from a more intact preparation, cerebellar slices, is broadly similar. It is unlikely that any current was generated by GABA (released by interneurones of the molecular layer), since all responses of Bergmann glia to this neurotransmitter are blocked by the $GABA_A$ receptor antagonist bicuculline (Müller *et al.* 1994) and the present experiments were all carried out in the presence of a $GABA_A$ antagonist, gabazine. We thus presume that the currents recorded

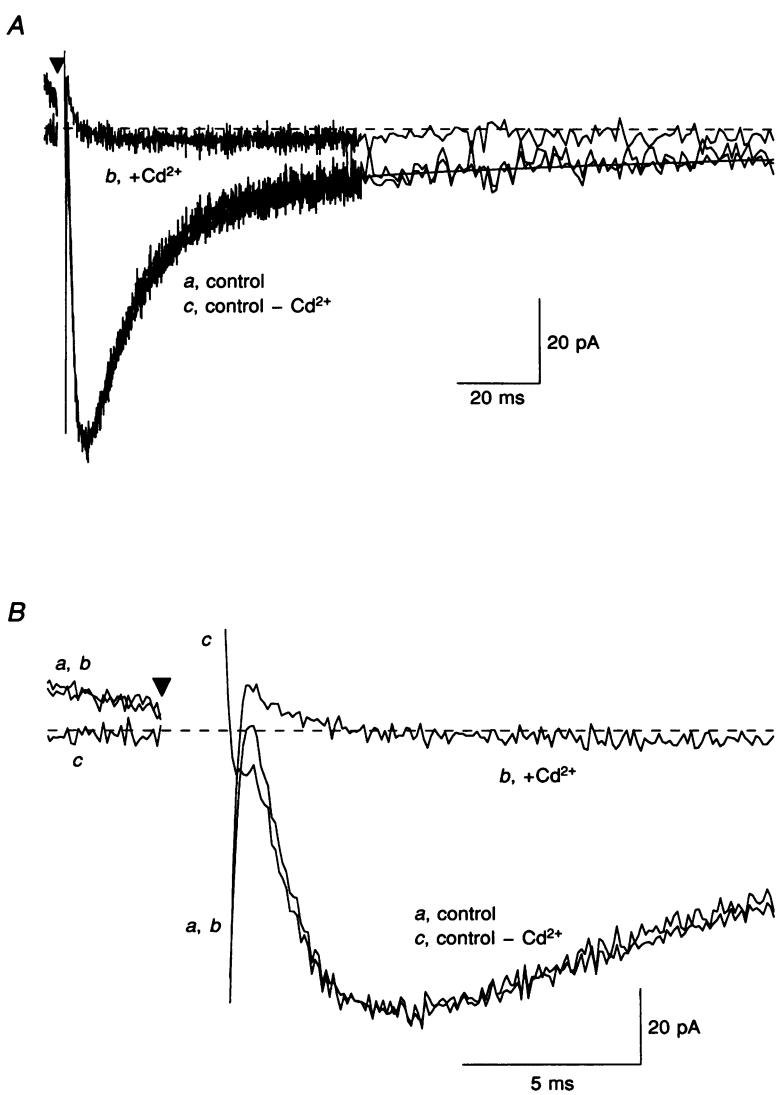

Figure 10. Kinetics of the putative uptake current obtained by pharmacological subtraction
A response with a small Cd^{2+}-insensitive component was chosen. The Cd^{2+}-insensitive component (*b*) was subtracted from the CNQX–APV-insensitive component (*a*, control) to obtain the best estimate of the putative uptake current (*c*, *a* − *b*). These 3 traces are displayed on two time bases (*A* slow, *B* fast). A bi-exponential fit is superimposed on the decay of trace *c* in *A*, with time constants of 14 ms (τ_1) and 240 ms (τ_2); the slower component was 15% of the total current amplitude extrapolated to the time of the peak. The dashed line in *A* represents the baseline current for traces *a* and *b*; the dashed line in *B* represents zero subtracted current. Before the stimulation of parallel fibres (▼), traces *a* and *b* are relaxing towards their baselines following the hyperpolarizing step used to probe the passive properties of the Bergmann glial cell. The sampling frequency was decreased from 10 kHz to 1 kHz midway through the decay of the response in *A*.

reflect the activity of the stimulated parallel fibres and the actions of the transmitter glutamate they release.

The extracellular field potential contributes to the membrane currents observed. In particular, it gives rise to a triphasic current reflecting the presynaptic action potentials, and it may induce an early outward current sensitive to CNQX.

The glutamate receptor antagonists, CNQX and DL-APV (or 7-Cl-Kyn), had complex actions on the currents induced in Bergmann glia by parallel fibre stimulation, reducing their amplitude and/or slowing their rise. Currents evoked by exogenous glutamate have been observed in other glial cells *in situ* (Clark & Mobbs, 1992), but their function is not known. Of key interest is whether activation of the glutamate receptors expressed in Bergmann glia occurs in response to synaptic activity. Unfortunately, owing to the complexity of conditions in the slice, we cannot exclude indirect mechanisms by which activation of ionotropic glutamate receptors in other cells may contribute to the currents observed. If direct activation of the glial AMPA/kainate receptors does occur, it is most likely to underlie the early inward current component whose inhibition by CNQX resulted in the consistent slowing of the rise of the currents recorded. Indirect mechanisms, for instance involving the accumulation of potassium ions in the extracellular space, are more likely to contribute to later and slower components.

We propose that the current fraction insensitive to CNQX–APV but blocked by Cd^{2+} reflects electrogenic uptake of synaptically released glutamate and not the activation of metabotropic receptors. This interpretation is strongly supported by the following: the block of the current by glutamate uptake blockers, inhibition by sodium replacement, inhibition by depolarization, and stimulation by intracellular nitrate. The lack of effect of metabotropic receptor antagonists argues against their involvement in generating the currents we recorded. Moreover, if a potassium conductance were modulated by metabotropic receptor activation (Schwartz, 1993), the amplitude of that modulation should increase with depolarization away from E_K, yet the current–voltage relation of the putative uptake current shows that it is inhibited by depolarization. This suggests that the recorded responses do not reflect modulation of a potassium conductance. The experiments introducing D-aspartate ions into the Bergmann glia would have provided strong confirmation of the uptake hypothesis if they had given positive results. The results were, instead, inconclusive. Intercellular variability may have masked a significant inhibition of the putative uptake current by intracellular D-aspartate; in addition, the degree of inhibition expected was unknown. Inhibition of uptake by internal 'agonist' (D-aspartate or glutamate) and sodium ions has been reported (Barbour *et al.* 1991; Takahashi, Sarantis & Attwell, 1996), but using higher concentrations of agonist than here. In addition, the dilution of the D-aspartate and sodium into the glial syncytium via gap junctions may reduce the effective concentration of these ions.

We conclude that a large fraction of the inward current induced in Bergmann glia following parallel fibre stimulation is generated by glutamate uptake. The type of transporter generating the responses recorded is likely to be primarily GLAST, since *in situ* hybridization suggests that it is strongly expressed in Bergmann glia. GLT-1 may contribute, as it is also expressed, though apparently to a lesser extent (Rothstein *et al.* 1994; Chaudhry *et al.* 1995).

A response component was insensitive to cadmium ions but blocked by TTX. The mechanism(s) generating this current are unknown, but a plausible hypothesis is that it is caused by the potassium efflux from parallel fibres during action potential propagation. However, Mennerick *et al.* (1996) suggest the Cd^{2+}-insensitive current is not generated by this mechanism in their preparation. The Cd^{2+}-insensitive current component decayed with a time constant of 480 ms. The response of a potassium-selective electrode to parallel fibre stimulation in the molecular layer (Shibuki & Okada, 1990) decayed with a time constant of about 1 s, at 35 °C.

Kinetics of the glutamate uptake current

The activation of the uptake current is rapid: it reaches 20% of its peak amplitude within 1·6 ms of the peak of the early triphasic current (which should correspond roughly to the peak of the compound action potential in parallel fibres) and rises to 80% of its peak activation within another 2·3 ms (without correction for the possible delays generated by cell filtering).

The rising phase of the uptake current could reflect one or more of the following processes: passage of the transporters through conformational changes between the glutamate-binding step and the current-generating step; the recruitment of additional 'extrasynaptic' carriers as glutamate spreads from its sites of liberation; or filtering by the imperfect voltage clamp in Bergmann glia. All processes could contribute.

There are also several possible determinants of the decay phase, whose principal time constant was 25 ms. It could reflect the unbinding of glutamate from the carriers, either close to equilibrium with a slowly decaying extracellular glutamate concentration or, if the glutamate transient is brief, as quickly as the carrier's affinity for glutamate allows. Alternatively, the uptake current we see may reflect the synchronous reaction of transporters that have been trapped in a particular conformation (e.g. with sodium ions bound, waiting for glutamate), leading to an intense current that relaxes to that associated with steady asynchronous transport. Such transient currents have been observed, notably in the case of presynaptic 5-HT transport in leech neurones, activated by photo-release of caged 5-HT (Bruns, Engert & Lux, 1993). Analogous experiments for glutamate transporters have not been reported, so this possibility cannot be evaluated.

Synaptic role of glutamate uptake

What conclusions is it possible to draw from the properties of the uptake current recorded here concerning the operation and role of glutamate transporters during synaptic transmission? The recording resolution in Bergmann glia is insufficient to detect uptake currents associated with transmission at individual parallel fibre–Purkinje cell synapses. This is because the uptake of one glutamate molecule is likely to involve the movement of just one or two electronic charge(s), compared with the thousands that flow during activation of a glutamate-gated channel. Thus, a unitary EPSC is detectable in a neurone, but the accompanying uptake current in a glial cell is not. Our results can only be interpreted with respect to the compound synaptic currents evoked by the stimulation we used, rather than more physiological unitary events. Since there is evidence that in Purkinje cells unitary parallel fibre currents have faster kinetics than do the compound currents (Häusser, 1994), the properties of synaptic transmission may differ between these two types of EPSC.

The uptake current activates near the beginning of the compound parallel fibre current, which can decay with time constants of 5–10 ms (Llano *et al.* 1991; Barbour *et al.* 1994). This would appear to argue against the hypothesis that only binding of glutamate to transporter sites, and not translocation, is fast enough to influence the synaptic current (Tong & Jahr, 1994). However, we do not know what fraction of the uptake-bound glutamate would need to be transported to generate the uptake current observed at early times. In addition, it is not even certain that generation of a current can be equated with a glutamate translocation step.

In summary, the uptake current activates near the beginning of the slow compound synaptic conductances with which it is associated. Its form and timing during activation of single synapses is unknown, but as unitary synaptic conductances are more rapid, uptake is likely to be less important than during the compound currents. The relative importance of binding to carriers and actual transport remains to be quantified.

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