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ACTIVATION OF GLUTAMATE RECEPTORS AND GLUTAMATE UPTAKE IN IDENTIFIED MACROGLIAL CELLS IN RAT CEREBELLAR CULTURES

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SUMMARY

1. Patch-clamp methods have been used to examine the action of excitatory amino acids on three types of glial cell in cultures of rat cerebellum, namely type-1-like astrocytes, type-2 astrocytes and oligodendrocytes. In addition we have examined glutamate sensitivity of the precursor cell (the O-2A progenitor) that gives rise to type-2 astrocytes and oligodendrocytes.

2. Glutamate (30 μM), quisqualate (3–100 μM), (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA, 10–30 μM) and kainate (10–500 μM) were applied to cerebellar type-2 astrocytes examined under whole-cell voltage clamp. Each of these agonists induced inward currents in cells held at negative membrane potentials. The currents reversed direction near 0 mV holding potential. *N*-Methyl-D-aspartate (NMDA, 30–100 μM) or aspartate (30 μM) in the presence of glycine (1 μM) did not evoke any whole-cell current changes in type-2 astrocytes.

3. The distribution of glutamate receptors in type-2 astrocytes was mapped with single- or double-barrelled ionophoretic pipettes containing quisqualate or kainate. Application of these agonists (current pulses 100 ms, 50–100 nA) to cells held at -60 mV evoked inward currents of 20–120 pA in the cell soma and 10–80 pA in the processes. Responses could also be obtained at the extremities of processes (~ 60 μm from the soma).

4. Quisqualate or kainate (at 30 μM) applied to O-2A progenitor cells from rat cerebellum or optic nerve induced whole-cell currents (quisqualate 20–30 pA; kainate 20–50 pA, holding potential, $V_h = -60$ mV) that reversed near 0 mV. In common with type-2 astrocytes, the progenitor cells did not respond to NMDA (30 μM).

5. Type-1-like astrocytes produced large inward currents to glutamate (30 μM). These currents remained inward-going at holding potentials as positive as +80 mV and were not accompanied by any apparent noise increase. This result can be explained by the presence of an electrogenic glutamate uptake carrier. In cells kept up to 4 days *in vitro*, quisqualate, kainate and NMDA each failed to produce any whole-cell current changes, indicating the absence of receptors in type-1-like astrocytes at this stage in culture. Furthermore the glutamate uptake currents in

type-1-like astrocytes were inhibited when external Na^+ was replaced by Li^+, although Li^+ was found to pass through the glutamate channel in type-2 astrocytes.

6. Oligodendrocytes in cerebellar cultures did not respond to glutamate, quisqualate or kainate indicating a lack both of a detectable electrogenic glutamate uptake mechanism and of glutamate receptor ion channels in these cells.

7. We conclude that, of the various macroglial cells, only the type-2 astrocyte and its progenitor cell possess 'fast' glutamate receptor channels in short-term (< 4 day) cultures. Detectable uptake currents were confined to type-1-like astrocytes. However, in older cultures (> 7 day) type-1-like astrocytes also developed glutamate receptor channels, in addition to their uptake currents. The possible involvement of glial glutamate receptors and glutamate uptake in neuronal–glial interaction is considered.

INTRODUCTION

Investigations characterizing the properties of mammalian glutamate receptor subtypes have been concerned, almost exclusively, with the receptor channels found in central neurones (see Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984; Cull-Candy & Ogden, 1985; Cull-Candy & Usowicz, 1987; Jahr & Stevens, 1987; Ascher & Nowak, 1988). However, glutamate also depolarizes glial cells (Bowman & Kimelberg, 1984) and this has led to the suggestion that these cells may also possess glutamate receptors. This remained uncertain in view of the fact that L-glutamate was known to depolarize Müller glial cells from salamander retina (Brew & Attwell, 1987) and type-1-like astrocytes from rat cerebellum (Cull-Candy, Howe & Ogden, 1988) by activating an electrogenic uptake mechanism. Recent electrophysiological experiments have indicated that glutamate receptors can occur in cortical astrocytes (Sontheimer, Kettenmann, Backus & Schachner, 1988) and the presence of glutamate receptors in cerebellar type-2 astrocytes has been proposed from neurochemical studies (Gallo, Giovannini, Suergiu & Levi, 1989). Furthermore, patch-clamp recording has demonstrated directly that, in cerebellar type-2 astrocytes, glutamate and related amino acids can evoke whole-cell and single-channel currents (Usowicz, Gallo & Cull-Candy, 1989).

In the present study the effects of glutamate receptor agonists have been examined on three types of glial cell from the mammalian central nervous system (CNS): type-1-like and type-2 astrocytes and oligodendrocytes. We have also looked at the effects of glutamate agonists on the precursor cell known to give rise to both oligodendrocytes and type-2 astrocytes, the so-called O-2A progenitor (Raff, Miller & Noble, 1983*a*). Two types of astrocyte-like cells have been described in the intact optic nerve of the adult rat; one has predominantly radially oriented processes while the other has predominantly longitudinally oriented processes (Miller, Fulton & Raff, 1989). Immunohistochemical studies of rat optic nerve with the monoclonal antibodies, HNK1, L2 and anti-NSP-4, have led to the suggestion that, *in vivo*, type-2 astrocytes may have longitudinal processes associated with nodes of Ranvier (ffrench-Constant & Raff, 1986). *In vivo* oligodendrocytes myelinate axons. It is thus thought that oligodendrocytes and type-2 astrocytes, which share a common lineage, may be intimately associated with the axons.

A preliminary report of some of our findings on glutamate receptors in glial cells has appeared (Cull-Candy, Mathie, Symonds & Wyllie, 1989).

METHODS

Isolation of cells

Cultures of cerebellar type-2 astrocytes were obtained as previously described (Gallo *et al.* 1989), with some modifications. Briefly, 8-day-old Sprague–Dawley rat pups were killed by cervical dislocation and decapitated. Cerebella were aseptically dissected and meninges removed. The tissue was chopped finely in two directions at right angles to each other on a McIlwain tissue chopper (micrometer 0·45 mm). Cerebellar cells were dissociated with trypsin and DNase and then plated on poly-L-lysine coated cover-slips (10 μg/ml, MW 53000) at a cell density of $1{\cdot}5 \times 10^6$ cells per dish. The cells were cultured in Eagle's basal medium (GIBCO) supplemented with 10% fetal calf serum, 2 mM-glutamine and 100 μg/ml gentamycin. This culture medium was changed after 2 days. Astrocytes with an antigenic phenotype similar to that of type-1 astrocytes isolated from rat optic nerve (Raff, Abney, Cohen, Lindsay & Noble, 1983*b*) were also present in cultures of cerebellar type-2 astrocytes. We refer to these cells as 'type-1-like' astrocytes, as it is not yet clear whether these cells are developmentally similar to type-1 astrocytes isolated from rat optic nerve. Because type-2 astrocytes divided infrequently in culture, while type-1-like astrocytes proliferated to form a confluent monolayer, electrophysiological recordings, except where otherwise stated, were made from cells after 3–5 days *in vitro* when individual cells could still be readily discerned. Some neurones were present in these cultures and could be easily identified from their morphology and by indirect immunofluorescence with the antibody RT97 (Wood & Anderton, 1981).

In some experiments recordings were made from astrocytes obtained from explant cultures of cerebellar cells as previously described (Cull-Candy *et al.* 1988). Cerebella, aseptically removed from 8-day-old rat pups, were chopped with fine scissors in Dulbecco's minimum essential medium (GIBCO), containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 units/ml). The tissue was then passed through a 200 μm nylon mesh to produce a suspension of cell clumps. This was then plated on cover-slips previously coated with poly-D-lysine. The explant culture technique used no enzymes and therefore allowed the examination of non-enzyme-treated cells. Such cultures contained clumps of neurones (mainly granule cells) as well as astrocytes.

Cerebellar O-2A progenitor cells were obtained from 1-day-old rat pups. These cells were isolated as described for type-2 astrocytes but were cultured in a conditioned serum-free medium obtained from cultures of confluent type-1-like astrocytes. Type-1 astrocytes release platelet-derived growth factor, which is necessary for O-2A progenitor cell division, which in turn controls the timing of oligodendrocyte differentiation (Lillien & Raff, 1990). Recordings were made from cerebellar O-2A progenitor cells within 3–5 days of plating. Recordings were also made from rat optic nerve progenitor cells (Lillien & Raff, 1990) that were kindly supplied by Dr Laura Lillien (Biology Department, UCL). These were obtained from 17-day-old rat embryos and were used 5–6 days after plating.

Oligodendrocytes were obtained from 1-day-old rat pups and isolated as described for type-2 astrocytes. However, these cells were plated on top of a confluent layer of type-1-like astrocytes and grown in a culture medium that did not contain any fetal calf serum. Oligodendrocytes were used 3–4 days after plating. Occasionally, recordings were made from oligodendrocytes that were present in the same cultures as type-2 astrocytes or O-2A progenitors.

Identification of cells

For electrophysiological experiments individual cover-slips were placed in a small recording chamber (volume 1 ml), and cells were identified morphologically under phase contrast optics (total magnification $\times 640$, water immersion). Type-2 astrocytes were process-bearing, phase-bright cells with a stellate morphology; type-1-like astrocytes were phase-dark and had a flattened polygonal, epithelioid morphology. O-2A progenitor cells were identified by their characteristic small size and bipolar (or occasionally multipolar) morphology and also by the fact that they tended to occur in clusters reflecting the fact that they continued to divide in our cultures. Oligodendrocytes had a characteristic appearance and possessed networks of radial processes (Raff, Mirsky, Fields, Lisak, Dorfman, Silberberg, Gregson, Liebowitz & Kennedy, 1978).

Our criteria for identification were confirmed by labelling cover-slips from the same cultures with rabbit antibodies to cow glial fibrillary acidic protein (GFAP, Dako Ltd.) which is characteristic of astrocytes (Bignami & Dahl, 1977; Raff, Fields, Hakomori, Mirsky, Pruss & Winter, 1979), mouse monoclonal antibodies to galactocerebroside (Gal-C) which is characteristic of oligodendrocytes (Raff *et al.* 1978) and the mouse monoclonal antibody LB1 (Levi, Gallo & Ciotti, 1986), which binds to the GD3 glycolipid expressed by type-2 astrocytes and O-2A progenitors (Curtis, Cohen, Fok-Seang, Hanley, Gregson, Reynolds & Wilkin, 1988). The antibodies were detected by immunofluorescent labelling (GFAP with fluorescein conjugated swine anti-rabbit immunoglobulins, Dako Ltd, Gal-C with fluorescein conjugated goat anti-mouse immunoglobulins, (Dako Ltd) and LB1 with a rhodamine conjugated goat anti-mouse immunoglobulin, Dako Ltd). Cells were viewed with a Zeiss Universal Incidence Fluorescence microscope under both phase contrast and fluorescence optics. Cells were identified as follows: type-2 astrocytes were GFAP positive–LB1 positive; type-1-like astrocytes were GFAP positive–LB1 negative; O-2A progenitors were GFAP negative–LB1 positive; and oligodendrocytes were Gal-C positive (see Raff, Abney & Fok-Seang, 1985; Raff *et al.* 1978). Gal-C and LB1 were kindly supplied by Dr Barbara Ranscht and by Dr Jim Cohen respectively.

Electrophysiological recordings and patch pipettes

Whole-cell recordings and single-channel current recordings from outside-out patches were made either with a List L/M-EPC 7 or Axopatch 1A patch clamp amplifier (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Patch-pipettes were made from hard (borosilicate), thick-walled (outside diameter 1·50 mm, inside diameter 0·86 mm) glass capillary tubing containing a filament (Clark Electromedical). When filled with intracellular solutions the pipettes had resistances of 5–10 MΩ. To reduce their electrical capacitance, pipette tips were coated with Sylgard 184 resin (Dow Corning) which was cured by passing the tip into a heated coil. Pipette tips were fire-polished with a heated platinum wire. Ionophoretic pipettes were made from hard (borosilicate) glass using a Campden moving coil microelectrode puller; they had resistances of ~ 200 MΩ, when filled with either 100 mM-kainate or 100 mM-quisqualate solutions.

When recording from type-1-like astrocytes it was necessary to correct membrane potentials for the voltage errors arising from the currents flowing across the series resistance of the pipette. Thus, for a series resistance, R_s, and a clamp current, i_c, a potential of amplitude $i_c R_s$ was subtracted from the command potential, V_{com}, to give the correct value of membrane potential, V_m. Thus $V_m = V_{com} - i_c R_s$. By plotting clamp current against membrane potential (corrected for series resistance) in the presence and absence of glutamate and subtracting the control current–voltage (I–V) curve from the glutamate I–V curve, the net glutamate-evoked I–V curve was obtained. Series resistances were typically in the range 12–22 MΩ and cell capacitances were between 15–30 pF for type-2 astrocytes and generally 50 pF or greater for type-1-like astrocytes.

Solutions

For whole-cell recording from type-2 astrocytes the patch pipettes were back-filled with an 'internal' solution of the following composition (mM): CsF, 110; CsCl, 30 (or CsCl, 140); NaCl, 4; EGTA, 5; K-HEPES, 10; $CaCl_2$, 0·5; pH 7·2. In some experiments on type-1-like astrocytes the 'internal' solution contained (mM): KCl, 140; $MgCl_2$, 2·0; EGTA, 0·5; K-HEPES, 5; ATP(Mg), 0·5; pH 7·2. When filled with these solutions pipettes had resistances in the range 5–10 MΩ. The cells, and the extracellular faces of outside-out patches, were bathed in an 'external' solution of composition (mM): NaCl, 150; KCl, 2·8, Na-HEPES, 10; $CaCl_2$, 1·0; pH 7·2. In some experiments NaCl was replaced by LiCl. For bath application of drugs, solutions were perfused through a fine plastic tube placed beneath the water immersion objective (flow rate 1·5 ml/min). Solutions were switched manually by means of a two-way Hamilton tap, while ensuring the bath was completely exchanged with control solution between applications. The following drugs were used: L-glutamate (Cambrian Chemicals Ltd), quisqualate (Tocris), (*S*)-α-amino-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA; Tocris), *trans*-D,L-1-amino-1,3-cyclopentane-dicarboxylic acid (*trans*-ACPD; Tocris), kainate (Sigma or Tocris), *N*-methyl-D-aspartate (NMDA; Cambridge Research Biochemicals), L-aspartate (Tocris), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris). All solutions were passed through a 0·22 μm filter before use.

RESULTS

Glutamate receptors in cerebellar type-2 astrocytes

Astrocytes (type-2 and type-1-like) were identified on the basis of their morphology, and by labelling the cells with the antibody to GFAP. Figure 1*A* is a

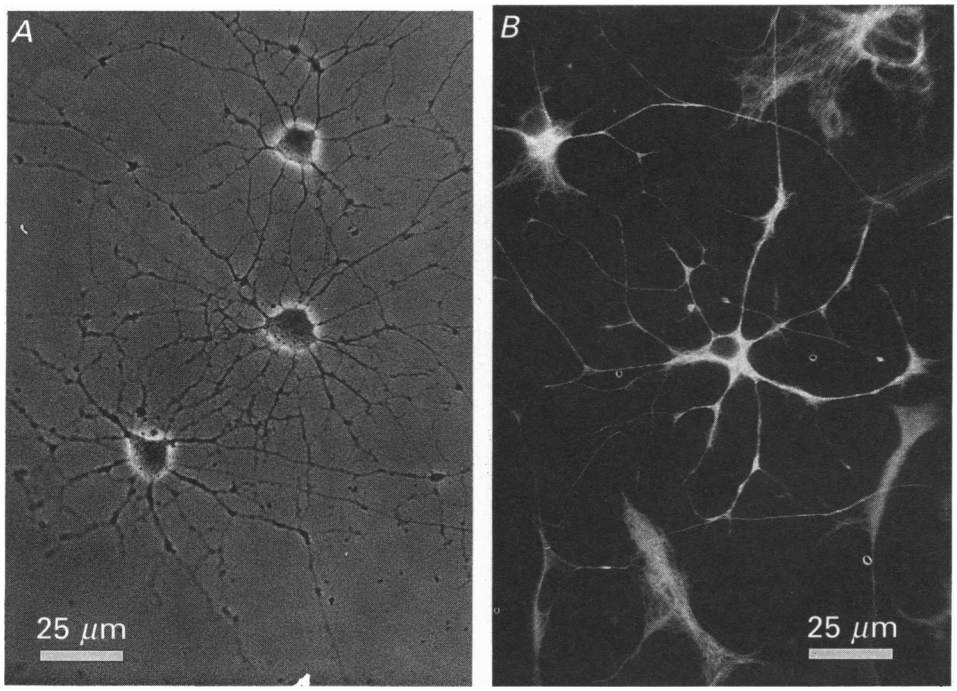

Fig. 1. *A*, photomicrograph of three cerebellar type-2 astrocytes seen under phase contrast optics. Note the phase-bright cell bodies and the stellate morphology. *B*, a field of astrocytes labelled with the antibody to GFAP and visualized with indirect immunofluorescence with fluorescein-labelled immunoglobulin. Note the fine extensive processes of the type-2 astrocyte (centre of field). The flat cells in the micrograph are type-1-like astrocytes. Cells were isolated from a 7-day-old rat pup and kept for 3 days *in vitro*.

photomicrograph of a group of three type-2 astrocytes under phase contrast optics. These cells usually occurred in groups and had a characteristic morphology in culture; their cell bodies were phase-bright ($\sim 15\ \mu$m in diameter) and gave rise to many fine processes of up to 100 μm in length. Figure 1*B* shows a field containing a type-2 astrocyte (centre of the field) and several type-1-like astrocytes stained with the antibody to GFAP and viewed with fluorescence optics. To confirm the reliability of our morphological identification of type-2 astrocytes, cells were double labelled with antibody to GFAP and with LB1. In all cases cells identified morphologically as type-2 astrocytes were GFAP positive–LB1 positive while those identified as type-1-like astrocytes were GFAP positive–LB1 negative.

Glutamate (30 μM), quisqualate (3–100 μM), AMPA (10–30 μM) and kainate (10–500 μM) each evoked large inward currents in type-2 astrocytes held at negative

membrane potentials. Typical whole-cell currents evoked by these agonists in cells held at −60 mV were in the range 40–80 pA for glutamate and quisqualate (30 μM), 40–100 pA for AMPA (30 μM) and 200–400 pA for kainate (100 μM). Figure 2*A* shows *I–V* plots for whole-cell responses to quisqualate (30 μM) and glutamate (30 μM); both

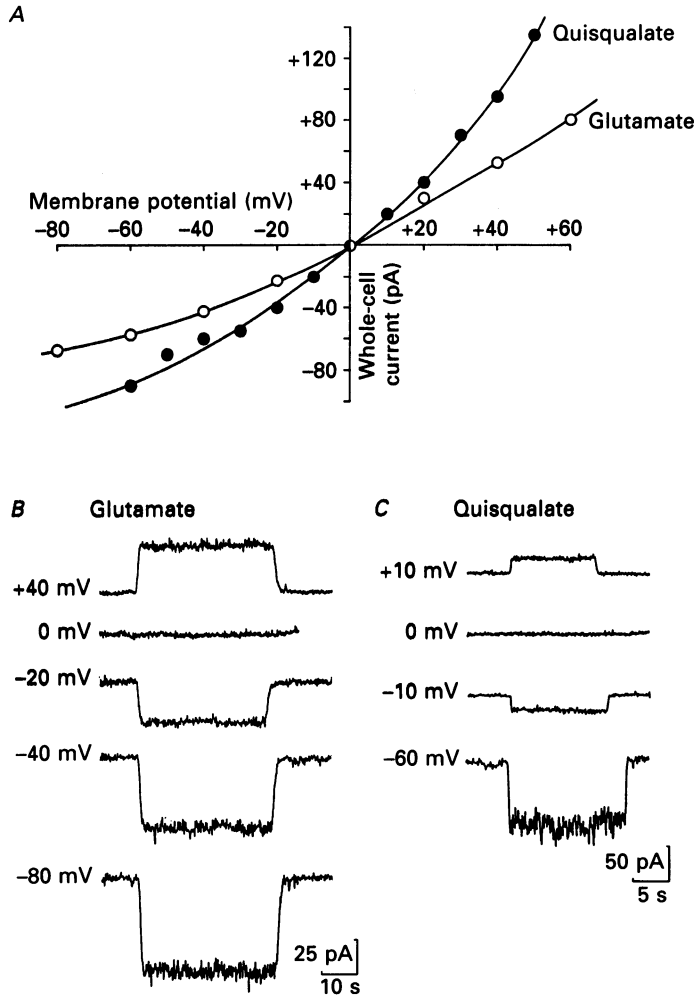

Fig. 2. *A*, current–voltage relationship for whole-cell currents evoked by 30 μM-glutamate (○) or 30 μM-quisqualate (●) in a type-2 astrocyte. Currents reversed near 0 mV holding potential and showed some outward rectification. *B*, whole-cell currents induced by 30 μM-glutamate in a type-2 astrocyte voltage clamped at +40, 0, −20, −40 and −80 mV. *C*, whole-cell currents evoked by 30 μM-quisqualate in a type-2 astrocyte at V_h values of +10, 0, −10, and −60 mV. Note the increase in noise during the application of glutamate and quisqualate.

curves show rectification. All agonists produced similar reversal potentials; pooling data gave a mean value of 0·4 ± 0·5 mV (mean ± S.E.M., $n = 25$ cells). Figure 2*B* and *C* shows examples of whole-cell currents produced by 30 μM-glutamate and 30 μM-

quisqualate applied to two different cells over a range of membrane potentials. The currents reversed near 0 mV membrane potential and were accompanied by a clear noise increase.

The application of NMDA (30 and 100 μM) in the presence of glycine (1 μM), which is known to potentiate the NMDA response in neurones (Johnson & Ascher, 1987), failed to evoke whole-cell current changes in type-2 astrocytes at either negative or positive membrane potentials ($n = 6$ cells). These experiments were all carried out in nominally Mg^{2+}-free solutions to eliminate the possibility of Mg^{2+} block of the channel, of the sort described for NMDA channels in neurones (Mayer, Westbrook & Guthrie, 1984; Nowak *et al.* 1984). In neurones, NMDA responses can be potentiated by reducing proton concentration below normal physiological levels (Traynelis & Cull-Candy, 1990). However, when the pH of the extracellular medium was changed from 7·2 to 8·6, NMDA (30 μM) still failed to produce any whole-cell current changes in type-2 astrocytes ($n = 5$ cells). Similarly, type-2 astrocytes in explant cultures (i.e. prepared without the use of enzymes) did not respond to NMDA, confirming the previous observation (Usowicz *et al.* 1989) that the lack of sensitivity to NMDA was not due to the normal enzyme treatment used in the cell dissociation (see Akaike, Kaneda, Hori & Krishtal, 1988; Allen, Brady, Swann, Hori & Carpenter, 1988). NMDA receptors were present in granule cells both in the enzyme-treated and explant cultures.

Distribution of quisqualate and kainate receptors in type-2 astrocytes

There is indirect evidence to suggest that type-2 astrocytes may be one of the cells which send processes to the nodes of Ranvier *in vivo* (ffrench-Constant & Raff, 1986), raising the possibility that receptors in these cells may be activated by glutamate released from axons. It was therefore of interest to investigate whether type-2 astrocytes possessed glutamate receptors in their processes and at their extremities. From earlier work, it was clear that receptors were present in patches removed from the cell soma (Usowicz *et al.* 1989). In the present experiments the distribution of glutamate receptors in type-2 astrocytes was mapped with ionophoretic pipettes containing quisqualate or kainate. In some experiments kainate and quisqualate were applied from adjacent barrels of double-barrelled pipettes. The cells were continuously perfused with control solution to reduce the background level of agonist and the diffusion of ionophoretically applied drug to other regions of the cell. Application of these agonists (current pulses 20–100 ms, 50–100 nA) evoked currents both in the cell soma (20–120 pA; $V_h = -60$ mV, $n = 22$ cells) and in the processes (10–80 pA; $n = 15$ cells).

Figure 3*A* shows a drawing of the cell soma and one of the main processes of a type-2 astrocyte in which receptor distribution was mapped with a kainate-filled micropipette positioned at various sites (labelled *a–f*). Responses to brief ionophoretic pulses of kainate could be obtained along the entire length of the process (positions *b*, *c*, *e* and *f*), as well as in the soma (position *a*). As expected, the responses rapidly disappeared when the ionophoretic pipette was either moved laterally away from the process (position *d*) or raised 5–10 μm above the process (not shown). This indicates that the responses in the processes did not result from diffusion of kainate onto the cell soma. We have not attempted to estimate the

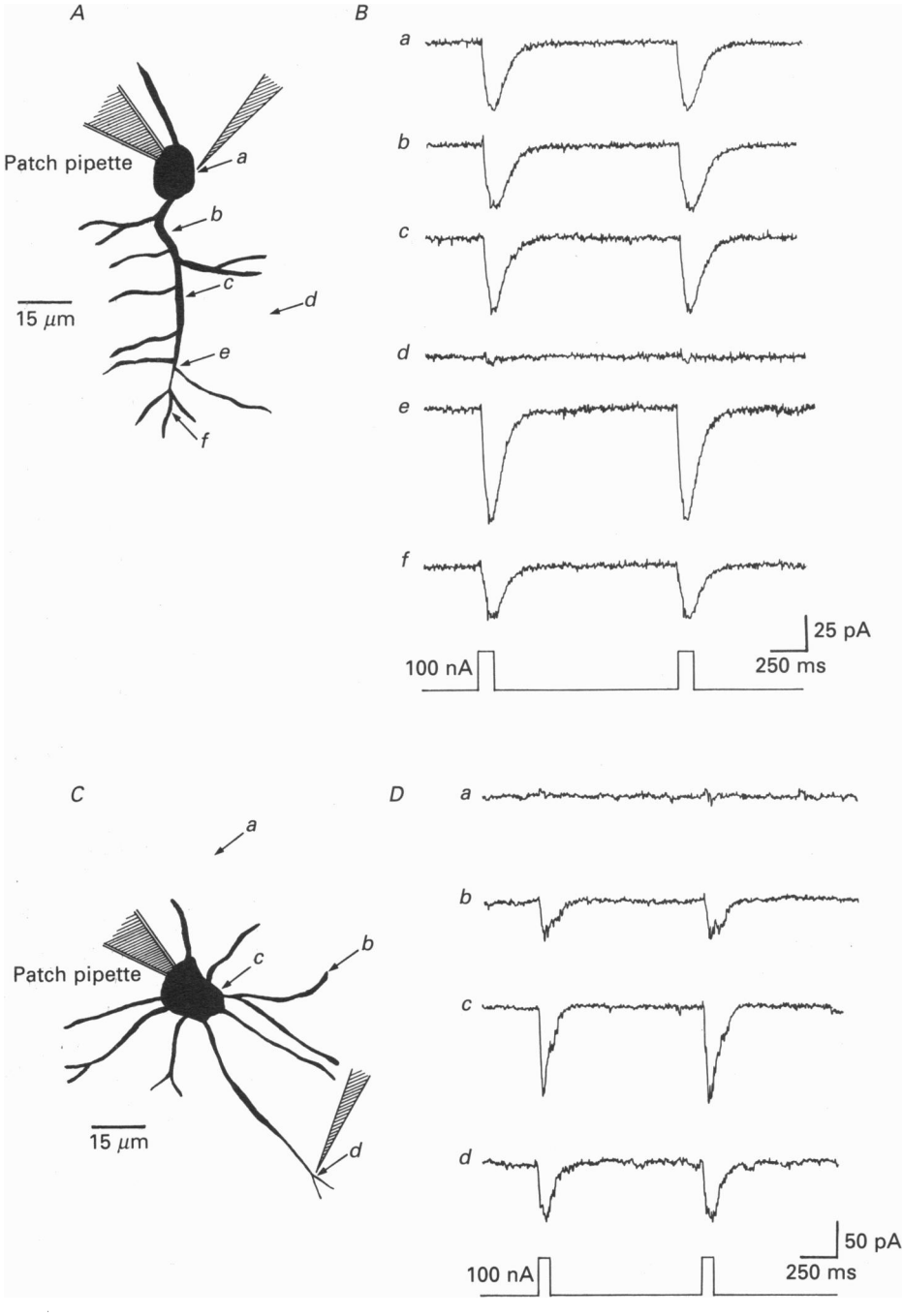

Fig. 3. *A*, drawing of part of a type-2 astocyte examined under whole-cell clamp. Receptor distribution was mapped with a kainate-filled ionophoretic micropipette positioned near the cell soma (*a*), or along the length of the main process (at *b*, *c*, *e* and *f*). *B*, ionophoretic application of kainate (pulse width 100 ms, amplitude 100 nA) evoked responses on the cell soma and along the entire length of the process. Note the large response obtained at

density of the receptors at the ends of the processes. However, the responses at positions *e* and *f* are similar to those obtained in the soma. As these sites are electrically remote from the soma, and the surface area of the process which the ionophoretically applied kainate can reach is small, it seems likely that the receptor density in the processes is greater than in the cell body.

Figure 3*B* shows an example of a similar experiment in which the quisqualate sensitivity of a type-2 astrocyte has been mapped. In this experiment quisqualate responses could be obtained on the soma (position *c*) and at the tip of a main process (position *d*, about 60 μm from the soma). Again no current was recorded when the ionophoretic pipette was moved away to a region free of processes (position *a*). Current–voltage relationships were obtained for responses to ionophoretically applied quisqualate and kainate, both in the cell soma and in the extremities of processes (in eight cells). In both situations the agonist evoked currents reversed direction near to 0 mV, implying reasonable voltage control in the cell processes, at least for low-frequency events. Experiments with double-barrelled ionophoretic micropipettes indicated that there was no clear differential distribution of quisqualate and kainate receptors within individual cells. Hence, areas of low or high sensitivity affected quisqualate and kainate responses to similar extents.

Glutamate receptors in O-2A progenitor cells

If glutamate receptors are involved in neurone–glia interactions before the second postnatal week in the rat, which is the time when type-2 astrocytes first appear (Miller, David, Patel, Abney & Raff, 1985), then glutamate receptors might also be expected to occur in the precursor cell that gives rise to type-2 astrocytes. We have therefore examined this possibility.

O-2A progenitor cells in culture had a bipolar or multipolar morphology and usually occurred in groups, probably because they continued to divide in the culture conditions used in the present study. Figure 4*A* and *B* shows a single field of cells in which O-2A progenitor cells have been identified immunohistochemically. Cells were labelled with the antibody to GFAP (Fig. 4*A*) and with the surface antibody LB1 (Fig. 4*B*), which binds to O-2A progenitors and type-2 astrocytes (Levi *et al.* 1986); type-2 astrocytes were not present in culture conditions used. The O-2A progenitor cells (arrowed) were identified as cells that were LB1 positive but lacked glial fibrillary acid protein. The remaining cells in the field (which are GFAP positive and LB1 negative) are type-1-like astrocytes. Although the morphology of O-2A progenitor cells was similar to that of neurones the majority of bipolar or multipolar cells in our progenitor cultures were LB1 positive. Moreover, O-2A progenitor cells occurred in groups and occasionally could be seen dividing.

In addition to studying the effects of glutamate agonists on cerebellar progenitor

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e; the response at *f* (approximately 60 μm from the soma) was similar to the response at the tip of the process (not shown). No response was obtained at *d*, an area free of processes. $V_h = -60$ mV. *C*, drawing of part of a type-2 astrocyte mapped with a quisqualate-filled micropipette positioned near to the cell soma (*c*) and close to the tips of two processes (*b*, *d*). *D*, ionophoretic application of quisqualate (pulse width 80 ms, current amplitude 100 nA) evoked responses in all cell regions examined. The response was lost when the pipette was positioned away from the cell (*a*). $V_h = -60$ mV.

cells, we have also recorded from rat optic nerve O-2A progenitors. Cultures made from rat optic nerve are particularly convenient for studying O-2A progenitors since the optic nerve contains no neuronal cell bodies; this avoids any possible ambiguities in distinguishing between neurones and O-2A progenitors. Furthermore, differ-

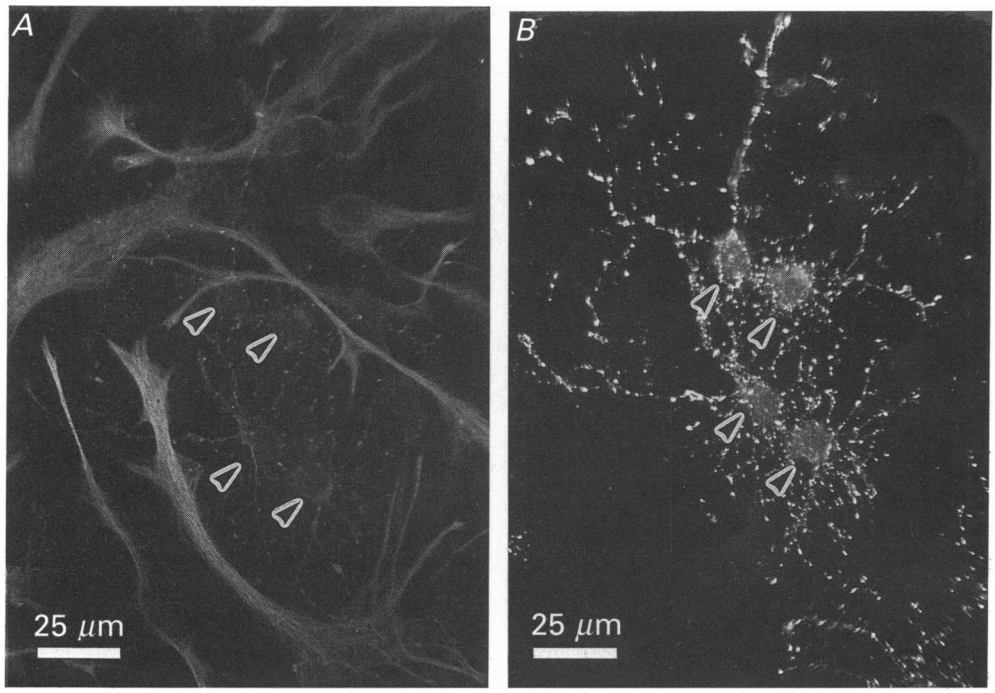

Fig. 4. *A*, photomicrograph of cells isolated from a 1-day-old rat cerebellum after 3 days *in vitro*, labelled with antibody to GFAP, and reacted with fluorescein-conjugated immunoglobulin. The labelled cells have a flattened epitheliod morphology and are type-1-like astrocytes. O-2A progenitor cells (arrowed), which are faintly visible, are GFAP negative. Under the culture conditions used no type-2 astrocytes developed. *B*, same field of cells as in *A*, labelled with the antibody LB1 and reacted with rhodamine conjugated immunoglobulin. O-2A progenitor cells are LB1 positive while type-1-like astrocytes remained unlabelled.

entiation of glial cells isolated from the rat optic nerve has been widely studied (for a review see Raff, 1989), and it was of interest to know if the expression of glutamate receptors by O-2A lineage cells was peculiar to the cerebellum.

Quisqualate or kainate applied to O-2A progenitor cells from rat cerebellum (n = 12 cells) or optic nerve (n = 8 cells) induced whole-cell currents in the range 20–30 pA for quisqualate (30 μM) and 20–50 pA for kainate (30 μM) ($V_h = -60$ mV). Figure 5*A* shows typical whole-cell current–voltage plots for responses to 30 μM-kainate in an optic nerve progenitor cell and to 30 μM-quisqualate in a cerebellar O-2A progenitor cell. Examples of whole-cell currents recorded over a range of potentials are shown in Fig. 5*B* (kainate, optic nerve progenitor) and *C* (quisqualate, cerebellar progenitor). As expected, the currents reversed near 0 mV and were accompanied by a noise increase, indicating the opening of ion channels. Figure 5*D* shows whole-cell

current recordings from a cerebellar progenitor cell exposed to 30 μM-NMDA or 30 μM-kainate. Kainate produced a large inward current (at −60 mV) with accompanying noise increase, while NMDA produced no detectable response. The lack of response to NMDA (observed in five other O-2A cells) resembled the situation in the type-2 astrocytes.

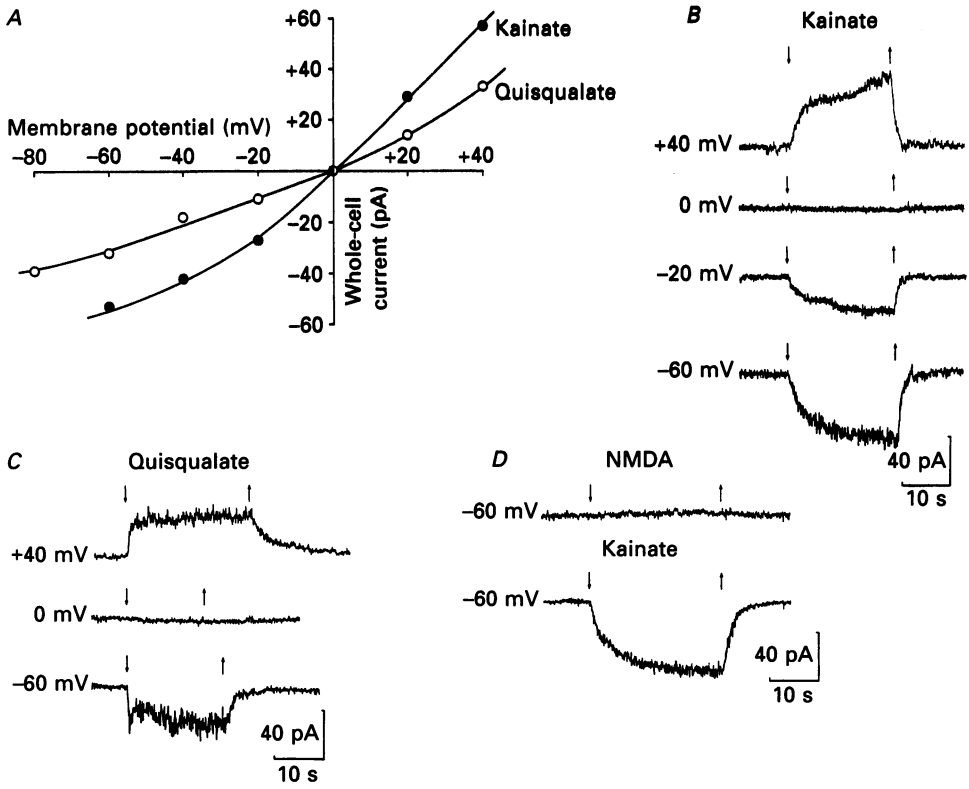

Fig. 5. *A*, current–voltage relationship for whole-cell currents evoked by 30 μM-kainate (●) in an optic nerve O-2A progenitor cell (same cell as in *B*) and by 30 μm-quisqualate (○) in a cerebellar O-2A progenitor cell (same cell as in *C*). *B*, whole-cell currents evoked by 30 μM-kainate (applied between arrows) in an optic nerve O-2A progenitor cell voltage clamped at +40, 0, −20 and −60 mV. Currents reversed direction at about 0 mV. Note the noise increase during kainate application. *C*, whole-cell currents evoked by 30 μM-quisqualate in a cerebellar O-2A progenitor cell voltage clamped at +40, 0 and −60 mV. Currents reversed direction near 0 mV. *D*, whole-cell current recordings from a single cerebellar progenitor cell voltage clamped at −60 mV and exposed to 30 μM-NMDA or 30 μM-kainate; NMDA failed to produce a response while kainate produced a large inward current and noise increase. For illustration, currents were low-pass filtered at 1 kHz (−3 dB).

Oligodendrocytes lack responses to glutamate

Oligodendrocytes in cerebellar cultures also develop from the O-2A progenitor cell (Levi *et al.* 1986; Levine & Stallcup, 1987). In our cultures, a majority of O-2A progenitors differentiated into oligodendrocytes rather than type-2 astrocytes, when

grown in low-serum medium (as previously described by Raff *et al.* 1983*a*). However, to be certain that the cells were oligodendrocytes and not type-2 astrocytes it was essential to use markers to identify the oligodendrocytes immunohistologically. Figure 6*A* shows a photomicrograph of an oligodendrocyte viewed under phase

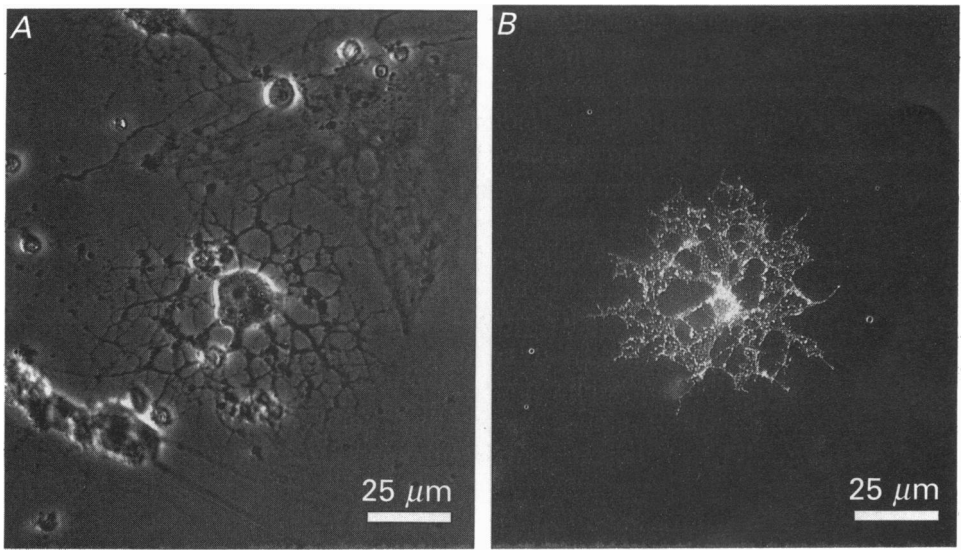

Fig. 6. *A*, photomicrograph of an oligodendrocyte seen under phase contrast optics. *B*, photomicrograph of an oligodendrocyte labelled with antibody to the glycolipid, galactocerebroside, which is characteristic of oligodendrocytes. Note the difference in process morphology between the oligodendrocyte and type-2 astrocytes (see Fig. 1). Oligodendrocytes possess intricate networks of radial processes whereas type-2 astrocytes possess long thin processes extending up to ~ 100 μm in length.

contrast optics while Fig. 6*B* shows an oligodendrocyte identified with the antibody to galactocerebroside, which specifically labels oligodendrocytes (Gal-C, Raff *et al.* 1978). In our cultures oligodendrocytes tended to have larger cell bodies than the type-2 astrocytes, and possessed less extensive, but more intricate, networks of radial processes.

Oligodendrocytes that were either cultured in low fetal calf serum (see above) or were present in cultures containing 10% fetal calf serum (which also contained type-1-like and type-2 astrocytes) produced no detectable response to glutamate, quisqualate or kainate (all at 30 μM, $n = 7$ cells). Furthermore we found no evidence for a glutamate uptake current in these cells. Similarly, oligodendrocytes acutely isolated from rat optic nerve do not express glutamate receptor channels (Barres, Koroshetz, Swartz, Chun & Corey, 1990).

Type-1-like astrocytes possess glutamate uptake currents

Astrocytes antigenically and morphologically similar to type-1 astrocytes found in cultures of rat optic nerve (Raff *et al.* 1983*b*) can be identified in cultures of rat cerebellar cells. These cells develop from their own precursor cell and, in culture, have

a flattened epithelioid, polygonal appearance and are phase-dark. Type-1-like astrocytes were identified immunohistochemically by labelling with antibody to GFAP, and by the fact that the cells were negative for the surface antibody label LB1 (see Fig. 4*A* and *B*); a further distinctive characteristic of these cells was their large capacitance (> 50 pF) and low input resistance (< 200 MΩ). The presence of an uptake current to glutamate has been observed in type-1-like cerebellar astrocytes (Cull-Candy *et al.* 1988). However, the possibility that part of this current may originate from the activation of glutamate receptors has not previously been examined.

During most of the present whole-cell recordings from type-1-like astrocytes the internal solution contained KCl (see Methods) rather than CsCl (unlike previous studies, see Cull-Candy *et al.* 1988), which we found to reduce the magnitude of the uptake current (data not shown). This may be expected if Cs^+ substitutes poorly for intracellular K^+ which has been shown to be involved in the glial uptake current in salamander glial cells (Barbour, Brew & Attwell, 1988; although see Schwartz & Tachibana, 1990). Application of glutamate (30 μM) to cells at negative holding potentials evoked large inward currents (> 500 pA at −60 mV, $n = 8$ cells). Furthermore, large clamp currents were required to hold the membrane potential away from the zero-current potential of the cell. It was therefore necessary to select for smaller cells to avoid using large holding currents to clamp the potential of the cell.

Figure 7*A* shows typical records of whole-cell uptake currents to glutamate in a cell held at −70, −41 and +28 mV (values corrected for pipette series resistance). The currents at −70 mV were roughly five times larger than those obtained in type-2 astrocytes in response to a comparable concentration of glutamate. As expected for an electrogenic glutamate uptake current (Brew & Attwell, 1987), the responses in Fig. 7*A* are accompanied by little apparent noise increase and remain inward-going at positive potentials (see also Cull-Candy *et al.* 1988). Figure 7*B* shows a current–voltage relationship for a type-1-like astrocyte in the presence of 30 μM-glutamate. From the *I–V* plot it is apparent that the currents remained inward-going at potentials as positive as +80 mV. However, as shown in Fig. 7*C*, quisqualate, kainate and NMDA (all at 30 μM) each failed to evoke a detectable current change when applied to this same cell (observed in seven other cells). It has previously been shown that these agonists are not taken up by the glutamate uptake carrier mechanism (see Balcar & Johnston, 1972, 1975; Brew & Attwell, 1987). We therefore found no evidence for glutamate receptors in type-1-like astrocytes maintained in culture for less than 4 days, although these cells produced large uptake currents to glutamate.

From neurochemical and electrophysiological studies it is known that several Na^+ ions are co-transported with each glutamate molecule in the carrier mechanism in glial cells (Balcar & Johnston, 1972; Brew & Attwell, 1987; for a review see Erecinska, 1987) and it was therefore of interest to determine whether the uptake carrier in type-1-like astrocytes showed selectivity for the ion which it co-transported. In particular Li^+ has previously been shown to be a poor substitute for Na^+ in amino acid transport systems (Peterson & Raghupathy, 1974). As shown in Fig. 8*A* and *B*, when we substituted Li^+ for external Na^+, the glutamate uptake

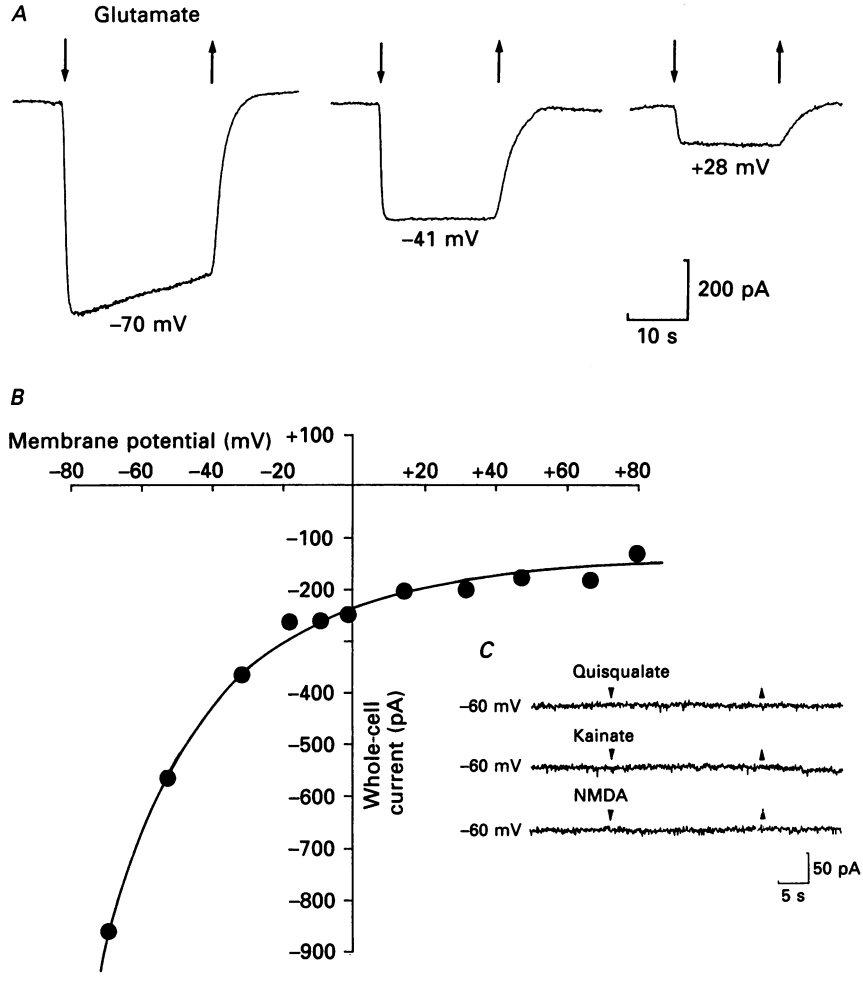

Fig. 7. *A*, whole-cell currents evoked by 30 μM-glutamate in a type-1-like astrocyte at holding potentials of −70, −41 and +28 mV (membrane potentials corrected for series resistance). The largest currents are subject to clamp errors of approximately 4 mV. Note the lack of noise increase during the application of glutamate. *B*, *I–V* relationship for whole-cell currents evoked by 30 μM-glutamate in a type-1-like astrocyte. The current decreased as the cell was held at more positive potentials; however, the current was still inward at a holding potential of +80 mV. *C*, quisqualate, kainate and NMDA (at 30 μM) each failed to evoke any current changes in a type-1-like astrocyte voltage clamped at −60 mV.

current was reduced by about 80% (n = 4 cells). Figure 8*A* shows an *I–V* plot for the glutamate uptake current in a cell bathed initially in external Na^+, and after the cell had been bathed for 10 min in Li^+ external solution (zero Na^+). Although the size of the current was reduced in Li^+ medium, the *I–V* relationship of the small residual current was apparently unchanged.

Li^+ was, however, able to substitute well for Na^+ and pass through glutamate receptor channels in type-2 astrocytes. Figure 8*C* shows a whole-cell *I–V* plot for a

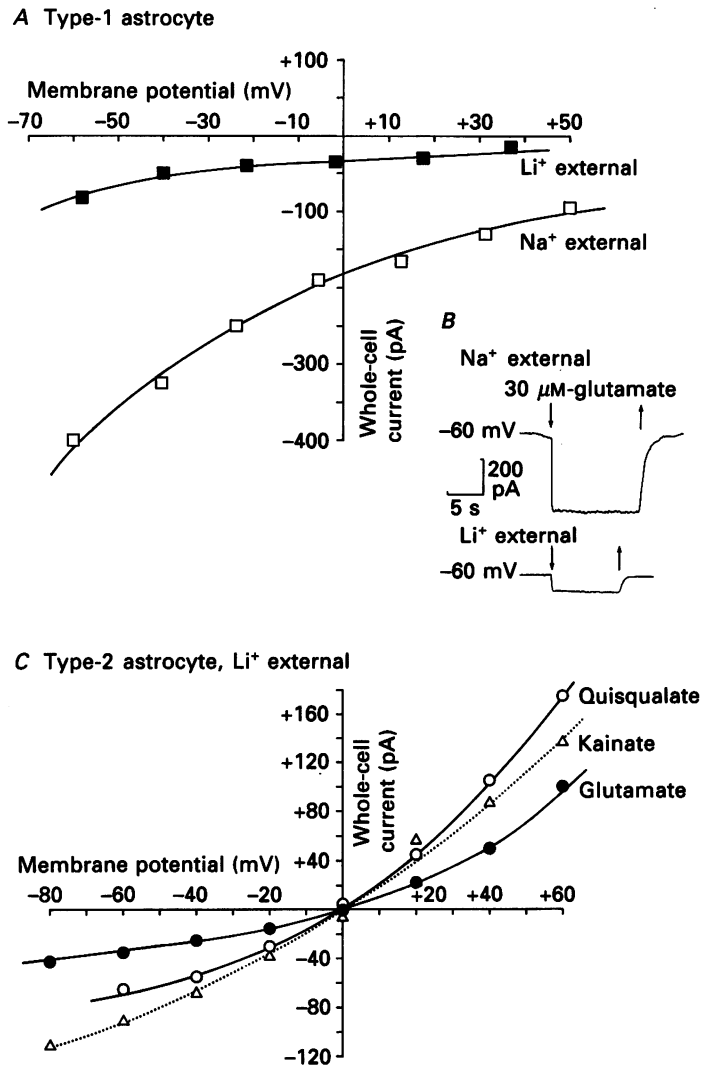

Fig. 8. *A*, *I–V* relationship for whole-cell currents induced by 30 μM-glutamate in a type-1-like astrocyte in Na^+-containing external solution (□) and after the cell had been perfused with Li^+ -containing external solution for 10 min (■). Substitution of Na^+ by Li^+ reduces the uptake current although the current is still inwards at positive potentials. *B*, examples of whole-cell currents evoked by 30 μM-glutamate (−60 mV same cell as *A*), in Na^+ external (upper trace) and Li^+ external (lower trace) bathing solutions. Exchange of external Na^+ by Li^+ reduced the uptake current from 400 to 80 pA. *C*, *I–V* relationship for whole-cell currents in a cerebellar type-2 astrocyte bathed in Li^+ external solution. Glutamate (●), kainate (△) and quisqualate (○), at 30 μM, each evoked whole-cell currents which reversed at 0 mV, indicating that Li^+ is able to pass through glutamate receptor channels in these cells.

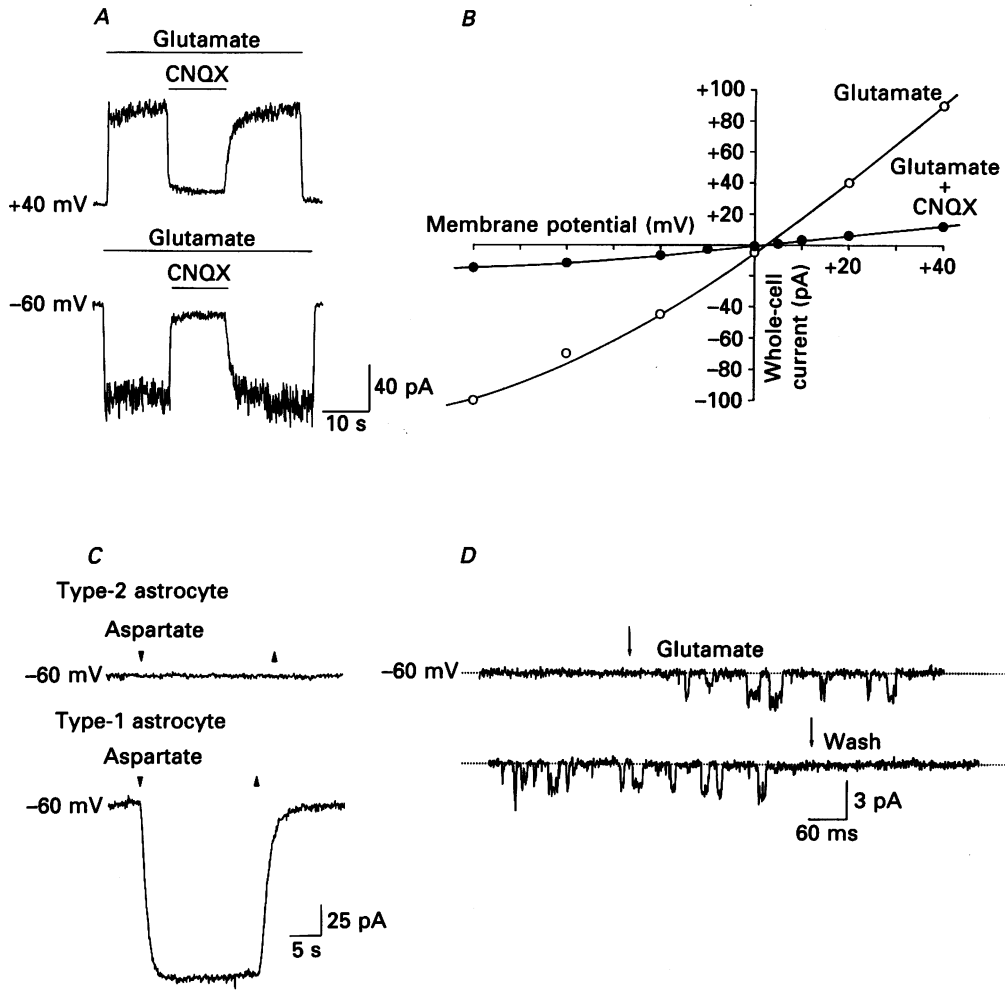

Fig. 9. Experiments to determine whether type-2 astrocytes have a detectable uptake current. *A*, whole-cell current traces at +40 and −60 mV. Glutamate (30 μM) and CNQX (30 μM) applications are indicated by bar. Note the rapid block of the glutamate current when CNQX and glutamate are present. The current at +40 mV during the application of CNQX is still outward (rather than the inward current expected for glutamate uptake) and in both traces currents in the presence of CNQX remain noisier than the baseline current. *B*, *I–V* plot for whole-cell currents (same cell as *A*) activated by 30 μM-glutamate (○) or 30 μM-glutamate and 30 μM-CNQX (●). CNQX inhibited glutamate currents by about 80 % but did not completely block them. Both glutamate and glutamate+CNQX currents reversed direction near 0 mV holding potential, indicating that the residual current in the presence of CNQX did not contain a large electrogenic uptake component. *C*, application of aspartate (30 μM) to a type-2 astrocyte (−60 mV) did not produce any current changes. However, perfusion of aspartate onto a type-1-like astrocyte on the same cover-slip (−60 mV) resulted in a large inward current with no accompanying noise increase, as expected if the aspartate current was generated by an electrogenic uptake carrier. *D*, current traces from an outside-out patch of membrane (−60 mV) excised from the cell soma of a type-2 astrocyte. Application of glutamate

cerebellar type-2 astrocyte exposed to glutamate, quisqualate and kainate in Li^+ external solution. Currents reversed at 0 mV and the amplitudes of the whole-cell currents were similar to those recorded in the normal Na^+-containing external solutions. Substitution of external Na^+ with Li^+ therefore proved a convenient method of discriminating between uptake currents and receptor-mediated currents in astrocytes.

Do type 2 astrocytes possess an electrogenic glutamate uptake carrier?

Since type-1-like astrocytes can produce a large uptake current to glutamate, it was necessary for us to determine what fraction, if any, of the glutamate response in type-2 astrocytes may also have resulted from the activation of an electrogenic uptake. If the glutamate current in type-2 astrocytes possesses a significant electrogenic component the whole-cell *I–V* relationship may be expected to reverse at potentials more positive than 0 mV. To examine this possibility the size of the receptor-mediated current was reduced with CNQX to increase the likelihood of identifying any uptake component. As shown in Fig. 9*A* and *B*, CNQX (30 μM), which is a non-NMDA antagonist in neurones (Honore, Davies, Drejer, Fletcher, Jacobsen, Lodge & Flemming, 1988) and also inhibits quisqualate and kainate whole-cell currents in type-2 astrocytes (Cull-Candy *et al.* 1989), greatly reduced glutamate-evoked currents in type-2 astrocytes (Fig. 9*A*). From the whole-cell current–voltage plot (Fig. 9*B*) the reversal potential for the residual glutamate-activated current remained near 0 mV (+4 mV in the example shown) in the presence of CNQX, suggesting that there was little, if any, contribution from an uptake current. Furthermore, although the residual glutamate current in the presence of CNQX shows a much reduced noise level at −60 mV, it is clearly outward-going at +40 mV (Fig. 9*A*).

In further support of the idea that type-2 astrocytes lacked a detectable electrogenic glutamate uptake carrier we were unable to detect a DC shift in the baseline current on a high recording gain when glutamate was applied to outside-out membrane patches removed from the soma of type-2 astrocytes. Figure 9*D* shows a typical example of such an experiment. Glutamate (30 μM) produced clear single-channel current openings exhibiting multiple conductance levels. However, the baseline current level (dotted line) remained unchanged throughout glutamate application.

Aspartate is an effective substrate for the glutamate carrier in the CNS (Balcar & Johnston, 1972), but is unlikely to activate receptor-mediated currents in cells lacking NMDA receptors. We have therefore examined the effect of L-aspartate (30 μM) on type-2 astrocytes, on a high recording gain. As shown in Fig. 9*C*, 30 μM-aspartate failed to evoke a detectable whole-cell current change ($n = 6$ cells) in type-2 astrocytes, while in type-1-like astrocytes in the same culture it gave large inward currents with no apparent noise increase (Fig. 9*C*, lower trace).

(30 μM) (at arrow) evoked single-channel currents which displayed multiple conductance levels, but there was no DC shift in the baseline current (indicated by the dotted line). Returning to the control solution (wash) resulted in a disappearance of the single-channel events with no change in DC level.

Glutamate responses in type-1-like astrocytes in longer term culture

We are currently examining glutamate responses in astrocytes maintained in culture for at least 7 days. After this period of time type-2 astrocytes seem to withdraw their processes and adopt a more flattened appearance and type-1-like astrocytes have formed a confluent monolayer as a result of rapid cell division. It was thus no longer possible to distinguish, unambiguously, between type-1-like and type-2 astrocytes on morphological grounds. Furthermore, since type-2 astrocytes only transiently express the LB1 antigen in culture (Lillien & Raff, 1990), by 7 days it was no longer possible to distinguish between type-1-like and type-2 astrocytes on the basis of antigenic phenotype. Nevertheless, it is likely that the rapidly dividing type-1-like astrocytes constitute the overwhelming proportion of cells in such cultures.

Preliminary results (data not shown) indicate that the majority of cells in these cultures responded to glutamate, quisqualate and kainate, generating inward currents at negative holding potentials. Glutamate-evoked currents in these cells were only partly blocked by CNQX suggesting that much of the glutamate current is due to electrogenic uptake. However, kainate currents are inhibited by the non-NMDA antagonist, CNQX, and are likely to result from activation of receptor channels. Therefore, at this stage in culture, these cells possess an electrogenic uptake carrier and express glutamate receptors. A more detailed account of these findings will be reported elsewhere.

DISCUSSION

From our experiments it appears that glutamate receptors are present both in cerebellar type-2 astrocytes (Usowicz *et al.* 1989), and in the O-2A progenitor cells (from cerebellum and optic nerve) that give rise to type-2 astrocytes, although they are absent from oligodendrocytes which are also derived from the same lineage (see also Cull-Candy *et al.* 1989). It therefore seems that these glutamate receptors cease to be expressed if the O-2A progenitor differentiates into an oligodendrocyte rather than a type-2 astrocyte. Both type-2 astrocytes and O-2A progenitor cells lacked NMDA receptors and in this respect differed from the neurones (mainly granule cells) present in some of the cerebellar cultures. A lack of response to NMDA has also been described in rat cerebral astrocytes (Sontheimer *et al.* 1988). Interestingly, our experiments indicate that in type-1-like astrocytes (which do not derive from the O-2A lineage) the glutamate response is generated solely by the activation of an electrogenic uptake mechanism in cells maintained in short-term ($\leqslant 4$ day) cultures. Thus it appears that in central glial cells in short-term culture the glutamate receptors are confined to O-2A lineage cells as previously proposed from neurochemical studies (Gallo *et al.* 1989) and electrophysiological experiments (Cull-Candy *et al.* 1989).

In some hippocampal (Cornell-Bell, Finkbeiner, Cooper & Smith, 1990) and cortical astrocytes (McNaughton, Lagnado, Socolovsky, Hunt & McNaughton, 1990) there is good evidence, from Ca^{2+} imaging experiments, that glutamate receptors may be linked to a second messenger system; such receptors may resemble the so-called glutamate 'metabotropic' receptor, which causes production of inositol-1,4,5-trisphosphate and diaclyglycerol through activation of phospholipase C (Sugiyama, Ito & Hirono, 1987). This has been proposed following the observations of

oscillations in intracellular Ca^{2+} in cultured hippocampal and cortical astrocytes exposed to glutamate, quisqualate and kainate. These oscillations may result from inositol phosphate production as the glutamate agonists produced an increase in inositol phospholipid break-down in these cells. However, there are various reasons for assuming that the glutamate responses that we have observed, in type-2 astrocytes and in the O-2A progenitors, were produced exclusively by the activation of 'fast' ion channels. First, single-channel currents evoked by glutamate, quisqualate and kainate were present in excised outside-out membrane patches (see also Usowicz *et al.* 1989) where most of the cytoplasmic components are expected to be lost. Responses linked to a second messenger system may be expected to undergo a gradual 'wash-out' during whole-cell recording. The responses in the present study were well maintained throughout the recording period (up to 1 h). Furthermore, we have been unable, so far, to detect resolvable single-channel currents under the patch pipette in cell-attached patches (not exposed to agonist) when the rest of the cell is exposed to either glutamate, quisqualate or *trans*-ACPD (*trans*-D,L-1-amino-1,3-cyclopentane-dicarboxylic acid), a selective agonist for the quisqualate 'metabotropic' receptor (Desai & Conn, 1990).

Interestingly, inhibitory GABA receptor channels are also present in mammalian astrocytes (from cerebral cortex) (Backus, Kettenmann & Schachner, 1988), as are a variety of other neurotransmitter receptors (reviewed by Murphy & Pearce, 1987). In several respects the responses of cerebral astrocytes to GABA parallel the glutamate responses observed in our experiments. Thus, two types of GABA-evoked whole-cell currents have been observed (Bormann & Kettenmann, 1988): at negative holding potentials GABA produces inward currents in both small round astrocytes and flat astrocytes. However, the currents recorded in the round astrocytes were accompanied by a noise increase whilst the currents in the flat cells showed no detectable increase in noise level. Although the distinction between type-1-like and type-2 astrocytes was not made, by analogy with our experiments it seems possible that the two types of GABA response may have been due to the activation of GABA receptor-operated channels in type-2 astrocytes (round cells) and the activation of an electrogenic GABA uptake mechanism in type-1-like astrocytes (flat cells) although this remains to be seen.

The functional significance of the glutamate receptors that occur in type-2 astrocytes and O-2A progenitor cells is unclear, although several interesting possibilities arise from the present and previous studies. Ultrastructure studies of CNS tissue have shown that astrocytic processes encircle nodes of Ranvier (Hildebrand, 1971) and there is indirect evidence that at least some of the perinodal astrocyte processes may belong to type-2 astrocytes (ffrench-Constant & Raff, 1986). Our ionophoretic mapping experiments indicate that glutamate receptors occur not only in the soma but also along the entire length of processes, including their extremities in type-2 astrocytes (e.g. Fig. 3). *In vivo* these astrocytic glutamate receptors would, therefore, be well placed to detect any axonal release or 'leakage' of glutamate. A Ca^{2+}-insensitive non-quantal 'leakage' of transmitter – acetylcholine in vertebrates, and glutamate in invertebrates – is known to occur at motor nerve terminals (see Katz & Miledi, 1977; Vyskočil, Nikolsky & Edwards, 1983; Antonov & Magazanik, 1988). Glutamate leakage has also been shown to occur from axons in regions distant from sites of synaptic contact such as in the giant axon of the squid

(Lieberman, Abbott & Hassan, 1989), where glutamate is thought to mediate axon to Schwann cell signalling, and also in the peripheral and central nerve trunks of vertebrates (Wheeler, Boyarski & Brooks, 1966; Weinreich & Hammerschlag, 1975) and invertebrates (Evans, 1974). This has led to the suggestion (Usowicz *et al.* 1989) that such receptors may be involved in neuronal glial signalling in white matter. If glutamate receptors are present in the processes of type-2 astrocytes *in vivo*, then activation of such receptors would result in ion movement across the glial cell membrane which may alter the concentrations of Na^+ and K^+ at the node of Ranvier, and hence the electrical excitability of the axon in the nodal region. Indeed, in kainic acid-lesioned rat hippocampus, kainate caused K^+ efflux from astrocytes and it has been suggested that this could be responsible for the late slow potential recorded in some neurones (MacVicar, Baker & Crichton, 1988). Conversely it has also been proposed that neuronal activity can influence glial cell function. In frog optic nerve the *I–V* relationship of glial cell sodium currents is shifted in a hyperpolarizing direction during axonal stimulation (Marrero, Astion, Coles & Orkand, 1989), possibly due to the axonal release of a mediator, or mediators, influencing the properties of the glial ion channels. However, the possibility that these changes in the electrical properties were caused by a change in the ionic environment around the glial cell could not be ruled out.

In addition to any neuronal–glial signalling interactions at the node of Ranvier, type-2 astrocytes may also play a role in the development and formation of the node. O-2A progenitor cells are thought to migrate within the CNS (Small, Riddle & Noble, 1987). It is possible that the glutamate receptors may allow the migratory O-2A progenitor cell to 'detect' the axon and establish a contact which could be crucial in determining the site of node formation. It also seems feasible that activation of glutamate receptors in the astrocytes or progenitor cells may act as a trigger for cell growth, since activation of glutamate receptors in neurones may serve a trophic function resulting in greatly enhanced neurite outgrowth (Pearce, Cambray-Deakin & Burgoyne, 1987; Balazs, Jorgenson & Hack, 1988; Patterson, 1988). In this respect it would clearly be of interest to determine whether calcium influx occurs in the type-2 astrocytes, either directly as a result of calcium permeation of glutamate channels, or indirectly from the activation of voltage-gated calcium channels, two types of which are known to be present in type-2 astrocytes (Barres, Chun & Corey, 1988). The recent finding that non-NMDA glutamate analogues can also activate ion channels in acutely dissociated O-2A progenitor cells (Barres *et al.* 1990) lends further support to the idea that such receptors may be of functional importance *in vivo*.

Unlike the glutamate currents in type-2 astrocytes or O-2A progenitor cells, those recorded in type-1-like astrocytes (up to 4 days *in vitro*) resulted from the activation of an electrogenic glutamate uptake carrier. The evidence for this is threefold: application of glutamate produced large inward currents with no detectable noise increase, the currents did not reverse direction at potentials as positive as +80 mV, and these currents were not activated by the glutamate analogues quisqualate, kainate or NMDA, which are not considered to be taken up by the high-affinity glutamate uptake mechanism (Balcar & Johnston, 1972, 1975; Brew & Attwell, 1987). Glutamate (and aspartate) uptake in the CNS is known to be dependent on extracellular Na^+ (Balcar & Johnston, 1972; for a review see Erecinska, 1987) and

inhibited by extracellular Li^+ (Peterson & Raghupathy, 1974), which has also been shown to inhibit the uptake of radiolabelled GABA into type-2 astrocytes (Gallo, Patrizio & Levi, 1990). Thus, our finding that replacement of external Na^+ with Li^+ greatly reduced the glutamate-evoked currents in type-1-like astrocytes is in agreement with the idea that the glutamate currents were predominantly or solely caused by electrogenic uptake of glutamate. The inability of Li^+ to substitute for Na^+ in the glutamate uptake carrier in type-1-like astrocytes suggests that the carrier is selective for the cation it co-transports along with glutamate and that the glutamate carrier present in type-1-like astrocytes may be somewhat similar to that characterized in salamander retinal glial cells (Schwartz & Tachibana, 1990). It has been proposed that electrogenic uptake carriers can operate in the reverse direction in some cells, allowing Ca^{2+}-insensitive release of neurotransmitter following depolarization (see Schwartz, 1987). Release of GABA from type-2 astrocytes has been suggested to occur via the activation of such a 'reverse uptake' mechanism following depolarization of cells by the glutamate agonists (Gallo *et al.* 1990). This suggestion for type-2 astrocytes is based on the fact that replacement of external Na^+ with Li^+ will inhibit kainate- and quisqualate-induced release of preaccumulated [^{3}H]GABA (Gallo *et al.* 1990). Our results would be consistent with this suggestion since Li^+ permeates well through glutamate channels in type-2 astrocytes causing sufficient glutamate-induced depolarization of the cells to permit 'conventional' transmitter release. Since we find that Li^+ inhibits uptake currents in other astrocytes, it seems possible that 'reverse uptake' would be similarly inhibited. We have not, however, looked directly for the presence of such a 'reversed' uptake in astrocytes.

In older cultures (> 7 days), where the majority of cells are type-1-like astrocytes, most cells responded to the non-NMDA agonists kainate and quisqualate. The kainate-evoked currents were antagonized by CNQX suggesting that the agonist-induced current resulted from receptor activation. Therefore, it appears that type-1-like astrocytes maintained in longer term cultures express glutamate receptor channels.

Finally, how similar are astrocytic and neuronal glutamate receptors? This is of some importance since studies of glutamate receptors expressed in oocytes, following injection of messenger (m) RNA, mainly use whole brain message which will be derived from both neurones and glia. Furthermore, a recently cloned putative kainate receptor (Gregor, Mano, Maoz, McKeown & Teichberg, 1989) appears to be of glial (cerebellar Bergmann glia) rather than neuronal origin (Somogyi, Eshhar, Teichberg & Roberts, 1990). Although molecular studies will be needed to determine similarities precisely, channel characteristics and pharmacology should give some clear insight into this question at a functional level. In this respect the non-NMDA receptor channels found in type-2 astrocytes have marked similarities to those found in neurones in terms of their multiple conductance levels (Usowicz *et al.* 1989) and the fact that they are blocked by the non-NMDA antagonist, CNQX (Cull-Candy *et al.* 1989). However, it remains to be seen whether neuronal and glial glutamate channels are similar in other respects.

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