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Glutamate-, Kainate- and NMDA-Evoked Membrane Currents in Identified Glial Cells in Rat Spinal Cord Slice

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Summary

The effect of L-glutamate, kainate and N-methyl-D-aspartate (NMDA) on membrane currents of astrocytes, oligodendrocytes and their respective precursors was studied in acute spinal cord slices of rats between the ages of postnatal days 5 and 13 using the whole-cell patch-clamp technique. L-glutamate (10^{-3} M), kainate (10^{-3} M), and NMDA ($2x10^{-3}$ M) evoked inward currents in all glial cells. Kainate evoked larger currents in precursors than in astrocytes and oligodendrocytes, while NMDA induced larger currents in astrocytes and oligodendrocytes than in precursors. Kainate-evoked currents were blocked by the AMPA/kainate receptor antagonist CNQX (10^{-4} M) and were, with the exception of the precursors, larger in dorsal than in ventral horns, as were NMDA-evoked currents. Currents evoked by NMDA were unaffected by CNQX and, in contrast to those seen in neurones, were not sensitive to Mg^{2+}. In addition, they significantly decreased during development and were present when synaptic transmission was blocked in a Ca^{2+}-free solution. NMDA-evoked currents were not abolished during the block of K^+ inward currents in glial cells by Ba^{2+}; thus they are unlikely to be mediated by an increase in extracellular K^+ during neuronal activity. We provide evidence that spinal cord glial cells are sensitive to the application of L-glutamate, kainate and transiently, during postnatal development, to NMDA.

Key words
Astrocytes – Oligodendrocytes – Development – Patch-clamp – Receptors

Introduction

Glial cells as well as neurones express a variety of ligand-gated ionic channels including glutamate-activated channels (for reviews see Hollmann and Heinemann 1994, Gallo and Russell 1995, Steinhäuser and Gallo 1996). A number of studies have found that the glutamate receptor agonists kainate or N-methyl-D-aspartate (NMDA) evoke different responses in glial cells and neurones (Berger *et al.* 1995). Generally, the sensitivity of glial cells to these agonists is smaller than that of neurones. It has been shown that, in glial cells, the glutamate receptor agonists kainate, quisqualate or AMPA mimic the glutamate response and that receptor activation leads to the opening of a cationic pore with an accompanying transient increase in intracellular Ca^{2+} and/or a block of K^+ channels (Enkvist *et al.* 1989, Glaum *et al.* 1990, Kim *et al.* 1994, Seifert and Steinhäuser 1995, Borges and Kettenmann 1995, Gallo *et al.* 1996, Porter and McCarthy 1996). Recently, functional NMDA receptors were described in cultured glial precursor cells (Wang *et al.* 1996).

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The majority of electrophysiological and pharmacological studies of glutamate receptors have been performed in glial cell cultures, and little information is available on the presence of different types of glial glutamate receptors *in situ*. With the development of the brain slice technique (Edwards *et al.* 1989), it has become possible to study glial cells in their natural cellular environment and to study glutamate-evoked membrane currents during postnatal development. On the other hand, it is necessary to keep in mind that in slice preparations, glutamate and its receptor agonists affect both neurones and glial cells, and that a complicated relationship between these cell types may occur. Studies in isolated optic nerve or in brain slices indicate that glutamate depolarizes glial cells (Orkand *et al.* 1981, Berger *et al.* 1992, Müller *et al.* 1992, Porter and McCarthy 1995). In slices of the mouse cerebellum, where Bergmann glial cells were studied with the whole-cell patch-clamp technique and confocal Ca^{2+} imaging, kainate induced an inward current which led to membrane depolarization, Ca^{2+} influx, and a decrease in resting K^+ conductance (Müller *et al.* 1992). In mouse or rat brain slices, patch-clamp studies revealed that the glutamate response of glial cells in the hippocampus and in the corpus callosum is mediated by a ligand-gated ion channel closely matching the pharmacology of the AMPA/kainate receptor (Steinhäuser *et al.* 1994, Berger 1995).

In acutely isolated slices of the mouse cerebellum, Müller *et al.* (1993) described inward currents in Bergmann glial cells induced by NMDA. Most properties of these currents were different from those described in neurones: the current-voltage relation was linear and the response was not affected by glycine or Mg^{2+}. Furthermore, a detectable change in cytosolic Ca^{2+} after the application of NMDA was not observed, while kainate induced a prominent change in Ca^{2+} in the same cell. A number of studies have also shown that ligand-gated channel expression can undergo developmental changes in cultured glial cells as well as in glial cells *in situ* (for review see Berger *et al.* 1995).

Our previous study of rat spinal cord slices, using the whole-cell patch-clamp technique as well as immunohistochemical methods, distinguished four types of glial cells during early postnatal days 3 to 18 (P3–P18). These cell types were immunohistochemically distinguished as astrocytes, oligodendrocytes and their respective precursors and had clearly different membrane current patterns (Chvátal *et al.* 1995). We have also demonstrated that glial cells in the rat spinal cord slice respond to the inhibitory neurotransmitters γ-aminobutyric acid (GABA) and glycine (Pastor *et al.* 1995). There is apparently no information about the glial response to kainate and NMDA in spinal cord *in situ*. The aim of this study was therefore to analyze current responses produced by the glutamate receptor agonists kainate and NMDA in glial cells in spinal cord slices during the first two postnatal weeks by means of the whole-cell patch-clamp technique. In addition, we investigated the pharmacological properties of kainate- and NMDA-evoked inward currents and their spatial distribution within the spinal cord slice.

Materials and Methods

Preparation of spinal cord slices and electrophysiological set-up

Young rats were sacrificed at postnatal days 5 to 13 (P5–P13) by decapitation. The spinal cords were quickly dissected and washed in artificial cerebrospinal fluid (ACF) at 8–10° C. A 4–5 mm long segment of the lumbar cord was embedded in 1.7% agar at 37° C (Agar Noble, Difco, Detroit, USA). Transverse 200 μm thick slices were made using a vibroslice (752M, Campden Instruments, U.K.). Slices were transferred onto a nylon net immersed in cold (5° C) ACF and slowly warmed to room temperature. For patch-clamp recording, slices were placed in a chamber mounted on the stage of a microscope (Axioskop FX, Carl Zeiss, Oberkochen, Germany) and fixed in the chamber using a U-shaped platinum wire with a grid of nylon threads (Edwards *et al.* 1989). The chamber was continuously perfused with oxygenated ACF. All experiments were carried out at room temperature (~22° C). Cell somata in the spinal cord slice were approached by the patch electrode using an INFRAPATCH system (Luigs & Neumann, Ratingen, Germany). The cells in the slice and recording electrode were imaged with an infrared-sensitive video camera (C2400-03, Hamamatsu Photonics, Hamamatsu City, Japan) and displayed on a standard TV/video monitor (GoldStar, Korea).

Selected cells with a membrane potential more negative than −60 mV had a clear, dark membrane surface and were located 5–10 μm below the slice surface. Positive pressure was applied to the recording pipette while it was lowered towards the slice. The cellular debris was thus blown away and the tip could be placed onto the surface of a cell soma. Membrane currents were measured with the patch-clamp technique in the whole-cell recording configuration (Hamill *et al.* 1981). Current signals were amplified with EPC-7 amplifier (List Electronics, Darmstadt, Germany), filtered at 3 kHz and sampled at 5 kHz by an interface (TIDA, HEKA Elektronik, Lambrecht/Pfalz, Germany) connected to an AT-compatible computer system, which also served as a stimulus generator.

Solutions and electrodes

The artificial cerebrospinal fluid (ACF) contained (in mM): NaCl 117.0, KCl 3.0, $CaCl_2$ 1.5, $MgCl_2$ 1.3, Na_2HPO_4 1.25, $NaHCO_3$ 35.0, D-glucose 10.0, L(+)-ascorbic acid sodium salt 0.2, thiourea 0.2. The solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 (Linde Technoplyn, Prague, Czech Republic) to maintain a final pH of 7.4. In pharmacological studies, 3 mM $MgCl_2$ or 0.5 mM $BaCl_2$ (Sigma Chemical Company, St. Louis, MO, USA) was dissolved in the bath solution; their addition was compensated for by reducing Na^+ concentration to maintain a constant ionic strength. Ca^{2+}-free ACF (0 mM $CaCl_2$) also contained 1 mM ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid (EGTA, Sigma Chemical Company). The following substances were added to the bath solution in the concentrations indicated in the text: L-glutamate, kainate, N-methyl-D-aspartate (NMDA, Sigma Chemical Company) and 6-cyano-7-nitroqinoxaline-2,3-dione (CNQX, Research Biochemicals International, Natick, MA, USA). All drugs were applied by continuous perfusion of the recording chamber (~2 ml volume) with a perfusion rate of 5 ml/min.

Recording pipettes (4–6 MΩ) were pulled from borosilicate capillaries (Kavalier, Otvovice, Czech Republic) using a Brown-Flaming micropipette puller (P-80/PC, Sutter Instruments Company, Novato, U.S.A.). The internal solution had the following composition (in mM): KCl 130.0, $CaCl_2$ 0.5, $MgCl_2$ 2.0, EGTA 5.0, HEPES 10.0. The pH was adjusted with KOH to 7.2.

Localization of glial cells

Glial cells within the spinal cord slice were localized during electrophysiological recording. An image of the slice was obtained with an infrared camera, then transferred to a PC AT *via* Video Galaxy Gamma TV/Video Interface (Aztech Systems Ltd., Singapore) and stored for analysis.

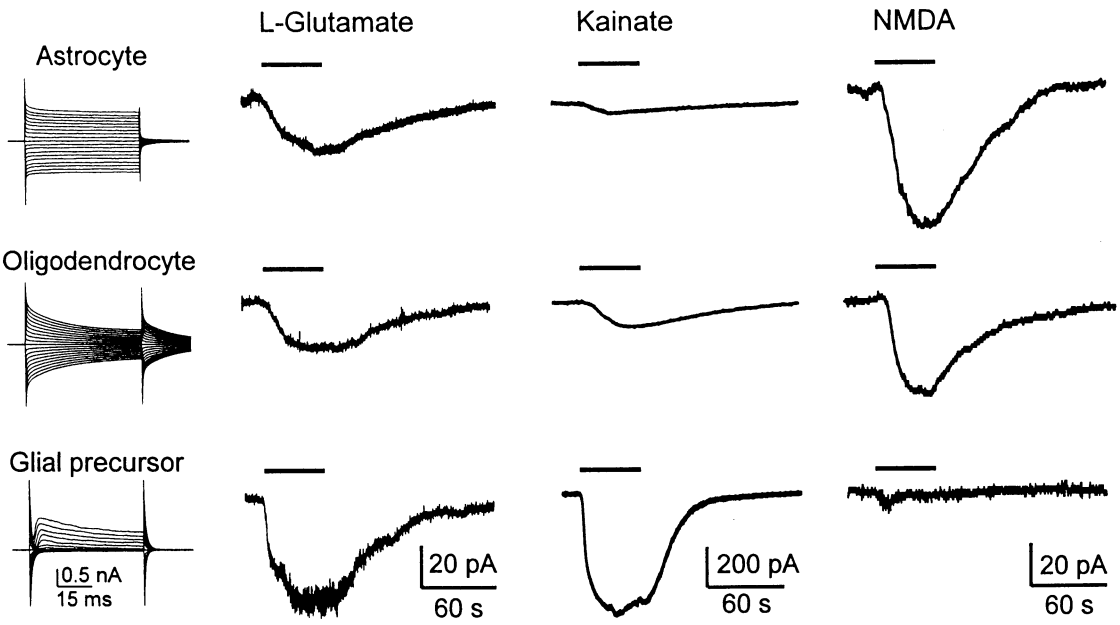

Fig. 1. *Membrane current patterns of an astrocyte, oligodendrocyte and glial precursor cell (left) and their typical responses to L-glutamate (10^{-3} M), kainate (10^{-3} M) and NMDA ($2x10^{-3}$ M). Cells from 6- to 7-day-old animals were voltage-clamped at −70 mV. To generate the typical current pattern for each type of cell shown on the left, the membrane was clamped for 50 ms to increasing de- and hyperpolarizing potentials ranging from −160 mV to +20 mV in 10 mV increment. Current traces are not corrected for leakage and capacitance currents. The horizontal bar indicates the time of application of the test substance. Note the difference in the calibrations for the ligand-activated currents.*

Identification of glial cells

Based on the membrane current patterns and morphological and immunohistochemical features, the following glial cells have been distinguished in rat spinal cord slice at P5–P13: astrocytes, oligodendrocytes, and their respective precursors (Chvátal *et al.* 1995, Pastor *et al.* 1995). Astrocytes displayed symmetrical passive non-decaying K^+-selective currents in both the depolarizing and hyperpolarizing direction (see Fig. 1). Oligodendrocytes could be identified by symmetrical passive but decaying K^+ currents in the depolarizing and

hyperpolarizing direction and by the presence of tail currents when the membrane current was clamped back to the holding potential. Glial precursor cells had a complex pattern of voltage-gated channels, namely Na^+, inwardly rectifying, delayed outwardly rectifying and A-type K^+ channels, and it was sometimes difficult to differentiate between them. Since the responses of these cells to glutamate receptor agonists did not differ substantially, we did not distinguish between the glial precursor cell types in the present study.

Statistical analysis

Results are expressed as the mean±S.E.M. Statistical analysis of the differences between groups was evaluated using the one-way analysis of variance (ANOVA) test. Values of $p<0.05$ were considered significant.

Linear regression was used to find the line that best predicts evoked inward current amplitudes as a function of the age of the animal. The p value was used to determine whether the slope of the linear regression was significantly different than zero. Values of $p<0.05$ were considered significant.

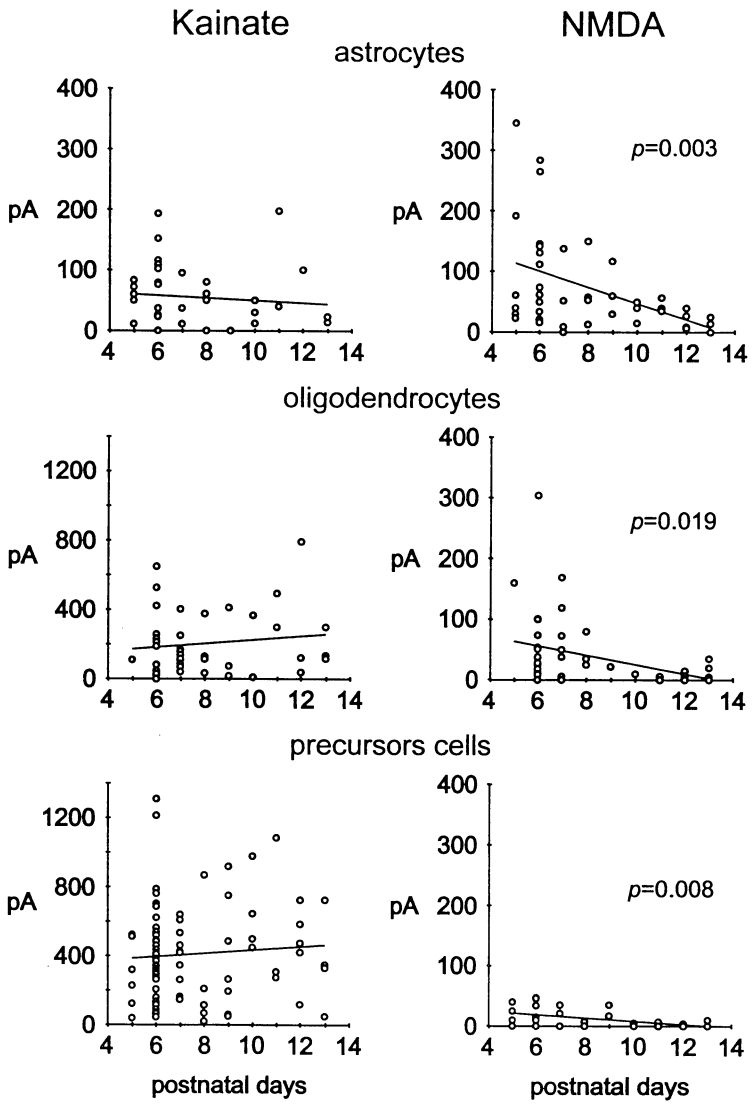

Fig. 2. *Kainate- (10^{-3} M) and NMDA-evoked ($2x10^{-3}$ M) inward currents in astrocytes, oligodendrocytes and glial precursor cells in the spinal cord during postnatal development. The amplitudes of the ligand-activated currents as a function of animal age and the corresponding linear regressions are shown for each type of glial cell. If the slope of the linear regression is significantly different than zero, the p value is indicated in the graph. Note the differences in scale of the amplitude plots of kainate-evoked currents in astrocytes and oligodendrocytes.*

Results

Inward currents evoked by L-glutamate, kainate, and NMDA

At a holding membrane potential of −70 mV, L-glutamate (10^{-3} M) evoked inward currents in all types of glial cells (Fig. 1). In astrocytes (n=20), 95 % of cells responded to the application of glutamate with an inward current of 52±8.0 pA. In oligodendrocytes (n=33), the mean amplitude of the current was 36±6.6 pA in 94 % of cells. In glial precursor cells (n=51), glutamate evoked currents of 54±5.1 pA in 96 % of cells. Glutamate-evoked inward currents in glial precursor cells were characterized by prominent single-channel noise.

Kainate (10^{-3} M) evoked inward currents in all types of glial cells (Fig. 1). In astrocytes (n=37), 84 % of cells responded to kainate application with an

inward current of 65±9.0 pA. In oligodendrocytes (n=44), kainate evoked currents with an amplitude of 179±23 pA in 98 % of cells. In 100% of glial precursor cells (n=85), kainate-evoked inward currents were significantly larger than in astrocytes and oligodendrocytes, with an amplitude of 411±29 pA. Kainate-evoked currents in all types of cells did not significantly change with age (Fig. 2).

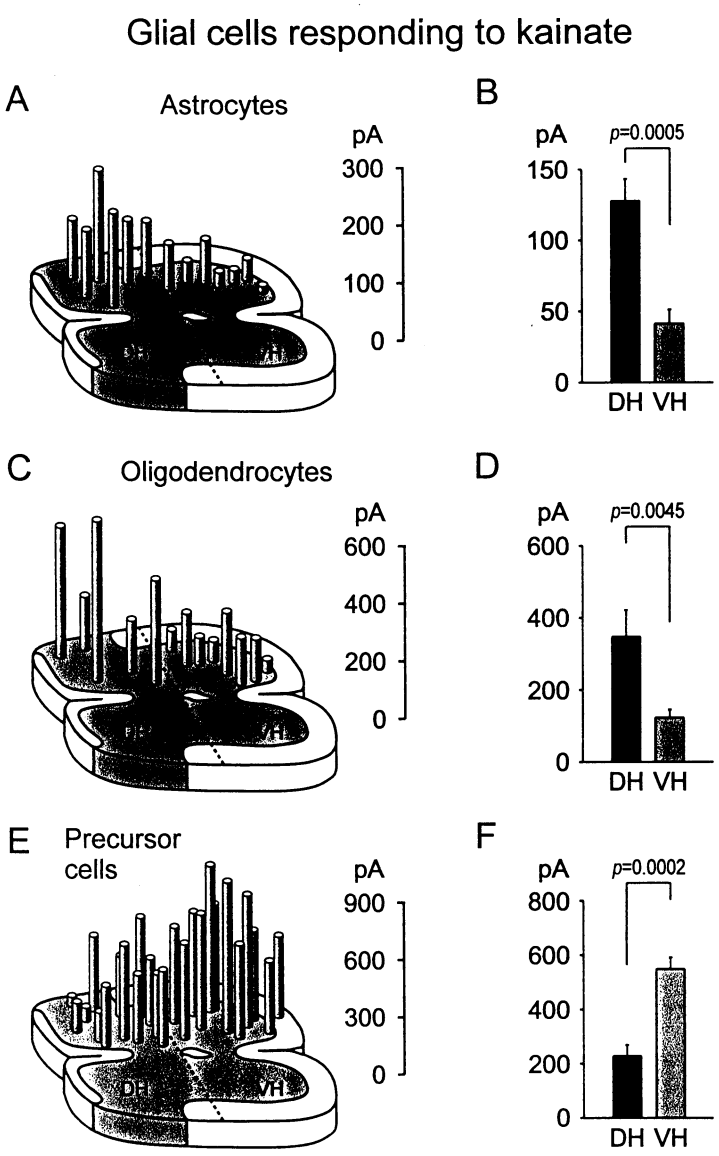

Fig. 3. *Location of astrocytes (A), oligodendrocytes (C), and precursor cells (E) with kainate-evoked (10^{-3} M) inward currents within the spinal cord slice at P6–P7. Each column represents the recorded inward current from an individual glial cell. Mean values ± S.E.M. for dorsal (DH) and ventral horn (VH) and significant differences between them are shown for each cell type in the graphs on the right (B, D, F).*

NMDA ($2x10^{-3}$ M) also evoked inward currents in all types of glial cells (Fig. 1). In astrocytes (n=42), 95 % of cells responded to NMDA application with an amplitude of 77±13 pA. In oligodendrocytes (n=45), the mean amplitude of the current was 49±9.6 pA in 84 % of cells. In glial precursor cells (n=30), NMDA evoked currents of 22±3.6 pA in 57 % of cells. As is evident in Fig. 2, NMDA-evoked inward currents in all glial cell types significantly decreased with age.

Kainate and NMDA responses are dependent on glial cell location

The location of glial cells responding to kainate and NMDA was studied in a group of animals of the same age (P6–P7) showing the largest responses. In both astrocytes and oligodendrocytes, kainate- and NMDA-evoked currents were significantly larger in dorsal horns than in ventral horns. The mean amplitude of kainate-evoked inward currents in dorsal horn astrocytes was 128±15 pA (n=6), while in ventral horns it was only 41.3±9.9 pA (n=7) (Figs 3A, 3B). Similarly, the mean amplitude of NMDA-evoked inward currents in astrocytes in dorsal horns was 179±31 pA (n=6), in ventral horns 56.2±6.6 pA (n=5) and in ventral horn white matter 8.3±4.6 pA (n=3) (Figs 4A, 4B).

Kainate-evoked currents in oligodendrocytes were also significantly larger in dorsal horns (348±73

pA, n=5) than in ventral horns (123±22 pA, n=8, Figs 3C, 3D). Similarly, the mean amplitude of NMDA-evoked currents in oligodendrocytes in dorsal horns was 121±34 pA (n=7), while in ventral horns it was 35±10 pA (n=8), and in ventral horn white matter only 4.8±2.1 pA (n=5) (Figs 4C, 4D).

In contrast to astrocytes and oligodendrocytes, kainate-evoked currents in glial precursor cells were significantly larger in the ventral horns of the spinal cord slice than in dorsal horns (Figs 3E, 3F). The mean amplitude of kainate-evoked inward currents in ventral horns was 549±43 pA (n=11), while in dorsal horns it was only 288±41 pA (n=13). NMDA-evoked currents in precursor cells were too small to study the differences between various regions.

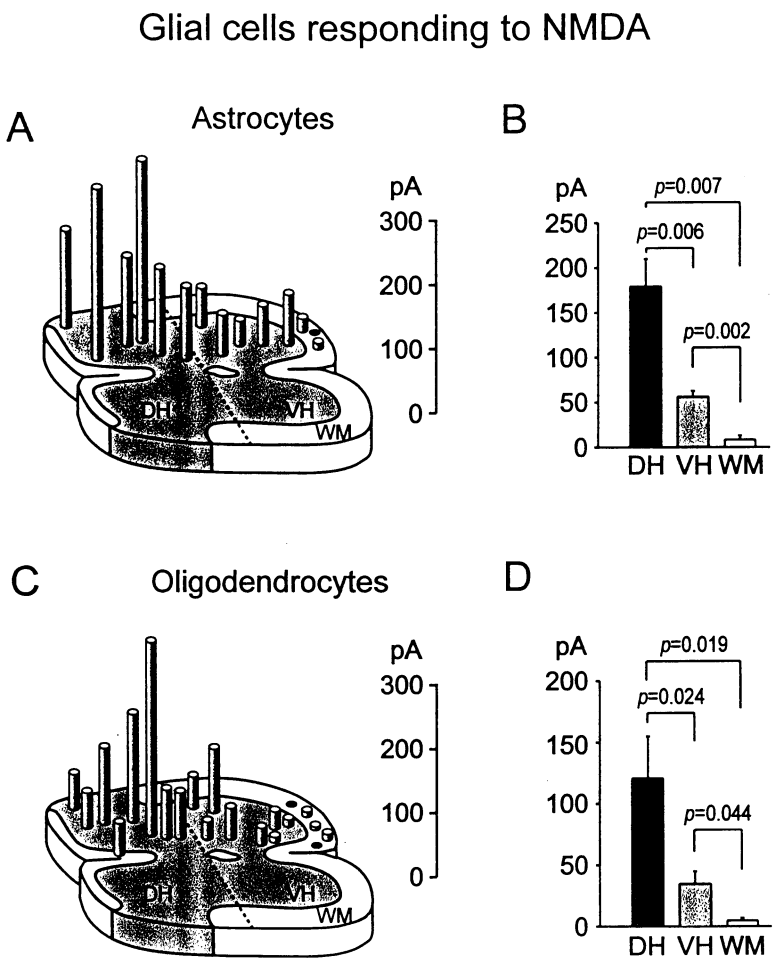

Fig. 4. *Location of astrocytes (A) and oligodendrocytes (C) with NMDA-evoked ($2x10^{-3}$ M) inward currents within the spinal cord slice at P6–P7. Each column represents the recorded inward current from an individual glial cell. Mean values ± S.E.M. for dorsal horns (DH), ventral horns (VH) and white matter (WM) and significant differences between them are shown for each cell type in the graphs on the right (B, D).*

Pharmacological properties of kainate- and NMDA-evoked inward currents

The pharmacological properties of kainate- and NMDA-evoked inward currents were studied in the dorsal horns of P6–P7 animals, where astrocytes and oligodendrocytes were most sensitive to both substances. We are aware of the fact that in our slice preparation, kainate as well as NMDA affected both neurones and glial cells, and thus inward currents recorded in glial cells might be due to an indirect effect of substances released by neurones (Fig. 5A).

To test whether the observed responses of glial cells are mediated through the release of glutamate from neurones, the presumed effect of glutamate on glial kainate receptors was blocked by the AMPA/kainate receptor antagonist, CNQX. Inward currents evoked by kainate (n=3) were reversibly blocked by CNQX (10^{-4} M) (Fig. 5B). In contrast to the application of kainate, we observed desensitization of NMDA-induced currents when NMDA was applied repeatedly (Fig. 5C). It was therefore not possible to compare NMDA responses after repeated applications. However, mean NMDA-evoked inward currents were not significantly affected by CNQX (10^{-4} M) application and their amplitude was 212±48 pA (n=4, three astrocytes, one oligodendrocyte) (Fig. 5D).

As was described by Inokuchi *et al.* (1992), a Ca^{2+}-free bathing solution prevents the release of glutamate and aspartate from neurones in the cat spinal cord slice. Therefore, in another series of experiments, we abolished the possible release of glutamate from neurones by blocking synaptic transmission using Ca^{2+}-free ACF containing EGTA (10^{-3} M). NMDA-evoked currents in Ca^{2+}-free

solution were not significantly different from those obtained under normal ACF and reached a mean amplitude of 120±52 pA (n=3, one astrocyte, two oligodendrocytes) (Fig. 5E).

All recordings in our experiments were performed in the presence of 1.3 mM Mg^{2+} in the bathing solution. Mg^{2+} is known to block NMDA-activated ion channels in a voltage-dependent manner in neurones (Mayer and Westbrook 1987). In additional experiments (n=3, two astrocytes, one oligodendrocyte) we therefore increased Mg^{2+} concentration up to 3.0 mM. The application of NMDA under high Mg^{2+} concentration did not evoke significantly smaller inward currents (127±12 pA, Fig. 5F).

To test whether the NMDA-evoked inward currents in glial cells are mediated through the increase in extracellular K^+ concentration caused by NMDA-evoked neuronal activity, we blocked K^+ inward currents in glial cells by Ba^{2+}. Recently Marrero and Orkand (1996) found that the addition of Ba^{2+} completely blocked glial cell depolarization during nerve stimulation in the frog optic nerve. Figure 5G shows that the application of Ba^{2+} ($5x10^{-4}$ M) did not block NMDA-evoked inward currents in the spinal cord glial cells (65±25 pA, n=3, one astrocyte, two oligodendrocytes).

We therefore suggest that glial kainate- and NMDA-evoked inward currents are mediated by the activation of ionotropic subpopulations of glutamate receptors located on glial cells.

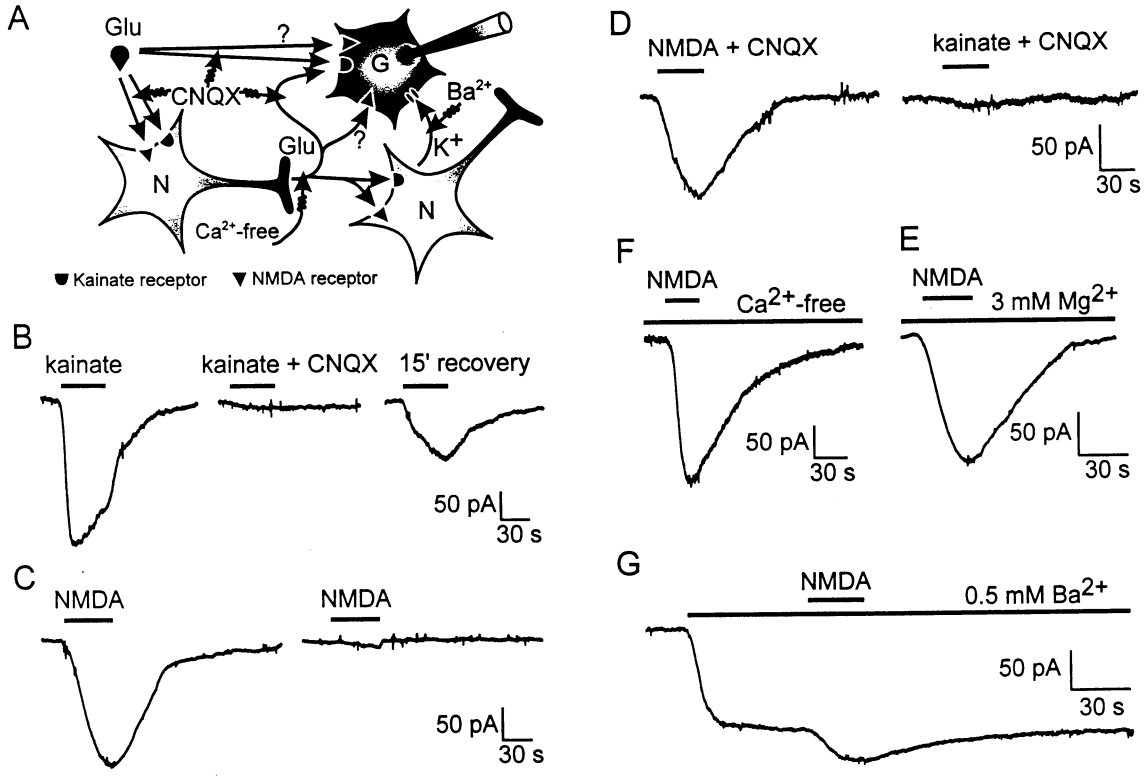

Fig. 5. *Pharmacological properties of kainate- and NMDA-evoked inward currents.*
A. Scheme of glutamate-evoked effects on neurones (N) and glial cells (G) in the spinal cord slice. Inward currents evoked in glial cells by glutamate (Glu), kainate or NMDA might result from direct activation of glial receptors and/or activation of neuronal receptors, which in turn may release glutamate and K^+. CNQX was used to block kainate receptors on glial as well as on neuronal cells, Ba^{2+} was used to block glial K^+ inward currents, and a Ca^{2+}-free bathing solution containing EGTA was used to block synaptic activity and release of glutamate from neurones. Activation of presumed NMDA receptors on glial cells is indicated by the question mark.
B. Kainate-evoked (10^{-3} M) inward current was blocked by CNQX (10^{-4} M). This effect of CNQX was reversible.
C. First and second application of NMDA ($2x10^{-3}$ M) to the same cell showing desensitization.
D. NMDA-evoked ($2x10^{-3}$ M) inward current was not blocked by CNQX (10^{-4} M) in contrast to kainate-evoked inward current recorded from the same cell.
E, F, G. Neither 5 min preincubation with Ca^{2+}-free ACF containing EGTA, 5 min preincubation with 3 mM of Mg^{2+} nor application of 0.5 mM of Ba^{2+} significantly affected NMDA-evoked inward currents. Membrane currents were recorded in dorsal horn of P6–P7 animals at a holding potential of −70 mV. In B, C and D the interval between applications was 5 min.

Discussion

L-glutamate, kainate and NMDA currents in glial cells

The effect of L-glutamate and the glutamate receptor agonists, kainate and NMDA, on glial cells in brain slices *in situ* has been described previously in several different regions of the CNS but not in the spinal cord (for review see von Blankenfeld *et al.* 1995). In the present study performed in the rat spinal cord, we have found that L-glutamate and its receptor agonists, kainate and NMDA, induced inward currents in all types of glial cells. In contrast to astrocytes and oligodendrocytes, an increase in single-channel noise was observed in glial precursor cells during application of L-glutamate, indicating the activation of ionic channels (see Fig. 1). In astrocytes and oligodendrocytes single-channel noise was not observed, and we therefore cannot exclude the possibility that Na^+-dependent glutamate uptake (Wyllie *et al.* 1991) and/or metabotropic glutamate receptors (Premkumar and Chung 1995) may contribute to the glutamate-evoked inward currents. The concentrations of glutamate and glutamate receptor agonists used in our study evoked maximal inward currents. The application of glutamate, i.e. a substance that activates the NMDA as well as the AMPA/kainate subpopulations of glutamate receptors, evoked smaller currents than the application of kainate. The explanation for this might be that in contrast to kainate, which is normally not present in the CNS, the concentration of glutamate is decreased in the CNS either by cellular uptake or by degradation. In our experiments, kainate induced larger inward currents in glial precursors than in astrocytes and oligodendrocytes. These currents were blocked by the AMPA/kainate receptor antagonist CNQX, as described for Bergmann glial cells (Müller *et al* 1992).

There is no compelling experimental evidence for the presence of NMDA receptors in glial cells (Berger *et al.* 1992, Gallo and Russell 1995, Steinhäuser and Gallo 1996). However, some experiments performed on glial cells in culture (Wang *et al.* 1996) or *in situ* (Kirchhoff *et al.* 1993, Müller *et al.* 1993, Steinhäuser *et al.* 1994, Porter and McCarthy 1995, Kirchhoff *et al.* 1996) indicate that such receptors might be present. It was also shown in the study of Conti *et al.* (1996) that astrocytes investigated in the cerebral cortex of adult rats express NR1 and NR2A/B subunits of native NMDA receptors. An immunoreaction product indicative of NR1 and NR2A/B expression was found in some distal astrocytic processes, but only rarely in astrocytic cell bodies (Conti *et al.* 1997). In Bergmann glial cells of mouse cerebellum and in protoplasmic astrocytes of the parietal and cingular neocortex of three-week-old mice *in situ*, NMDA induced inward currents in the range of 28–300 pA (Kirchhoff *et al.* 1993, Müller *et al.* 1993). These currents were distinct from the currents observed in neurones: they were Mg^{2+}- and glycine-insensitive, and they were also present during the application of the AMPA/kainate receptor antagonist CNQX. Similarly, we have found in our experiments that NMDA-induced inward currents, which were significantly larger in astrocytes and oligodendrocytes than in glial precursor cells, were not affected by the presence of CNQX. In addition, NMDA-evoked inward currents in our experiments were Mg^{2+}-insensitive, indicating that they have pharmacological properties of the NMDA responses described previously in glial cells in cerebellum (Müller *et al.* 1993). As calculated from the affinity of the neuronal NMDA receptor to Mg^{2+}, an increase in Mg^{2+} concentration from 1.3 to 3 mM used in our experiments, resulted in 93% inhibition of neuronal NMDA receptor. The lack of sensitivity of the presumed glial NMDA receptor to Mg^{2+} might be explained by the presence of the $\epsilon 3$ receptor subunit, which was found in mouse cerebellum to be resistant to Mg^{2+} block (Kutsuwada *et al.* 1992). We did not test the sensitivity of the NMDA-evoked currents to glycine in our experiments, because glycine receptors are found in spinal cord glial cells (Pastor *et al.* 1995) and their activation would interfere with our measurements. In Müller cells of the retina, which belong to the radial glial cells, NMDA application activated channels with a pharmacological profile identical to those seen in neurones (Uchihori and Puro 1993).

In the study of Porter and McCarthy (1995), confocal microscopy and the use of calcium-sensitive fluorescent dyes revealed that the application of NMDA to hippocampal slices evoked Ca^{2+} transients in astrocytes of the CA1 and CA3 stratum oriens. These astrocytic responses were obtained in the presence of tetrodotoxin to block neurone action potentials. In accordance with these findings, we have observed in our experiments that NMDA-evoked currents were present even when synaptic activity was blocked by Ca^{2+}-free bathing solution containing EGTA.

Another possible explanation for the effect of NMDA might be that glial cells are responding to an elevated extracellular K^+ concentration, due to the release of K^+ from activated neurones. The application of NMDA in our experiments evoked substantial inward currents only in astrocytes and oligodendrocytes, while an increase in K^+ concentration in ACF from 3 mM to 55 mM evoked similar inward currents in all types of glial cells, including glial precursor cells (Chvátal *et al.* 1995). Moreover, we found in our study larger NMDA-evoked currents in the first postnatal week, while K^+-evoked inward currents did not change during postnatal development. Ba^{2+} was shown to completely block

glial cell depolarization caused by an increase in $[K^+]_e$ during nerve stimulation in the frog optic nerve (Marrero and Orkand 1996). NMDA-evoked currents in our study were, however, not blocked by the application of Ba^{2+}.

Our study therefore indicates that glial cells in the spinal cord might express NMDA receptors with different pharmacological properties than those described in neurones.

Developmental changes and specific localization of kainate and NMDA responses

Our results indicate that kainate-activated currents did not significantly change in any type of glial precursor cell during early postnatal development, while NMDA-evoked inward currents in astrocytes and oligodendrocytes significantly decreased with age. Developmental changes in GABA- and glycine-evoked inward currents in the rat spinal cord were also observed in astrocytes and in glial precursor cells (Pastor *et al.* 1995). Our results are also in agreement with receptor autoradiography experiments performed in the developing mouse spinal cord (Gonzalez *et al.* 1993), which demonstrated that NMDA-sensitive [^{3}H]glutamate binding levels peaked around P6–P8 and then declined in adults. In mixed cultures of cerebellar glial cells, glutamate responses were found in O-2A progenitors but not in oligodendrocytes (Wyllie *et al.* 1991). On the other hand, in corpus callosum brain slices, cells of the oligodendrocyte lineage of 6- to 13-day-old mice did not show developmental regulation of glutamate receptors (Berger *et al.* 1992). This could be explained by the earlier maturation of the murine CNS. Similarly, there was no apparent developmental regulation from P6 to P20 of the amplitude or the pharmacological profile of the NMDA-induced responses in mouse Bergmann glial cells *in situ* (Müller *et al.* 1993, Berger *et al.* 1995). It appears that during postnatal development, NMDA receptors in the rat spinal cord are expressed in a narrow time-window, most likely during the formation of neuronal synapses. During postnatal development, changes were observed not only in the number of expressed ligand-gated channels, but also in their permeability. In glial precursor cells of the rat dentate gyrus, for instance, the variation of the reversal potentials of currents through AMPA receptors showed a significant negative correlation with aging, indicating that the permeability of AMPA receptors to Ca^{2+} decreased between P2 and P15 (Backus and Berger 1995).

We observed significant regional differences in the kainate and NMDA responses of glial precursor cells, astrocytes and oligodendrocytes. Kainate-evoked currents in glial precursors were largest in the ventral horns, while kainate- and NMDA-evoked currents in astrocytes and oligodendrocytes were larger in the dorsal horns than in the ventral. Quantitative receptor autoradiography, immunoblotting and immunohistochemistry revealed that during early postnatal life, non-NMDA and NMDA receptors in the rat spinal cord are present at high concentrations throughout the whole spinal gray matter, i.e. the dorsal and ventral horn (Kalb *et al.* 1992, Gonzalez *et al.* 1993, Jakowec *et al.* 1995). During the first 3 weeks after birth, however, the level of receptors in the ventral horn dramatically decreases, and both non-NMDA and NMDA receptors become largely restricted to the substantia gelatinosa, a dorsal horn structure involved in pain perception. These studies furthermore revealed a transient high level of expression of both non-NMDA and NMDA receptors only in the developing ventral horn during activity-dependent development of motor neurones (Jakowec *et al.* 1995).

Functional implications

The function of the glial glutamate receptors, which are transiently expressed in the dorsal horn of the spinal cord during development, is not clear. It has been postulated by several authors (von Blankenfeld and Kettenmann 1991, Müller *et al.* 1992) that ligand-gated glial receptors are involved in the morphological establishment of synapses. Moreover, these receptors may be involved in the formation of perineuronal nets and the ensheathment of synapses (Brückner *et al.* 1997). Indeed, it has been shown in the substantia gelatinosa of lumbar spinal cord that the growth of axons and dendrites of propriospinal projecting neurones begins prenatally and that the major period of neuronal maturation and elaboration of dendritic arbors, presumably accompanied by synaptogenesis, occurs around P10 (Bicknell and Beal 1984). We have observed in our experiments that the amplitudes of NMDA-evoked currents in glial precursor cells were significantly lower than those evoked by kainate. These findings could be explained in two ways. First, in contrast to AMPA/kainate receptors, the majority of NMDA receptors may be present in glial precursor cell processes and thus the clamp control by the patch pipette may be significantly limited. Second, the overall density of NMDA receptors may be much lower than that of AMPA/kainate receptors. The amplitudes of NMDA-evoked inward currents in astrocytes and oligodendrocytes decrease during development, which might indicate that, in contrast to kainate receptors, presumed NMDA receptors in glial cells are involved in synapse maturation. The effects of glutamate, kainate, AMPA and NMDA on glial cells may also include blockage of K^+ conductance and a concomitant impairment of proliferation (DeCoursey *et al.* 1984, Chiu and Wilson 1989), as well as an intracellular Ca^{2+} increase, which may be the signal for a cascade of effects that in turn could modulate neuronal functions (Berger *et al.* 1995). It was also suggested by Conti *et al.* (1996) that glial NMDA receptors can mediate part of the neurone-glia signaling mechanisms that regulate

gene expression and responses to pathological elevations of glutamate and that, in addition, they may participate in the mechanisms subserving activity-dependent cortical plasticity. Such neurone-glia interactions at synaptic regions could be important for long-term modification of signal transduction.

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