Molecular Neurobiology

ISSN0893-7648/1992/5/1:31–43/$2.60

Glutamate and GABA Receptors in Vertebrate Glial Cells

*Gabriela v. Blankenfeld and Helmut Kettenmann**

Department of Neurobiology, University of Heidelberg,
Im Neuenheimer Feld 345, 6900 Heidelberg, FRG

Contents

*Author to whom all correspondence and reprint requests should be addressed.

Abstract

Glial cells of the central nervous system express receptors for the main inhibitory and excitatory neurotransmitters, GABA and glutamate. The glial GABA and glutamate receptors share many properties with the neuronal $GABA_A$ and kainate/quisqualate receptors, but are molecularly and, in some aspects, pharmacologically distinct from their neuronal counterparts. The functional role of these receptors is as yet speculative: They have been proposed to control proliferation of astrocytes, serve to balance ion changes at GABAergic synapses, or they could enable the glial cell to detect neuronal synaptic activity.

Index Entries: Glutamate, GABA; glial cells; transmitter receptors; oligodendrocyte; astrocyte; neuron–glia interaction.

Introduction

The Neurotransmitters GABA and Glutamate Depolarize Glial Cells

Glial cells have been classically described as the electrically passive elements in the nervous system. Whenever physiologists recorded from cells with a highly negative membrane potential and no apparent electrical activity, they regarded these elements as glial cells. In the late 1960s and the early 1970s, a few scientists noticed that these cells could be depolarized by neurotransmitters (e.g., Krnjevic and Schwartz, 1967; Hösli et al., 1978,1979). Such responses did not fit into the scheme of the glial cell as an electrically passive element, and these responses were thus interpreted as electrogenic uptake currents for GABA or glutamate or indirect depolarizations caused by K^+ release from adjacent neurons (Krnjevic and Schwartz, 1967; Hösli et al., 1978,1979). Two technical improvements advanced our knowledge about glial transmitter receptors: First, the availability of identified purified populations of cultured glial cells excluded the influence from adjacent neurons on the recorded activity. Both macroglial cell types, astrocytes and oligodendrocytes, exhibited intrinsic responses to GABA and glutamate (Bowman and Kimelberg, 1984; Kettenmann et al., 1984; Tang and Orland, 1986; Gilbert et al., 1984). In contrast, mammalian Schwann cells or microglial cells were unresponsive in cell culture (D. Hoppe and H. Kettenmann, unpublished results). Second, the application of the patch-clamp technique to these identified cells demonstrated the existence of the receptors and has made it possible to identify the associated single-channel currents in the glial cells. It was demonstrated that the GABA-induced depolarization in cortical astrocytes and oligodendrocytes and their precursors was exclusively caused by the activation of a ligand-gated ion channel, and this response was unaffected by blockers of GABA uptake (Gilbert et al., 1984; Kettenmann and Schachner, 1985). In contrast, there is a diversity among glial cells with respect to the response to glutamate. In cultures of mammalian astrocytes from rat cortex, the current (and thus the concomitant depolarization) was caused by ligand-gated channels (Sontheimer et al., 1988), whereas in mammalian astrocytes from cerebellum cultured for up to four days (Wyllie et al., 1991) and isolated amphibian Müller cells, the inward current was generated by electrogenic glutamate uptake (Brew and Attwell, 1987). In this review, however, we will focus on receptor-mediated processes.

Glial Glutamate Receptors

Classification of Glutamate Receptors

Glutamate receptors, in general, are classified by their selective agonists, *N*-methyl-D-aspartate

(NMDA, quisqualate, and kainate (for review *see* Barnard and Henley, 1990). NMDA and kainate receptors are ionotropic, i.e., they possess an intrinsic ion channel. The quisqualate receptors are subdivided, one that also gates an ion channel and another that activates phosphoinositide turnover (Sugiyama et al., 1987; for review *see* Schoepp et al., 1990). According to their specific agonists, they are named AMPA (D,L-α-amino-hydroxy-5-methyl-4-isoxalone proprionic acid) and ACPD (1-aminocyclopentane-1*S*, 3R-dicarboxyclic acid) receptor, respectively. A variety of parameters served as effector systems to study the properties of glial glutamate receptor. These included changes in the membrane potential and membrane current (Bowman and Kimelberg, 1984; Enkvist et al. 1988; Sontheimer et al., 1988; Kettenmann and Schachner, 1985), an increase in inositol phosphate formation (Pearce et al., 1986, 1990) and intracellular Ca^{2+} (Cornell-Bell et al., 1990a; Jensen and Chiu, 1990; Glaum et al., 1990; Ahmed et al., 1990), and transmitter release (Gallo et al., 1987a,b).

Ionotropic Glutamate Receptors in Glial Cells

The glutamate-induced currents in cultured astrocytes from rat cortex (Fig. 1) reversed at a membrane potential of about 0 mV as expected for a cation-selective ligand-gated ion channel. This was substantiated by the finding that current noise increased at potentials positive or negative from the reversal potential (Sontheimer et al., 1988). The resulting current to voltage-curve was linear and depended strongly on the transmembrane Na^+/K^+- but not on the Ca^{2+}- or Cl^--gradient. These findings indicated that glutamate opens a Na^+/K^+ channel similar to the kainate/quisqualate receptor described in neurons (Sontheimer et al., 1988). To better isolate the glutamate-induced current, these studies were performed in the presence of K^+ channel blockers; in contrast, currents observed in the absence of such blockers reversed at a substantially more negative potential, indicating that glutamate, in addition to opening the glutamate-gated channel, may also activate K^+ channels in astrocytes (Sontheimer et al., 1988).

In a study with type-2 cerebellar astrocytes, Usowicz and coworkers (1989) demonstrated the activation of single-channel currents by glutamate with similar properties as central neurons (Fig. 1). At least five subconductance levels ranging from 6 to 47 pS were distinguished. These single-channel currents reversed close to 0 mV and showed a linear current to voltage-relation as found for the whole cell currents. The glutamate-gated channel was not only expressed in mature glial cells, but also by progenitors to the glial O-2A cell lineage from rat optic nerve (Barres et al., 1990b). Glutamate-induced currents were not detected in freshly isolated astrocytes from rat optic nerve (Barres et al., 1990a). Wyllie et al. (1991) investigated the expression of glutamate receptors in mixed cultures of cerebellar glial cells. They found the expression of glutamate receptors in type-2 astrocytes, O-2A progenitor cells and in type-1 astrocytes after eight days in culture, but not in oligodendrocytes. This finding implies that the receptors may also play a functional role during development.

Pharmacology Classifies the Glial Ionotropic Receptors as of the Kainate/Quisqualate Subtype

The physiological properties of the glial response classified the glial ionotropic glutamate receptor as a kainate/quisqualate type. This was substantiated by a number of pharmacological studies in which the effect of glutamate was compared with selective agonists. The common observation was that NMDA and other selective agonists for the NMDA receptor subtype did not elicit any physiological response in glial cells, even under conditions that facilitate NMDA currents (Backus et al., 1989; Barres et al., 1990b; Wyllie et al., 1991). Non-NMDA receptor agonists, such as kainate and quisqualate, were both effective. It is, however, likely that astrocytes possess two different ionotropic glutamate receptors

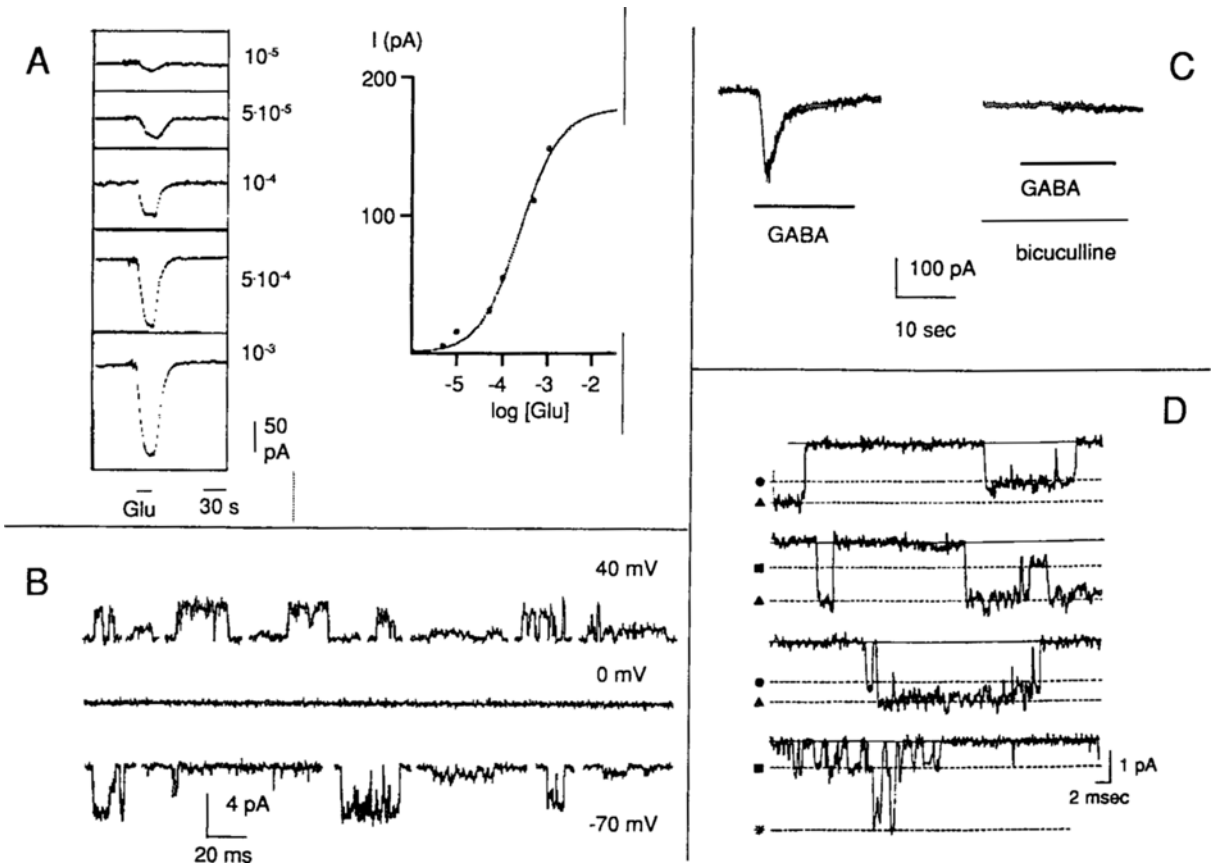

Fig. 1. Whole cell and single-channel currents from astrocytes activated by GABA and glutamate. (A) Dose–response curve of glutamate-activated currents recorded from cultured cortical astrocytes (from Sontheimer et al., 1988). (B) Single-channel currents recorded from type 2 astrocytes in hippocampal cultures in the presence of glutamate. The three current traces correspond to different membrane potentials as indicated (from Usowicz et al., 1989). (C) Whole cell currents activated by GABA can be blocked by the $GABA_A$ receptor antagonists bicuculline. The recording was obtained from an oligodendrocyte cultured from cortex (from Blankenfeld et al., 1991). (D) Single-channel currents activated by GABA. The recordings were obtained from cultured cortical astrocytes (from Bormann and Kettenmann, 1988).

since Gallo et al. (1987a,b) observed that kynurenic acid antagonized the effect of kainate, but not that of quisqualate. The specific ligands may partially act on both receptors since coapplication of kainate and quisqualate resulted in a smaller current response than application of kainate alone (Backus et al., 1989). In summary, astrocytes express kainate and quisqualate, but not NMDA receptors.

Glutamate Receptors Are Linked to Inositol Phospholipid Metabolism

Glutamate, kainate, and quisqualate stimulated Ca^{2+} fluxes and IP1 (inositol-1-phosphate) formation, indicating an activation of the phosphoinositide metabolism (Pearce et al., 1986,1990) in neonatal cortical rat astrocytes.

Whereas the inositide breakdown after kainate and, in part, after glutamate were mediated by a membrane depolarization, the effect of quisqualate seemed to be exerted via the metabotropic quisqualate receptor (Pearce et al., 1986,1990; Nicoletti et al., 1990). Robertson et al. (1990) suggested that the glutamate-induced phosphoinositide breakdown was coupled via a G protein. An interaction between the ionositol phosphate and cAMP second messenger systems were indicated by the finding that elevation of the internal cAMP level resulted in a concentration-dependent inhibition of the glutamate-induced phosphoinositide lipid hydrolysis (Robertson et al., 1990).

Receptor Activation Leads to Ca^{2+} Waves in Astrocytes

With the use of fluorescent Ca^{2+} indicators several groups have described that the application of glutamate receptor agonists cause an increase in cytosolic Ca^{2+} (Cornell-Bell et al., 1990a; Jensen and Chiu, 1990; Glaum et al., 1990; Ahmed et al., 1990). The percentage of astrocytes responding to glutamate agonists with a rise in Ca^{2+} varied among different brain regions (Glaum et al., 1990). The increase in Ca^{2+} can be attributed to a release from intracellular stores or influx through voltage-activated Ca^{2+} channels in the plasma membrane. These pathways were differentially activated by the different glutamate receptor agonists. The kainate-induced Ca^{2+} rise was almost completely abolished in Ca^{2+}-free external solutions, indicating an influx via Ca^{2+} channels. In contrast, glutamate and quisqualate triggered a rise in cytosolic Ca^{2+} under the condition of Ca^{2+} free external solutions, suggesting the release of Ca^{2+} from internal stores (Cornell-Bell et al., 1990a; Cornell-Bell and Finkbeiner, 1991; Jensen and Chiu, 1990; Glaum et al., 1990; Ahmed et al., 1990).

In all investigations, it was found that quisqualate and glutamate frequently induced Ca^{2+} oscillations with a frequency in the seconds range. In contrast, kainate never induced such oscillations. Long-lasting oscillations were abolished when extracellular Ca^{2+} was removed (Cornell-Bell et al., 1990a; Jensen and Chiu, 1990), indicating an involvement of Ca^{2+} channels or a depletion of internal stores under these conditions. Intracellular Ca^{2+} waves propagate so smoothly to neighboring cells that participating cells appeared to function as a syncytium (Cornell-Bell and Finkbeiner, 1991).

Glutamate Receptor Activation Induces Swelling and Filopodia Protrusion in Astrocytes

Astrocytes started to swell after application of glutamate, aspartate, or quisqualate as determined by the uptake of radioactively labeled glucose (Chan et al., 1990). Since swelling was not induced by kainate, this response is not merely linked to the membrane depolarization and thus the mechanism of this response remained unresolved. Surprisingly, the glutamate-induced swelling was antagonized by the NMDA receptor antagonists ketamine and MK-801, suggesting furthermore that a mechanism was activated that is not fully compatible with the activation of the ligand-gated channel (Chan and Chu, 1989; Chan et al., 1990). This study illustrates that the effect of glutamate on glial cells is far from being fully understood.

An even more complex morphological change could be activated by glutamate, namely, the rapid formation of filopodia as observed in cultured hippocampal astrocytes (Fig. 2; Cornell-Bell et al., 1990b). The pharmacology of this event matched the pharmacology of the electrophysiological data, i.e., it could be mimicked by kainate and quisqualate, but not by NMDA. The link between receptor activation and the morphological change was assumed to be mediated via inositol lipid hydrolysis and a reorganization of the actin network (Cornell-Bell et al., 1990b), but not

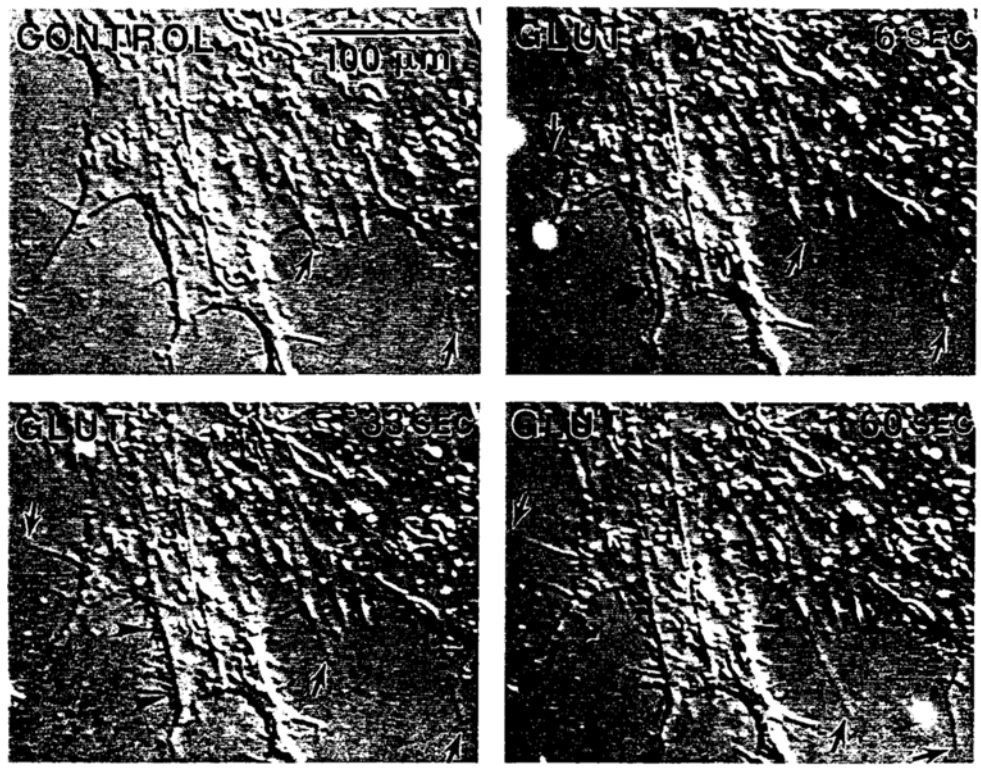

Fig. 2. Filopodia formation by astrocytes (from Cornell-Bell et al., 1990b, Fig. 2).

by the depolarization *per se*. Such membrane reorganization could occur at synaptic regions where glial membranes are exposed to transmitter released from active synapses.

Receptor Activation Reduces Proliferation

The proliferative state of the astrocytes was also linked to glutamate receptors. Addition of glutamate or quisqualate inhibited the incorporation of [methyl-^{3}H]thymidine in rat cerebral astrocytes without affecting the viability of the cells (Condorelli et al., 1989; Nicoletti et al., 1990). This inhibiting effect on cell proliferation displayed a similar pharmacology to the glutamate-induced swelling, suggesting that the quisqualate receptor is linked to cell division, but not the kainate receptor. NMDA did not affect proliferation, which is in line with previous observations that NMDA receptors are not expressed by astrocytes (Condorelli et al., 1989). AMPA was not effective in reducing the ^{3}H-thymidine incorporation, indicating the involvement of the ACPD receptor subtype. This was further supported by the finding that excitatory amino acids reduced the thymidine incorporation with a similar pharmacologic profile than the phosphoinositide lipid hydrolysis (Nicoletti et al., 1990).

Molecular Biology of Glial Glutamate Receptors

Recent advances in molecular biology has led to the isolation and cloning of different types of putative glutamate receptors. One gene product, a 49-kDa polypetide that contains a specific kainate binding site, is exclusively located in cere-

bellar Bergmann glial cells in close proximity to established glutamatergic synapses. The sequence of the protein has been determined, but reconstitution experiments have yet failed to demonstrate that the protein forms an ion channel (Gregor et al., 1989). An antibody recognizing the kainate-binding proteins also stained for a 93-kDa protein in Bergmann glia (Ortega et al., 1991). In the light of these results it is likely that glial and neuronal glutamate receptors are distinct from one another.

Glial GABA Receptors

The GABA Receptors in Astrocytes and Oligodendrocytes Share Properties with the Neuronal $GABA_A$ Receptor

Astrocytes and oligodendrocytes are depolarized by GABA (Kettenmann et al., 1984; Gilbert et al., 1984). The ionic mechanism leading to the depolarization was identified as an opening of Cl^- channels (Fig. 1; Kettenmann et al., 1987). Single-channel recordings from astrocytic GABA receptor Cl^- channels showed no differences to their counterparts in neurons when comparing kinetic behavior and conductance levels (Bormann and Kettenmann, 1988). The pharmacological characterization of the GABA response indicated more similarities to neuronal $GABA_A$ receptors: GABA-induced depolarizations were mimicked by the $GABA_A$ receptor agonist muscimol, blocked by the Cl^- channel blocker picrotoxin, and by the GABA antagonist bicuculline (Fig. 1; Kettenmann and Schachner, 1985). GABA responses in glial cells were augmented by the barbiturate pentobarbital and the benzodiazepine agonists diazepam or flunitrazepam (Backus et al., 1988) and modulated by steroids (Chvatal and Kettenmann, 1991). The expression of a $GABA_A$ receptor has also been described on skate Müller (glial) cells (Malchow et al.,1989) and oligodendrocytes and their precursors (Blankenfeld et al., 1991). Recordings of GABA responses from astrocytes of hippocampal slices (MacVicar et al., 1989; Steinhäuser et al., 1991a) and oligodendrocytes from the corpus callosum slice (Berger et al., 1991a) demonstrated that the glial response is not a cell culture artefact.

Glial and Neuronal Receptors Are Pharmacologically Distinct

One striking difference between astrocytic and the neuronal receptors became apparent when the influence of benzodiazepines on astrocytic GABA responses was studied. Inverse agonists (such as DMCM) augmented the GABA response in astrocytes, whereas in neurons and cells of the oligodendrocyte lineage the response was decreased (Fig. 3; Backus et al., 1989; Bormann and Kettenmann, 1988). There were, however, also differences between cells of the oligodendrocyte lineage on one hand and astrocytes and neurons on the other: The dose–response relation in astrocytes indicated two binding sites for GABA with strong allosteric interactions as previously described for the neuronal $GABA_A$ receptor (Bormann and Kettenmann, 1988). In contrast, the receptor of oligodendrocytes has either only a single binding site or two sites with no apparent allosterical interaction (Blankenfeld et al., 1991).

Molecular Biology of Glial GABA Receptors

The molecular composition of glial GABA receptors is so far unresolved, but first hints of a molecular understanding of glial GABA receptors are emerging. *In situ* hybridization revealed that a specific α subunit, α_2, is confined to the Bergmann glial cell layer (Wisden et al., 1989). Antibodies directed against $GABA_A$ receptor subunits recognized a single 63-kDa protein selective for astrocytes (Ventimiglia et al., 1990). In addition, different recombinant receptors could be composed that mimic the difference in benzodiazepine pharmacology and allosteric interactions as described above (Fig. 3; Blankenfeld et al., 1990).

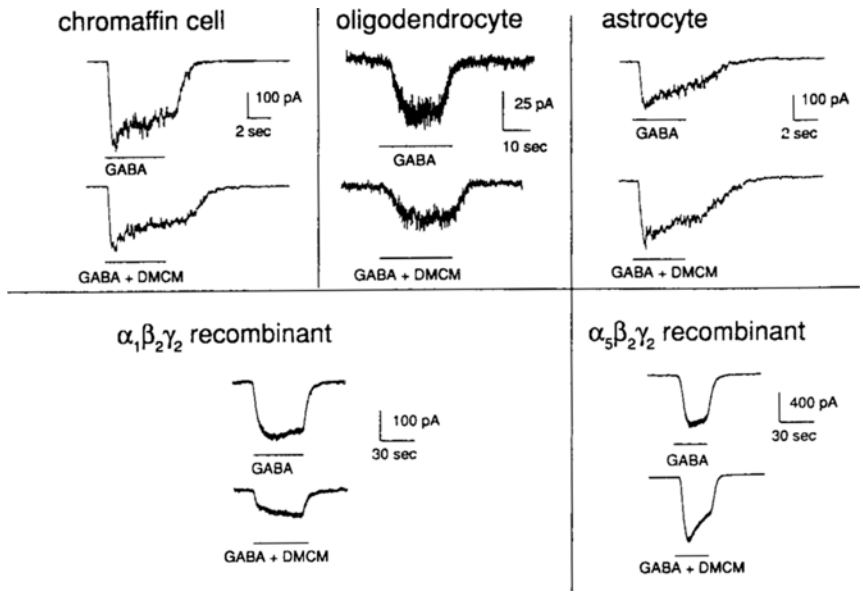

Fig. 3. Comparison of recombinant GABA receptors with those expressed in astrocytes, chromaffin cells, and oligodendrocytes. Inward currents were activated by appli-cation of GABA (50 μM for chromaffin cells and astrocytes, 1 μM for oligodendrocytes and recombinant receptors) and compared to responses in the presence of DMCM (10 μM). In chromaffin cells, oligodendrocytes and fibroblasts transfected with cDNA encoding the $\alpha_1\beta_2\gamma_2$ subunits, GABA responses were increased in the presence of DMCM. In contrast, in astrocytes and fibroblasts transfected with cDNA encoding the $\alpha_5\beta_2\gamma_2$, subunits GABA responses were increased in the presence of DMCM (with modifications from Blankenfeld et al., 1990,1991; Bormann and Kettenmann, 1988).

Receptor Activation Leads to an Efflux of Cl^-

In contrast to most neurons, astrocytes and oligodendrocytes taken from different tissues and species are depolarized by GABA. This difference is caused by the increase in intracellular Cl^- in glial cells far above the passive distribution. In oligodendrocytes, the Cl^- inward transport was primarily owing to the activity of a bumetanide-sensitive $Na^+/K^+/Cl^-$ transport system. The mechanism of Cl^- transport across the astrocytic membrane involved $Na^+/K^+/2Cl^-$-cotransport and Cl^-/HCO^-_3-exchange and was thus more complex than the mechanism in oligodendrocytes (Kettenmann, 1990; Kimelberg, 1990). Thus, opening of GABA-gated Cl^- channels led to an efflux of Cl^- from the glial cells as determined with ion-selective microelectrodes (Hoppe and Kettenmann, 1989). This difference in ion flux direction between neurons and glial cells could be of functional importance as discussed below.

Functional Importance: The Receptors and Its Transmitters as Links in Neuron–Glia Interaction

GABA Receptors Could Be Involved in Cl^- Homeostasis

In neurons, activation of GABA receptors commonly leads to an influx of Cl^- into the cell resulting in the hyperpolarizing inhibitory postsynaptic potentials (ipsp), in contrast to glial

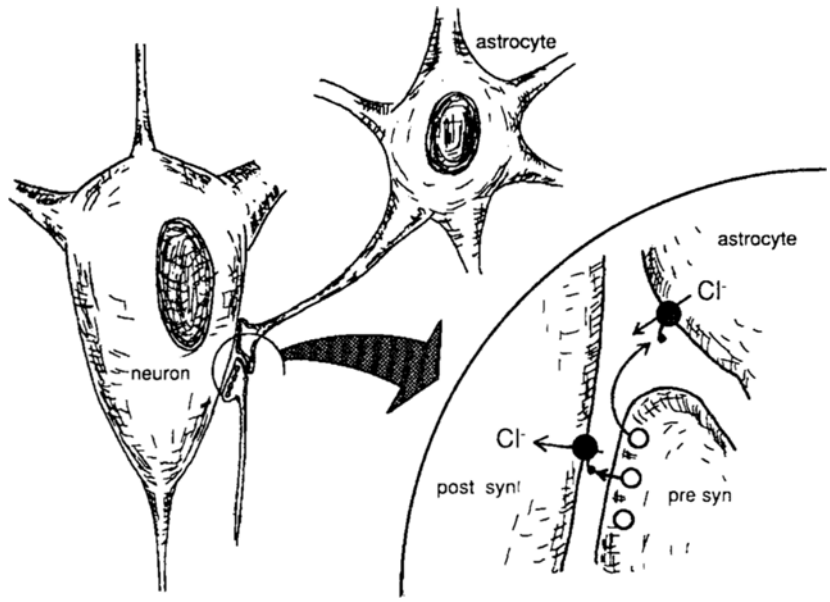

Fig. 4. Hypothesis for function: Cl^- homoeostasis. An astrocytic end-foot is assumed to be in close proximity to GABAergic synapse. The magnified view illustrates that GABA release from the presynaptic terminal (pre syn) leads to activation of GABA receptors in the postsynaptic membrane (post syn) and in membranes of adjacent astrocytes. Whereas Cl^- enters the neuron, it flows out of glial cells.

cells where the membrane potential was depolarized. Since astrocytic processes are found in close vicinity to synapses, the efflux of Cl^- from adjacent astrocytic processes through GABA-activated Cl^- channels could serve to maintain a constant extracellular Cl^- at the synaptic cleft (Fig. 4; Bormann and Kettenmann, 1988; MacVicar et al., 1989). The astrocytic GABA receptor may thus serve to buffer extracellular Cl^- levels at synaptic regions. This would reveal an additional function of astrocytes, that of providing Cl^- homeostasis, beside their well-known involvement in the regulation of extracellular K^+.

Synaptic Activity Could Influence Glial Cells in the Vicinity via Glial Receptor Activation

The glial GABA or glutamate receptors could, however, serve more complex functions: Since there is increasing evidence that glial cells interact with neurons during the development of the nervous system (e.g., Hatten, 1990), these receptors could serve for the detection of active neuronal synapses. The glial cell possesses the main excitatory (glutamate) and the main inhibitory neurotransmitter (GABA) system of the brain. Synaptic activity could trigger activation of these receptors, inducing glial cells to extend processes (Fig. 5). The formation of filopodia in astrocytes triggered by glutamate provides a hint in this direction. It is thus an intriguing hypothesis that glial processes, by interacting with synapses, could thus determine their survival or annihilation. Such a neuron–glia interaction is strengthened by the observation that the transplantation of astrocytes into the adult cat visual cortex reestablished ocular dominance plasticity (Müller and Best, 1989). Neuron–glia interactions at synaptic regions could thus be important for long-term modification of signal transduction resulting in complex functions, such as "memory."

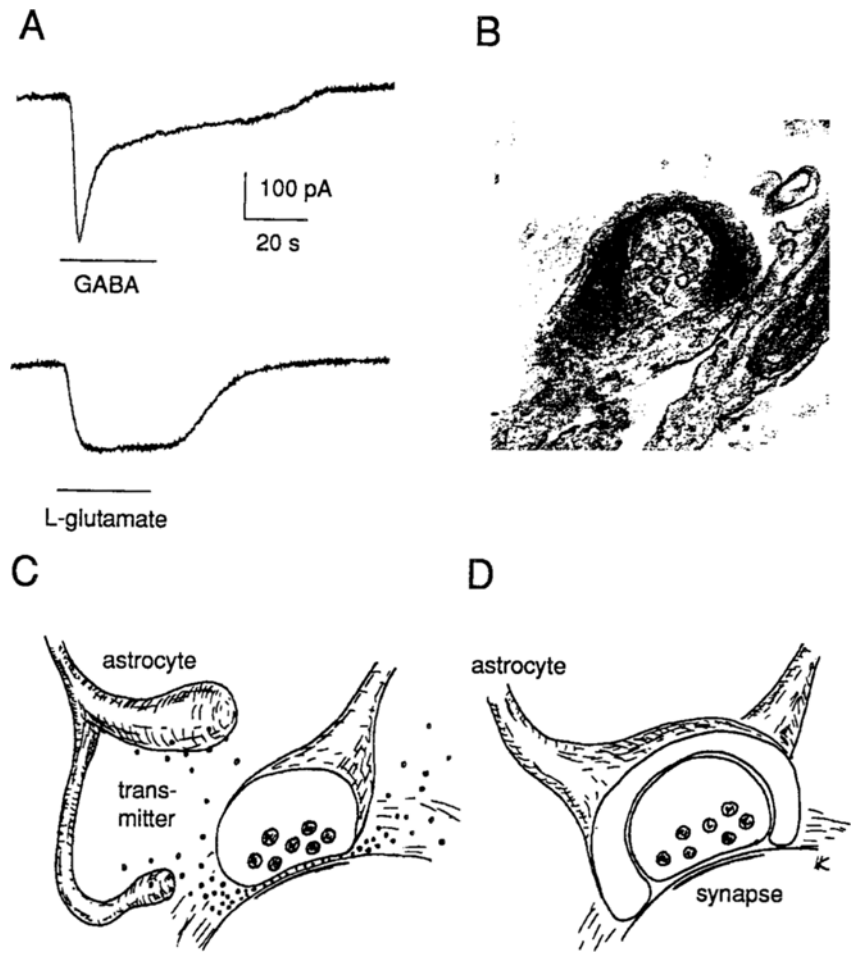

Fig. 5. Hypothesis for function: Target detection (A) Glial cells from an intact tissue, the hippocampal slice, were shown to respond to the neurotransmitters GABA and glutamate (with modifications from Steinhäuser et al., 1991a). Current recordings were obtained from a glial cell clamped to –70 mV. The transmitters were applied as indicated by bars. (B) Glial cells are in close apposition with synaptic areas. A glial cell was physiologically identified and subsequently dye-labeled for inspection in the electron microscope. A glial process is depicted that surrounds a presynaptic terminal. The process can be recognized by the dark labeling of the dye reaction product (with modifications from Steinhäuser et al., 1991b). (C, D) In this hypothetical model, an active synapse activates glial transmitter receptors on adjacent glial membranes. Activation of astrocytic receptors could trigger the outgrowth of processes (C). These processes could interact with the synaptic region, stabilize it, and confine the transmitter release to the synaptic cleft (D). The glial transmitter receptors thus function as target detectors.

Glial Cells Can also Release GABA and Glutamate: A Feedback Loop?

One of the early functions of glial cells recognized by physiologists was the uptake of neurotransmitters from the extracellular space. This function is important for the rapid clearance of the extracellular space from the excess transmitter and was especially attributed to astrocytes, which can form intimate contacts with synaptic areas. Efficient uptake systems for GABA and glutamate have been characterized (e.g., Henn et al., 1974; Schon and Kelly, 1975). Moreover, astrocytes can also release GABA or glutamate. GABA release is triggered by glutamate and related agonists (Gallo et al., 1987a,b), glutamate release by

swelling of astrocytes (Kimelberg et al., 1990). Since application of extracellular glutamate itself induced swelling, the interaction between these responses may be highly complicated. These mechanisms could provide ways of information transfer between glial cells themselves and between glial cells and neurons. At present, we are still at the brink of understanding the complex interplay of glial cells among each other, and with their neuronal partners. One notion, however, emerges: To fully understand neurotransmission, the contribution of the glial cells has to be included.

Conclusions and Future Directions

The studies summarized above have opened more questions than they have answered. They have, however, clearly shown that the receptor proteins from the brain can originate from both, glial cells and neurons. Thus, biochemical and molecular biological studies on transmitter receptors obtained from bulk-isolated material can no longer be simply attributed to neurons. The central question for neurobiologists working on glial receptors is: What role do glial receptors play in brain function?

To address this question, glial cells need to be studied in more intact systems, such as brain slices, where the anatomical relations are, at least in part, intact. The application of the patch-clamp technique and ion activity measurements using dyes to brain slices has opened the possibility of studying rapid ion and membrane changes in glial cells in concert with their neuronal partners (Berger et al., 1991a,b; Steinhäuser et al., 1991a,b). It may become possible to provide partial answers to this question by carefully designing experiments involving the two partners in the brain, the neurons, and the glial cells.

References

Ahmed Z., Lewis C. A., and Faber D. S. (1990) Glutamate stimulates release of Ca^{2+} from internal stores in astroglia. *Brain Res.* 516, 165–169.

Backus K. H., Kettenmann H., and Schachner M. (1988) Effect of benzodiazepines and pentobarbital on the GABA-induced depolarization in cultured astrocytes. *Glia* 1, 132–140.

Backus K. H., Kettenmann H., and Schachner M. (1989) Pharmacological characterization of the glutamate receptor in cultured astrocytes. *J. Neurosci. Res.* 22, 274–282.

Barnard E. A. and Henley J. M. (1990) The non-NMDA receptors: Types, protein structure and molecular biology. *TIPS* 11, 500–507.

Barres B. A., Koroshetz W. J., Chun L. L. Y., and Corey D. P. (1990a) Ion channel expression by white-matter glia: The type-1 astrocyte. *Neuron* 5, 527–544.

Barres B. A., Koroshetz W.J ., Swartz K. J., Chun L. L. Y., and Corey D. P. (1990b) Ion channel expression by white matter glia: The O–2A glial progenitor cell. *Neuron* 4, 507–524.

Berger T., Walz W., Schnitzer J., and Kettenmann H. (1991a) GABA and glutamate-activated currents in glial cells of the mouse corpus callosum slice. *J. Neurosci. Res.*, in press.

Berger T., Schnitzer J., and Kettenmann H. (1991b) Developmental changes in the membrane current pattern, K buffer capacity, and morphology of glial cells in the corpus callosum sclice. *J. Neurosci.* 11, 3008–3024.

Blankenfeld G. von, Ymer S., Pritchett D. B., Sontheimer H., Ewert M., Seeburg P. H., and Kettenmann H. (1990) Differential benzodiazepine pharmacology of mammalian recombinant $GABA_A$ receptors. *Neurosci. Lett.* 115, 269–273.

Blankenfeld G.von, Trotter J., and Kettenmann H. (1991) Expression and developmental regulation of a $GABA_A$ receptor in cultured murine cells of the oligodendrocyte lineage. *Eur. J. Neurosci.* 3, 310–316.

Bormann J. and Kettenmann H. (1988) Patch-clamp study of γ-aminobutyric acid receptor Cl^- channels in cultured astrocytes. *Proc. Natl. Acad. Sci. USA* 85, 9336–9340.

Bowman C. L. and Kimelberg H. K. (1984) Excitatory amino acid directly depolarize rat brain astrocytes in primary culture. *Nature* 311, 656–659.

Brew H. and Attwell D. (1987) Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells. *Nature* 327, 707–709.

Chan P. H. and Chu L. (1989) Ketamine protects cultured astrocytes from glutamate-induced swelling. *Brain Res.* 487, 380–383.

Chan P. H., Chu L., and Chen S. (1990) Effects of MK-801 on glutamate-induced swelling of astrocytes in primary cell culture. *J. Neurosci. Res.* **25**, 87–93.

Chvátal A. and Kettenmann H. (1991) Effect of steroids on GABA induced currents in cultured mouse astrocytes. *Pflügers Arch.* **419**, 263–266.

Condorelli D. F., Ingrao F., Magri G., Bruno V., Nicoletti F., and Avola R. (1989) Activation of excitatory amino acid receptors reduces thymidine incorporation and cell proliferation rate in primary cultures of astrocytes. *Glia* **2**, 67–69.

Cornell-Bell A. H. and Finkbeiner S. M. (1991) Ca^{++} waves in astrocytes. *Cell Calcium* **12**, 185–204.

Cornell-Bell A. H., Finkbeiner S. M., Cooper M. S., and Smith S. J. (1990a) Glutamate-induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science* **247**, 470–473.

Cornell-Bell A. H., Thomas P. G., and Smith S. J. (1990b) The excitatory neurotransmitter glutamate causes filopodia formation in cultured hippocampal astrocytes. *Glia* **3**, 322–334.

Enkvist M. O., Holopainen I., and Akerman K. E. O. (1988) The effect of K and glutamate receptor agonists on the membrane potential of suspensions of primary cultures of rat astrocytes as measured with a cyanine dye, DiS-C-(5). *Brain Res.* **462**, 67–75.

Gallo V., Suergiu R., Giovannini C., and Levi G. (1987a) Glutamate receptor subtypes in cultured cerebellar neurons: Modulation of glutamate and gamma-aminobutyric acid release. *J. Neurochem.* **49**, 1801–1809.

Gallo V., Suergiu R., and Levi G. (1987b) Functional evaluation of glutamate receptor subtypes in cultured cerebellar neurones and astrocytes. *Eur. J. Pharmacol.* **138**, 293–297.

Gilbert P., Kettenmann H., and Schachner M. (1984) γ-Aminobutyric acid directly depolarizes cultured oligodendrocytes. *J. Neurosci.* **4**, 561–569.

Glaum S. R., Holzwarth J. A., and Miller R. J. (1990) Glutamate receptors activate Ca^{2+} mobilization and Ca^{2+} influx into astrocytes. *Proc. Natl. Acad. Sci. USA* **87**, 3454–3458.

Gregor P., Mano I., Maoz I., McKeown M., and Teichberg V. I. (1989) Molecular structure of the chick cerebellar kainate-binding subunit of a putative glutamate receptor. *Nature* **342**, 689–692.

Hatten M. E. (1990) Riding the glial monorail: A common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. *Trends Neurosci.* **13**, 179–184.

Henn F. A., Goldstein M. N., and Hamberger A. (1974) Uptake of the neurotransmitter candidate glutamate by glia. *Nature* **249**, 663,664.

Hoppe D. and Kettenmann H. (1989) GABA triggers a Cl^- efflux from cultured mouse oligodendrocytes. *Neurosci. Lett.* **97**, 334–339.

Hösli A. L., Andres P. F., and Hösli E. (1978) Neuron–glia interactions: Indirect effect of GABA on cultured glial cells. *Exp. Brain Res.* **33**, 425–434.

Hösli A. L., Andres P. F., and Hösli E. (1979) Action of amino acid transmitters on cultured glial cells of the mammalian peripheral and central nervous system. *J. Physiol.* **75**, 655–659.

Jensen A. M. and Chiu S. Y. (1990) Fluorescence measurement of changes in intracellular calcium induced by excitatory amino acids in cultured cortical astrocytes. *J. Neurosci.* **10**, 1165–1175.

Kettenmann H. (1990) Chloride channels and carriers in cultured glial cells, in *Chloride Channels and Carriers in Nerve, Muscle, and Glial Cells* (Alvarez-Leefmans F. J. and Russell J. M., eds.), Plenum, New York, pp. 193–208.

Kettenmann H., Backus K. H., and Schachner M. (1984) Aspartate, glutamate and gamma-aminobutyric acid directly depolarize cultured astrocytes. *Neurosci. Lett.* **52**, 25–29.

Kettenmann H., Backus K. H., and Schachner M. (1987) γ-Aminobutyric acid opens Cl^- channels in cultured astrocytes. *Brain Res.* **404**, 1–9.

Kettenmann H. and Schachner M. (1985) Pharmacological properties of GABA, glutamate and aspartate induced depolarizations in cultured astrocytes. *J. Neurosci.* **5**, 3295–3301.

Kimelberg H. K. (1990) Chloride transport across glial membranes, in *Chloride Channels and Carriers in Nerve, Muscle, and Glial Cells* (Alvarez-Leefmans F. J. and Russell J. M., eds.), Plenum, New York, pp. 159–191.

Kimelberg H. K., Goderie S. K., Higman S., Pang S., and Waniewski R. A. (1990) Swelling-induced release of glutamate, aspartate, and taurine from astrocyte cultures. *J. Neurosci.* **10**, 1583–1591.

Krnjevic K. and Schwartz S. (1967) Some properties of unresponsive cells in the cerebral cortex. *Exp. Brain Res.* **3**, 306–319.

MacVicar B. A., Tse F. W., Crichton S. A., and Kettenmann H. (1989) GABA-activated Cl^- channels in astrocytes of hippocampal slices. *J. Neurosci.* **9**, 3577–3583.

Malchow R. P., Qian H. H., and Ripps H. (1989)

gamma-Aminobutyric acid (GABA)-induced currents of skate Muller (glial) cells are mediated by neuronal-like $GABA_A$ receptors. *Proc. Natl. Acad. Sci. USA* **86**, 4226–4230.

Müller C. M. and Best J. (1989) Ocular dominance plasticity in adult cat visual cortex after transplantation of cultured astrocytes. *Nature* **342**, 427–430.

Nicoletti F., Magri G., Ingrao F., Bruno V., Catania M. V., Dell'Albani P., Condorelli D. F., and Avola R. (1990) Excitatory amino acids stimulate inositol phospholipid hydrolysis and reduce proliferation in cultured astrocytes. *J. Neurochem.* **54**, 771–777.

Ortega A., Eshar N., and Teichberg V. I. (1991) Properties of kainate receptor/channels on cultured Bergmann glia. *Neurosci.* **41**, 335–349.

Pearce B., Albrecht J., Morrow C., and Murphy S. (1986) Astrocyte glutamate receptor activation promotes inositol phospholipid turnover and calcium flux. *Neurosci. Lett.* **72**, 335–340.

Pearce B., Morrow C., and Murphy S. (1990) Further characterisation of excitatory amino acid receptors coupled to phosphoinositide metabolism in astrocytes. *Neurosci. Lett.* **113**, 298–303.

Robertson P. L., Bruno G. R., and Datta S. C. (1990) Glutamate-stimulated, guanine nucleotide-mediated phosphoinositide turnover in astrocytes is inhibited by cyclic AMP. *J. Neurochem.* **55**, 1727–1733.

Schoepp D., Bockaert J., and Sladeczek F. (1990) Pharmacological and functional characteristics of metabotropic excitatory amino acid receptors. *TIPS* **11**, 508–515.

Schon F. and Kelly J. S. (1975) Selective uptake of (^{3}H) beta-alanine by glia: Association with the uptake system for GABA. *Brain Res.* **86**, 243–257.

Sontheimer H., Kettenmann H., Backus K. H., and Schachner M. (1988) Glutamate opens Na^+/K^+ channels in cultured astrocytes. *Glia* **1**, 328–336.

Steinhäuser C., Berger T., Jabs R., Frotscher M., and Kettenmann H. (1991a) Voltage- and ligand-gated membrane current pattern of identified glial cells in the hippocampal slice, in *Slow Activity Changes in the Brain* (Haschke W. and Speckmann E.-J., eds.), Birkhäuser, Boston, in press.

Steinhäuser C., Berger T., Frotscher M., and Kettenmann H. (1991b) Heterogeneity in the membrane current pattern of identified glial cells in the hippocampal slice: A combined patch-clamp and ultrastructural study (submitted for publication).

Sugiyama H., Ito I., and Hirono C. (1987) A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature* **325**, 531–533.

Tang C. M. and Orkand R. K. (1986) Glutamate depolarization of glial cells in Necturus optic nerve. *Neurosci. Lett.* **63**, 300–304.

Usowicz M. M., Gallo V., and Cull-Candy S. G. (1989) Multiple conductance channels in type-2 cerebellar astrocytes activated by excitatory amino acids. *Nature* **339**, 380–383.

Ventimiglia R., Grierson J. P., Gallombardo P., Sweetnam P. M., Tallman J. F., and Geller H. M. (1990) Cultured rat neurons and astrocytes express immunologically related epitopes of the $GABA_A$/benzodiazepine receptor. *Neurosci. Lett.* **115**, 131–136.

Wisden W., McNaughton L. A., Darlison M. G., Hunt S. P., and Barnard E. A. (1989) Differential distribution of GABA receptor mRNAs in bovine cerebellum-localization of γ2 mRNA in Bergmann glia layer. *Neurosci. Lett.* **106**, 7–12.

Wyllie D. J. A., Symonds C. J., and Cull-Candy S. G. (1991) Activation of glutamate receptors and glutamate uptake in identified macroglial cells in rat cerebellar cultures. *J. Physiol.* **432**, 235–255.