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The role of glial cells in synaptic function

Alberto Bacci, Claudia Verderio, Elena Pravettoni and Michela Matteoli*

CNR-Cellular and Molecular Pharmacology and 'B. Ceccarelli' Centers, Department of Medical Pharmacology, University of Milano, via Vanvitelli 32, 20129 Milano, Italy

Glial cells represent the most abundant cell population in the central nervous system and for years they have been thought to provide just structural and trophic support to neurons. Recently, several studies were performed, leading to the identification of an active interaction between glia and neurons. This paper focuses on the role played by glial cells at the level of the synapse, reviewing recent data defining how glia is determinant in synaptogenesis, in the modulation of fully working synaptic contacts and in synaptic plasticity.

Keywords: astrocytes; synaptogenesis; synaptic transmission; glia–neuron interaction

1. INTRODUCTION

Glial cells make up a large percentage of the cell population in the brain. According to their localization and their functional properties, glial cells can be divided into at least three categories: Schwann cells (in the peripheral nervous system) and oligodendrocytes (in the central nervous system (CNS)), which insulate axons and allow the fast and efficient propagation of the action potential; microglial cells, which have several common aspects with immune cells and serve this function in the brain; and finally, astrocytes, which represent the most abundant subclass of glial cells in the brain and which are responsible for extracellular K^+ homeostasis and metabolic support to neurons.

In the past, the main role of glial cells was thought to be that of providing structural and trophic support to neurons, keeping them stuck together ('glia' is the Greek word for glue) and providing them with factors essential for their survival. On the other hand, the function of transmitting and processing information was attributed exclusively to neurons. This concept has changed dramatically in the last few years. It is now widely believed that glial cells and neurons, which are intimately juxtaposed throughout the nervous system, are functionally interacting. It is also clear that the key site of neuron–glia interaction is the synapse, which is the most specialized structure responsible for transmitting and processing information between neurons. In this review, we will focus on recently reported data which suggest the existence of a close dialogue between neurons and glia. We will also report the results of studies aimed at defining how this dialogue may influence and modulate synaptogenesis and synaptic functionality.

2. ASTROCYTES IN NEURONAL DEVELOPMENT AND SYNAPTOGENESIS

One of the first steps in brain development is the migration of neurons from their birthplace to their final destination, a phenomenon which has been extensively studied in the neocortex and the cerebellum (Sidman & Rakic 1973). It has been reported (Komuro & Rakic 1996) that the movement of granule cells in cerebellar microexplant cultures is controlled by the occurrence of transient cytosolic calcium fluctuations, which, by activating an intracellular signal cascade, appear to regulate the rate of neuronal migration. The migration of cerebellar neurons occurs along radial glial fibres and it has been demonstrated that neuregulin-erbB receptor-mediated signalling is crucial for neuronal migration along glia (Rio *et al.* 1997). In the developing cerebral cortex, neurons regulate radial glia functions, and in turn glial cells direct neuronal migration and differentiation. This is accomplished by neuronal secretion of a soluble neuregulin acting on glial erbB2 receptor, which is responsible for maintenance and elongation of radial glial processes, along which neurons can migrate (Anton *et al.* 1997).

Glial cells appear to influence not only neuronal migration, but also the formation of synapses between neurons. This process is defined by the differentiation of a presynaptic compartment, containing clustered synaptic vescicles, and by the maturation of a postsynaptic compartment, characterized by the presence of neurotransmitter receptors in the plasmamembrane facing the active zone (Hall & Sanes 1993). A role of glial cells in promoting synaptogenesis has been suggested by data obtained on primary cultures of cortical and retinal neurons (Nakanishi *et al.* 1994; Pfrieger & Barres 1997) using functional approaches, such as electrophysiology and calcium imaging, to detect the establishment of fully differentiated synapses. In particular, Pfrieger & Barres (1997) used an immunopanning method to purify retinal ganglion cells at 99.5% purity, to place neuronal cultures devoid of glial cells. Under these experimental conditions a few synapses were established, displaying normal ultrastructural morphology but little spontaneous electrical activity and high failure rate. In co-cultures with neuroglia, few failures were detected and the frequency and amplitude of spontaneous synaptic events were both strongly enhanced. The same results were obtained using

*Author for correspondence (michelam@farma.csfic.mi.cnr.it).

astrocytic- or oligodendrocytic-conditioned media added to the glia-free neuronal cultures, suggesting that a diffusible factor originating from glial cells was acting on neurons promoting synapse formation. Similar results have been obtained recently on hippocampal neurons growing in the absence of glial cells, or co-cultured with hippocampal or cortical astrocytes. It has been found that most of the neurons grown in contact with astrocyte monolayers displayed a full synaptic functionality, detectable in the form of bursts of excitatory postsynaptic currents (EPSCs), even after 3–5 days in culture, a developmental stage in which neurons growing without neighbouring glial cells displayed no or few miniature synaptic events. The homotypic co-culture (hippocampal neurons grown on hippocampal astrocytes) seemed to be more efficient in fastening synapse formation. Moreover, differently from the case of retinal cells, a strict proximity between neurons and astrocytes appeared to be relevant in supporting the enhanced synaptogenesis, possibly by facilitating the delivery of trophic factors by astrocytes to neurons (A. Bacci and M. Matteoli, unpublished data). The nature of the factors involved in fastening synapse formation is still unknown. However, in a recent demonstration a glial-conditioned medium displayed a neurotrophic activity, which was mostly attributable to low-molecular weight fractions, containing high levels of L-serine. When added to hippocampal neurons, L-serine was found to improve neuronal survival and neurite outgrowth (Mitoma *et al.* 1998).

3. ASTROCYTE-OPERATED UPTAKE OF GLUTAMATE FROM THE SYNAPTIC CLEFT

Glutamate is the major excitatory neurotransmitter in the brain. The duration of the glutamatergic synaptic response is determined by receptor desensitization and by the lifetime of the neurotransmitter in the synaptic cleft. Both neurons and astrocytes express glutamate transporters and take up glutamate, even though astrocytes appear to display a higher capacity to remove glutamate from the extracellular space. In a recent study, Rothstein and collaborators (1996) have demonstrated the crucial importance played by glial-operated removal of glutamate. Taking advantage of the different subtypes of glutamate carriers expressed by neurons and astrocytes, the authors have selectively impaired the expression of either glial (GLAST and GLT-1) or neuronal (EAAC1) transporters using antisense oligonucleotides. They found that a block of the glial, but not of the neuronal, glutamate uptake resulted in a drastic elevation of glutamate, leading to neuronal degeneration due to excitotoxicity.

The crucial role of glial transporters in removing functionally relevant amounts of synaptically released glutamate is supported by anatomical and functional data. Ultrastructural studies have demonstrated that the astrocytic glutamate transporters are preferentially localized in those membrane areas which face synaptic regions, whereas they are virtually absent from areas with lower demand (i.e. vascular endothelium, pial surface and apposed astrocytic membranes) (Chaudry *et al.* 1995; Derouiche 1997). Conversely, a localization on the extrasynaptic plasma membrane of the postsynaptic neurons has been demonstrated for the neuronal

glutamate transporters EAAC1 (Coco *et al.* 1997) and EAAT4 (Dehnes *et al.* 1998). Both neuronal transporters appeared to be enriched on the spine neck and on the dendritic shaft, but not on the postsynaptic site at the level of the spine head. Given their localization, neuronal glutamate transporters may play a role in the uptake of neurotransmitter during glutamate spillover, following intense presynaptic stimulation, whereas glial transporters may play a major role in removing relevant amounts of glutamate from the synaptic cleft (figure 1). Interestingly, the uptake of glutamate analogues into glial processes surrounding the synapses has been found to be more efficient for nerve terminals adapted to high-level tonic activity, suggesting that glial-operated glutamate metabolism may adapt to the activity pattern of a given synapse (Gundersen *et al.* 1995). Not only the functionality but even the expression of glial glutamate transporters seems to be regulated by the presence of neurons. Gegelashvili *et al.* (1997) showed that GLT-1 is not expressed by pure astroglial cultures, even though GLT-1 protein and mRNA are induced either in glia–neuron co-cultures or by treatment of pure glial cultures with neocortical neuron-conditioned medium. The authors hypothesize that a soluble factor, released by neurons, differently regulates the expression of GLT-1 and GLAST in cultured astroglia. The molecular nature of this factor needs to be elucidated. However, glia–neuron co-cultures grown in the presence of transmitter release inhibitors should clarify whether this soluble factor is represented by neuronal-released glutamate.

Glutamate carriers present on the membrane of glial cells are able to respond to glutamate released at the synapse. Mennerick and co-workers (1994, 1996), using an electrophysiological approach, performed simultaneous recordings from an astrocyte and a neuron grown on a micro-island. When an autaptic current was elicited in the neuron, an inward current could be detected in the astrocyte, corresponding to the Na^+-coupled electrogenic glutamate uptake. When glutamate uptake in the astrocyte was blocked by pharmacological tools, or by clamping the astrocyte membrane at positive voltages, the astrocyte current could not be further recorded. In parallel, a dramatic prolongation of the postsynaptic response was detected. The electrogenic glutamate uptake in astrocytes, coupled with synaptic activation, has recently also been recorded in rat cerebellar (Clark & Barbour 1997) and hippocampal slices (Bergles & Jahr 1997).

All together, these data indicate that synaptically released glutamate activates astrocytic carriers and, in turn, the carrier function influences the decay of the synaptic response. A direct demonstration that this mechanism may have a major effect on the transmission of signals in a neuronal network comes from results of experiments recently carried out on cultured hippocampal neurons and on the mouse visual system. Experiments performed on hippocampal cultures have revealed that neuronal synchronized activity, detectable as oscillations of cytosolic calcium coincident with membrane potential burst firing (Bacci *et al.* 1999), was strictly dependent on the presence of glial cells, which direct the occurrence of repetitive burst firing by strictly controlling the glutamate concentration and lifetime in the extracellular medium (reported in abstract form in
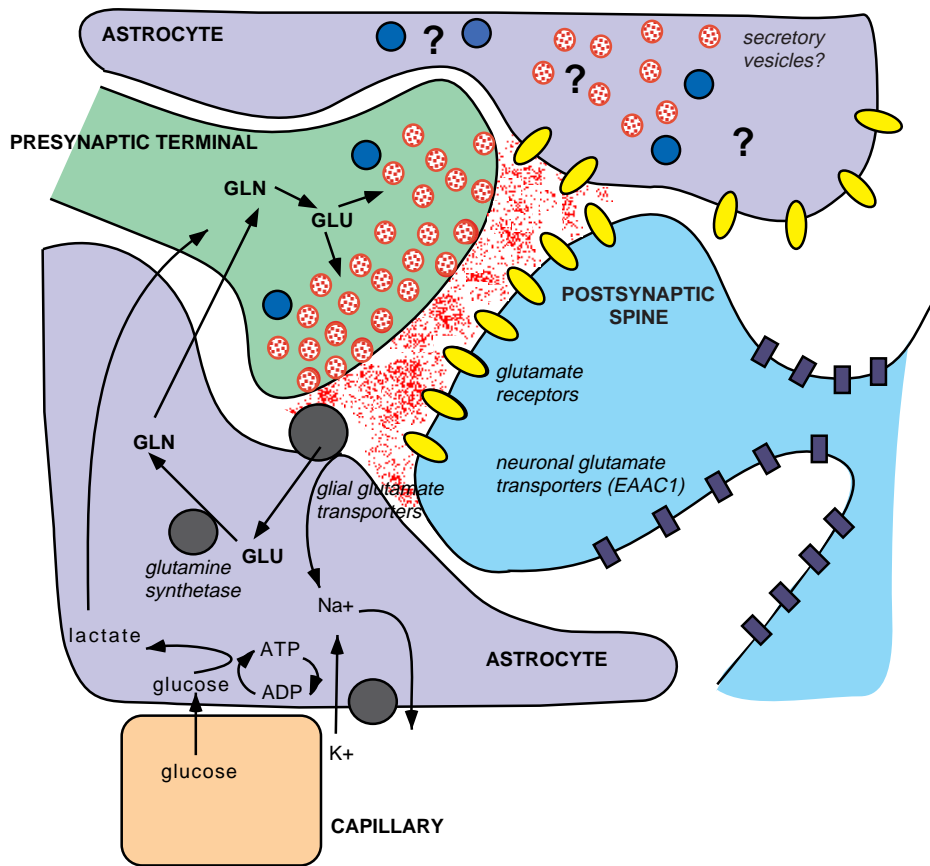
Figure 1. Schematic representation of cellular pathways by which astrocytes may influence neuronal function. Synaptically released glutamate is taken up by astrocyte transporters and converted to glutamine by the enzyme glutamine synthetase. Glutamine is then provided to neurons for the synthesis of new molecules of glutamate. Glutamate is co-transported with Na^+, causing an increase in Na^+ concentration which leads to the activation of a Na^+/K^+ ATPase. This activation stimulates the glycolysis, which is taken up by astrocytes from the blood vessels, with consequent lactate production. Lactate is then released to neurons, which use it as a preferential energy substrate. The presence of glutamate receptors on the astrocyte membrane and the possible presence of secretory vesicles in the astrocyte cytoplasm are indicated. The scheme also depicts the localization of the neuronal glutamate transporters EAAC1 on the spine neck of the postsynaptic neurons. See text for details and references. GLU, glutamate; GLN, glutamine.

Bacci *et al.* 1997; C. Verderio, unpublished data). In line with this observation, Harada *et al.* (1998) showed that mutant mice lacking GLAST transporters display reduced electroretinogram potentials, suggesting that GLAST is required for normal neurotransmission between photoreceptors and bipolar cells. The glia-operated removal of glutamate may therefore end up controlling the synchronous activity occurring in a neuronal network. It is conceivable to hypothesize that the astrocyte-operated removal of glutamate influences synaptic function by keeping glutamate at low levels, thus avoiding glutamate receptor desensitization and enhancing neuronal sensitivity to glutamate.

4. METABOLIC COUPLING BETWEEN NEURONS AND GLIA

Glial cells are metabolically coupled to neurons. A tight and regulated association takes place at the level of synaptic contacts, where large amounts of energy are required to support a prolonged synaptic transmission. This is also suggested by the presence of several mitochondria in the presynaptic terminal. Glucose, the main energy source for the brain, crosses the blood−brain barrier and enters astro-

cytes, which contact the blood vessels by end-feet terminals. Astrocytes are then responsible for transferring the metabolic substrate to the neuropil (for a review, see Tsacopoulos & Magistretti 1996) (figure 1).

During the last few years, many studies have been performed to determine whether astrocytes simply release glucose to neurons or deliver metabolically active intermediates. Poitry-Yamate *et al.* (1995) showed that in the retina Müller cells, radioactive glucose is converted to lactate, which is then released in the extracellular space. Measuring the formation of carbon dioxide, the authors showed that neurons strongly prefer lactate as a substrate for oxidative metabolism, suggesting that glial cells supply neurons with their preferred energy source. Glutamate uptake, performed by the astrocytic carriers, is also involved in the cycle which leads to glucose uptake into astrocytes and to lactate release in the extracellular space. Indeed, glutamate is transported into astrocytes coupled to the inward transport of 2−3 Na^+ per glutamate molecule (Bouvier *et al.* 1992). The increased concentration of Na^+ in astrocytes leads to the activation of the Na^+/K^+ ATPase, which stimulates glycolysis, i.e. glucose use and lactate production (Tsacopoulos & Magistretti 1996). It could be said that astrocytes fuel neurons in a

glutamate-dependent way and therefore by a synaptic activity-operated mechanism (Pellerin & Magistretti 1994). Synaptic function and its energy supply from the glial cells appear therefore to be tightly coupled (Pfrieger & Barres 1996). This idea is further supported by a recent paper (Sibson et al. 1998) in which the stoichiometry between oxidative glucose metabolism and glutamate-neurotransmitter cycling in rat neocortex in vivo has been reported. By using ^{13}C NMR the authors measured the rates of tricarboxilic acid cycle and glutamine synthesis, and found that the stoichiometry between the two cycles is close to 1:1, thus demonstrating that glutamatergic synaptic activity accounts for more than 80% of total glucose oxidation. Their data suggest that the synaptic glutamate release might be the key step for cortical glucose consumption.

One of the most important aspects of the metabolic coupling between glia and neurons is represented by the glutamate–glutamine cycle. Glutamate released in the synaptic cleft is taken up by astrocytes and is converted to the non-excitatory amino acid glutamine. Glutamine is then released in the extracellular space, taken up by neurons and converted back to glutamate in the presynaptic terminals (figure 1). Glutamate is then available to fill new vesicles and ready to exert its excitatory action during neurotransmission (Pfrieger & Barres 1996). A key molecule in this cycle is represented by the enzyme glutamine synthetase, which is selectively expressed in glial cells and which is co-localized with GLAST glutamate transporters in the retina (Derouiche & Rauen 1995). The overlapping subcellular distribution of GLAST and glutamine synthetase indicates a close correlation between uptake of released glutamate at the synapse and its conversion to glutamine in glial cells. Using a morphological approach, Laake et al. (1995) studied the ultrastructural distribution of both glutamate and glutamine in rat hippocampal slices after specific pharmacological inhibition of astrocytic glutamine synthetase by methionine sulphoximine (MSO). When glutamine synthetase activity was impaired by drug application, glutamine immunoreactivity was strongly reduced in astrocytes. In parallel, an increase of glutamate concentration in glial processes and a consequent reduction of glutamate immunoreactivity in presynaptic neurons was found to take place. These data, which were in line with the results previously obtained by Pow & Robinson (1994) in cultured rabbit retinae, supported the hypothesis of a glutamate–glutamine shuttle between glia and neurons, and suggested that the major source of glutamate in neurons derives from glutamine supplied by glial cells.

A demonstration that the glutamate–glutamine cycle between astrocytes and neurons plays a relevant role in neuronal synaptic functionality comes from the results of recent experiments obtained in primary cultures of hippocampal neurons. In this model, the spontaneous neuronal oscillations' synchronous activity was found to undergo a progressive decay, up to a complete block, when astrocytic glutamine synthetase was blocked by MSO. Neuronal synchronous activity could be rescued by the application of exogenous glutamine (Verderio et al. 1998), suggesting that glial-operated glutamine delivery to the presynaptic terminal is essential for the long-term maintenance of a fully working synaptic transmission.

This concept is further sustained by the demonstration that blocking glial metabolism with fluorocitrate or fluoroacetate, which are taken up selectively by glial cells and act as 'suicide' substrates for the enzyme aconitase rapidly stopping the Krebs cycle in astrocytes and therefore glutamine production, results in an impairment of synaptic transmission in rat hippocampus (Keyser & Pellmar 1994, 1997).

5. ASTROCYTES IN SYNAPTIC PLASTICITY

Synaptic plasticity takes place in a variety of regions in the CNS, including: hippocampus, where long-term potentiation (LTP) and long-term depression (LTD) have been associated with learning and memory (Malenka 1994); neocortex, where phenomena of plasticity have been correlated with the development of the visual system (for review, see Post & Weiss 1997); and cerebellum, where long-term changes in synaptic transmission control sensory-motor learning and eyeblink conditioning (for review, see Kim & Thompson 1997). Whereas the mechanisms regulating the induction of neuroplasticity phenomena seem to be essentially clear, those related to their expression are still a matter of debate. Most of the experiments on the long-term enhancement or depression of synaptic transmission have focused on isolated neuronal functions with little attention payed to adjacent glial cells. We report here results from studies which support the possibility of an active role played by astrocytes in synaptic plasticity. These contributions suggest that astrocytes may participate to plasticity phenomena via at least three different mechanisms.

Astrocytes, by the activation of inward-rectifying potassium channels present on their plasma membrane, play a major role in keeping extracellular K^+ concentration low, preventing therefore a chronic depolarization of neurons during prolonged activity. A recent paper by Janigro et al. (1997) showed that extracellular caesium application causes interictal-like bursting and prevents maintenance of LTD in CA1 hippocampal area. This effect was attributable to a direct action of caesium on glial inward-rectifying potassium channels. Reduction of K^+ uptake in glia resulted therefore in alterations of plasticity phenomena.

In a report by McCall et al. (1996), the authors analysed the effect of glial fibrillary acidic protein (GFAP)-null deletion in neuronal physiology. GFAP belongs to the family of intermediate filament structural proteins and is predominantly expressed by astrocytes of the CNS. The onset of expression of this protein represents one of the key events during astrocyte differentiation. Using GFAP-null mice, the authors demonstrated that the baseline synaptic transmission in the CA1 hippocampal area is not affected in mutant mice, whereas LTP is enhanced, implying a role of astrocytes in LTP. Also LTD is altered in GFAP-mutant mice. Shibuki and collaborators (1996) demonstrated that mice devoid of GFAP, though developing as normal and displaying a regular synaptic transmission, show a high LTD deficiency associated with a dramatic impairment of eyeblink conditioning, thus suggesting that GFAP is required for communications between Bergmann glia and Purkinje cells during LTD induction and maintainance. The observations that astrocytic GFAP deficiency leads to

the enhancement of LTP and to the impairment of LTD may support the possibility that GFAP directly participates in the changes of glial processes juxtaposed to the synaptic terminals during LTP expression. Indeed, a quantitative electron microscopy study in rat hippocampus (Wenzel *et al.* 1991) showed that significant changes in the ramifications of astrocyte processes occur after repeated high-frequency stimulations of the perforant pathway, resulting in an increase in the density of glial processes, which were in a closer apposition to the synaptic cleft in the potentiated synapses.

In the CNS, synaptic plasticity seems to be dependent also on the production of nitric oxide (NO), which is thought to act as a messenger, enhancing transmitter release from the presynaptic terminal (Holscher 1997). The presence of the nitric oxide synthetase enzyme (NOS) in astrocytes has been detected in the CAl hippocampal region and its induction has been reported to be enhanced after hippocampal injury (Stojkovic *et al.* 1998). Another study, performed on cultured cerebellar astroglia (Baltrons & Garcia 1997), showed that glial AMPA receptors are calcium permeable and that their activation stimulates NO production in astrocytes, suggesting therefore a role in glutamate-dependent synaptic plasticity in the cerebellum.

6. MESSAGES RUNNING FROM GLIA TO NEURONS

In the last few years, a growing number of studies focused on calcium signalling in glial cells (for a review, see Verkhratsky & Kettenmann 1996). Glial cells generate calcium waves or oscillations, following a variety of stimulations, including neurotransmitters, hormones and mechanical or electrical stimulations. Since glial cells are not equipped with ion channels to generate and propagate action potentials, they use intracellular calcium elevations and spreading calcium waves as signalling tools in the CNS. One of the first pieces of evidence that calcium waves in cultured astrocytes are elicited by glutamate came from a report by Cornell-Bell and collaborators (1990). These authors showed that when glutamate was applied, astrocytes responded with oscillations of cytosolic free calcium. This activity was found to be dependent on two types of glutamate receptors, one preferring quisqualate and the other preferring kainate, inducing calcium release from intracellular stores or promoting calcium influx from the plasma membrane, respectively. Calcium waves were found to propagate between adjacent astrocytes, suggesting a long-range signalling system.

Calcium oscillations in astrocytes represent a plastic phenomenon, as demonstrated by Pasti *et al.* (1995), who studied long-term changes in the response to glutamate in cultured cortical astrocytes. Glutamate application caused calcium oscillations in astrocytes and a further application of glutamate was able to induce a response characterized by enhanced frequency. The potentiation of the response was long-lasting, required the activation of the metabotropic glutamate receptors coupled to inositol triphosphate production, and was impaired by NOS inhibitors. In a more recent paper (Pasti *et al.* 1997), the same authors detected a comparable plastic phenomenon in astrocytes present in cortical slices, on stimulation with metabotropic glutamate receptor

agonists as well as on synaptic stimulation. This long-lasting enhancement of calcium oscillations in astrocytes reveals that cellular memory in the CNS is not a unique feature of neurons.

Calcium elevations in astrocytes appear to lead to glutamate release from the same cells. Indeed, calcium transients induced in glial cells by stimulation with bradykinin or with mechanical or electrical stimulations were associated with subsequent calcium elevations and with the activation of a slow inward current (SIC) in neighbouring neurons in culture. Both calcium transients and SIC were elicited by released glutamate, as demonstrated by their inhibition with the NMDA receptor blocker AP-5. (Parpura *et al.* 1994; Araque *et al.* 1998). These data indicate that calcium fluctuations in astrocytes are associated with release of glutamate. PGE2-stimulated release of glutamate from astrocytes has recently been demonstrated in both culture systems and acute hippocampal slice preparations. PGE2 was found to induce an increase in astrocytic free calcium concentration and a consequent delayed calcium response in the adjacent neurons. Once again, astrocyte-induced neuronal response was dependent on the activation of neuronal ionotropic glutamate receptors (Bezzi *et al.* 1998). Glutamate, however, is not the only molecule released by astrocytes following and modulating neuronal synaptic transmission. Indeed, beta-adrenergic stimulation (but neither alpha-adrenergic nor vasoactive intestinal peptide (VIP) stimulation) of astrocytes in culture (Do *et al.* 1997) was found to result in release of homocysteic acid, which exerts an excitatory effect on neurons mainly through NMDA receptors. Homocysteic acid is exclusively present in glial cells and, given its effect on neuronal cells, has been defined as a 'gliotransmitter'.

Even though the secretory properties of astrocytes are well established, a clear demonstration of the presence of a typical regulated secretory pathway in glial cells has not yet been provided. Recently, however, the presence of large, dense core vesicles containing the marker for regulated secretion secretogranin II has been demonstrated in cultured astrocytes. Secretogranin II was found to be released by stimulation with secretagogues inducing increases in the levels of intracellular calcium (F. Calegari, S. Coco, M. Bassetti, C. Verderio, M. Matteoli and P. Rosa, unpublished data), indicating that astrocytes possess a typical pathway for regulated secretion. It will be important to define the identity of molecules possibly co-stored with secretogranin II in the lumen of astrocyte large dense core vesicles.

7. CONCLUSIONS

Glial cells, by lying in close associations with neurons all over the CNS, play a primary role in promoting neuronal migration, differentiation and synaptogenesis. By actively uptaking neurotransmitter released during synaptic activity, glial cells avoid receptor desensitization and maintain a high signal-to-noise ratio, possibly helping neurons to set the timing of synchronous activity. Astrocytes fuel neurons with the energy source lactate and with the neurotransmitter precursor glutamine, allowing neurons to function efficiently during prolonged synaptic activity. All these processes appear to be finely regulated by neuronal activity. Neurons, while exerting

their function of transmitting and processing information, send messages to the adjacent glial cells, which in turn react by providing neurons with all the necessary aids and by generating negative or positive feedback. A third element should therefore be added to the typical scheme of the synapse, as formed by two neurons contacting each other: the ensheathing glial cells, sensing and modulating synaptic transmission.

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