Leech Giant Glial Cell: Functional Role in a Simple Nervous System

JOACHIM W. DEITMER,* CHRISTINE R. ROSE, THOMAS MUNSCH, JOACHIM SCHMIDT, WOLFGANG NETT, HANS-PETER SCHNEIDER, AND CHRISTIAN LOHR

Abteilung für Allgemeine Zoologie, FB Biologie, Universität Kaiserslautern, Kaiserslautern, Germany

ABSTRACT   The giant glial cell in the central nervous system of the leech *Hirudo medicinalis* has been the subject of a series of studies trying to link its physiological properties with its role in neuron–glia interactions. Isolated ventral cord ganglia of this annelid offer several advantages for these studies. First, single giant glial cells can easily be identified and are quite accessible to electrophysiological and microfluorometric studies. Second, only two giant macroglial cells are located in the neuropil of each ganglion, rendering them well suited for studying neuron–glia interactions. Third, many neurons can be identified and are well known with respect to their physiology and their roles in controlling simple behaviors in the leech. This review briefly outlines the major recent findings gained by studying this preparation and its contributions to our knowledge of the functional role of glia in nervous systems. Emphasis is directed to glial responses during neuronal activity and to the analysis of intracellular Ca^{2+} and H^+ transients mediated by neurotransmitter receptors and ion-driven carriers. Among its numerous properties, the leech giant glial cell prominently expresses a large K^+ conductance, voltage-dependent Ca^{2+} channels, ionotropic non-NMDA glutamate receptors, and an electrogenic, reversible Na^+-HCO_3^- cotransporter. *GLIA 28:175–182, 1999.* © 1999 Wiley-Liss, Inc.

INTRODUCTION

The first demonstration of neuronal cell bodies in the leech CNS goes back to Ehrenberg (1836), when he was "at the threshold of detecting cellular structures" as living units of multicellular organisms (see Kettenmann and Rudolph, 1998). Half a century later, Gustav Retzius performed his impressive anatomical studies on annelid nervous systems, including that of the medicinal leech (Retzius, 1891). The fine structure of the CNS of the medicinal leech *Hirudo medicinalis* L. was studied by Coggeshall and Fawcett (1964), whose work still serves as the major reference for the ultrastructure of the leech CNS.

The first physiological studies on leech glial cells were performed by Kuffler and coworkers (Kuffler and Potter, 1964; Kuffler and Nicholls, 1966). Because of their good accessibility in contrast to most vertebrate glial cells, leech glial cells were among the first preparations used for studying physiological properties and functions of glial cells in general. It was found that the leech glial cells are electrically coupled and that their resting potential is mainly determined by the K^+ permeability of the plasma membrane, properties later also found to apply to astrocytes (see Duffy et al., 1995; Giaume and McCarthy, 1996).

MORPHOLOGY

With the exception of small microglial cells, the two giant glial cells are the only cells in leech ganglia whose
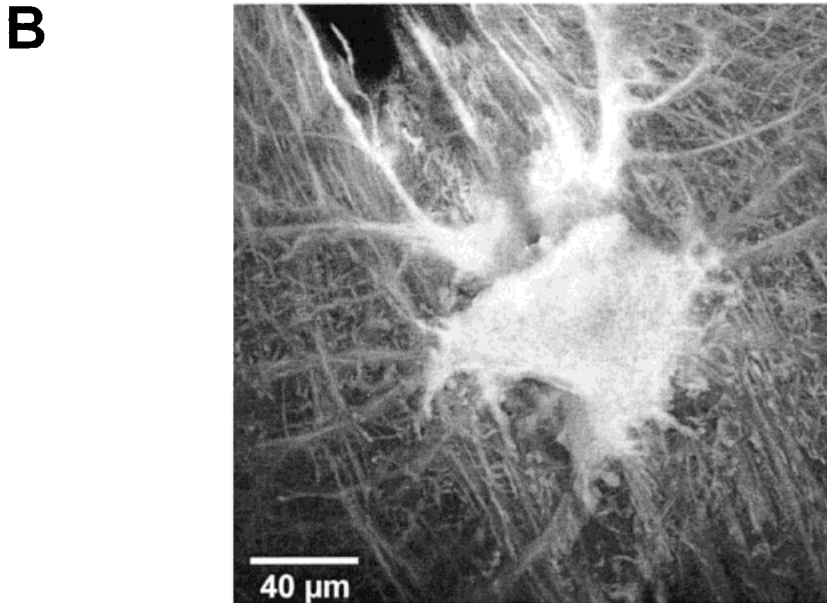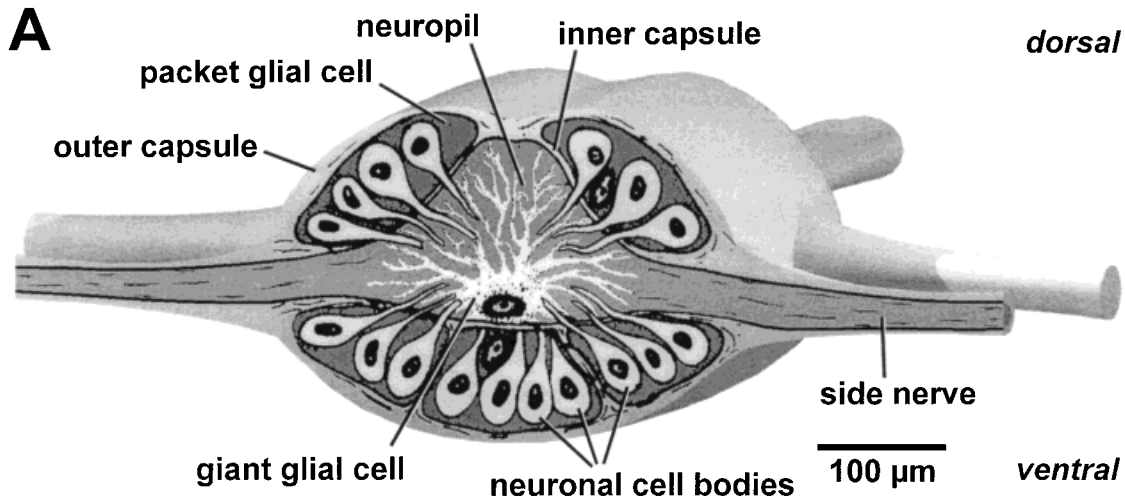
Fig. 1. **A:** Schematic diagram of a segmental ganglion with a giant glial cell in the neuropil in cross section (modified from Kuffler & Potter, 1964). **B:** Posterior giant glial cell, filled with the fluorescent dye Oregon green BAPTA-1. The cell was projected from a stack of 45 confocal images.

cell bodies are located in the neuropil (Fig. 1A), one in the anterior part and one in the posterior part. They are surrounded by a meshwork of dendrites, axons, and synaptic contacts provided by neurons, the cell bodies of which are located in the ganglionic periphery (Coggeshall and Fawcett, 1964). The other types of macroglial cells in leech ganglia are two connective glial cells that surround the axons and six packet glial cells that ensheathe the cell bodies of neurons.

In adult ganglia, the cell body of each giant glial cell measures 80–100 µm in diameter (Munsch and Deitmer, 1992; Lohr and Deitmer, 1997a, 1999). Numerous, extensively branching cell processes ("dendrites") ex-

tend across the entire width (300–350 µm) of the neuropil (Fig. 1B), reaching into the immediate vicinity of neuronal cell processes (Coggeshall and Fawcett, 1964; Lohr and Deitmer, 1997a; Riehl and Schlue, 1998).

The giant glial cells are electrically and dye coupled with each other and with their neighboring packet and connective glial cells by gap junctions (Kuffler and Potter, 1964; Coggeshall, 1974; Lohr and Deitmer, 1997a). Thus, the macroglial cells build a syncytium within the whole nerve cord that is likely to function as a pathway for small molecules, such as metabolites or messengers.
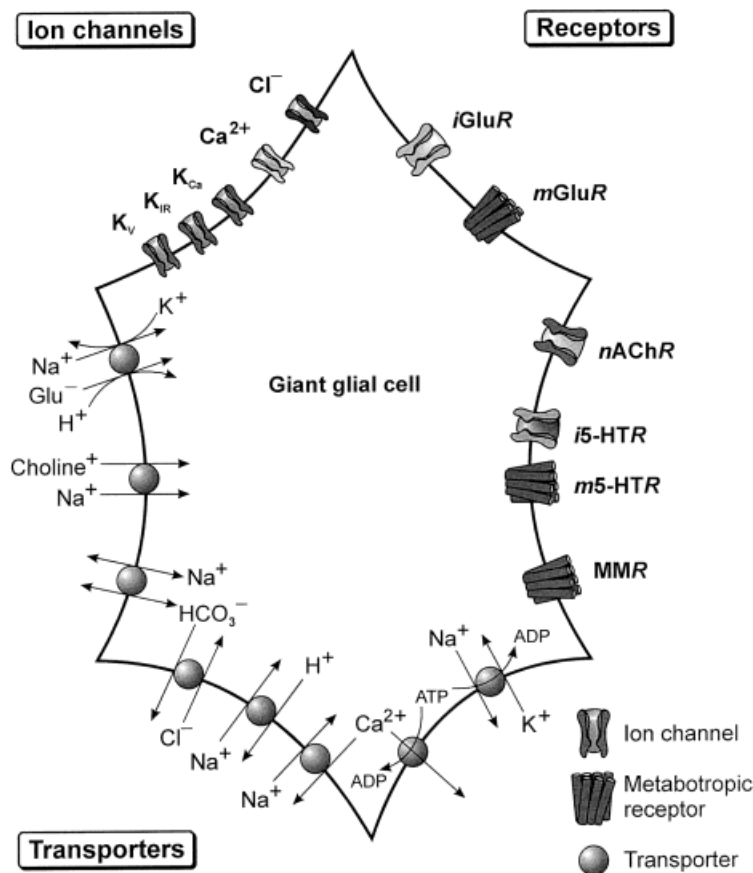
Fig. 2. A selection of ion channels, receptors, and transporters that have been identified in the leech giant glial cell. Abbreviations: iGlu$R$ ionotropic glutamate receptor; mGlu$R$ metabotropic glutamate receptor; nAChR nicotinic acetylcholine receptor; i5-HTR ionotropic 5-hydroxytryptamine receptor; m5-HTR metabotropic 5-hydroxytryptamine receptor; MMR myomodulin receptor.

Although the anterior and the posterior giant glial cells may differ somewhat in size and shape, no differences have so far been detected in their physiological properties and functions. Therefore, we usually do not differentiate between these two cells, and refer to either cell as "the giant glial cell."

RESTING MEMBRANE POTENTIAL

The giant glial cell is equipped with a large set of ion channels, receptors, and transporters (Fig. 2). It is characterized mainly by a large K^+ conductance (Kuffler and Potter, 1964), and different types of K^+ channels have been identified (Müller and Schlue, 1997; Nett and Deitmer, 1998a). The resting membrane potential of the giant glial cell varies between -50 mV and -75 mV. Two observations indicate that this wide range reflects a physiological property and is not necessarily due to cell damage caused by the insertion of a microelectrode. First, the cells often alkalinize in parallel to membrane depolarization (Deitmer and Schneider, 1995, 1998). A damaged cell, in contrast, always acidifies owing to the inward movement of acid equivalents along the electrochemical H^+ gradient across the cell membrane. Second, lower resting membrane potentials are not correlated with a decreased membrane input resistance, as would be expected for damaged cells (J.W. Deitmer, H.P. Schneider, and J. Schmidt, unpublished). Instead, the more depolarized membrane potentials appear to be caused by a decreased K^+ conductance. Third, addition of 5-hydroxytryptamine (5-HT; 10–50 µM) to the bathing medium evokes a sustained membrane hyperpolarization to -70 mV in healthy cells, whereas damaged cells hyperpolarize only transiently in response to 5-HT.

pH REGULATION

Intracellular pH regulation in the leech giant glial cell in situ was first studied in the late 1980s (Deitmer and Schlue, 1987, 1989), at the time when the first reports on pH regulation in cultured mammalian astrocytes were published (Chesler and Kraig, 1987). In leech glial cells, three major pH_i-regulating processes have been described (Fig. 2): the Na^+–HCO_3^- cotransport, the Na^+–H^+ exchange, and the Cl^-–HCO_3^- exchange (Deitmer and Schlue, 1987, 1989; Deitmer, 1991; Szatkowski and Schlue, 1994). It turned out that

TABLE 1. Effects of neurotransmitters and neuromodulators on membrane potential and ion activities of the giant glial cell (in nominally CO_2/HCO_3-free saline)*

	Glutamate	Kainate	Aspartate	5-Hydroxy-tryptamine	Acetylcholine/carbachol	Myomodulin
E_m	dep.	dep.	dep.	hyp.	dep.	hyp.
Ca_i^{2+}	↑	↑	↑	↑	↑	—
Na_i^+	↑	↑	↑	—	↑	n.d.
K_i^+	↓	↓	n.d.	—	↓	n.d.
pH_i	↓	↓	↓	—	↓	—

*dep., Depolarization; hyp., hyperpolarization; ↑, increase; ↓, decrease; —, no effect; n.d., not determined.

the electrogenic Na^+–HCO_3^- cotransport is the dominant pH_i-regulating transport in this cell. It has been estimated that it is at least 10 times as active as the Na^+–H^+ exchange at pH_i of 7.0 and in the presence of 5% CO_2/24 mM HCO_3^- (Deitmer and Schneider, 1995, 1998). This estimation was made under the assumption that Na^+–H^+ exchange is the dominant acid extruder in the nominal absence of CO_2/HCO_3^- in the saline. Because the Na^+–HCO_3^- cotransport has a very high affinity for HCO_3^-, it shifts acid/base equivalents across the cell membrane, even in the nominal absence of CO_2/HCO_3^-, though much more slowly than in the presence of added HCO_3^-. Apparently, the HCO_3^- in the saline derived from air-CO_2, amounting to submillimolar concentrations, is sufficient to drive the Na^+–HCO_3^- cotransport (Deitmer and Schneider, 1998). It is likely, therefore, that Na^+–HCO_3^- cotransport is significantly more active than was previously estimated.

The major features of the Na^+–HCO_3^- cotransporter in the leech giant glial cell, which is found in many types of glial cells (see Deitmer and Rose, 1996) are the following. 1) The cotransport is electrogenic, with a stoichiometry of 2 HCO_3^- and 1 Na^+. 2) The cotransport is reversible and operates in the inward or outward direction and shifts the pH_i until the cotransport is at its electrochemical equilibrium near −75 mV. 3) Hence, the actual membrane potential determines the intraglial pH value via the electrogenic cotransporter; membrane depolarization alkalinizes, and membrane hyperpolarization acidifies the glial cell. 4) The cotransport is inhibited by the stilbene compound DIDS but is not blocked by inhibitors of the carbonic anhydrase. 5) Stimulation of the glial Na^+–HCO_3^- cotransport can also change the pH in the extracellular spaces; and a change in the glial membrane potential may result in a small but significant pH_e shift, opposite that occurring in the glial cells.

Because the Na^+–HCO_3^- cotransport carries base equivalents into and out of the glial cell, it functions as an acid extruder and acid loader, depending on the glial membrane potential, as discussed above. Therefore, the significance of the acid loader Cl^-–HCO_3^- exchange and the acid extruder Na^+–H^+ exchange should be reinvestigated. The electroneutral Na^+–H^+ exchange can be blocked by amiloride, but has not been further analyzed in detail because of its minor impact on pH_i

regulation in the leech glial cell under physiological conditions.

CALCIUM REGULATION

Changes in intracellular Ca^{2+} play a central role in the contribution of glial cells to signalling processes in nervous systems. The release of calcium from intracellular stores and the spread of calcium waves from one glial cell to another have been studied extensively in mammalian astrocytes (Cornell-Bell et al., 1990; Charles et al., 1991) and have been summarized in several recent reviews (Verkhratsky and Kettenmann, 1996; Verkhratsky et al., 1998; Deitmer et al., 1998).

The leech giant glial cell is the only invertebrate glial cell in which calcium signalling and calcium regulation have been studied in any greater detail. The main mechanism by which the low resting Ca^{2+} concentration of 30–70 nM (Munsch et al., 1994; Nett and Deitmer, 1998b) is maintained is a plasmalemmal Ca^{2+}-ATPase. Inhibition of the Ca^{2+}-ATPase by injection of orthovanadate into the glial cell results in a rise of intracellular Ca^{2+} by several hundred micromoles/liter. Removal of Na^+, in contrast, leads to a moderate intracellular Ca^{2+} increase of 30 nM. Na^+–Ca^{2+} exchange appears to become significant only when the Ca^{2+}-ATPase is inhibited and/or following a large cytoplasmic Ca^{2+} load (Nett and Deitmer, 1998b). Intracellular organelles, such as the endoplasmic reticulum and mitochondria, may also contribute to the regulation of intracellular Ca^{2+}, but little is known about these mechanisms in the giant glial cell.

The giant glial cell expresses functional, voltage-dependent Ca^{2+} channels (Munsch and Deitmer, 1992, 1995; Hochstrate et al., 1995) which are activated at membrane potentials more positive than −50 mV. Hence, membrane depolarizations beyond −50 mV evoke large and rapid Ca^{2+} transients in the giant glial cell. Membrane depolarizations of this amplitude can be obtained by raising the external K^+ concentration above 10 mM or by activating neurotransmitter receptors such as nicotinic acetylcholine receptors (Ballanyi and Schlue, 1988; Hochstrate and Schlue, 1995) or ionotropic, non-NMDA glutamate

receptors (Munsch et al., 1994, Munsch and Deitmer, 1997).

specific behaviors and what role glial cells may play in controlling these behaviors.

NEURON–GLIA INTERACTION

The leech giant glial cell responds both to electrical stimulation of ganglionic side nerves and to bath application of a variety of neurotransmitters, including glutamate, acetylcholine, 5-HT, and GABA, with membrane potential changes (Table 1; see Deitmer and Rose, 1996; Deitmer et al., 1998). Side nerve stimulation can result in membrane hyperpolarization and/or in depolarization, depending on previous activation and value of the membrane potential (J.W. Deitmer, H.P. Schneider, and J. Schmidt, unpublished observations). Cells with a resting potential more positive than -60 mV respond to the nerve stimulation with a sustained hyperpolarization. Subsequent stimulations, at membrane resting potentials negative to -60 mV, usually induce membrane depolarizations. Sometimes multiphasic membrane potential changes are recorded, indicating that more than one mechanism can affect the glial membrane potential during neuronal activity (Schmidt et al., 1999; J.W. Deitmer, H.P. Schneider, and J. Schmidt, unpublished observations).

We have recently found that the activity of a single neuron can elicit a change in the membrane potential in the giant glial cell (Schmidt and Deitmer, 1999). Brief stimulation of the neurosecretory Leydig neuron evokes a hyperpolarization of the glial cell membrane. Suppression of the spontaneous activity in the Leydig neuron, in contrast, causes a small, reversible membrane depolarization in the giant glial cell. This suggests that the glial membrane potential might be partially determined by the firing rate of the two Leydig neurons in each ganglion.

Leydig neurons contain a peptide of the myomodulin family (Keating and Sahley, 1996) that has been sequenced recently (Wang et al., 1998). Both bath application of the molluscan myomodulin A and of the leech myomodulin-like peptide directly hyperpolarize the glial membrane or cause an outward current in a voltage-clamped giant glial cell (Schmidt and Deitmer, 1999; F.C. Britz, J. Schmidt, C. Lohr, W. Nastainzcyk, and J.W. Deitmer, in preparation). This suggests that the effect of Leydig cell activity on the glial cell might be mediated by a (co-)released myomodulin-like peptide.

Preliminary studies have shown that the giant glial cell responds to the activation of reflex arcs during certain behavioral patterns with membrane potential shifts (Deitmer and Kristan, 1999). Stimulation of neural networks underlying the whole-body shortening reflex induced a membrane hyperpolarization in the giant glial cell, whereas activation of the oscillatory network underlying swimming behavior has no significant effect on the glial membrane potential in the semi-intact leech preparation. Further studies are needed to establish whether glial cells may change their properties prior to, during, or in response to

CALCIUM AND pH SIGNALING

Nerve root stimulation results not only in membrane potential shifts but also in intra- and extracellular calcium and pH changes (Rose et al., 1995; Rose and Deitmer, 1995a,b; Lohr and Deitmer, 1999). In contrast to Ca^{2+} signaling in mammalian astrocytes, Ca^{2+} transients in leech giant glial cells are mostly due to Ca^{2+} influx from the extracellular space rather than to Ca^{2+} release from intracellular stores. So far only glutamate is known to cause intracellular Ca^{2+} release mediated by the activation of metabotropic receptors (Lohr and Deitmer, 1997b). This Ca^{2+} transient is evoked via inositol-trisphosphate ($InsP_3$) production and $InsP_3$-sensitive Ca^{2+} release, resulting in a cytoplasmic Ca^{2+} rise of 10–30 nM. This is much smaller than $InsP_3$-mediated Ca^{2+} transients observed in vertebrate glial cells, where Ca^{2+} rises by one to two orders of magnitude (Jensen and Chiu, 1990; Brune and Deitmer, 1995), and indicates that the leech giant glial cell has a relatively low intracellular Ca^{2+} storage capacity.

Ionotropic, non-NMDA glutamate receptors that are activated by low concentrations of kainate or AMPA (5–100 µM) not only mediate a membrane depolarization and hence the opening of voltage-gated Ca^{2+} channels but are themselves permeable to Ca^{2+} (Munsch et al., 1994; Munsch and Deitmer, 1997). Similar properties have been reported for mammalian glial cells (Burnashev et al., 1992; Müller et al., 1992).

5-HT hyperpolarizes the leech glial membrane by raising the K^+ permeability (Walz and Schlue, 1982), presumably by a metabotropic pathway. In addition, 5-HT leads to a rise in Ca^{2+} (Munsch and Deitmer, 1992). This Ca^{2+} increase is dependent on the presence of external Ca^{2+} and is not affected by a depletion of intracellular calcium stores by cyclopiazonic acid. This suggests that the Ca^{2+} increase might be due to Ca^{2+} influx from the extracellular space rather than to intracellular Ca^{2+} release (Munsch and Deitmer, 1992; Lohr and Deitmer, 1999). It is as yet unclear which channels mediate this Ca^{2+} influx (therefore, two receptor symbols are indicated for 5-HT in Fig. 2).

When applied focally, both glutamate and 5-HT evoke Ca^{2+} responses not only on the cell soma but also on glial cell processes, suggesting the presence of neurotransmitter receptors in the membrane of the glial dendrites (Lohr and Deitmer, 1997a, 1999). As was mentioned above, the glial cell processes are probably of particular functional importance; they get into close contact with synaptic domains in the neuropil.

Neuronal activity elicits a rise in intraglial Ca^{2+} in the cell body and in the dendrites of the giant glial cell (Rose et al., 1995; Lohr and Deitmer, 1999). These Ca^{2+} transients are suppressed by CNQX, an inhibitor of ionotropic glutamate receptors. The stimulation-induced Ca^{2+} transients in the glial dendrites show a

heterogeneous spatial distribution. In some dendritic processes the Ca^{2+} changes reach large amplitudes, whereas the Ca^{2+} changes are much smaller or even absent in other cell processes (Lohr and Deitmer, 1999). Because the giant glial cell possesses Ca^{2+}-dependent K^+ channels (Müller and Schlue, 1997), locally restricted Ca^{2+} transients in the glial dendrites might alter the K^+ conductance in defined regions. Hence, the spatial K^+ buffering capacity would be improved, particularly where neuronal activity is high.

Nerve root stimulation elicits changes in the intracellular pH in neurons and glial cells of the leech (Rose and Deitmer, 1995a,b). Whereas neurons always acidify, pH changes in glial cells depend on the actual membrane potential and on the buffer system used. In HEPES-buffered saline the glial cytoplasm acidifies, whereas in CO_2/HCO_3^--buffered saline the intraglial pH often increases. This variability is due to the electrogenic Na^+–HCO_3^- cotransporter, which is stimulated in the inward direction when the glial membrane is depolarized and which is significantly more active in the presence of CO_2/HCO_3^- (Rose and Deitmer, 1994).

Nerve root stimulation elicits a fast and transient alkalinization followed by an acidification in the extracellular spaces in CO_2/HCO_3^--free saline (Rose and Deitmer, 1995a,b). In the presence of CO_2/HCO_3^-, the transient alkalinization is greatly reduced, presumably because the Na^+–HCO_3^- cotransporter is moving base equivalents into the glial cells. This CO_2/HCO_3^--dependent effect can be reversed by voltage clamping the glial cell to suppress the stimulation-induced membrane depolarization (Rose and Deitmer, 1994). This indicates that the activity of the glial electrogenic Na^+–HCO_3^- cotransporter can itself lead to (or suppress) pH changes not only in the glial cell itself but also in the extracellular spaces. Again, the changes in the glial membrane potential seem to be pivotal for the direction of intraglial and extracellular pH shifts.

GLIAL ROLE IN ION HOMEOSTASIS AND UPTAKE OF NEUROTRANSMITTERS

In recent years, the role of glial cells in the development of nervous systems, in the maintenance of neuronal function, and in the modulation of synaptic efficacy has been analyzed in different preparations (for review see Vernadakis, 1996). The leech giant glial cell has been shown to serve as a buffer system for K^+ that is released along axons during the propagation of action potentials; a mechanism that maintains the neuronal excitability and is known as "spatial potassium buffering" (Kuffler and Nicholls, 1966; Walz, 1982). K^+ uptake by leech glial cells can be improved by the activation of neurotransmitter receptors (Foster et al., 1992).

Besides K^+, protons (H^+) play a major role in the glia-to-neuron communication. The glial Na^+–HCO_3^- cotransporter is a prominent modulator of the acid/base environment of the neurons and may influence neuro-

nal excitability (see above; see also Deitmer and Rose, 1996).

The leech giant glial cell takes up metabolites such as amino acids or neurotransmitters from the extracellular space, and some of these metabolites are supplied to neurons (Globus et al., 1973; Kai-Kai and Pentreath, 1981). The glycogen metabolism is altered by neuronal activity and neurotransmitter application, an effect that is mediated by cyclic AMP (Pentreath and Kai-Kai, 1982; Pennington and Pentreath, 1987). A role for intracellular Ca^{2+} and pH in modulating glial metabolism, however, has been proposed, but this needs further evidence.

Two neurotransmitter uptake systems have been demonstrated in the leech giant glial cell (Fig. 2), an electrogenic, sodium-dependent glutamate transporter (Deitmer and Schneider, 1997) and a choline transporter that is presumably also sodium-dependent (Adamic, 1975; Wuttke and Pentreath, 1990). Choline is a degradation product of the neurotransmitter acetylcholine cleaved by acetylcholinesterases. Because choline itself is a potent ligand at acetylcholine receptors (Wallace, 1981; Ballanyi and Schlue, 1989), it has to be efficiently removed from the synaptic cleft.

The electrogenic glutamate transporter of the leech giant glial cell is driven by the electrochemical Na^+ gradient. It presumably cotransports H^+ as an additional ion, reflected by the intraglial acidification following stimulation of the transporter by glutamate or aspartate (Deitmer and Schneider, 1997). Hence, its transport capability depends on both the membrane potential and the pH_i. It remains to be elucidated what role the pH_i-regulating Na^+–HCO_3^- cotransporter plays in the transport of acid/base equivalents during glutamate uptake.

PERSPECTIVES

The leech giant glial cell has been a useful model to promote our understanding of neuron–glia interactions on the level of single, identified cells. There are numerous parallels to vertebrate glial cells, above all the rich endowment with neurotransmitter receptors and transport systems in the cell membrane. The most promising aspect of the leech nervous system and its giant glial cell is perhaps its accessibility to studies on the role of glial cells in neural circuits underlying behaviors. It will be of great interest to establish whether glial cells are more directly involved in maintaining such neural circuits.

Furthermore, our extensive knowledge on many neural circuits and synaptic connectivity in the leech CNS might also help us to understand glial functions during synaptic transmission. Of particular interest is the neuronal influence on the glial membrane potential that largely determines the activity of the electrogenic transporters, which, in turn, help to modify the neuronal environment, including that of local synaptic domains. The leech giant glial cell may also be useful in

the future to unravel further glial contributions to information processing in nervous systems.

ACKNOWLEDGMENTS

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