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Pathways of neuron-astrocyte interactions and their possible role in neuroprotection

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Abstract Astrocytes are the most numerous cell type within the central nervous system. Early during development they act as guiding structures for migratory neurons; later they are not only the main source for nutrients and growth factors in the brain, but they are also communication partners of neighboring neurons. For this purpose astrocytes are equipped with several types of transmitter receptors and the capacity to release neuroactive substances. In addition, they form an extended syncytium via gap junction channels which allows fast intercellular signaling pathways. The pivotal involvement of astrocytes in brain function during disease situations is the topic of many studies.

Here, we will review the role of astrocytic gap junctions, astroglial metabolism and neuron-astrocyte signaling. Identification of the molecular mechanisms of these three functions will improve our understanding of neuroprotection.

Key words Astrocytes · Metabolism · Gap junction · Signaling · Neuron-glia communication

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Abbreviations

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
Cx – connexin
FGF – fibroblast growth factor
GFAP – glial fibrillary acidic protein
GJC – gap junctional communication
GSH – glutathione
MPTP – 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine
NO – nitric oxide
ROS – reactive oxygen species
SNAP-25 – synaptosomal-associated protein of 25 kDa
SNARE – soluble N-ethylmaleimide-sensitive factor attachment protein receptor

Introduction

Substantial neuronal losses are found in acutely injured as well as in chronically diseased brain. Uncovering the molecular mechanisms leading to this specific death of neurons will help to develop novel pharmaceuticals for neuroprotection. Many studies focusing on ischemic insults suggest pathological increases of intracellular calcium concentration after overexcitation and enhanced free radical production as primary cause of neuronal cell death (Choi, 1995; Lipton, 1999; Love, 1999; Simonian and Coyle, 1996). Strategies blocking neuronal calcium entry routes in stroke patients, however, did not reveal significant improvement of disease progression. Macroglial cells, i. e., astrocytes and oligodendrocytes, seem to be more resistant to damage of the central nervous system (CNS), although necrotic or apoptotic glial cell death has also been observed. In contrast, several studies even suggest a beneficial role for astrocytes (Ahlemeyer et al., 2000; Blanc et al., 1998; Desagher et al., 1996; Ye and Sontheimer, 1999). The distinction of necrosis and apoptosis as two versions of neural cell death caused by cellular swelling or programmed cell

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death, respectively, seems to be more and more problematic since similar criteria of dying cells are found in both situations. It is more appropriate to classify neural cell death according to defined fatal molecular pathways (Yuan and Yankner, 2000; Hengartner, 2000). However, any future neuroprotective approach has not only to identify molecular reaction steps of death initiation, propagation and finishing, but it has also to consider interaction between several brain cell types and intercellular communication pathways. In practice, this means, we have to detect pathological imbalances of extracellular neural signaling and subsequent intracellular reactions. To prevent cell death we have to interfere with both. Neural cell death often extends over larger though focused areas of the brain. How neural cells communicate to each other and with their bystanders, in particular astrocytes, during the final steps of their life is largely unknown.

In the past astroglial cells have been regarded as passive elements of the nervous system serving mainly as guiding structures during brain development, as metabolic support for neighboring neurons or as regulators of extracellular ion homeostasis.

Recent studies, however, show that astrocytes are bidirectional communication partners in the CNS, receiving signals from neighboring neurons and responding to them with release of neuroactive substances (Araque et al., 1999) (Fig. 1). Astrocytes are perfectly suited to fulfill a pivotal role in cellular communication. Their plexi of fine processes enwrap synaptic terminals thereby being in close apposition to neuronal signal transmission (Ventura and Harris, 1999; Grosche et al., 1999). In addition, they express receptors for almost all neurotransmitters and neuromodulators (Verkhratsky et al., 1998). Increase of intracellular calcium concentration and/or membrane depolarization in astrocytes are common responses to neuronal activity. Calcium is released from intracellular stores after activation of heptahelical, G-protein coupled receptors such as metabotropic glutamate receptors or β2 adrenergic receptors. Another entry route is via ligand- or voltage-gated Ca^{2+} permeable ion channels such as AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptors or Ca^{2+} channels (Verkhratsky et al., 1998; Verkhratsky and Kettenmann, 1996; Gallo and Ghiani, 2000; Condorelli et al., 1999). In addition, astrocytes are metabolically coupled

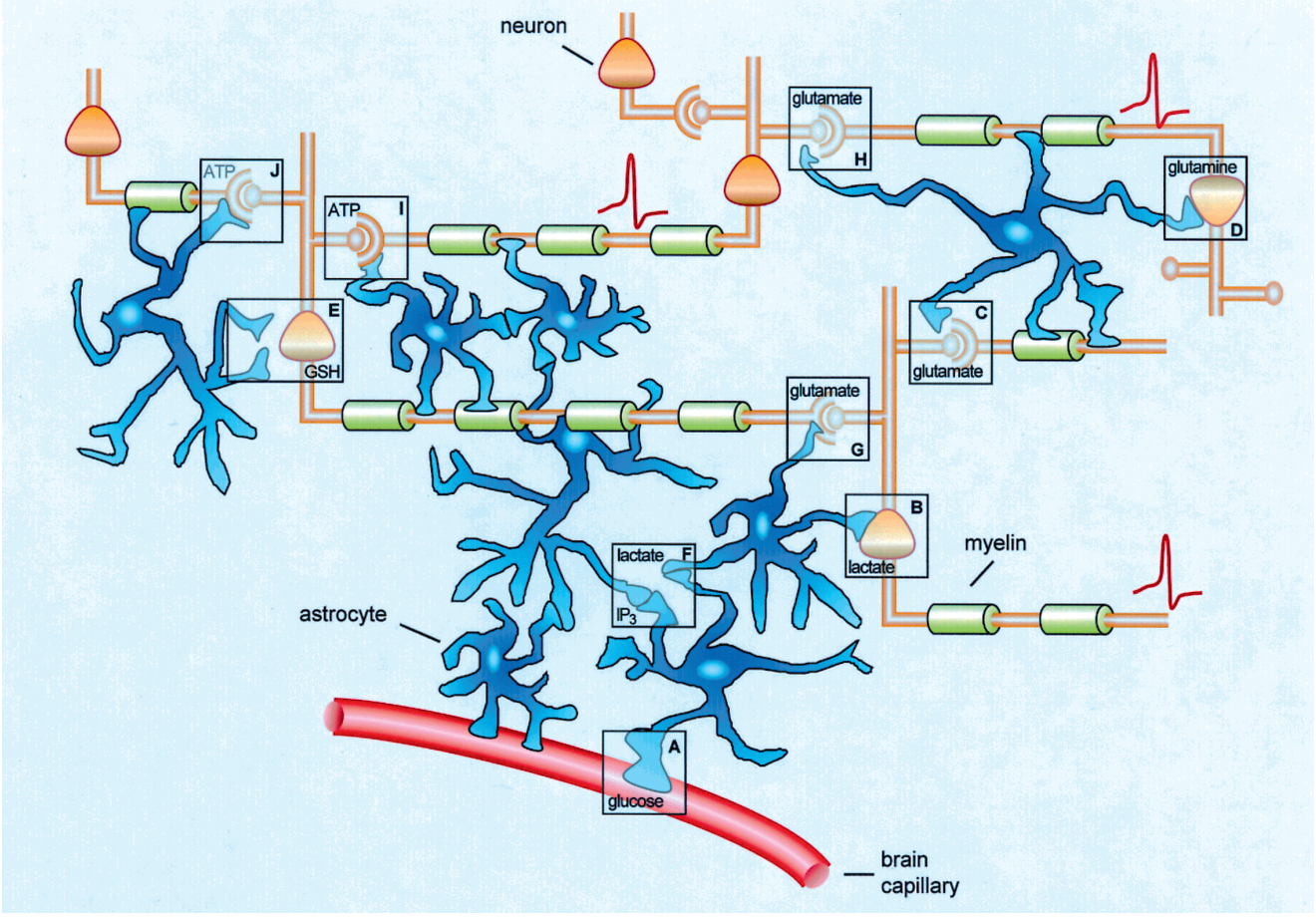

Fig. 1 *Communication pathways of the neuron-glia network.* Astrocytes and neurons form an intimate communication network. The interaction involves the exchange of metabolites extracellularly or intracellularly via gap junctions as well as activation of transmitter receptors on both sides. While neurons use electrical activity along their axons as an information pathway over long distances up to meters in human, signaling of astrocytes is spatially more restricted to the order of millimeters within their gap junction coupled syncytium. The boxes outline individual interaction pathways which are described in more detail in Fig. 2.

to neuronal activity. Glutamate released during synaptic transmission is taken up by astrocytes, and can be converted to lactate, which in turn can be utilized by neurons to produce ATP (Magistretti, 2000; Pellerin et al., 1998).

In the following we will review how astrocytes as part of the neuron-glia network could transmit extra-, intra- and intercellular signals (Fig. 1) while and after sensing synaptic transmission or the metabolic status of neighboring cells, and we will speculate how their physiological properties might be modulated to prevent neural cell death.

Metabolism of astrocytes and their metabolic cooperation with neurons

The brain is one of the most metabolically active organs of the human body. Although the brain contributes only up to 2 % to the body mass, about 20 % of the glucose and the oxygen consumed by the body are used in the human brain (Clarke and Sokoloff, 1999). In addition to glucose metabolism, an intense amino acid metabolism occurs in brain due to protein turnover and the synthesis and degradation of amino acids and amino acid-derived neurotransmitters. Based on the observation that astrocytes are positioned between brain capillaries and neurons, it was already Camillo Golgi who speculated more than one century ago that glial cells provide nutrition to neurons (Somjen, 1988). During the last two decades evidence has been growing that especially between astrocytes and neurons an intensive metabolic exchange occurs. Astrocytes express key enzymes of several metabolic pathways which are not expressed in neurons, including enzymes involved in amino acid metabolism, carbohydrate metabolism and taurine synthesis (glutamine synthetase, glutamate-glutamine cycle (Norenberg and Martinez-Hernandez, 1979); glycogen phosphorylase, glycogen mobilization (Pfeiffer et al., 1990; Reinhart et al., 1990); pyruvate carboxylase, anaplerotic synthesis of oxaloacetate (Yu et al., 1983; Shank et al., 1985; Cesar and Hamprecht, 1995); cysteine sulfinate decarboxylase, synthesis of taurine (Tappaz et al., 1994; Almarghini et al., 1991); glycine cleavage system, glycine metabolism (Sato et al., 1991; Verleysdonk et al., 1999). Due to this cell type compartimentation energy is not wasted by equiping every brain cell type with every metabolic pathway. The dependence of neurons on metabolites or intermediates of metabolic pathways of astrocytes strongly suggest a metabolic cooperation between the neighboring cell types of the brain rather than competition of these cell types for extracellular substrates. However, the loss of independence is the price the different cell types in the brain have to pay for a close coupling due to specialization for different functions.

Evidence on metabolite trafficking between astrocytes and neurons has recently been summarized for the metabolism of carbohydrates (Tsacopoulos and Magistretti, 1996; Wiesinger et al., 1997), metabolism of glutamate and glutamine (Hertz et al., 1999; Daikhin and Yudkoff, 2000), as well as for the defense against xenobiotics and oxidative stress (Cooper, 1998; Dringen, 2000). Examples for three types of metabolic cooperation of astrocytes and neurons will be mentioned here, i) the supply of the energy substrate lactate to neurons, ii) the recycling of neuronal glutamate by the glutamate-glutamine cycle, and iii) the supply by astrocytes of precursors for neuronal glutathione (GSH) synthesis.

Supply of substrates for ATP production to neurons

Astrocytes produce and release in the presence of a variety of exogenous precursors metabolic intermediates which are good substrates for the energy metabolism of neurons (Medina et al., 1999). Especially lactate, the product of glycolytic glucose metabolism in astrocytes (Walz and Mukerji, 1988), the product of the catabolism of other hexoses (Wiesinger et al., 1997) as well as of astrocytic glycogen mobilization (Dringen et al., 1993), has been considered as an important nutrient for oxidative ATP production in neurons (Fig. 2 A and B) (Wiesinger et al., 1997; Magistretti et al., 1999; Medina et al., 1999; Schurr et al., 1999). Glucose utilization is spatially and temporally in register with neuronal activity in active brain areas. Changes in cerebral blood flow, oxygen consumption and metabolic intermediates like lactate and glucose can be imaged via positron emission tomography, functional magnetic resonance imaging and magnetic resonance spectroscopy (Magistretti and Pellerin, 1999; Magistretti and Pellerin, 1996). Other energy metabolites produced in astrocytes are ketone bodies derived from fatty acids (Auestad et al., 1991) or leucine (Bixel and Hamprecht, 1995). After release from astrocytes, ketone bodies are utilized by neurons (Lopes-Cardozo et al., 1986; Edmond et al., 1987). Consequently, the metabolic properties of astrocytes allow the conversion of a variety of different substrates to a few metabolites such as lactate and ketone bodies which are valuable fuel material in the oxidative pathway of energy production in neurons.

Metabolic coupling via glutamate

After release from glutamatergic neurons, the neurotransmitter glutamate is rapidly inactivated by cellular uptake (Clements et al., 1992), predominantly into astrocytes (Bergles and Jahr, 1998; Pellerin and Magistretti, 1994). This glutamate uptake has a major influence on the astrocytic metabolism. Na^+ ions are co-transported and increase intra-astrocytic Na^+ concentration which activates the Na^+/K^+ ATPase. Subsequently, Na^+/K^+-ATPase activation stimulates glycolysis, i. e., glucose utilization and lactate production (Magistretti, 2000; Chatton et al., 2000). The glutamate-glutamine cycle is a pathway for glutamate to be returned to neurons (Fig. 2 C and D) (Hertz et al., 1999; Daikhin and Yudkoff, 2000). Glutamate is amidated to glutamine by the ATP-consuming reaction catalyzed by glutamine

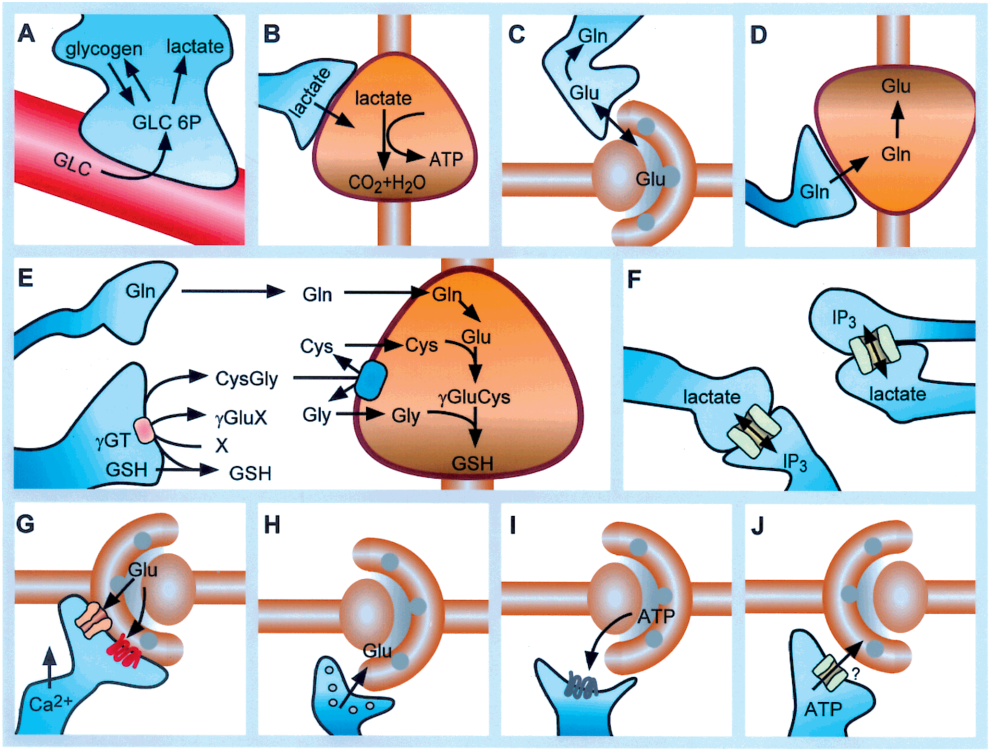

Fig. 2 *Neuron-astrocyte interaction pathways.* **A, B** Supply of the energy fuel lactate from astrocytes to neurons. Lactate is generated and relased by astrocytes from glucose received from brain capillaries or by glycogen mobilization and is utilized in neurons as substrate for oxidative ATP production. **C, D** Glutamate-glutamine cycle. The neurotransmitter glutamate is released into a synapse from a glutamatergic neuron and is subsequently taken up into astrocytes. Glutamine synthetase in astrocytes converts glutamate into glutamine, which is released and used by neurons as a precursor for glutamate. **E** Supply of glutathione precursors from astrocytes to neurons. The GSH released from astrocytes is substrate of the astroglial ectoenzyme γ-glutamyl transpeptidase (γGT). X represents an acceptor of the γ-glutamyl moiety transferred by γGT from GSH. The second product, the dipeptide CysGly, is most likely hydrolyzed by a neuronal ectopeptidase to cysteine and glycine. In addition, glutamine is released from astrocytes and used by neurons as a precursor for the glutamate necessary for GSH synthesis. **F** Astroglial networks. Gap junction channels connect neighbouring astrocytes allowing the transfer of second messengers or small metabolites such as inositoltrisphosphate, glucose or lactate, respectively. **G** Activation of astroglial glutamate receptors. Spillover of synaptically released glutamate can be sensed by neighboring astrocytes. Activation of metabotropic glutamate receptors leads to transient increases of astroglial $[Ca^{2+}]_i$. **H** Glutamate release from astrocytes. Increases of $[Ca^{2+}]_i$ induce glutamate release from astrocytes, which also express a number of proteins known from presynaptic release vesicles. **I, J** Neuron-glia signaling via ATP. Synaptically released ATP activates astroglial purinoceptors of the P2 type. Conversely, ATP can be released from astrocytes and modulate synaptic transmission. The involvement of Cx hemichannels is under debate.

synthetase, an enzyme which is present in astrocytes but absent from neurons (Norenberg and Martinez-Hernandez, 1979). Glutamine which has no neurotransmitter action is released from astrocytes and rapidly taken up into neurons. There, hydrolysis of glutamine to glutamate is catalyzed by the phosphate-activated glutaminase (Kvamme et al., 2000). Consequently, regeneration of the neurotransmitter glutamate and metabolic coupling during energy production are important tasks of the cooperation between astrocytes and neurons.

Defense against reactive oxygen species and neuronal GSH synthesis

Reactive oxygen species (ROS) are continuously generated during oxidative metabolism. An increased production of ROS and/or a decrease in the antioxidative capacity of cells cause oxidative stress which can compromise essential cellular functions. Compared to other organs the brain produces ROS at a high rate but apparently has less antioxidative capacity (Cooper, 1997; Dringen, 2000). That might lead to the oxidative stress which has been considered as an important factor influencing at least the progression of neurodegenerative diseases, i. e., Parkinson disease, Alzheimer disease and amyotrophic lateral sclerosis (Bains and Shaw, 1997; Schulz et al., 2000).

Astrocytes are considered to play an important role in the defense against ROS. Containing the highest levels of various antioxidants in the brain (Peuchen et al., 1997; Wilson, 1997; Dringen, 2000), astrocytes support other brain cell types in the defense against ROS-induced toxicity of various compounds and treatments (Dringen, 2000). Evidence is growing that GSH plays an important role in the detoxification of ROS in brain and that a compromised glutathione system is an important factor facilitating progression of neurodegenerative disorders (Dringen, 2000; Schulz et al., 2000). In this context, the availability of precursors for the synthesis of GSH in neurons appears to be crucial. Evidence obtained recently on several model systems suggests that extracellular precursors for neuronal GSH synthesis are provided by neighboring astrocytes (Fig. 2 E) (Dringen et al., 2000). Glutamine, the release of which is part of the

glutamate-glutamine cycle between neurons and astrocytes, is the best extracellular glutamate precursor for neuronal GSH synthesis (Kranich et al., 1996). However, only the availability of extracellular cystein limits GSH synthesis in neurons (Dringen et al., 1999). Cysteine as well as glycine can be provided from astrocytes by release of GSH (Dringen et al., 1997) and consecutive cleavage of the tripeptide by the action of γ-glutamyltranspeptidase and an ectopeptidase (Dringen et al., 2000). Consequently, precursors for all three amino acids necessary for neuronal glutathione synthesis are provided by astrocytes to neurons.

Gap junctional communication and astrocytic networks

Astrocytes form an extended glial syncitium in which neurons are intimately embedded (Fig. 1). Functional tests and electrophysiological recordings performed in primary cultures and in brain slices have demonstrated that the cytoplasms of astrocytes are highly coupled to each other by gap junctions. Occasionally, astrocytes can even be coupled to neurons and oligodendrocytes (heterotypic coupling) (Venance et al., 1995; Froes et al., 1999; Alvarez-Maubecin et al., 2000). The use of low molecular weight intercellular tracers (Lucifer yellow, biocytin), which pass through junctional channels, demonstrates that several tens of astrocytes are coupled in cortical, cerebellar and hippocampal slices (Müller et al., 1996; Konietzko and Muller, 1994; D'Ambrosio et al., 1998). This high degree of intercellular communication has led to the proposal that astrocytes are organized as networks which might be subjected to remodeling and to some plasticity (Giaume and McCarthy, 1996). Besides the passive diffusion of intercellular tracers, gap junction channels in astrocytes were shown to be permeable to endogenous signaling molecules, such as inositol trisphosphate (Leybaert et al., 1999), glucose and its metabolites, glutamate, glutamine and lactate (Fig. 2F) (Giaume and Venance, 1998; Tabernero et al., 1996; Medina et al., 1999). Accordingly, the participation of astrocytes in various brain functions should not only be considered as contributed by individual elements but also by co-ordinate groups of communicating cells (Fig. 1).

Gap junction channels are composed of two hemichannels, named connexons, each of them being a hexamer of structural subunit proteins, termed connexins (Cxs). Cxs are organized around a relatively large hydrated pore which allows the diffusion of ions and small molecules up to 1–1.2 kDa (Bruzzone et al., 1996). In the CNS, Cxs are widely expressed in neurons, in glial and ependymal cells (Dermietzel, 1998). Astrocytes express high levels of Cxs from the late embryo to adult stages; in particular, the prevalent Cx43 which is detected early during mammalian brain development and remains constant throughout adulthood (Dermietzel et al., 1989). In mature astrocytes *in situ* and in late primary cultures, Cx30 also is found abundantly (Kunzelmann et al., 1997; Nagy et al., 1999). Since these two Cxs are not found in neurons, they are considered as astrocytic Cxs (Rozental et al., 2000 a; Rozental et al., 2000 b). Other Cxs (Cx40; Cx45; Cx26) have been detected as well in astrocytes studied *in vitro* or *in situ* (Bruzzone and Giaume, 1999; Alvarez-Maubecin et al., 2000), but these Cxs are also expressed in other brain cell types.

Neurons regulate astrocytic network

The extent and the shape of astrocytic networks are up- or down-regulated by neurons as a consequence of a change in biophysical properties and/or the number of junctional channels. Such neuronal control of gap junctional communication (GJC) in astrocytes occurs in multiple situations. First, neurons release bioactive molecules, including neurotransmitters, peptides, lipids, which stimulate astrocytic receptors and then modify the activity (short-term regulation) and/or the expression (long term regulation) of Cx channels (Giaume and McCarthy, 1996). Second, functional studies and Cx43 immunoblots performed with cocultures (neurons and astrocytes) and pure cultures of astrocytes have demonstrated that GJC in astrocytes depends on synaptic activity in neurons (Rouach et al., 2000). In addition, an enriched environment known to promote structural and biochemical changes in neurons was recently found to increase gene expression in the mouse brain (Rampon et al., 2000). A number of these genes being related to neuronal structure and synaptic activity may play an important role in learning and memory performance. Interestingly, the transcript for Cx30, an astrocytic gap junction protein (Kunzelmann et al., 1997; Nagy et al., 1999) was also enhanced in these experiments. This observation suggests that the enhanced expression of genes involved in the formation of new synapses and the strengthening of existing synapses are associated with an increase in the level of gene expression of an astrocytic Cx. Finally, neuronal destruction or nerve injury was also shown to alter astrocytic GJC. For instance, Cx43 redistribution is observed *in situ* after kainic acid-induced neuronal lesions (Hossain et al., 1994) and peripheral transection of the facial nerve (Rohlmann et al., 1993).

Neurodegenerative brain pathologies and connexin expression in astrocytes

In the last decade, several attempts have been performed to determine whether changes in gap junction properties in neurons, as well as in glial cells, are associated with nervous system diseases (Rozental et al., 2000 a). There are now several evidences indicating that expression of the astroglial connexin, Cx43, is affected in neurodegenerative brain pathologies. For instance, an elevation of Cx43 was described at sites of amyloid plaques in temporal cortices of Alzheimer patients. By electron microscopy, Cx43 was localized at astrocytic gap junc-

tions and by light microscopy, cortical areas containing numerous β/A4 amyloid plaques were found to exhibit increased immunostaining density for Cx43, while some plaques corresponded exactly to sites of intensified Cx43 immunoreactivity (Nagy et al., 1996). In complement to this observation, the expression of Cx43 and the induction of GJC were reported to occur in PC12 cells overexpressing a C-terminal 97 amino acid fragment containing β/A4 amyloid peptide of the amyloid precursor protein (Lynn et al., 1995). In Huntington's diseased human brain, characterized by neuronal death in the basal ganglia, the distribution of Cxs was also studied (Vis et al., 1998). While the pattern of distribution of Cx26 and Cx32 was similar in normal and diseased brains, the immunoreactivity for Cx43 antibodies was changed in the caudate nucleus, but not in the globus pallidus. Indeed, in the caudate nucleus the Cx43 expression in astrocytes was increased, became located in plaques and was associated with a high enhancement of GFAP (glial fibrillary acidic protein) staining. Finally, analysis of Cx43 expression and functional test for GJC were carried out with a rat MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-model of Parkinson disease (Rufer et al., 1996). This study indicated that in treated animals the level of Cx43 expression was transiently enhanced in the striatum in parallel with an increase in the number of GFAP positive cells. Unilateral administration of FGF-2 (fibroblast growth factor 2), which has potent trophic effects on developing and toxically impaired dopaminergic neurons crucially affected in Parkinson disease, resulted in a further increase in Cx43-positive punctata. More recently, reevaluation of GJC in striatal astrocytes was conducted using neurobiotin instead of Lucifer yellow, as an intercellular tracer. In this study, GJC between astrocytes studied in brain slices was detected demonstrating that connexin channels are functional in this structure (Hamon et al., 2001) and, accordingly, the incidence of MPTP lesion on Cx43 expression and GJC in astrocytes should now be reinvestigated.

Astrocytic gap junctions: their good and bad incidence on neuronal survival

A large amount of data indicate that astrocytes serve neurotrophic and neuroprotective roles (Mattson et al., 1997). For some of these functions, experimental evidence for GJC contribution has been provided. Indeed, inhibitors of GJC in astrocytes, such as the volatile anesthetic halothane and the long chain alcohol octanol, were shown to reduce the extent of brain injuries during ischemia and spreading depression (Saito et al., 1997; Rawanduzy et al., 1997). Moreover, the incidence of astrocytic GJC in neuroprotection has been investigated by comparing neuronal vulnerability to oxidative stress in the presence of communicating and non-communicating astrocytes (Blanc et al., 1998). Following exposure to oxidative insults, the blockade of GJC in astrocytes resulted in a markedly enhanced generation of intracellular peroxides and neuronal death. In addition, the peak elevation of neuronal Ca^{2+} induced by oxidative stress was larger during uncoupling treatment, suggesting that astrocytic GJC was also involved in Ca^{2+} homeostasis as previously reported (Giaume and Venance, 1998). These observations suggest the existence of a link between astrocytic GJC and neuronal vulnerability induced by oxidative insults. Astroglial intercellular communication decreases neuronal vulnerability to injury by a mechanism involving stabilization of cellular Ca^{2+} homeostasis and dissipation of oxidative stress. From immunohistological studies performed *in vivo* after kainic acid lesion, it was proposed that massive neuronal loss alone, or in conjunction with direct actions of excitotoxins on astrocytes, precipitates an astrocytic reaction accompanied by the redistribution of Cx43 (Ochalski et al., 1995). Reorganization of GJC within the astrocytic networks may be neuroprotective by isolating the lesion site from the healthy syncytium. In order to generalize these statements it remains to be established in other models of neurotoxicity whether this neuroprotective role is a general property of GJC in astrocytes.

Conversely, GJC could also play an important role in the propagation of death signals in astrocytic networks, which secondarily may affect neuronal fate. Although GJC in astrocytes are significantly reduced during ischemic conditions, junctional channels were reported to remain open in dying astrocytes (Cotrina et al., 1998 a). Consequently, free exchange of intracellular messengers between dying and potentially viable astrocytes might contribute to secondary expansion of ischemic lesions, which in turn could affect bystander neurons. This suggests that GJC may connect ischemic astrocytes in an evolving infarct with the surrounding neurons. Recently, these observations were strengthened by showing that hypoxia also reduces GJC and dephosphorylates Cx43 in cultured astrocytes (Li and Nagy, 2000). Furthermore, in focal ischemia the analysis Cx43 distribution demonstrated changes in location and phosphorylation status of this protein (Li et al., 1998) which suggests differential functional status of astrocytic gap junction channels at the ischemic center and the periphery. Finally, GJC in astrocytes were shown to propagate and amplify cell injury by allowing intercellular diffusion of death signals (Lin et al., 1998). Indeed, although overexpression of the human proto-oncogene bcl2 in C6 glioma cells increased their resistance to injury, the relative resistance of $bcl2^+$ cells to calcium overload, oxidative stress and metabolic inhibition was altered when they communicated via gap junctions to more vulnerable cells, like $bcl2^-$ cells and astrocytes. Through this communicating pathway dying cells killed adjacent cells that would otherwise have escaped injury. It was proposed that such process could account for the secondary propagation of brain injury in cerebral ischemia (Lin et al., 1998).

Release of neuroactive substances by astrocytes upon glial stimulation

The diversity of neuron-astrocyte communication is further enhanced by the astroglial capacity to release neurotransmitters, allowing a bi-directional communication pathway (Fig. 2 G-J). So far, two different transmitters released from glial cells upon stimulation have been identified, glutamate and ATP (Fig. 2 H and J) (Innocenti et al., 2000; Parpura et al., 1994; Guthrie et al., 1999; Wang et al., 2000).

Release of glutamate can be evoked by application of many substances increasing intracellular calcium of glial cells either selectively such as noradrenalin and glutamate via receptor activation or unspecifically by ionomycin, mechanical or electrical stimulation (Parpura and Haydon, 2000; Hassinger et al., 1996; Innocenti et al., 2000). Interestingly, it has been shown that in hippocampal brain slices glutamate released during neuronal activity causes $[Ca^{2+}]_i$ increases in neighboring astrocytes (Porter and McCarthy, 1996). Suprathreshold $[Ca^{2+}]_i$ by itself is necessary and sufficient to induce glutamate release from astrocytes. The Ca^{2+} dependence suggests an exocytotic release mechanism. This is further substantiated by enhanced glutamate release in cultures of astrocytes incubated with the black spider venom α-latrotoxin which induces vesicle fusion to cellular membranes (Parpura et al., 1995). Glutamate release is also blocked in astrocytes after incubation with bafilomycin A1 which eliminates the electrochemical gradient maintained by a V-ATPase across vesicular membranes and after injection of the Botulinum neurotoxin B which cleaves the vesicle protein synaptobrevin (Araque et al., 2000). Several other SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins involved in vesicular release such as synaptotagmin, synaptophysin, synapsin I or SNAP-25 (synaptosomal-associated protein of 25 kDa) have been found in astrocytes as well (Maienschein et al., 1999). We need, however, further ultrastructural investigation of astrocytes in tissue culture as well as *in situ* to visualize glial release vesicles.

The mechanism of astroglial ATP release is poorly understood. Similar to glutamate, ATP release can be achieved by several means, e. g., glutamate receptor activation and mechanical or electrical stimulation (Charles et al., 1991; Cotrina et al., 1998 c; Hassinger et al., 1996). In contrast to glutamate, however, the release of ATP seems to be Ca^{2+}-independent (Wang et al., 2000; Salter and Hicks, 1995). Gap junction channels are thought to modulate the ATP signaling. Induced expression of glial connexins leads to an increase in ATP release (Cotrina et al., 1998 b). Upon mechanical or electrical stimulation of astrocytes in culture or in retinal whole mount preparations (Newman and Zahs, 1997; Newman and Zahs, 1998), ATP is released at such high amounts that purinergic receptors on neighboring cells are activated thereby propagating a signal wave (Scemes et al., 2000; Fam et al., 2000). Theoretical calculations exclude a pure point source of ATP at the place of stimulation. It is suggested that neighboring cells prolong the signal by regenerative ATP release (Guthrie et al., 1999). The simplest model of astroglial ATP release postulates that connexin hemichannels could function as a release pore for ATP (Cotrina et al., 2000). Subsequently, released ATP propagates a signal wave via activation of P2-type purinoceptors (Fam et al., 2000; Cotrina et al., 2000). For astrocytes of the dorsal spinal cord, it was shown that the P2Y1 subtype of purinoceptor was necessary and sufficient for Ca^{2+} wave propagation although the presence of other P2Y purinoceptors could not be excluded (Fam et al., 2000). P2Y1 and other purinoceptors may participate in pathological situations. P2Y purinoceptor activation of astrocytes can stimulate trophic signaling pathways via activation of extracellular signal-regulated protein kinase (Neary et al., 1999) or induce changes in gene expression (Priller et al., 1998). Proliferation and differentiation are prerequisits for astroglial scar formation during insults like hypoxia, ischemia or brain injuries. Noxious stimuli inducing ATP release from damaged cells could cause these reactive responses by eliciting Ca^{2+} waves and regenerating ATP signals. During inflammatory events P2Y purinoceptors also play a role. Receptor antagonists downregulate interleukin-1 β-stimulated expression of nitric oxide, tumor necrosis factor-α and interleukin-6 (John et al., 1999; Liu et al., 2000).

Very recently, nitric oxide (NO) has been suggested as another glial signaling molecule released upon mechanical stimulation (Willmott et al., 2000). Released NO induces intercellular Ca^{2+} waves which are mediated via cGMP and cGMP-dependent protein kinase and involve intracellular ryanodine receptor-linked stores.

Conclusions

Altogether these findings indicate that astrocytes and neurons form a highly complex structure with a multitude of interdependencies. Within astrocytic networks, GJC are tightly associated to the functional status of surrounding neurons where they likely contribute to their fate. Moreover, GJC not only provide important intercellular signaling pathways, but they play also a prominent role for the trafficking and distribution of energy substrates provided by the astrocytic metabolism to nurture the brain. In addition, extracellular signals either due to neuronal activity or released from astrocytes themselves modulate energetic metabolism and the extent of connectivity in astrocytes. Conversely, synaptic transmission is influenced by intracellular Ca^{2+} waves through the astroglial syncytium and by released transmitters such as glutamate, ATP or NO. These multicellular signaling cascades are linked to and drastically affected during pathological situations. Depending upon the situation, up- or down-regulation of such a pathway could either help the neurons to survive or on the contrary contribute to their killing.

Prospectives

Future therapeutical strategies will certainly address the modulation of the astrocytic support functions in brain metabolism which will interfere with the neuron-glia interaction. It can be envisaged that either extracellular growth factor application or intracellular induction of enzymes affecting either signaling pathways, GJCs or gene expression could be beneficial. However, it will be very important to apply very selectively neuroprotective strategies, with either regional or/and cell-type specificity. Whether tools provided by gene technology such as viral vector systems with inducible and/or cell-type specific promoters will reach broad acceptance remains to be demonstrated. In recent studies, the delivery of glial cell line-derived growth factor (GDNF, a member of the tumor growth factor β superfamily) to the facial nucleus in mice after nerve axotomy or to the lateral ventrical in gerbils after induction of transient global ischemia was shown to prevent the death of adult motoneurons or CA1 pyramidal neurons, respectively (Hottinger et al., 2000; Yagi et al., 2000). In another recent study, the induction of a synthetic glucocorticoid-promoter driven glucose transporter (GLUT-1) decreased glutamatergic excitotoxicity in culture and expression of the transgene could also be achieved *in vivo* (Ozawa et al., 2000). The ways of investigation drawn by these works will certainly contribute to assess the potential role of neuron-astrocyte interactions in neuroprotection.

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