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Excitotoxicity in glial cells

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Abstract

Excitotoxicity results from prolonged activation of glutamate receptors expressed by cells in the central nervous system (CNS). This cell death mechanism was first discovered in retinal ganglion cells and subsequently in brain neurons. In addition, it has been recently observed that CNS glial cells can also undergo excitotoxicity. Among them, oligodendrocytes are highly vulnerable to glutamate signals and alterations in glutamate homeostasis may contribute to demyelinating disorders. We review here the available information on excitotoxity in CNS glial cells and its putative relevance to glio-pathologies.

Keywords: Glutamate; Transporter; Receptor; Cell death; Oligodendrocyte; Demyelination

1. Introduction

To understand how aberrant glutamate signaling can cause excitotoxicity, we will first summarize current knowledge of glutamate receptors and transporters and their expression in glial cells. Subsequently, we will provide a description of what is known about excitotoxicity in neurons and mention its putative relevance to neurodegenerative diseases of the central nervous system (CNS). Then, the evidence for excitotoxicity in glial cells will be discussed as well as the cell death mechanisms activated by over-activation of glutamate receptors. This information will finally be placed in the context of CNS diseases in which glial cell excitotoxicity may be a component of the etiology.

2. Glutamate signalling in the CNS

The main determinants of glutamate signaling are glutamate receptors and transporters. Glutamate activates ionotropic receptors, which gate membrane ion channels permeable to cations and metabotropic receptors, which are coupled to G proteins (for reviews, see Michaelis, 1998; Dingledine et al., 1999). Molecular cloning has revealed that each receptor subtype is composed of several subunits with high homology within each receptor class. Thus, functional AMPA receptors are formed by GluR1–4, kainate receptors by GluR5–7 and KA1–2, and NMDA receptors by NMDAR1 and NMDAR2A–D subunits (Dingledine et al., 1999; Lerma, 1999). Kainate receptors are activated by AMPA and kainate and are best isolated in the presence of GYKI53655, a selective AMPA receptor antagonist (Lerma, 1999).

Glutamate uptake from the extracellular space is essential to shaping excitatory postsynaptic currents (Auger and Attwell, 2000) and to prevent excitotoxic death due to overstimulation of glutamate receptors (Rothstein et al., 1996). At least five glutamate transporters have been cloned (Danbolt, 2001). Of these, glutamate transporter 1 (GLT-1), which is located almost exclusively in astrocytes (Conti and Weinberg, 1999), exhibits the highest level of expression and is responsible for most of the total glutamate transport (Danbolt, 2001).

3. Glutamate receptors and transporters in glial cells

Functional glutamate receptors and transporters are expressed in gray as well as in white matter tracts (for recent reviews, see Matute et al., 1999; Verkhratsky and Steinhäuser, 2000; Danbolt, 2001).

Ionotropic glutamate receptors, mainly of the AMPA and kainate type, are expressed by astrocytes, oligodendrocytes (Table 1) and their precursors. Activation of these receptors

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Table 1
Glutamate receptor subunits in glial cells

	Oligodendrocytes		Astrocytes
	In vitro	In situ	In situ
AMPA receptors			
GluR1	–	–	+
GluR2	–	–	w
GluR3	+	w	+
GluR4	+	w	–
Kainate receptors			
GluR5	–	–	+
GluR6	+	+	+
GluR7	+	+	+
KA1	+	+	+
KA2	+	+	+

Oligodendrocyte cultures were derived from perinatal rat optic nerves. Findings in situ correspond to those observed in the adult rat optic nerve and in bovine corpus callosum. No NMDA receptor subunits were detected in these preparations. w, weak expression.

on glial cells produces a large variety of biological responses, including the release of neurotransmitters and growth factors, which suggests that they may have an active role in brain signaling and repair. The receptors expressed by glial cells have the same general properties as those present on neurons. However, they are edited to a lesser extent (Matute et al., 1999) and therefore are more permeable to Ca^{2+} (Burnashev, 1996).

Glutamate transporters are also present in astrocytes, oligodendrocytes and their precursors. Thus, the main transporters expressed by astrocytes are GLT-1 and glutamate aspartate transporter (GLAST), whereas oligodendrocytes in situ have only GLAST. Finally, the neuronal transporter, excitatory amino acid carrier 1 (EAAC1), is present in a subpopulation of adult oligodendrocyte progenitor cells, and may inactivate glutamate released in synapses formed between neurons and these cells (Bergles et al., 2000). It thus appears that all macroglial cells differentially express the three major glutamate transporters present in the CNS.

4. Enhanced glutamate signals can lead to excitotoxic cell death

Excitotoxicity is a phenomenon whereby prolonged activation of excitatory amino acid receptors leads to cell death. It was first described in the late 1950s by Lucas and Newhouse (1957), who observed that sustained exposure to glutamate destroys retinal neurons. Later, Olney and Sharpe (1969) found that this vulnerability is shared by all central neurons bearing excitatory amino acid receptors, and they hypothesized later that glutamate receptor overactivation might be a primary cause of the neuronal loss associated with many neurological diseases. Since then, this idea has been substantiated by a growing body of evidence implicating glutamate excitotoxicity in acute injury to the CNS and chronic neurodegenerative diseases (Choi, 1988; Lipton and Rosenberg, 1994).

NMDA receptors, which are highly permeable to calcium and distributed widely on CNS neurons, are the major initiators of excitotoxicty. However, activation of Ca^{2+}-permeable AMPA or kainate receptors can also trigger neuronal cell death (reviewed in Weiss and Sensi, 1999). Moreover, antagonists of these receptors display a higher protective efficacy than NMDA receptor antagonists in some experimental neurodegenerative paradigms (Gill and Lodge, 1997). This can be explained by the partial overlap between the restricted patterns of both Ca^{2+}-permeable AMPA or kainate receptors and that of neuronal death in pathologies such as epilepsy and ischemia, in which excitotoxicity is involved.

5. Excitotoxicity in glial cells: types involved

Many studies carried out over the last few years have shown that, in addition to neurons, glial cells can die by excitotoxicity. The main glial cell types vulnerable to excitotoxicity belong to the oligodendrocyte lineage. However, there is evidence that sustained activation of ionotropic glutamate receptors can also kill astrocytes and microglia.

5.1. Astrocytes

Although astrocytes express functional glutamate receptors, they are generally resistant to excitotoxic insults, and their vulnerability to this cell death mechanism varies from one region to another. Thus, prolonged exposure of cultured hypothalamic astrocytes to high concentrations of glutamate and AMPA/kainate receptor agonists does not affect their viability (Prieto and Alonso, 1999). In contrast, similar insults are harmful to neocortical astrocytes, a feature that is greatly potentiated by blocking AMPA receptor desensitization (David et al., 1996). Therefore, over-activation of AMPA receptors can be rapidly lethal to astrocytes but desensitization normally limits this toxicity.

It is important to distinguish the excitotoxicity that is initiated by receptor activation from other gliotoxic actions of glutamate. Thus, it has recently been shown that in astrocytes brief exposure to glutamate results in cell swelling, whereas sustained incubation injures these cells via oxidative stress (Chen et al., 2000). Instead of an excitatory mechanism, glutamate-induced gliotoxicity is mediated predominantly by a reduction in the glutathione content, and its effects are almost completely blocked by anti-oxidants and glutamate transporter inhibitors.

5.2. Microglia

Microglia appear to be the glial cell type least susceptible to excitotoxicity. This is due to the fact that microglial cells only express glutamate receptors when they are

reactive, as occurs in the post-ischemic brain (Gottlieb and Matute, 1997) and in Alzheimer's disease (Kingham et al., 1999). Thus, senile plaques in the Alzheimer's brain are characterized by activated microglia and immunoreactivity for the peptide chromogranin A. Incubation of primary cultures of rat brain-derived microglia with this peptide triggers nitric oxide production followed by enhanced microglial glutamate release. In turn, this causes microglial death, which is reduced by ionotropic glutamate receptor antagonists (Kingham et al., 1999).

5.3. Oligodendrocytes

The first evidence that oligodendrocytes are highly vulnerable to glutamate was obtained in primary cultures a few years ago (Oka et al., 1993). After a 24-h exposure to glutamate, oligodendroglial death was comparable to that described in neurons. In contrast to these cells, oligodendroglial toxicity was not mediated by glutamate receptors but rather by a transporter-related mechanism involving the inhibition of cysteine uptake, which results in gluthatione depletion and cellular vulnerability to toxic free radicals (Oka et al., 1993). This toxicity appears to occur predominantly in young cultures, since similar neurotoxic effects of glutamate have been observed in the early stages of neuronal cortical cultures (Choi et al., 1987; Schubert and Piasecki, 2001).

More recently, it was shown that prolonged activation of glutamate receptors is toxic to cells of an oligodendroglial cell line (Yoshioka et al., 1996) and to oligodendrocytes in vitro (Matute et al., 1997; McDonald et al., 1998). Strikingly, oligodendrocytes, which do not express NMDA receptors (Table 1), are highly vulnerable to glutamate excitotoxicity. Thus, in culture a short exposure to agonists

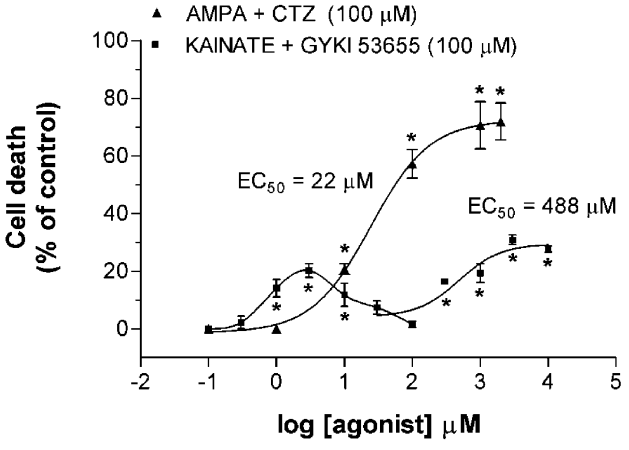

Fig. 1. Dose–response toxicity curves for oligodendrocytes after activation of AMPA and kainate receptors. Three distinct receptor types trigger excitotoxicity: AMPA, and high- and low-affinity kainate receptors. AMPA receptors were activated by AMPA applied in conjunction with cyclothiazide (100 μM). Selective activation of kainate receptors was achieved in the presence of GYKI53655 (Lerma, 1999). Adapted from Sánchez-Gómez and Matute (1999). * $P<0.05$.

of AMPA and kainate receptor can cause oligodendrocyte death (Fig. 1). This toxicity is directly related to Ca^{2+} influx subsequent to receptor activation, and it is greatly attenuated in the absence of Ca^{2+} in the culture medium (Matute et al., 1997; Sánchez-Gómez and Matute, 1999).

Excitotoxic oligodendroglial death has also been observed in the isolated spinal dorsal column (Li and Stys, 2000), and following infusion of AMPA/kainate receptor agonists onto the optic nerve (Matute et al., 1997; Matute, 1998) and into the subcortical white matter (McDonald et al., 1998). In all instances, damage to oligodendrocytes is unlikely to be secondary to neuronal excitotoxic death since gray matter was not present or carefully avoided in these preparations and excitotoxic death was prevented by specific receptor antagonists.

6. Excitotoxicity and calcium homeostasis

Ionotropic glutamate receptors mediating excitotoxicity are permeable to Ca^{2+}, a property that varies from one receptor subclass to another. High concentrations of extracellular glutamate generated, for instance, after traumatic and ischemic CNS injury result in overstimulation of AMPA, kainate and NMDA receptors and, as a consequence, in the influx of Na^+ and Ca^{2+} ions through the pores formed by these receptors. Na^+ influx can in turn trigger a secondary increase in the intracellular Ca^{2+} concentration through the activation of voltage-gated Ca^{2+} channels and reverse operation of Na^+/Ca^{2+} exchange (Lee et al., 1999). In neurons, glutamate toxicity is greatly reduced by NMDA receptor antagonists; however, AMPA or kainate receptors are also relevant to neuronal excitotoxicity (Weiss and Sensi, 1999). In contrast, glutamate excitotoxicity in glial cells is exclusively mediated by AMPA and kainate receptors.

In oligodendrocytes, selective activation of AMPA and kainate receptors leads to Ca^{2+} influx (Fig. 2), an effect that is totally abolished by non-NMDA receptor antagonists or by removing Ca^{2+} from the culture medium. Blockade of voltage-gated Ca^{2+} channels reduces substantially the amplitude of the Ca^{2+} current triggered by AMPA receptor activation, but not that initiated by low- and high-affinity kainate receptors (Alberdi et al., in press). In contrast, inhibition of the plasma membrane Na^+/Ca^{2+} exchanger attenuates weakly the rise in $[Ca^{2+}]_i$ caused by activation of kainate receptors. Surprisingly, excitotoxicity triggered by glutamate receptor over-activation is not reduced by calcium channel blockers or by inhibition of the Na^+-Ca^{2+} exchanger (Fig. 3). This indicates that Ca^{2+} influx via AMPA and kainate receptors alone is sufficient to initiate cell death in oligodendrocytes, and it does not require the entry of calcium via other routes such as voltage-activated calcium channels or the plasma membrane Na^+-Ca^{2+} exchanger.

Overall, these results are similar to those observed in neurons, indicating that rapid Ca^{2+} influx through Ca^{2+}-permeable AMPA/kainate channels may result in mitochon-

drial Ca^{2+} overload and in the production of oxygen radicals, which leads to neuronal death (Carriedo et al., 1998).

There are several features that may render oligodendrocytes vulnerable to over-activation of AMPA and kainate receptors. First, the subunits that constitute the receptors endow them with higher Ca^{2+} permeability. Thus, AMPA receptors in oligodendrocytes are homomeric and/or heteromeric entities formed by subunits GluR3 and GluR4 (Table 1), and lack GluR2, a configuration which allows Ca^{2+} entry (Hollmann and Heinemann, 1994). In addition, a major constituent of kainate receptors in oligodendrocytes is the unedited version of GluR6, which again displays higher calcium permeability (Burnashev, 1996). Second, oligodendrocytes do not express several of the Ca^{2+}-binding proteins present in neurons which makes cells more resistant to excitotoxicity (Baimbridge et al., 1992).

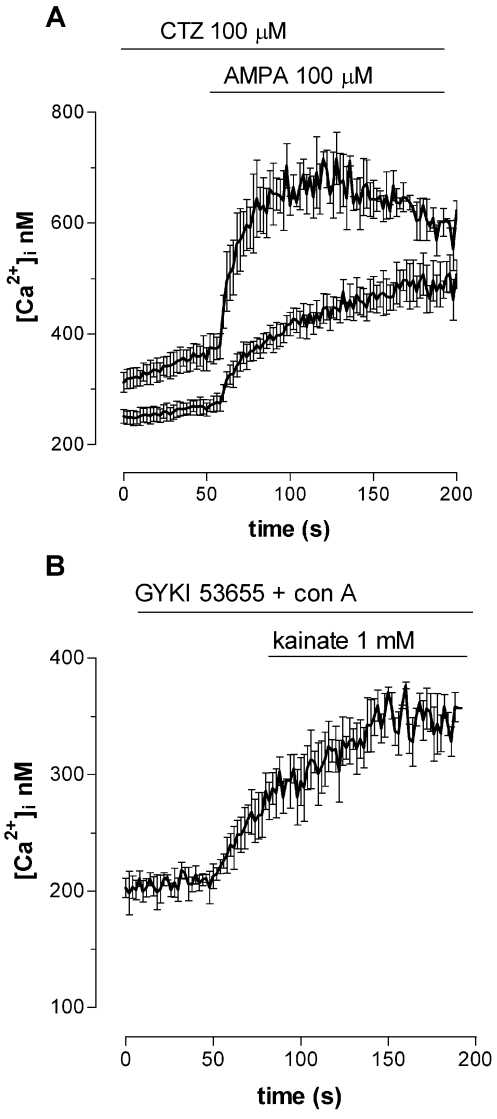

Fig. 2. AMPA and kainate receptor activation causes Ca^{2+} influx into oligodendrocytes. (A) Two populations of cells respond differently to AMPA in the presence of cyclothiazide (100 μM). (B) Responses to kainate (1 mM) applied together with concanavalin A (20 μM) and GYKI53655 (100 μM). These responses are absolutely dependent on the presence of extracellular Ca^{2+}.

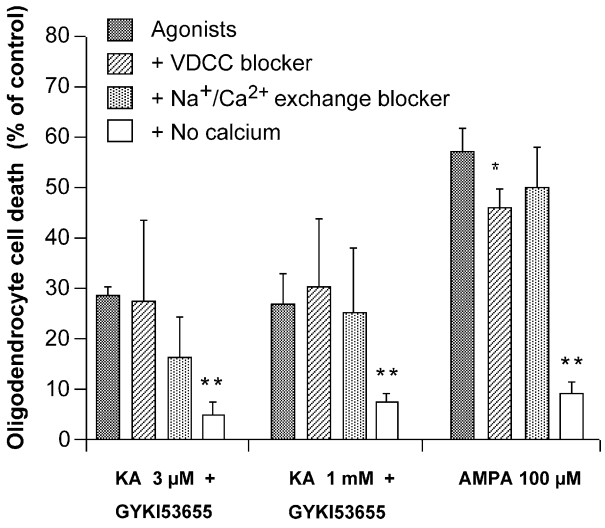

Fig. 3. Ca^{2+} influx through AMPA or kainate receptors is the major trigger of oligodendrocyte death. Excitotoxicity triggered by selective activation of AMPA or kainate receptors is greatly reduced in the absence of extracellular Ca^{2+}, but virtually unaltered by voltage-dependent Ca^{2+} channel blockers (VDCC; $LaCl_3$, 100 μM), and by preventing the reverse mode of operation of the Na^+/Ca^+ exchange with a selective blocker (KB-R7943; 10 μM). GYKI53655 was applied at 100 μM; AMPA and kainate receptors were activated in the presence of cyclothiazide (100 μM) and concanavalin A (20 μM), respectively. $^*P<0.05$, $^{**}P<0.01$.

7. Ca^{2+} overflow and mitochondrial function

The types of excitotoxic cell death observed depend on the intensity and duration of exposure and involve two temporally distinct phases of necrosis and apoptosis, a feature that relies on mitochondrial physiology. Mitochondria accumulate much of the Ca^{2+} that enters the cell during excitotoxic insult, and blockade of this process prevents cell death (Stout et al., 1998; Rego et al., 2001). Thus, it seems that mitochondrial Ca^{2+} accumulation is a critical event leading to the dysfunction of this organelle. Mitochondrial depolarization, increased production of oxygen free radicals, and release of pro-apoptotic factors that activate caspases have all been reported as a consequence of excitotoxic stimuli (Luejtens et al., 2000; Atlante et al., 2001). The relative contribution of these events determines necrosis or apoptosis (Ankarcrona et al., 1995).

The mechanisms by which Ca^{2+} accumulation in mitochondria causes dysfunction of this organelle are still poorly understood. One topic that has received much attention is the translocation of cytochrome *c* from the mitochondria to the cytoplasm, whereby it can activate caspases and trigger apoptosis. Alternatively, cytochrome *c* release can also increase free radical production by the mitochondrial electron transport chain at the levels of complexes I, III, and ubiquinone, causing non-specific cellular damage and

necrotic-like morphology (Murphy et al., 1999). Depending on the levels of cytochrome *c* released by mitochondria, the amount of ATP may be insufficient to complete the apoptotic program. Because of this, cells can die by apoptosis or necrosis, as it is indeed observed in excitotoxic models (Bonfoco et al., 1995).

Two main alternatives have been proposed to explain how cytochrome *c* is released into the cytoplasm. First, direct permeabilization of the mitochondrial outer membrane and, second, opening of the mitochondrial permeability transition pore and subsequent swelling of the mitochondrial matrix and outer membrane rupture (Green and Reed, 1998). In both scenarios, the family of Bcl-2 proteins is likely to play a critical role. This family of proteins comprises pro-apoptotic and anti-apoptotic members. Excitotoxic mitochondrial dysfunction is usually prevented by the anti-apoptotic members of this family such as Bcl-2 and Bcl-xl (Lawrence et al., 1996), whereas pro-apoptotic members such as Bad or Bax have been reported to be involved in excitotoxin-induced mitochondrial dysfunction (Xiang et al., 1998).

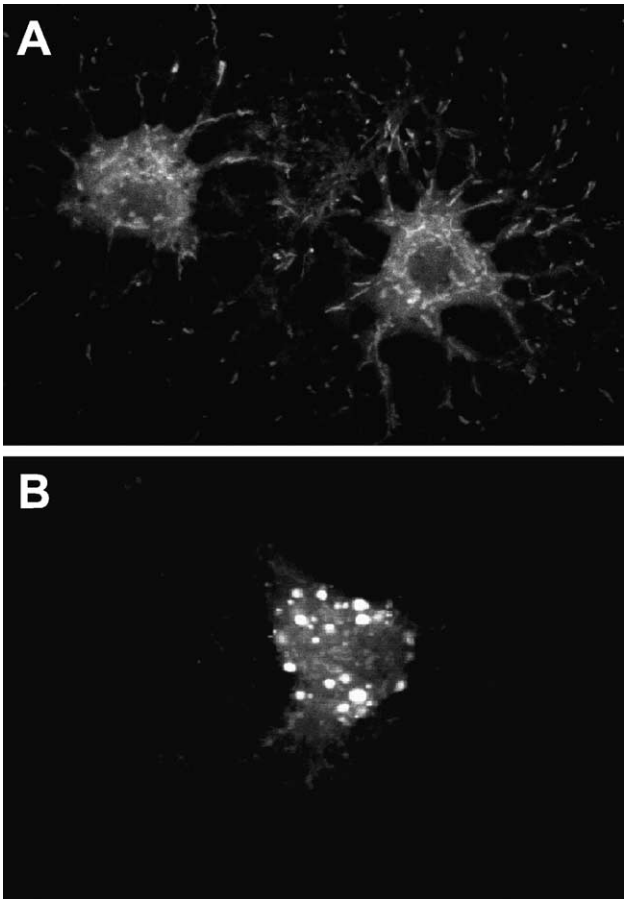

Fig. 4. Over-activation of AMPA and kainate receptors in oligodendrocytes causes mitochondrial damage. Oligodendrocyte cultures (A, control) were treated with kainate (B; 10 μM, 30 min). Staining with the voltage-sensitive fluorescence probe cloromethyl-dihydro-tetramethylrosamine reveals great alterations in mitochondrial function in the treated oligodendrocytes (B).

Mitochondrial alterations subsequent to excitotoxic insult in oligodendrocytes share features with those observed in neurons, in that they can trigger apoptotic and necrotic death (Sánchez-Gómez et al., 2001). Thus, mild insults lead to apoptosis of oligodendrocytes, which can be blocked by Bcl-2, and prompt activation of caspases including caspase-3, and alteration of mitochondrial function (Sánchez-Gómez et al., 2001). The latter can be detected soon after stimulation (Fig. 4).

8. Relevance of oligodendroglial excitotoxicity to disease

8.1. Hypoxia-ischemia related diseases

Excitotoxicity appears to be the predominant mechanism underlying ischemic damage (for a recent review, see Lee et al., 1999). Like neurons, differentiated oligodendrocytes in mixed glial cultures are very sensitive to transient oxygen and glucose deprivation. After 1 h under these conditions, the viability of oligodendrocytes is severely impaired, an effect that is attenuated by AMPA/kainate antagonists (McDonald et al., 1998). This indicates that glutamate released from astrocytes may initiate the excitotoxic cascade.

Immature oligodendrocytes appear to be even more sensitive to ischemic injury than their more mature counterparts (Fern and Möller, 2000). Strikingly, oxygen and glucose withdrawal kills immature oligodendrocytes in less than an hour; this represents the highest sensitivity to ischemic injury of any CNS cell type studied to date. Cell death is prevented by the removal of Ca^{2+} from the culture medium and by AMPA/kainate receptor antagonists, but not by the blockade of other potential sources of Ca^{2+} influx (Fern and Möller, 2000). The mechanism of Ca^{2+} influx and cell death in immature oligodendrocytes is therefore similar to, although more rapid than, that reported in mature cells of this lineage (Yoshioka et al., 1996; Matute et al., 1997; McDonald et al., 1998). That the observations of Fern and Möller (2000) were made with pure cultures of oligodendrocytes indicates that these cells release glutamate under ischemic conditions. A possible mechanism for such release may be reverse glutamate transport, given the fact that these cells express glutamate transporters (Domercq et al., 1999) that can function in reverse during ischemia (Rossi et al., 2000). This raises the possibility of a fatal autologous glutamate feedback resulting in oligodendrocyte death (Fern and Möller, 2000).

Consistent with the observations in vitro, permanent middle cerebral artery occlusion and brief transient global ischemia induce rapid oligodendroglial death, and in certain brain regions these cells are more vulnerable than neurons (Mandai et al., 1997; Petito et al., 1998). Interestingly, a few days after the insult there is an increase in the number of

oligodendroglial cells in areas bordering the infarcted area (Mandai et al., 1997), as well as of immature oligodendrocytes in periventricular areas (Gottlieb et al., 2000). This suggests that ischemic damage to oligodendroglia can be repaired, at least in part, by generating new oligodendrocytes.

Severe neuropathological alterations in the white matter and optic nerve have been described in disorders in which excitotoxicity appears to be an etiological component. Indeed, increasing evidence indicates that oligodendrocytes are vulnerable to anoxic and ischemic injury (reviewed by Stys, 1998). Perhaps the most dramatic cases of hypoxia-ischemia-related diseases with white matter damage are cerebral palsy and periventricular leucomalacia. These two conditions are characterized by isolated foci or diffuse areas of gliotic tissue that appear to be formed as a consequence of oligodendrocyte cell death, possibly caused by glutamate toxicity (Kinney and Armstrong, 1997).

8.2. Acquired immunodeficiency syndrome (AIDS) dementia

In addition to ischemia, viral infections can also result in excitotoxicity. Human immunodeficiency virus-1 (HIV-1) infection causes cognitive dysfunction in a large proportion of AIDS patients, a syndrome that may be due to neuronal excitotoxic damage (reviewed by Lipton, 1998). Interestingly, HIV encephalitis is characterized by myelin palor, among other features, suggesting that oligodendrocytes may also die as a result of excitotoxins. This possibility is supported by the fact that glutamate homeostasis is severely altered in this disease (Kaul et al., 2001). This may be due to inhibition of glutamate uptake by eicosanoids and free radicals produced by HIV-infected brain mononuclear phagocytes (Lipton, 1998) or as a consequence of excessive glutamate release from astrocytes (Bezzi et al., 2001). Thus, prolonged exposure to increased levels of glutamate may trigger glutamate receptor-mediated damage and/or oxidative stress to oligodendrocytes.

8.3. Demyelinating diseases

Excitotoxins like kainate applied to the optic nerve cause AMPA/kainate receptor-mediated histological damage (Matute, 1998). This includes disruption of the typical arrangement of interfascicular oligodendrocytes and gliosis. Importantly, long-term effects of chronic excitotoxicity result in atrophy as well as signs of profound demyelination. This finding raises the question whether excitotoxicity may underlie some of the damage observed in demyelinating diseases (Matute et al., 2001).

The idea proposed above has received support from recent data obtained using experimental autoimmune encephalomyelitis, the most reliable experimental model of multiple sclerosis (Steinman, 1999). In this model, it has been observed that the neurological symptoms associated with experimental autoimmune encephalomyelitis are ameliorated by AMPA/kainate receptor antagonists (Smith et al., 2000; Pitt et al., 2000). The improvement in the clinical score was underlined by an increase in oligodendrocyte survival and reduced axonal damage (Pitt et al., 2000). Surprisingly, the survival of spinal cord motor neurons in experimental autoimmune encephalomyelitis was also significantly improved after treatment with glutamate receptor antagonists which, in turn, ameliorated the clinical outcome of chronic relapsing experimental autoimmune encephalomyelitis (Smith et al., 2000).

A question opened by these remarkable findings is whether glutamate levels are increased in the CNS of patients with demyelinating disorders. Indeed, the concentration of excitatory amino acid in cerebrospinal fluid (CSF) is higher in patients with acute rather than silent MS and in controls (Stover et al., 1997). Potential cellular sources contributing to enhanced glutamate levels in CSF include activated microglia, which can release glutamate via the reverse glutamate transporter, a process that it is potentiated under pathological conditions (Noda et al., 1999). In addition, oxidative stress may also contribute to the increase in glutamate concentrations in the extracellular space, since free radicals reduce the efficiency of glutamate transporters (Volterra et al., 1994). In particular, this mechanism may impair the functioning of glutamate transporters in oligodendrocytes (Domercq et al., 1999). Consistent with this possibility, the neurological deficit resulting from experimental autoimmune encephalomyelitis is generally reduced by trial therapies intended to diminish the concentration of reactive oxygen species (Smith et al., 1999).

9. Conclusions

Like neurons, glial cells are sensitive to excitotoxicity. Among them, oligodendrocytes display great vulnerability to over-activation of AMPA and kainate receptors. Cell death is initiated by alterations in calcium homeostasis which, in turn, lead to mitochondrial damage. The vulnerability of oligodendrocytes to glutamate signals raises the possibility that excitotoxicity may be a component in the etiology of CNS demyelinating disorders. A thorough understanding of oligodendroglial excitotoxicity may open the way for new pharmacological strategies for the treatment of demyelinating disorders.

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References

Alberdi, E., Sánchez-Gómez, M.V., Marino, A., Matute, C., 2002. Ca^{2+} influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes. Neurobiol. Dis. 9, 234–243.

Ankarcrona, M., Dypbukt, J.M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S.A., Nicotera, P., 1995. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 15, 961–973.

Atlante, A., Calissano, P., Bobba, A., Giannattasio, S., Marra, E., Passarella, S., 2001. Glutamate neurotoxicity, oxidative stress and mitochondria. FEBS Lett. 497, 1–5.

Auger, C., Attwell, D., 2000. Fast removal of synaptic glutamate by postsynaptic transporters. Neuron 28, 547–558.

Baimbridge, K.G., Celio, M.R., Rogers, J.H., 1992. Calcium-binding proteins in the nervous system. Trends Neurosci. 15, 303–308.

Bergles, D.E., Roberts, J.D., Somogyi, P., Jahr, C.E., 2000. Glutamatergic synapses on oligodendrocyte precursor cells in hippocampus. Nature 405, 187–191.

Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., Vescovi, A., Bagetta, G., Kollias, G., Meldolesi, J., Volterra, A., 2001. CXCR4-activated astrocyte glutamate release via TNFa: amplification by microglia triggers neurotoxicity. Nat. Neurosci. 4, 702–710.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., Lipton, S.A., 1995. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc. Natl. Acad. Sci. U. S. A. 92, 7162–7166.

Burnashev, N., 1996. Calcium permeability of glutamate-gated channels in the central nervous system. Curr. Opin. Neurobiol. 6, 311–317.

Carriedo, S.G., Yin, H.Z., Sensi, S.L., Weiss, J.H., 1998. Rapid Ca^{2+} entry through Ca^{2+}-permeable AMPA/kainate channels triggers marked intracellular Ca^{2+} rises and consequent oxygen radical production. J. Neurosci. 18, 7727–7738.

Chen, C.J., Liao, S.L., Kuo, J.S., 2000. Gliotoxic action of glutamate on cultured astrocytes. J. Neurochem. 75, 1557–1565.

Choi, D.W., 1988. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1, 623–634.

Choi, D.W., Maulucci-Gedde, M., Kriegstein, A.R., 1987. Glutamate neurotoxicity in cortical cell culture. J. Neurosci. 7, 357–368.

Conti, F., Weinberg, R.J., 1999. Shaping excitation at glutamatergic synapses. Trends Neurosci. 22, 451–458.

Danbolt, N.C., 2001. Glutamate uptake. Prog. Neurobiol. 65, 1–105.

David, J.C., Yamada, K.A., Bagwe, M.R., Goldberg, M.P., 1996. AMPA receptor activation is rapidly toxic to cortical astrocytes when desensitization is blocked. J. Neurosci. 16, 200–209.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F., 1999. The glutamate receptor ion channels. Pharmacol. Rev. 51, 7–61.

Domercq, M., Sánchez-Gómez, M.V., Areso, P., Matute, C., 1999. Expression of glutamate transporters in rat optic nerve oligodendrocytes. Eur. J. Neurosci. 11, 2226–2236.

Fern, R., Möller, T., 2000. Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J. Neurosci. 20, 34–42.

Gill, R., Lodge, D., 1997. Pharmacology of AMPA antagonists and their role in neuroprotection. Int. Rev. Neurobiol. 40, 197–232.

Gottlieb, M., Matute, C., 1997. Expression of ionotropic glutamate receptor subunits in glial cells of the hippocampal CA1 area following transient forebrain ischemia. J. Cereb. Blood Flow Metabol. 17, 290–300.

Gottlieb, M., Domercq, M., Matute, C., 2000. Altered expression of the glutamate transporter EAAC1 in neurons and immature oligodendrocytes after transient forebrain ischemia. J. Cereb. Blood Flow Metabol. 20, 678–687.

Green, D.R., Reed, J.C., 1998. Mitochondria and apoptosis. Science 281, 1309–1316.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Kaul, M., Garden, G.A., Lipton, S.A., 2001. Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 410, 988–994.

Kingham, P.J., Cuzner, M.L., Pocock, J.M., 1999. Apoptotic pathways mobilized in microglia and neurones as a consequence of chromogranin A-induced microglial activation. J. Neurochem. 73, 538–547.

Kinney, H.C., Armstrong, D.D., 1997. Perinatal neuropathology. In: Graham, D.I., Lantos, P.L. (Eds.), Greenfield's Neuropathology. Arnold, London, pp. 537–600.

Lawrence, M.S., Ho, D.Y., Sun, G.H., Steinberg, G.K., Sapolsky, R.M., 1996. Overexpression of Bcl-2 with herpes simplex virus vectors protects CNS neurons against neurological insults in vitro and in vivo. J. Neurosci. 16 (2), 486–496.

Lee, J.M., Zipfel, G.J., Choi, D.W., 1999. The changing landscape of ischaemic brain injury mechanisms. Nature 399, 7–14.

Lerma, J., 1999. Kainate receptors. In: Jonas, P., Monyer, H. (Eds.), Handbook of Experimental Pharmacology. Ionotropic Glutamate Receptors in the CNS, vol. 141. Springer-Verlag, Heidelberg, pp. 275–307.

Li, S., Stys, P.K., 2000. Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J. Neurosci. 20, 1190–1198.

Lipton, S.A., 1998. Neuronal injury associated with HIV-1: approaches to treatment. Annu. Rev. Pharmacol. Toxicol. 38, 159–177.

Lipton, S.A., Rosenberg, P.A., 1994. Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med. 330, 613–622.

Lucas, D.R., Newhouse, J.P., 1957. The toxic effect of sodium L-glutamate on the inner layers of the retina. Arch. Ophthalmol. 58, 193–201.

Luejtens, C.M., Bui, N.T., Sengpiel, B., Münstermann, G., Poppe, M., Krohn, A.J., Bauerbach, E., Krieglstein, J., Prehn, J.H.M., 2000. Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome *c* release and a secondary increase in superoxide production. J. Neurosci. 20, 5715–5723.

Mandai, K., Matsumoto, M., Kitagawa, K., Matsushita, K., Ohtsuki, T., Mabuchi, T., Colman, D.R., Kamada, T., Yanagihara, T., 1997. Ischemic damage and subsequent proliferation of oligodendrocytes in focal cerebral ischemia. Neuroscience 77, 849–861.

Matute, C., 1998. Characteristics of acute and chronic kainate excitotoxic damage to the optic nerve. Proc. Natl. Acad. Sci. U. S. A. 95, 10229–10234.

Matute, C., Sánchez-Gómez, M.V., Martínez-Millán, L., Miledi, R., 1997. Glutamate receptor-mediated toxicity in optic nerve oligodendrocytes. Proc. Natl. Acad. Sci. U. S. A. 94, 8830–8835.

Matute, C., Domercq, M., Fogarty, D.J., Pascual de Zulueta, M., Sánchez-Gómez, M.V., 1999. On how altered glutamate homeostasis may contribute to demyelinating diseases of the CNS. In: Matsas, R., Tsacopoulos, M. (Eds.), The Function of Glial Cells in Health and Disease: Dialogue Between Glia and Neurons. Plenum, New York, pp. 97–108.

Matute, C., Alberdi, E., Domercq, M., Pérez-Cerdá, F., Pérez-Samartín, A., Sánchez-Gómez, M.V., 2001. The link between excitotoxic oligodendroglial death and demyelinating diseases. Trends Neurosci. 24, 224–230.

McDonald, J.W., Althomsons, S.P., Hyrc, K.L., Choi, D.W., Goldberg, M.P., 1998. Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. Nat. Med. 4, 291–297.

Michaelis, E.K., 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Prog. Neurobiol. 54, 369–415.

Murphy, A.N., Fiskum, G., Beal, M.F., 1999. Mitochondria in neurodegeneration: bioenergetic function in cell life and death. J. Cereb. Blood Flow Metab. 19, 231–245.

Noda, M., Nakanishi, H., Akaike, N., 1999. Glutamate release from microglia via glutamate transporter is enhanced by amyloid-beta peptide. Neuroscience 92, 1465–1474.

Oka, A., Belliveau, M.J., Rosenberg, P.A., Volpe, J.J., 1993. Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. J. Neurosci. 13, 1441–1453.

Olney, J.W., Sharpe, L.G., 1969. Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Science 166, 386–388.

Petito, C.K., Olarte, J.P., Roberts, B., Nowak, T.S., Pulsinelli, W.A., 1998. Selective glial vulnerability following transient global ischemia in rat brain. J. Neuropath. Exp. Neurol. 57, 231–238.

Pitt, D., Werner, P., Raine, C.S., 2000. Glutamate excitotoxicity in a model of multiple sclerosis. Nat. Med. 6, 67–70.

Prieto, M., Alonso, F., 1999. Differential sensitivity of cultured tanycytes and astrocytes to hydrogen peroxide toxicity. Exp. Neurol. 155, 118–127.

Rego, A.C., Ward, M.W., Nicholls, D.G., 2001. Mitochondria control AMPA/kainate receptor-induced cytoplasmic calcium deregulation in rat cerebellar granule cells. J. Neurosci. 21 (6), 1893–1901.

Rossi, D.J., Oshima, T., Attwell, D., 2000. Glutamate release in severe brain ischaemia is mainly by reversed uptake. Nature 403, 316–321.

Rothstein, J.D., Dykes-Hoberg, M., Pardo, C.A., Bristol, L.A., Jin, L., Kluncl, R.W., Kanai, Y., Hediger, M.A., Wang, Y., Schielke, J.P., Welty, D.F., 1996. Knock out of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–686.

Sánchez-Gómez, M.V., Matute, C., 1999. AMPA and kainate receptors each mediate excitotoxicity in oligodendrocyte cultures. Neurobiol. Dis. 6, 475–485.

Sánchez-Gómez, M.V., Alberdi, E., Ibarretxe, G., Torre, I., Matute, C., 2001. Activation of AMPA and kainate receptors triggers apoptotic and necrotic oligodendroglial death in vitro. Soc. Neurosci. Abs. 27, 57214.

Schubert, D., Piasecki, D., 2001. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. J. Neurosci. 21, 7455–7462.

Smith, K.J., Kapoor, R., Felts, P.A., 1999. Demyelination: the role of reactive oxygen and nitrogen species. Brain Pathol. 9, 69–92.

Smith, T., Groom, A., Zhu, B., Turski, L., 2000. Autoimmune encephalomyelitis ameliorated by AMPA antagonists. Nat. Med. 6, 62–66.

Steinman, L., 1999. Assessment of animal models for MS and demyelinating disease in the design of rational therapy. Neuron 24, 514–551.

Stout, A.K., Raphael, H.M., Kanterewicz, B.I., Klann, E., Reynolds, I.J., 1998. Glutamate-induced neuron death requires mitochondrial calcium uptake. Nat. Neurosci. 1 (5), 366–373.

Stover, J.F., Pleines, U.E., Morganti-Kossmann, M.C., Kossmann, T., Lowitzsch, K., Kempsky, O.S., 1997. Neurotransmitters in cerebrospinal fluid reflect pathological activity. Eur. J. Clin. Invest. 27, 1038–1043.

Stys, P.K., 1998. Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics. J. Cereb. Blood Flow Metab. 18, 2–25.

Verkhratsky, A., Steinhäuser, C., 2000. Ion channels in glial cells. Brain Res. Rev. 32, 380–412.

Volterra, A., Trotti, D., Tromba, C., Floridi, S., Racagni, G., 1994. Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. J. Neurosci. 14, 2924–2932.

Weiss, J.H., Sensi, S.L., 1999. Ca^{2+}–Zn^{2+} permeable AMPA or kainate receptors: possible key factors in selective neurodegeneration. Trends Neurosci. 23, 365–371.

Xiang, H., Kinoshita, Y., Knudson, C.M., Korsmeyer, S.J., Schawrtzkroin, P.A., Morrison, R.S., 1998. Bax involvement in p53-mediated neuronal cell death. J. Neurosci. 18 (4), 1363–1373.

Yoshioka, A., Bacskai, B., Pleasure, D., 1996. Pathophysiology of oligodendroglial excitotoxicity. J. Neurosci. Res. 46, 427–437.