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Excitotoxicity and the putative involvement of excitatory amino acids in neurodegenerative diseases

André Nieoullon, Laurence Had-Aissouni, Lydia Kerkerian-le Goff

Laboratoire de Neurobiologie Cellulaire et Fonctionnelle CNRS, Université de la Mediterranée II, Marseille, France

Summary
Excitatory amino acids (EAA) represent major brain neurotransmitters. They are present in numerous neuronal systems and thus are involved in almost all aspects of normal and pathological brain activity. Changes in EAA transmission have been associated with the functional impairments characterizing major neurological disorders, including epilepsy and schizophrenia. There is also a suspicion that EAA systems underlie the neuronal death associated not only with acute CNS insults, such as in ischemia or post-traumatic lesions, but also with neurodegenerative diseases such as ALS, Huntington's disease and Parkinson's disease. The neurotoxicity of EAA, referred to as excitotoxicity, is presumably mediated primarily through an excess of EAA synaptic receptor stimulation. Indeed, overstimulation of the ionotropic NMDA or AMPA/kainate receptor subtypes has been shown to produce an intense membrane depolarisation and further a massive increase in intracellular calcium leading to cell damage. The extreme diversity and specific pattern of expression of EAA receptor subunits could account for the differential vulnerability of certain brain areas to such excitotoxic processes. In addition, it is now believed that besides abnormalities in receptor functioning or in release processes, alterations in EAA transmission may result from dysfunction of the EAA uptake system, which represents the mechanism for EAA removal from the synapse. From the five transporter proteins cloned, termed EAAT1-5, the primarily glial transporters EAAT1 and EAAT2 have been shown to mediate the bulk of EAA uptake in the brain and it has then been suggested that they play a major role in the prevention of excitotoxic processes. In this respect, the degeneration of motor neurons in ALS has been associated with altered expression or inactivation of EAAT2. Moreover, recent evidence has been provided that pharmacological alteration of glutamate transport can also induce astrocyte degeneration, as observed in neurodegenerative insults, but through a mechanism independent of stimulation of EAA receptors. Thus, one can obviously consider that these EAATs can represent a key target for further development of new neuroprotective agents.

Keywords: neurodegenerative diseases – signalling pathways – excitatory amino acids – neurotoxicity – striatal neurodegeneration

INTRODUCTION

Glutamic acid (Glu) represents a major neurotransmitter in the brain. Its powerful excitatory effects on neurones were shown more than forty years ago (Curtis et al. 1959). Strong depolarizing effects were also demonstrated for other dicarboxylic acids, thus leading to a definition of endogenous excitatory amino acids (EAA) as general putative neurotransmitters acting at excitatory synapses. EAA have been identified as neurotransmitters in numerous neuronal systems and are considered to mediate transmission at more than 20% of total brain synapses. The entire brain is submitted to influences of EAA, from the spinal cord to cerebellum, hippocampus and telencephalon, suggesting their involvement in most brain functions. For instance, due to their signalling role

in cortical and hippocampal efferent pathways, EAA probably contribute to cognitive, limbic and motor aspects of behavioural processes.

Apart from having a key role in excitatory transmission, EAA have also been shown to contribute to neurodegenerative processes. This hypothesis was first based on the observation of the neurotoxic effects of exogenous Glu administration on retina (Lucas and Newhouse 1957) and has been further extensively documented over the last thirty years through numerous in vivo and in vitro experiments (see Doble 1999). Evidence has been now accumulated that acute neurodegenerative processes associated with ischemia, stroke or hypoglycemia involve at least partly a cytotoxic action of EAA. EAA may be also involved in certain chronic neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and possibly Huntington's chorea. However, the way in which an essential neurotransmitter becomes cytotoxic remains to be elucidated and experiments are still in progress to further characterize the actual mechanisms of excitotoxicity. This short review paper will focus, among the numerous existing hypotheses, on the recent proposal that dysfunction of the physiological removal of EAA from the brain synapses, which normally contributes to end the synaptic signalisation, may be a main mechanism of excitotoxicity.

MAIN FEATURES OF EAA TRANSMISSION

EAA and particularly Glu contribute to fast signalling pathways. They are released by specific nerve terminals which store the neurotransmitter in synaptic vesicles using a vesicular transporter. Exocytosis may be the way of neurotransmitter release which was shown to depend on extracellular calcium concentration. However, the exocytosis of synaptic vesicles from nerve terminals may not be the only mechanism underlying Glu release since it was recently suggested that calcium-dependent exocytosis occurs from astroglia. Moreover, Glu may be also released by non vesicular mechanisms, including a carrier-mediated release.

Glu is considered to be primarily produced from glutamine by a glutaminase. Thus, glutamine acts as a Glu precursor. Another source of Glu in the nerve terminals is from the extracellular space. Indeed, following neuronal release, Glu is removed from the extracellular space by neurons and glial cells. In astrocytes, Glu is converted from glutamine. Glutamine is thought to be produced by the glial cells, released in the extracellular space, taken up by the neurons and reconverted into Glu. Interestingly, some of the difficulties we have in characterizing the presynaptic aspects of glutamatergic transmission could be solved by the recent identification of a "Glu neuron-related protein" called EAT-4 in *Caenorhabditis elegans* which could act as a brain-specific inorganic phosphate (BNPI) transporter presenting strong sequence similarities with EAT-4. Indeed, EAT-4 mutants cause rather selective alteration in glutamatergic transmission (see Lee et al. 1999), whereas it may increase such glutamatergic transmission through enhancement of Glu synthesis linked to increased cytoplasmic phosphate concentrations in nerve terminals. The demonstration of the subcellular localization of BNPI in synaptic vesicles and the fact that this protein mediates the transport of Glu into synaptic vesicles support a specific role for this protein, now called VGLUT1 as a Glu vesicular transporter (Bellochio et al. 1998, 2000) and provide the evidence that it could be used to define the glutamatergic phenotype in neurons (Takamori et al. 2000).

Glu and more generally EAA have been shown to act on different subsets of EAA receptors. Based on the preferential action of selective agonists, the classification first discriminated "NMDA" and "non-NMDA" ionotropic receptors which were themselves initially separated in "quisqualate" and "kainate" receptors. Later on, three subclasses of metabotropic EAA receptors coupled to G protein (metabotropic receptors or *mgluR*) were characterized involving either phospholipase C or adenylate cyclase as effector (for reviews see Bigge 1999, Roberts 1995). However, because quisqualate can interfere with the metabotropic receptors linked to the G proteins, the so-called "quisqualate receptors" are currently identified on the basis of the action of a more selective agonist called "AMPA". Thus, the pharmacological classification of the ionotropic EAA receptors is based on the distinction between NMDA, kainate and AMPA receptors. Recent advances in molecular biology have shown that about twenty subunits or proteins can contribute to the EAA receptors. Since the ionotropic receptors represent heteromeric association of five subunits, many subtypes of the receptors with specific functional properties co-exist in the brain with respect to tissue-specific expression of the different subunits. Four subunits identified as *gluR1* to *gluR 4* can contribute to the AMPA receptors, five subunits called *gluR 5 to 7* and *ka 1* and *2* to the kainate receptors, and again five subunits (*nmdaR 1* and *nmdaR 2A to 2D*) to the NMDA receptors. Finally, seven subtypes of *mgluR* have been identified, subdivided into in Groups I, II and III with respect to the second messenger system coupled to the receptor and the process of coupling. *In situ* hybridization methods have revealed that the brain

expression of EAA receptors is very wide, thus highlighting the key influence of EAA in most brain functions from motor to limbic and cognitive aspects of behaviour, as above mentioned. Moreover, specific expression of different subsets of EAA receptors during development suggests a critical influence of EAA on neuronal plasticity and brain wiring.

The EAA ionotropic receptors have been shown to undergo rapid desensitization when overstimulated. Such a mechanism could represent a protection against the cytotoxic effects of Glu and other EAA involving strong receptor stimulation. In fact, rapid and efficient removal of the neurotransmitter from extracellular space is necessary for high signal – to - noise ratio in synaptic transmission and, further, to prevent excitotoxic injury. Glu removal from the synaptic cleft and maintenance of low extracellular levels of the neurotransmitter is achieved by a specific high affinity uptake process which primarily involves the surrounding astrocytes (see Nicholls and Attwell 1990). This uptake process is dependent on extracellular sodium concentration and shows a relative specificity toward Glu since other EAA and particularly aspartate are also taken up. Specific substrate inhibitors such as PDC (L-trans-pyrrolidine-2,4-dicarboxylate) and THA and competitive blockers like DHK and TBOA, have been produced. They represent useful pharmacological tools to block Glu uptake and to further analyse the characteristics of the transport system (see Barks and Silverstein 1994, Vandenberg 1998).

Cellular function, which involves both astrocytic and neuronal elements, is accomplished by a variety of specific transporter proteins that allow co-transport of Glu with sodium ions and concomitant counter-transport of potassium ions using the electrochemical gradient for sodium and potassium as the driving force which is maintained through the Na/K ATPase pumps. Thus, the transport is electrogenic and contributes to depolarize the cells. Molecular biology studies were the first to contribute to the identification of three subtypes of EAA transporters in rodents called GLT1 (Pines et al. 1992), GLAST (Storck et al. 1992) and EAAC1 (Kanai and Hediger 1992). GLT1 and GLAST were identified in astrocytes whereas EAAC1 was shown to be neuronal.

Later on, molecular cloning of human DNA revealed the existence of five transporter subtypes which are partly homologous (about 90%) to those identified in the rodents, called EAAT1 (GLAST), EAAT2 (GLT1), EAAT3 (EAAC1), EAAT4 and EAAT5 (see Arriza et al. 1997). The exact topology of these transporters is still debated. These transmembrane proteins could exhibit eight to ten transmembrane segments and both N- and C-terminals are intracellular. It is proposed that they form homo oligomeric complexes which may contribute to the transporter pore. Interestingly, the transporter proteins show one or more consensus phosphorylation sites which may be involved in regulatory processes.

In vitro studies have suggested that such regulatory mechanisms could involve PKA, the activation of which activates Glu transport (Casado et al. 1993, Lortet et al. 1999), presumably through GLT1. PKA activation was also shown to increase GLT1 and GLAST expression (see Danbolt 2001). The contribution of PKC is under investigation.. The regional expression of the transporter proteins showed an uneven distribution. GLAST expression was shown to predominate in the cerebellum but this transporter is also present in the hippocampus, striatum and thalamus. GLT1 is the most abundant transporter in the forebrain. It is expressed at high levels in the hippocampus, cortex, striatum and spinal cord but at low levels in the regions where GLAST is abundant.

EAAC1 distribution appears to be more even in the forebrain and also to involve the cerebellum and hippocampus. EAAT4 expression is mostly concentrated in the cerebellum and that of EAAT5 in the retina. Moreover, a developmental profile of the expression of certain transporters was evidenced, in such a way that EAAC1 is predominantly expressed during embryogenesis. GLAST and especially GLT1 expression is low during the early stages of development and increases during the synaptogenesis period to reach adult levels.

Recent in vitro studies have shown that the differentiation of astrocytes is concomitant with an increased expression of GLAST and GLT1, in agreement with in vivo observations. Finally, it is worth noting that GLT1 could be in vivo the major transporter involved in the clearance of Glu at extracellular level in the striatum, as shown using the antisense oligonucleotide methodology (Rothstein et al. 1996). Interestingly enough, the rate of the uptake process could be regulated through intervention of diffusible messengers such as NO or arachidonic acid which can be produced through activation of EAA receptors. However, other regulatory mechanisms involving, for instance, neurotransmitters have also been identified. Consequently, these cellular messengers could contribute to regulate the EAA transmission by influencing at post-translational level the Glu uptake rate. Thus, regulating the uptake process could represent a critical mechanism to exert a fine tuning of the EAA transmission.

EAA TRANSMISSION AND NEUROTOXICITY

As mentioned above, evidence has been obtained that Glu and EAA are cytotoxic. For example, monosodium glutamate administration was initially shown to affect the integrity of brain areas not protected by the blood brain barrier (see Olney 1988). Such a toxic effect of Glu was demonstrated in vitro on brain slices and cell cultures and was shown to involve the EAA receptors and an excess of excitatory processes (see Kim and Choi 1987). Consequently, EAA were suspected to contribute to degenerative mechanisms associated with both acute and chronic pathological states. In this respect, the origin of the excess of Glu in pathological conditions is still a matter of debate.

For a long time, the hypothesis was put forward that an excess of Glu release could contribute to the excitotoxic effect of excitatory transmission but we and others postulated that a deficit in Glu transport could likely be at the origin of an excess of extracellular Glu. Evidence has been provided that the increase in extracellular Glu in ischemia, stroke or hypoglycemia may result mainly from reversed uptake due to a deficit in energetic processes (see Rossi et al. 2000). Moreover, such a pathological situation could be associated with subtle changes in transporter expression. For example, GLT1 expression was reported to be decreased 24hours after hypoxia-ischemia in the newborn striatum, although a transient expression in neurons was observed (Martin et al. 1997), thus illustrating a possible involvement of EAA transporters in both deleterious and protective mechanisms.

Defective transport has been also implicated in neurodegenerative diseases, such as ALS, based initially on the post-mortem observation of reduced Glu uptake in the CNS (Rothstein et al. 1992). Thereafter, a dramatic reduction of the EAAT2 protein was reported to occur in the spinal cord and motor cortex of patients with ALS in the absence of change in EAAT2 mRNA levels (Bristol and Rothstein 1996), presumably due to aberrant splicing (Lin et al. 1998). It remains unclear whether the GLT1 down-regulation is a cause or consequence of the motor neuron death. However, the finding that pharmacological alteration of Glu uptake by THA, or antisense oligonucleotide-induced downregulation of GLT1 and GLAST induced motor neuron degeneration in organotypic spinal cord cultures, favours the idea that defective transport can trigger neurodegeneration (Rothstein et al. 1996). Interestingly, in patients suffering Huntington's chorea a reduction in EAAT2 protein expression was shown at striatal level despite astrocyte proliferation (Arzberger et al. 1997). In this connection, we recently provided the first in vivo evidence that chronically altering EAA transport through continuous intrastriatal administration of PDC in the adult rat results in striatal neurodegeneration (Lievens et al. 2000). Interestingly, this study also suggested that Glu uptake alteration may be deleterious to the quiescent astrocytes but not to reactive astrocytes, as shown in the histological and immunocytochemical examination of the lesion site.

This hypothesis was further investigated in primary cultures of striatal astrocytes (Had-Aissouni et al. 2000). PDC treatment was found to induce selective death of differentiated versus undifferentiated astrocytes. This effect was found to develop slowly, and treatments for two or 14 days induce a similar death rate. Finally, although PDC application produced a large increase in extracellular Glu levels (probably through heteroexchange), excitotoxicity does not mediate the astrocyte death. For instance, the gliodegeneration was not prevented by pharmacological blockade of the EAA receptor subtypes nor mimicked or potentiated by EAA receptor agonists. Finally, uptake blockers (TBOA) that do not mediate heteroexchange did not induce astrocytic death. Thus, we postulate that the cell death induced by PDC may result from depletion of intracellular Glu store and subsequent metabolic insult.

CONCLUSIONS

EAA play a key role in central excitatory transmission and are involved in motor, limbic and cognitive behavioural processes, as shown by the severe impairments resulting from pharmacological blockade of synaptic receptors. Such experimental data further suggest that a deficit in EAA transmission could be the source of functional alterations of major brain functions such as memory and learning, stressing the necessity to better understand the synaptic mechanisms involved in the neurotransmitter action at cellular and molecular levels.

Conversely, evidence has been accumulated for the cytotoxicity of an excess of extracellular EAA. However, excitotoxicity still remains a working hypothesis and the mechanisms mediating excitotoxic damages are not fully understood. In particular, additional studies are required to determine whether defective Glu transport is the primary event leading to cell death in neurodegenerative insults and to specify the molecular cascade underlying the resulting cytotoxicity. In experimental studies, the use of EAA receptor antagonists has been shown, under certain conditions, to efficiently limit tissue destruction but, although promising, this strategy has

not yet proven sufficient benefit in clinical trials. Thus, if our working hypothesis that EAA transporters may play a key role in some degenerative diseases is correct, the uptake process would represent a possible target for development of new compounds acting as neuroprotective agents.

REFERENCES

Arriza J. L., Eliasof S., Kavanaugh M. P., Amara S. G., Proc. Natl. Acad Sci. USA 94: 4155, 1997.

Arzberger T., Krampfl K., Leimgruber S., Weindl A., J. Neuropathol. Exp. Neurol. 56: 440, 1997.

Barks J. D. E. and Silverstein F. S., Mol. Chem. Neuropathol. 23: 201, 1994.

Bellochio E. E., Hu H., Pohorille A., Chan J., Pickel V. M., Edwards R. H., J. Neurosci. 18: 8648, 1998.

Bellochio E. E., Reimer R. J., Freneau R. T., Edwards R. H., Science 289: 847,2000.

Bigge C. F., Curr. Opin. Chem. Biol. 3: 441, 1999.

Bristol L. A. and Rothstein J. D., Ann. Neurol. 39: 676,1996.

Casado M., Bendahan A., Zafra F., Danbolt N. C., Aragon C., Gimenez C., Kanner B. I., J. Biol. Chem. 268: 27313, 1993.

Curtis D. R., Phillips J. W., Watkins J. C., Nature 183: 611, 1959.

Danbolt N. C., Progr. Neurobiol. 65: 1, 2001.

Doble A., Pharmacol. Ther. 81: 163, 1999.

Had-Aissouni L., Ré D., A□t-Mamar H., Samuel D., Shimamoto K., Kerkerian-Le Goff L., Birman S., Rev. Neurol., Spec. issue 24-17 May, Abstract P202, 2000.

Kanai Y. and Hediger M. A., Nature 360: 467, 1992.

Kim J. P. and Choi D. W., Neuroscience 23: 423, 1987.

Lee L. Y., Sawin E. R., Chalfie M., Horvitz H. R., Avery L., J. Neurosci. 19: 159, 1999.

Lievens J. C., Bernal F., Forni C., Mahy N., Kerkerian-Le Goff L., Glia 29: 222, 2000.

Lortet S., Samuel D., Had-Aissouni L., Masmejan F., Kerkerian-Le Goff L., Pisano P., Neuropharmacology 38: 395, 1999.

Lucas D. R. and Newhouse J. P., AMA Arch. Ophtalmol. 58: 193, 1957.

Martin L. J., Brambrink A. M., Lehmann C., Portera-Cailliau C., Koelher R., Rothstein J. D., Traystman R. J., Ann. Neurol. 42: 335, 1997.

Nicholls D. and Attwell D., Trends Pharmacol. Sci. 11: 462, 1990.

Olney J. W., In D. Lodge (ed): Excitatory amino acids in health and disease. John Wiley 1988, p. 337.

Roberts P. J., Neuropharmacology 34: 813, 1995.

Rossi D. J., Oshima T., Attwell D., Nature 403: 316, 2000.

Rothstein J. D., Dykes-Hoberg M., Pardo C. A., Bristol L. A., Jin L., Kunel R., Kanai Y., Hediger M. A., Wang Y., Scielke J., Welty D. F., Neuron 16: 675, 1996.

Rothstein J. D., Martin L. J., Kuncl R. W., New England J. Med. 326: 1464, 1992.

Takamori S., Rhee J. S., Rosenmund C., Jahn R., Nature 407: 141, 2000.

Vanderberg R. J., Clin. Exp. Pharmacol. Physiol. 25: 393, 1998.

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Address:
André Nieoullon, Laboratoire de Neurobiologie Cellulaire et Fonctionnelle CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France; nieoullon@lncf.cnrs-mrs.fr