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Effects of antagonists of glutamate receptors on pro-inflammatory cytokines in the brain cortex of rats subjected to experimental autoimmune encephalomyelitis

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Inflammatory cytokines and glutamate neurotoxicity have been proposed as major determinants accompanying the demyelination and axonal degeneration observed during the course of MS. The present study using the animal model of MS known as experimental autoimmune encephalomyelitis (EAE) demonstrates that pharmacological inhibition of ionotropic NMDA glutamate receptors by their antagonists (amantadine and memantine) suppresses neurological symptoms of disease in EAE rats and reduces expression of pro-inflammatory cytokines in the brain. Conversely, antagonists of group I metabotropic glutamate receptors, mGluRs (LY 367385 and MPEP), do not affect the inflammatory process and the neurological condition of EAE rats.

1. Introduction

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are autoimmune diseases in which the immune system attacks the central nervous system (CNS). Characteristic features of the disease are demyelinating areas in the white matter of the spinal cord and brain, which lead to disturbances in nerve transmission and further to disability. The primary pathological hallmark of MS is the appearance of inflammatory lymphocytes infiltrating the CNS from the peripheral circulation. In demyelinating lesions, the presence of lymphocytes, macrophages and activated microglia has been observed in the proximity of perivascular area. This suggests that these types of cells are involved in the process of demyelination (Cuzner et al., 1998). Macrophages and activated microglia generate free radicals, release excitatory amino acids, activate the complement pathway and produce pro-inflammatory cytokines and antibodies against proteins of the myelin sheath. All of these factors can damage myelin and oligodendrocytes and consequently induce injury of axons and death of neurons. Entry of leukocytes into the CNS leads to elevated concentrations of glutamate in the brain and spinal cord fluid (Groom et al., 2003).

Glutamate is the major excitatory amino acid neurotransmitter in the mammalian CNS. Glutamate signaling is mediated by a number of pre- and post-synaptically-located ionotropic glutamate receptors (iGluRs), metabotropic glutamate receptors (mGluRs) and excitatory amino acid transporters (EAAEs). The iGluRs are divided into three classes; AMPA, KA and NMDA. The members of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) class and the KA (kainite) class are expressed in both neurons and oligodendrocytes. Excitotoxic damage of oligodendrocytes mediated by no-NMDA glutamate receptors was observed in vitro (Yoshioka et al., 1996; Matute et al., 1997). The iGluRs of the NMDA class (N-methyl-D-aspartate) are expressed in neurons, microglia and astrocytes (Verkhratsky and Kirchoff, 2007). In vitro experiments show that agonists of glutamate receptors like glutamate, kainate and AMPA are toxic to neurons and oligodendrocytes at low concentrations (Ikonomidou and Turski, 1996; McDonald et al., 1998).

Metabotropic glutamate receptors (mGluRs) regulate a variety of intracellular signaling systems via activation of GTP-binding proteins (Schoepp et al., 1999). There are eight subtypes of mGluRs, which are classified into three main groups. Group I includes mGluR1 and mGluR5, which are coupled to phosphoinositide hydrolysis, while the mGluRs of group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to adenyl cyclase (Schoepp et al., 1999). Group I mGluRs (mGluR1 and mGluR5) are predominately located postsynaptically in neurons, but they are also present in astrocytes and microglia where they have been shown to be implicated in inflammatory processes (Bordli and Ugolini, 1999). Glial mGluRs can regulate glial function and may be involved in interactions between glia and neurons in physiological and pathological conditions by modulating the release of both glutamate and postsynaptic responses (Bruno et al., 1998; Biber et al., 1999). Moreover, mGluRs may play a role in neuroinflammatory disorders as specific mGluR agonists have the ability to regulate production

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of chemokines by astrocytes in vitro and improve the rate of neurological deficits during EAE course in vivo (Besong et al., 2002). Changes in group I mGluR expression in the CNS have been observed in animal models of inflammatory disease as well as in clinical inflammation (Mills et al., 2001; Geurts et al., 2003). Both subtypes of group I mGluRs (mGluR1 and mGluR5) are capable of modulating excitatory synaptic transmission. Antagonists of the mGluRs exhibit neuroprotective potential and administration of mGluRs has been proposed as a therapeutic strategy for treatment of numerous brain pathologies (Bordli and Ugolini, 1999; Bruno et al., 2001; Popoli et al., 2004). The neuroprotective effects were observed after pharmacological blockade of mGluR1 by its antagonist LY 367385 and blockade of mGluR5 by MPEP in different models of neurodegenerative diseases (Faden et al., 2001; Popoli et al., 2004).

Agents that specifically block the pathological stimulation of iGluRs (mainly of the NMDA class) or group I mGluRs might be expected to restore physiological function to synaptic nerve transmission and produce positive disease-modifying effects.

Our previous studies indicate a role of glutamate, glutamate receptors and transporters in the pathogenesis of EAE (Mitosek-Szewczyk et al., 2008; Sulkowski et al., 2009). Moreover, it was shown that glutamate and its NMDA receptors are involved in the mechanism of loss of blood–brain barrier (BBB) integrity which has been observed in EAE rats (Paul and Balton, 2002). Pharmacological investigations strongly suggest the involvement of NMDA and group I mGluRs in the pathogenesis of EAE. Administration of MK-801 (an antagonist of NMDA receptors) was found to significantly improve the neurological status of EAE rats (Bolton and Paul, 1997).

Memantine is an NMDA receptor antagonist which is structurally distinct from MK-801. Memantine blocks receptor activation by binding to an ion channel site. Memantine is a derivative of aminoadamantane (also known as amantadine). Both of these compounds have been used in clinical treatments of dementia and Parkinson's disease (Danysz et al., 1997) with good tolerance. A reduction in the relapse rate in MS patients treated with amantadine was also reported (Plaut, 1987). It is significant that the aminoadamantanes were found to be better tolerated by experimental animals in comparison with MK-801. Pharmacological blockade of iGluRs by MK-801 has limited clinical use because it blocks fast excitatory transmission and has several side effects. Hence, there are high expectations for the use of mGluRs and their antagonists as targets for development of new neuroprotective strategies in efforts to treat MS/EAE. However, memantine should be used in the clinic very carefully, because of the reports of its side effects (Villoslada et al., 2009).

The present study was undertaken to determine whether the glutamate receptor antagonists amantadine and memantine (antagonists of NMDA receptors), LY 367385 (an antagonist of mGluR1) and MPEP (an mGluR5 antagonist) have an effect on the expression of pro-inflammatory cytokines and modify the inflammatory processes occurring during the course of EAE. There is a link between inflammation and glutamate-mediated excitotoxicity, in which the interactions of the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) could be involved. Both enzymes are tightly coupled to the excitotoxic death of neurons and oligodendrocytes (Rose et al., 2004). Therefore the expression levels of these enzymes in EAE pathology and after treatment with glutamate receptors antagonists are also examined.

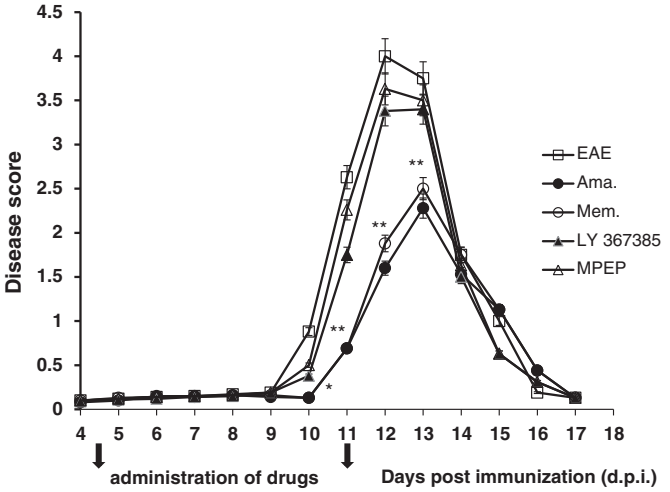

Fig. 1. Scores of the neurological symptoms during the acute phase of EAE and after treatment with antagonists of glutamate receptors. Antagonist doses were as follows: amantadine 100 mg/kg b.w./day, memantine 60 mg/kg b.w./day, LY 367385 10 mg/kg b.w./day and MPEP 10 mg/kg b.w./day. These doses were administered from 5 to 11 d.p.i. The values indicate neurological score ± SD. Results are combined data from five to eight animals in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with untreated EAE rats.

2. Materials and methods

2.1. Animal model

Female Lewis rats weighing about 190–200 g were used throughout the study. All procedures were carried out in accordance with ethical guidelines for care and use of laboratory animals and were approved by the Local Care of Experimental Animal Committee. To induce EAE, we immunized rats subcutaneously in both hind feet with an inoculum containing guinea pig spinal cord homogenate emulsified in Freund's complete adjuvant (CFA) containing 5.5 mg/mL *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI, USA). Control group received inoculum containing CFA without spinal cord homogenate.

The rats were housed in environmentally-controlled conditions and were provided with free access to food and water. During the experiment, the animals were fed a standard laboratory diet. Body weights and neurological deficits were determined daily according to the following scale: 0 = no signs of neurological deficits, 1 = flaccid tail, 2 = impairment of fighting reflex and/or loss of muscle tone

Table 1
Characterization of the EAE animal model and clinical parameters in EAE rats and in rats after treatment with antagonists of glutamatergic receptors.

	EAE	Amantadine	Memantine	LY 367385	MPEP
Number of animals with clinical symptoms (%)	95.95	100	100	97.9	100
Number of animals with severe EAE (%)	73.9	62.7*	63.6*	73.6	70.0
Lethality (%)	4.05	0	0	2.1	0
Inductive phase (days)	10.6 ± 2.4	12.1 ± 1.3*	12.2 ± 2.1*	10.8 ± 1.4	10.5 ± 1.5
Body weight at 12 d.p.i. (g)	140.5 ± .5	165.5 ± 1.3*	160.2 ± 3.5*	141.2 ± 4.0	135.5 ± 5.0
Maximal CI (score)	4.5 ± 0.3	2.4 ± 0.4*	2.6 ± 0.6*	4.1 ± 0.6	3.9 ± 0.5
Cumulative CI (score)	28.6 ± 4.7	19.6 ± 1.6*	20.1 ± 2.4*	26.98 ± 0.5	27.6 ± 0.7
Duration of disease (days)	21.4 ± 1.8	17.9 ± 1.5*	16.8 ± 2.3*	20.5 ± 1.8	21.0 ± 1.4
Number of animals	198	63	63	48	48

The values represent the means ± SD.
* $P < 0.05$, significantly different compared with EAE rats.