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Anticonvulsant action of an antagonist of metabotropic glutamate receptors mGluR5 MPEP in immature rats

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Abstract

Antagonists of type I of metabotropic glutamate receptors exhibit anticonvulsant action in adult as well as immature rodents. To know the anticonvulsant profile of a specific mGluR5 antagonist MPEP in developing rats, two models of epileptic seizures were used. MPEP (10, 20 or 40 mg/kg i.p.) suppressed in a dose-dependent manner epileptic afterdischarges induced by electrical stimulation of sensorimotor cortical area in three age groups (12, 18 and 25 days old). The anticonvulsant action was more expressed in the youngest group than in older animals so that in 25-day-old rats an additional dose of 80 mg/kg was used. In contrast to this marked anticonvulsant action, MPEP at a dose of 40 mg/kg i.p. in 18-day-old rat pups and at doses of 40 and 80 mg/kg in 25-day-old rat pups did not affect episodes of spike-and-wave rhythm elicited by low doses of pentetrazol. Our results delineate the profile of the anticonvulsant action of MPEP and confirm the higher efficacy of this antagonist at early developmental stages in comparison with prepubertal animals.

Keywords: Seizures; Ontogeny; Rat; Metabotropic glutamate receptor; MPEP

1. Introduction

L-Glutamate as the main excitatory neurotransmitter in the mammalian brain exhibits its effects by means of ionotropic and metabotropic receptors (Hollmann and Heinemann, 1994). Both classes of receptors have been implicated in epileptic activity (for review see Dingledine et al., 1990; Wong et al., 1999; Doherty and Dingledine, 2002). Ionotropic glutamate receptors have been at the center of attention for many years and their antagonists were examined as possible antiepileptic drugs (Chapman, 1991). In spite of marked anticonvulsant action of ionotropic glutamate receptor antagonists, serious side effects prevented their clinical use (Troupin et al., 1986). An increasing number of studies have therefore focused on possible anticonvulsant action of drugs influencing metabotropic glutamate receptors (Doherty and Dingledine, 2002; Moldrich et al., 2003; Nagaraja et al., 2004). Metabotropic glutamate receptors are G-protein coupled receptors linked to second messenger pathways. They are classified into three distinct groups according to their sequence homology, pharmacology and signal transduction (group I, II and III mGluRs). Group I mGluRs are coupled to the phosphoinositide/Ca^{2+} cascade. Group II and III mGluRs are negatively coupled to adenylate cyclase (Conn and Pin, 1997). Eight mGluR subtypes have been described; group I comprise mGluR 1 and 5, group II mGluR 2 and 3, and group III mGluR 4, 6, 7 and 8. Generally, in vitro

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studies demonstrated that group I mGluR agonists promote seizure-like activity, whereas group II and III agonists are considered antiepileptic (Wong et al., 1999).

The group I mGluRs have the potential to play an important role in initiating epileptic discharges, converting these from interictal to ictal events and maintaining the discharge for several hours even following termination of agonist activation of mGluR (Thuault et al., 2002). The group I mGluRs can modulate the duration and amplitude of epileptic discharges. In spite of predominant postsynaptic localization, there is evidence of a role of presynaptic mGlu5-type receptors in the regulation of neuronal glutamate release in the mammalian central nervous system (Thomas et al., 2001).

2-Methyl-6-(phenylethynyl)-pyridine (MPEP) was described as a potent, selective non-competitive antagonist for the type I metabotropic glutamate receptor (subtype 5, presynaptic mGluR5) active after systemic administration (Gasparini et al., 1999, 2002). It exhibited anticonvulsant activity after systemic administration in adult (Chapman et al., 2000; Barton et al., 2003; Nagaraja et al., 2004) as well as in immature rodents (Mareš and Mikulecká, 2004). This non-amino acid-like antagonist has no appreciable activity at the closely related mGluR1 (Gasparini et al., 1999). The present study was based on our previous data demonstrating efficacy of MPEP against motor seizures induced by pentetrazol without serious side effects in developing rats (Mareš and Mikulecká, 2004). The aim of our study was to further delineate a profile of anticonvulsant action of MPEP in immature rats. Two models of epileptic seizures were used: cortical epileptic afterdischarges (ADs) and a low-dose pentetrazol (PTZ) model of absence seizures.

Electrical stimulation of the sensorimotor cortical region offers different endpoints to be measured. Stimulation elicits clonic movements of the head and forelimbs, synchronous with individual stimuli (i.e. direct activation of the motor system). If stimulation intensity is sufficient, an epileptic afterdischarge will appear after the end of stimulation. These ADs formed by spike-and-wave rhythm are accompanied by clonic seizures similar to movements directly elicited by cortical stimulation. Clonic jerks are synchronous with individual sharp elements in EEG, i.e. they indicate a spread of epileptic activity in the motor system. Using high stimulation intensities, a transition of spike-and-wave ADs into another type of seizure could appear (Mareš et al., 2002). This second type of AD was not studied in the present experimental series. Cortical epileptic ADs represent a valuable model for testing the action of anticonvulsant drugs because of more than one endpoint (Voskuyl et al., 1992; Kubová et al., 1996; Krupp and Löscher, 1998). Cortical ADs can be regularly elicited in rat pups from early developmental stages (Mareš et al., 2002).

Low doses of PTZ produce seizures considered to model absence seizures with EEG characterized by episodes of spike-and-wave rhythm and behavioral arrest with minor motor correlates, usually rhythmic twitches of vibrissae (Snead, 1992). Under our conditions, this activity started approximately 5 min after the injection, and was stable for at least the next 20 min. It was suppressed by valproate, ethosuximide and benzodiazepines (Brabcová et al., 1993; Mareš, 1998).

Both types of epileptic seizures used in the present study are characterized by EEG spike-and-wave rhythm generated in the cerebral cortex or more probably in the thalamocortical system. Receptors of mGluR5 type are localized in neuropil in the rat cerebral cortex either postsynaptically (Shigemoto et al., 1993) in dendrites of pyramidal as well as nonpyramidal cells (López-Bendito et al., 2002) or both post- and presynaptically (Romano et al., 1996). This type of metabotropic glutamate receptor was demonstrated also in thalamus (Shigemoto et al., 1993; Romano et al., 1996). The different behavioral pattern might be due to different spread of epileptic activity. Therefore these two models could bring additional information about the spectrum of anticonvulsant action of MPEP.

2. Methods

The anticonvulsant action was studied in Wistar male rats (Institute of Physiology, Academy of Sciences, Czech Republic) 12 (P12), 18 (P18), and 25 (P25) days old. The day of birth was defined as P0, the litters were culled to 8–10 pups. The animals were bred in an environment with regulated light and temperature (12:12 h light/dark cycle, lights on at 6:00 a.m., 22±1 °C) and they received a standard diet ad libitum. The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic to be in line with the Animal Protection Law of the Czech Republic (fully compatible with European Community Council directives 86/609/EEC).

2.1. Experiment 1: cortical epileptic afterdischarges (ADs)

All three age groups were examined in this series. Surgical preparation was performed under ether anesthesia. Silver cortical electrodes were implanted epidurally. The coordinates were recalculated from the adult brain (values in parentheses) on the basis of bregma–lambda distance (8 mm was taken as an adult value). Two stimulation electrodes were put over the right sensorimotor area (AP = −1 and +1 mm, L = 2 mm); recording electrodes over left sensorimotor area (AP = 0 mm, L = 2 mm), left parietal area (AP = 3 mm, L = 3 mm),