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Age-dependent anticonvulsant action of antagonists of group I glutamate metabotropic receptors in rats

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Summary Metabotropic glutamate receptors (mGluR) may represent a perspective target for anticonvulsant therapy but spectrum of their anticonvulsant effects is not sufficiently known. Our study was aimed at comparison of anticonvulsant actions of antagonists of mGluR1 and mGluR5 subtypes in immature rats. Seven-, 12-, 18- and 25-day-old animals were pretreated with mGluR1 antagonist AIDA (1—20 mg/kg i.p.) or mGluR5 antagonist MTEP (5—40 mg/kg i.p.) 30 min before pentetrazol administration (100 mg/kg s.c.). Two types of motor seizures were elicited: minimal, clonic seizures (mS) and generalized tonic—clonic seizures (GTCS). mS could be induced only in 18- and 25-day-old rats, and their incidence was decreased to 0—50% by nearly all doses of either drug in 18- but not in 25-day-old rats. GTCS were observed in all age groups; higher doses of both antagonists specifically suppressed the tonic phase in 7-, 12- and 18-day-old rats. The highest efficacy was found in 12-day-old rats; seizure severity was significantly decreased even by the 10-mg/kg dose of MTEP and the 2-mg/kg dose of AIDA in this age group. In addition, MTEP tended to suppress also the clonic phase in 7-day-old rats. Time course of action studied in 12-day-old animals demonstrated much longer action of MTEP (more than 4 h) than of AIDA (less than 1 h). Administration of AIDA but not MTEP resulted in a paradoxical shortening of latencies of seizures even at time intervals when the incidence of the tonic phase of GTCS was decreased. Both mGluR antagonists exhibit specific anticonvulsant action in rat pups during the first 3 postnatal weeks.

Introduction

Antagonists of ionotropic glutamate receptors of AMPA and especially NMDA type exhibit not only marked anticonvulsant action (for review Chapman, 1991) but also strong unwanted side effects (Vanderschuren et al., 1998). Previous studies demonstrated that both effects are present also in immature animals—anticonvulsant activity (Velíšek et al., 1991; Kubová et al., 1997; Mareš et al., 1997, 2004), locomotor hyperactivity and ataxia (Mikulecká and Mareš, 2002) and neuronal death in developing brain (Kaindl and Ikonomidou, 2007). Because of these side effects attention has now been shifted to the second group of glutamate

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receptors—metabotropic ones (for review Alexander and Godwin, 2006).

Metabotropic glutamate receptors are homodimers and they are classified into group 3 of G-protein coupled receptors. They are divided according to sequentional homology, transducers and pharmacology into three groups with eight subtypes (Conn and Pin, 1997). We started with studying drugs influencing group I of these receptors. Group I is formed by mGluR1 and mGluR5 subtypes, they are bound with G_q protein and dissociation of G-protein activates hydrolysis of phosphoinositol (Gasparini et al., 2002; Moldrich et al., 2003). Agonists of this group exhibit proconvulsant or convulsant activity, antagonists may represent potential anticonvulsant drugs (Conn and Pin, 1997; Ritzén et al., 2005; Alexander and Godwin, 2006). Data for adult animals demonstrated anticonvulsant efficacy of antagonists of either subtype in some in vivo and in vitro models (Chapman et al., 1999, 2000; Thuault et al., 2002; Barton et al., 2003; Moldrich et al., 2003; Sayin and Rutecki, 2003; Stoop et al., 2003; Nagaraja et al., 2004; Shannon et al., 2005) but negative data were also published (Nagaraja et al., 2004; Löscher et al., 2006). In agreement with these negative data, a role of mGluR5 type of metabotropic receptors in generation of epileptic seizures could not be demonstrated in mGluR5 knockout mice and MTEP did not suppress seizures induced by convulsant drugs with different mechanisms of action in wild type mice (Witkin et al., 2008). In addition to the published data, antagonists of both subtypes of group I metabotropic glutamate receptors can be administered systemically and they are commercially available.

Recently we demonstrated anticonvulsant action of systemically administered mGluR5 antagonist MPEP in two different tests—motor seizures elicited by pentetrazol (Mareš and Mikulecká, 2004) and cortical epileptic afterdischarges induced by rhythmic electrical stimulation of the sensorimotor cortical area (Lojková and Mareš, 2005). In spite of the fact that high doses must be used to have an anticonvulsant effect in both models (Mareš and Mikulecká, 2004; Lojková and Mareš, 2005) no serious side effects on motor performance and spontaneous behavior were found (Mareš and Mikulecká, 2004). MPEP is specific for mGluR5 (Gasparini et al., 1999) if low doses are used but with high doses is there a possibility of a nonspecific action on NMDA receptors (Movsesyan et al., 2001) as well as on noradrenergic transporter (Heidbreder et al., 2003; Ritzén et al., 2005). Availability of a more specific mGluR5 antagonist MTEP (Lea and Faden, 2006) offered a possibility to give a definitive answer to these doubts. Because of involvement of both subtypes of group I metabotropic glutamate receptors in modulation of epileptiform activity demonstrated in vitro (Stoop et al., 2003) as well as due to some differences between the effects of mGluR5 and mGluR1 antagonists described in adult brains (at least as their neuroprotective action is concerned—Pellegrini-Giampietro, 2003), a competitive antagonist of mGluR1 AIDA was included into present study.

Motor seizures elicited by a subcutaneous injection of pentetrazol (PTZ) were chosen as a test. If an appropriate dose of PTZ is administered, control rats in all age groups exhibit generalized tonic—clonic seizures (GTCS) with a loss of righting reflexes and animals 18 and more days old exhibit at first minimal, predominantly clonic seizures with preserved righting ability and only after a longer latency GTCS (Velíšek et al., 1991; Mareš and Mikulecká, 2004). Minimal clonic seizures (mS) represent a model of human myoclonic seizures (Löscher and Schmidt, 1988; Mareš and Zouhar, 1988), GTCS are generally accepted as a model of human generalized seizures of the grand mal type (Löscher and Schmidt, 1988).

Methods

The experiments were approved by Animal Care and Use Committee of the Institute of Physiology to be in agreement with Czech Animal Protection Law and European Commission Council directives 86/609/EEC).

Animals

Experiments were performed in 7-, 12-, 18- and 25-day-old male Wistar albino rats (the day of birth was counted as 0). Rat pups were taken from their mothers just before the pretreatment. If the interval between the two injections was longer than 15 min the pups were returned to their mothers. Each animal was used only once, after the experiments the rats were killed with an ether anesthesia overdose.

Experimental procedure

Animals were pretreated either with MTEP (3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine, Ascent Scientific, UK) in doses from 5 to 40 mg/kg i.p. (similar to doses used by Löscher et al., 2006) or with AIDA ((*R*, *S*)-1-aminoindan-1,5-dicarboxylic acid, Tocris, UK) in doses from 1 to 20 mg/kg i.p. (Schachtman et al., 2003 and Kłodzińska et al., 2004 used 4 and 8 mg/kg, respectively, as the highest dose, we added two higher doses because of lack of effect of the 4-mg/kg dose in some age groups) and 15 min later pentetrazol (pentamethylenetetrazol, PTZ, Sigma, St. Louis, MO) was injected subcutaneously in a dose of 100 mg/kg. The 15-min interval was chosen according to our data on the effects of the two drugs in another model—repeatedly elicited cortical epileptic afterdischarges (Lojková et al., submitted). Animals were then observed in isolation for 30 min and incidence and pattern as well as latency of seizures were recorded. Seizure severity was quantified by means of a 5-point scale (1, isolated myoclonic jerks; 2, incomplete minimal seizures; 3, minimal clonic seizures; 4, generalized seizures without a tonic phase; 5, generalized tonic—clonic seizures—Pohl and Mareš, 1987). In addition, all other behavioral phenomena were registered. In the second series of experiments, time profile of anticonvulsant action of both drugs was studied in 12-day-old rats. The doses of MTEP (40 mg/kg i.p.) and AIDA (10 mg/kg i.p.) were chosen because of their efficacy at the 15-min interval. Different intervals between the administration of a single dose of MTEP and/or AIDA and PTZ (again 100 mg/kg s.c.) were used according to the current results (from 15 min to 8 h for MTEP and from 15 min to 3 h for AIDA). All age and dose groups consisted of 7—10 rats, age-matched control groups injected only with PTZ were substantially larger (more than 20 rats) because control animals from four different experiments were pooled.

Statistics

Incidence of the two seizure types was evaluated by means of Fisher exact test, severity and latencies of seizures in individual age groups by means of ANOVA on Ranks with post hoc pairwise comparisons by means of Tukey test (SigmaStat® SPSS). The level of statistical significance was set at 5%.