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Research report

Different action of a specific NR2B/NMDA antagonist Ro 25-6981 on cortical evoked potentials and epileptic afterdischarges in immature rats

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ABSTRACT

Ro 25-6981 maleate is a highly selective and activity-dependent antagonist of NMDA ionotropic glutamate receptors containing NR2B subunit (NR2B/NMDARs). The aim of our study was to investigate the influence of Ro 25-6981 administration in developing rats on physiological (single and paired pulse cortical interhemispheric evoked potentials) and epileptic brain activity (cortical afterdischarges (ADs)).

Electrophysiological experiments were performed in animals with epidurally implanted electrodes at postnatal days (P) P12, P18, and P25. The drug was injected intraperitoneally at a dose of 1 or 3 mg/kg. Control animals were injected with saline (1 ml/kg). Single interhemispheric responses were evoked with 0.5-ms biphasic pulses with intensities increasing from 0.4 to 5 mA, paired-pulse responses were elicited by twofold threshold intensity. The ADs were elicited by series of 15-s of 1-ms pulses at 8-Hz frequency. Firstly, six stimulations with stable suprathreshold intensity repeated at 30-min intervals were used to determine the time course of Ro 25-6981 effects against ADs in P12 animals. Secondly, similar experiment was performed in all age groups of animals but with 20-min intervals as well as a further experiment using stimulations with stepwise intensities increasing at 10-min intervals from 0.2 to 15 mA.

Pretreatment with the 3-mg/kg (but not the lower) dose of Ro 25-9681 decreased significantly the amplitude of single responses evoked with higher stimulation intensities in P12 and P18 animals. Both doses affected responses in P25 animals, only the 1-mg/kg dose was more efficacious than the 3-mg/kg one. Paired pulse responses were not affected by either dose of Ro 25-6981 in any age group.

Ro 25-9681 clearly influenced the duration of ADs only in P12 animals. The 1-mg/kg dose did not change the duration of ADs whereas the 3-mg/kg dose suppressed progressive prolongation of ADs with repeated stimulations. This effect was seen even 110-min after the drug injection.

The modification of ADs, i.e. stimulations with stepwise increasing intensities (10 min intervals) was used to demonstrate possible dependence on activity. The Ro 25-6981 was administered immediately after the 4-mA stimulation (i.e. when rats experienced six ADs on the average). The 3-mg/kg dose resulted in shorter ADs after high stimulation intensities in P12. There were no significant effects in older animals, only a tendency to ADs shortening was observed in P25 rats.

In conclusion, our results indicate that Ro 25-6981 as a selective antagonist of NR2B/NMDARs exhibit age- and activation-dependent anticonvulsant action at early postnatal development. In contrast, the influence of Ro 25-6981 on physiological excitability induced by single pulse stimulation of sensorimotor cortex does not depend on age. This compound may thus represent a useful antiepileptic agent in immature brain since its action against ADs prolongation can be observed even 110 min after the single administration of the drug.

1. Introduction

Seizures occur more often in children than in adults (Sayin et al., 2004; Jiang et al., 2007). Since the early-life seizures may have serious neurological, behavioral and cognitive consequences, it is of great importance to develop age-specific anticonvulsants and know their targets (Isaeva et al., 2006; Jiang et al., 2007).

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The involvement of NMDARs in numerous physiological (neuronal growth, migration, excitability, memory) and pathological processes (excitotoxicity, epilepsy, neurodegeneration – Waxman and Lynch, 2005; Jiang et al., 2007) make them the most intensively studied ionotropic glutamate receptors. NMDARs are heteromeric complexes of four subunits surrounding central ion channel. Seven different NMDA receptor subunits were determined, i.e. NR1, NR2A-D, NR3A and B. Functional NMDA receptors consist usually of two glycine binding NR1 subunits and two NR2A-D glutamate binding subunits that participate in the formation of di- (e.g., NR1/NR2A; NR1/NR2B) as well as triheteromeric (e.g. NR1/NR2B/NR2A) receptors (Köhr, 2006; Paoletti et al., 2013; Szczurowska and Mareš, 2013). In addition to postsynaptic NMDARs, they are also present on presynaptic membranes of both, excitatory and inhibitory axon terminals (auto- or heteroreceptors) as well as on the glial cells. Presynaptic and glial NMDARs can contribute significantly to the effects of activation of cortical NMDARs (Conti et al., 1997).

Soon after birth, developing cortical neurons of rodents exhibit high expression of NMDA receptors containing NR2B subunit that allow greater calcium influx through their ion channel (Cull-Candy et al., 2001). During the first postnatal week in rats, expression of NR2A subunit increases and it begins to take over for the function of NR2B/NMDARs. Nevertheless, overall expression level of NR2B subunit remains relatively stable and does not change dramatically throughout development (Liu et al., 2004a). It was demonstrated that overactivation of NR2B-containing NMDARs is involved in excitotoxicity, seizures and neuronal death (Zhou and Baudry, 2006; Jiang et al., 2007; Vizi et al., 2013). Several reports confirmed that blockade of NR2B/NMDARs by selective antagonists can suppress seizures (Wang and Bausch, 2004; Bandyopadhyay and Hablitz, 2006; Mareš and Mikulecká, 2009; Ghasemi and Schachter, 2011; Choo et al., 2012; Di Maio et al., 2013) and, as an advantage, can display neuroprotective effects (Kiss et al., 2012; Vizi et al., 2013).

Ifenprodil is commonly used as NR2B/NMDARs antagonist (Chazot, 2004; Gogas, 2006; Mareš and Mikulecká, 2009). However, another compound, Ro 25-6981 maleate, has been reported to be more potent in blocking of NR2B/NMDARs than ifenprodil, yet it is structurally related to it (Fischer et al., 1997). Possible anticonvulsant action of Ro 25-6981 was tested in both, in vitro and in vivo models of seizures and epilepsy (Hellier et al., 2009; Burket et al., 2010). In our study, we decided to investigate action of Ro 25-6981 in the in vivo models of physiological and epileptic brain activity in three age groups of developing rats.

Here, we present effect of selective and activity-dependent blocker of NR2B/NMDARs, Ro 25-6981, on excitability of the immature brain in single pulse and paired pulse cortical evoked potentials as well as in electrically elicited cortical epileptic afterdischarges, which are considered as a model of myoclonic seizures (Kubová et al., 1996).

2. Material and methods

2.1. Animals

The experiments were carried out in male albino rats of Wistar strain (breeding of the Institute of Physiology Academy of Sciences, Prague) at postnatal days P12, P18 and P25. The day of birth was counted as zero. Animals were housed under standard conditions (food and water ad libitum, 12:12 h light:dark cycle, temperature $22 \pm 1\,^{\circ}C$).

Each age group was formed by control animals treated with saline and two groups treated with different doses of Ro 25-6981. Every age and dose group consisted of eight animals.

The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic to be in agreement with the Animal Protection Law of the Czech Republic (fully compatible with European Community Council directives 86/609/EEC).

2.2. Drugs

Ro 25-6981 maleate ((αR, βS)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol maleate) was purchased from Tocris Bioscience. Ro 25-6981 was freshly dissolved in saline (1 mg/ml) before beginning of each experiment. The drug was administered intraperitoneally in doses of 1 or 3 mg/kg. Control animals received saline (1 ml/kg). The doses were chosen according to previously published literature (Boyce et al., 1999; Kosowski and Liljequist, 2004; Higgins et al., 2005; Kos et al., 2011).

2.3. Surgery

Animals were anesthetized with ether and flat silver stimulation and recording electrodes were implanted epidurally. Surgery was performed as described previously (Mareš et al., 2013), i.e. two stimulation electrodes over right sensorimotor cortex, three recording electrodes over left hemisphere and the fourth over right occipital area. Both ground and indifferent electrodes were placed over the cerebellum. The animals were allowed to recover for at least 1 h and only then, they were connected to the recording system.

2.4. Electrophysiology

2.4.1. Stimulation and EEG recording

For stimulation of sensorimotor cortex, A-M Systems of isolated pulse stimulator Model 2100 with a constant current output was used. For recording of all electrophysiological signals, TDT Open Project Program (Tucker-Davis Technologies) was used. All obtained signals were amplified (RA16PA preamplifier and Pentusa Base Station, Tucker-Davis Technologies, FL, USA) and digitalized at 2 kHz for evoked potentials and at 1 kHz for epileptic afterdischarges.

2.4.2. Single pulse evoked potentials

Single 1-ms pulses with intensities increasing from 0.4 to 5.0 mA (0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mA) were applied. First cycle of stimulations was a control one, then Ro 25-6981 or saline were injected and 20 min later the second stimulation series started. The software automatically averaged five subsequent responses (at each of 13 different current intensities used) and the amplitude was measured between peaks of N1 (first negative) and P2 (second positive) waves (Fig. 1a). First positive wave could not be used because it was often distorted by stimulation artifact.

2.4.3. Paired pulse evoked potentials

The threshold stimulation intensity was found for each animal and double times this intensity was used to elicit paired responses with interpulse intervals from 50 to 1000 ms. Two cycles of stimulations were again performed: first before administration of the drug, and the second 20 min after Ro 25-6981 or saline administration. Amplitude of the first (A1) and second (A2) response was again measured between peaks of N1 (first negative) and P2 (second positive) waves (Fig. 1b). The A2/A1 ratio was calculated for each interval.