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Research report

Effects of mGluR5 and mGluR1 antagonists on anxiety-like behavior and learning in developing rats

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ABSTRACT

Antagonists of group I metabotropic receptors exhibit anxiolytic action in adult rats. In immature animals we demonstrated anticonvulsant action of MPEP and AIDA, antagonists of group 5 and group 1, respectively. However, there are no developmental data on anxiolytic-like and learning actions of both compounds.

This study investigated whether the anticonvulsant dose range of MPEP and AIDA affects anxiety-like behavior and learning ability in immature rats.

Animals at 12, 18 and 25 postnatal (P) days received MPEP in doses of 10, 20 or 40 mg/kg i.p., AIDA in doses of 10 or 20 mg/kg i.p. In P18 and P25 rats anxiety-like behavior and locomotor activity were tested in the light–dark box and open-field test at 15 (1st session) and 60 (2nd session) minutes after drug administration. Learning ability of P12, P18, and P25 animals was examined in the homing response test 15 min after drug administration.

Both antagonists exhibited anxiolytic-like action in the 1st session, effects in the 2nd session were less marked. In the open-field test both antagonists increased locomotion only in P18 animals. Age-dependent changes were found in the homing response test, the return latency being longer only in P12 animals. While MPEP in doses of 20- and 40-mg/kg in P12 and 40-mg/kg in P18 rats prolonged the homing response, AIDA did not affect the homing behavior.

Both MPEP and AIDA exert anxiolytic-like effect also in immature rats. Except for the youngest animals no changes in learning ability in the homing response test were found.

1. Introduction

Metabotropic glutamate receptors, which are divided into three groups and eight subtypes (group I consists of mGluR1 and mGluR5, group II of mGluR2 and mGluR3 and group III of mGluR4, mGluR6, mGluR7 and mGluR8) [28] participate in various physiological and pathological processes. They can be a target for the therapy in neurologic and psychiatric disorders [7,24]. We focused our attention on the group I antagonists—there are commercially available antagonists of both subtypes of this group: the mGluR5 antagonist MPEP (2-methyl-6-(phenylethynyl)-pyridine) and the mGluR1antagonist AIDA ((R,S)-1-aminoindan-1,5-dicarboxylic acid).

Among the effects of antagonists influencing metabotropic glutamate receptors, anxiolytic and antidepressant actions were demonstrated [44]. MPEP exerted anxiolytic-like effect in several conditioned and non-conditioned tests of anxiety-like behavior such as the elevated-plus maze, social exploration, Geller–Seifter test, fear-potentiated startle or Vogel-conflict [4,36,37,39]. Similarly, mGluR1 antagonists may also be involved in regulating anxiety-like behavior [27] although to date, their effects have not been investigated so often as the action of mGluR5 antagonists. AIDA exhibited anxiolytic-like effects in conflict drinking and elevated-plus maze tests [17].

In adult rodents, systemic administration of MPEP impaired behavioral response in various hippocampal-dependent learning and memory tests such as radial arm maze [5], spontaneous alternation and instrumental learning [15] or conditioned taste aversion test [34]. However, MPEP was found to show no effect in other spatial learning tasks (for review see Ref. [35]).

Clinical research demonstrated that approximately half of epilepsies start in infancy and childhood [13]. Considering that our laboratory is focused on developmental aspects of epilepsies and antiepileptic drugs, we studied drugs acting at metabotropic glutamate receptors as potential anticonvulsants. Till now, anticonvulsant effects of MPEP were repeatedly proven [18,19,23]. In our previous paper we demonstrated that doses of 20 and 40 mg/kg exhibit anticonvulsant action without compromising motor performance in immature rats [23]. Further, AIDA at doses of 10 and 20 mg/kg had also anticonvulsant properties in rats 12 and 18 days old [22].

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Since there are no data available on possible action of MPEP and/or AIDA on various behavioral indicators in immature rats, our attention in this study was focused on effects of both drugs on anxiety-like behavior, learning ability and locomotor activity. To study behavioral effects of MPEP and AIDA, we used the dose range proven to be anticonvulsant [19,22,23], i.e. higher doses than those exhibiting behavioral effects in adult rodents [4,17,27,35,37,44]. Suppression of anxiety-like behavior can be considered as positive side effect whereas compromised learning ability might represent an important unwanted side effect.

First, to study the anxiolytic-like effect, we used a modification of light–dark test [38] based on the tendency of animals to explore a novel environment in contrast to the tendency to avoid a brightly lit arena. Time spent in a light box is considered to be the main behavioral indicator of anxiolytic-like effect. Second, to assess effects of both antagonists in immature rats, the homing response as a simple spatial learning test was used [1]. Cooperation between olfactory and/or visual cues is considered to be an important factor in mastering the homing response [32]. The shortening of homing latency as well as traveled distance observed with repeated exposure can be used as an indicator of improving spatial learning [43]. Third, in order to assess the effect of both drugs on locomotor activity, which can affect the behavioral performance in the above-mentioned tests, the animals were observed in the open-field test.

2. Materials and methods

2.1. Animals

Wistar albino rats (Charles River, Breeding of the Institute of Physiology, Academy of Sciences, Prague) were used. The colony room was maintained under controlled temperature (22 ± 1 °C) and humidity (50–60%) with a 12/12 h light/dark cycle (lights on at 6:00 a.m.). Food and water were provided ad libitum (with the exception of the test period). On day 1 (birth counted as day 0) the pups were randomly fostered and each litter was adjusted to ten males. The animals were weaned at postnatal day (P) 28. In the present study total 460 animals were used. In the light–dark (LD) test, the total 50 and 24 animals at P18 were used for testing MPEP and AIDA effects, respectively; the total 46 and 32 animals at P25 were used for testing either drug. Each age and MPEP dose group consisted of 12–14 animals, analogous AIDA groups consisted of 8–12 rats. For testing the effects of MPEP and AIDA in the homing response (HR) test P12 (n = 53 and n = 34), P18 (n = 39 and n = 36) and P25 (n = 33 and n = 32) animals were used. Each age and dose group consisted of 10–14 animals. The total 120 of P18 and P25 animals were used in the open-field (OF) test; each age and dose group consisted of 10 rats, the same control animals being used for both drugs in the HR and OF tests. To avoid the possible litter effect only one animal from a litter was assigned to a specific treatment group [14]. The experiments were approved by Animal Care and Use Committee of the Institute of Physiology ASCR to be in agreement with Animal Protection Law of the Czech Republic and European Community Council directives 86/609/ECC.

2.2. Drugs

The mGluR5 antagonist MPEP (2-methyl-6-(phenylethynyl)-pyridine) was purchased from Sigma (St. Louis, MO, USA) and mGluR1 antagonist AIDA ((R,S)-1-aminoindan-1,5-dicarboxylic acid) from Tocris (Bristol, UK). Both drugs were freshly dissolved in physiological saline and injected intraperitoneally (i.p.). MPEP was administered in the doses of 10, 20 or 40 mg/kg, AIDA in doses of 10 or 20 mg/kg. High doses were used in these experiments because only such doses are efficient as anticonvulsants in immature rats [19,20,22,23]. Decision to use only two doses of AIDA was based on the outlined U-shape of dose–response curve in the model of cortical epileptic afterdischarges [20].

2.3. Behavioral testing

2.3.1. Light–dark test

The light–dark test apparatus consisted of two boxes; a light one (27 cm × 27 cm × 27 cm) and a black one (27 cm × 18 cm × 27 cm). The boxes were connected by a doorway (7 cm × 7 cm) through which rats could traverse. The light box floor, divided in 9 squares, was illuminated with a 60-W lamp placed at the height of 40 cm above the floor. A single rat was placed into the middle of the black box facing the light box and then the black box was covered with a lid. The boxes were cleaned with water and dried after each animal. The behavioral testing was carried out 15 min (1st session) and 60 min (2nd session) after the drug administration, with the aim to examine the effects at the time of maximal anticonvulsant action and at the time when the anticonvulsant action of MPEP could still be demonstrated [23]. The rat behavior in the light box was registered on a videotape for 5 min. The following parameters were evaluated: time spent in the light box, number of transitions between light and dark boxes and squares crossed in the light box.

2.3.2. Homing response test

The apparatus consisted of two transparent plexiglass boxes connected through a small opening (diameter 4 cm). A "home" box (34 cm × 34 cm × 24 cm) was divided in two equal parts by a transparent wall. Soiled bedding, collected from the cage where a dam and litter lived, was placed to the part of the "home" box located farther from a starting box. Then the tested pups were placed into this part. The pups were allowed to acclimate to their new environment for 20 min before testing. The start box for P18 and P25 rats was identically sized as the home box; that for P12 rats was 20 cm × 20 cm × 24 cm. The testing started 15 min after the drug administration. A single pup was removed from the "home" part and placed in the center of the start box. A correct response was defined as the pup entering the "home" box with all feet within 60 s. If the pup failed to enter the "home" box within 60 s, it was gently pushed toward the opening; maximum latency of 60 s was assigned. Twelve trials with 60 s inter-trial interval were chosen for P12 rats, and ten trials with 180 and 300 s inter-trial intervals for P18 and P25 rats, respectively. (The number of total trials, the inter-trial intervals and the number of trials for the correct homing response were chosen according to a pilot experiment.) After the completion of testing, the pups were placed in their maternal cage. The following behavioral criteria were evaluated: mean latency to homing, ratio of correct responses (the latency <60 s) to the total number of trials (correct/total responses × 100) and occurrence of five consecutive correct homing responses (HR acquisition). The start box was cleaned with water and dried after each trial; both boxes were cleaned and dried between individual litters.

2.3.3. Open-field test

Spontaneous locomotor activity was monitored by a videocamera in the open-field arena (48 cm × 48 cm × 30 cm). The floor of the arena was divided into 16 squares. Each rat was tested for 5 min starting 15 min (1st session) and 60 min (2nd session) after the drug administration. The rat was placed into the left corner of the arena and the total number of squares crossed was registered. The experimental arena was carefully wiped after each animal exposure.

2.4. Statistics

Statistical analysis was performed with a program SigmaStat (SYSTAT, Inc.). The data from LD and OF tests were analyzed using two-way ANOVA, with one between groups' factor (treatment) and one within-subject factor (repeated sessions). Since the data from the HR test did not meet the assumption of equal variance of parametric ANOVA, the Kruskal–Wallis analysis with post hoc Dunn's method was applied. The differences in homing response acquisition between control and dose groups were evaluated by means of a Chi^2-test. Statistical significance $P < 0.05$ was accepted.

3. Results

3.1. Effect of MPEP on anxiety-like behavior

Results are summarized in Fig. 1.

In P18 rats, the analysis of the time spent in the light box revealed a significant main effect of MPEP treatment [$F(3,46) = 3.45$, $P = 0.024$] and session [$F(1,46) = 14.75$, $P = 0.0004$]. In both sessions, the animals treated with MPEP spent more time in the light box than controls; however, only the 20 mg/kg dose increased this time significantly. The doses 10 and 20 mg/kg significantly decreased the time spent in light box in the 2nd session as compared to the 1st session. Similarly, there was a significant main effect of MPEP treatment [$F(3,46) = 3.97$, $P = 0.013$] and session [$F(1,46) = 16.86$, $P = 0.0002$] on the transitions from the dark to the light box. MPEP increased transitions compared to the controls; a subsequent analysis showed that the two lower doses of MPEP significantly increased such transitions in the 1st session whereas the change outlined in the 2nd session did not reach the level of statistical significance. The dose of 10 mg/kg significantly decreased the transition in the 2nd session as compared to the 1st one. Finally, there was a significant main effect of the MPEP treatment [$F(3,46) = 5.89$, $P = 0.002$] as well as session [$F(1,46) = 43.03$, $P < 0.0001$] on the squares crossed. All MPEP doses significantly increased the number of squares crossed in the light box in 1st session; no effect was found in 2nd session. All doses of MPEP decreased the number of squares crossed in the 2nd session as compared to the 1st one.