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Different effects of two *N*-methyl-D-aspartate receptor antagonists on seizures, spontaneous behavior, and motor performance in immature rats

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ABSTRACT

Typical *N*-methyl-D-aspartate (NMDA) receptor antagonists exhibit anticonvulsant action and unwanted effects, even in developing rats. Therefore, we studied the actions of the low-affinity, noncompetitive antagonist memantine and the NR2B-specific antagonist ifenprodil. Seizures (minimal clonic and generalized tonic–clonic) were elicited with pentylenetetrazol (100 mg/kg subcutaneously) in rats 7, 12, 18, and 25 days old pretreated with memantine (2.5–40 mg/kg intraperitoneally) or ifenprodil (10–60 mg/kg intraperitoneally). The effects of both drugs were studied in open field and motor performance tests in 12-, 18-, and 25-day-old rats. Memantine suppressed generalized tonic–clonic seizures in all age groups; minimal seizures were potentiated. Ifenprodil abolished the tonic phase of generalized tonic–clonic seizures in 7-, 12-, and 18-day-old rats only; minimal seizures remained untouched. Memantine induced locomotor hyperactivity and compromised motor performance in all age groups. Ifenprodil exerted these effects only in 12-day-old rats; older animals were less active in open field tests. Memantine exhibits both anti- and pro-convulsant and behavioral effects typical of NMDA antagonists. Ifenprodil exerted the same effects in 12-day-old rats, but its anticonvulsant action in 18-day-old rats was accompanied by a decrease in locomotion.

1. Introduction

Both competitive and noncompetitive antagonists of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors exert potent anticonvulsant activity (for review, see [1]) but also side effects: hyperlocomotion and the most serious effect, derangement of cognitive processes. On the basis of these unwanted effects, models of schizophrenia have been proposed [2–4]. We studied the anticonvulsant activity of NMDA receptor antagonists in various models of epileptic seizures in immature rats and confirmed the marked anticonvulsant activity at early developmental stages (for review, see [5]). It is in agreement with the higher efficacy of NMDA receptor agonists [6] and with the overexpression of NMDA receptors in the second week of postnatal life in rats [7–9]. Unfortunately, there are very strong, unwanted effects on motor performance in immature rats, too [10]. Data on cognitive derangement in immature rodents are lacking. In addition, dizocilpine (MK-801) induces neuronal death in immature brain [11], thus making the clinical use of high-affinity antagonists untenable. Therefore, attention is now focused on antagonists with different modes or sites of action on NMDA receptors [12].

Among the low-affinity NMDA receptor antagonists, memantine (1,3-dimethyl-5-aminoadamantane) was recently approved for treatment of Alzheimer dementia (for review, see [13]). Its preclinical actions and possible clinical applications have been reviewed by Parsons et al. [14]. Its anticonvulsant activity was described more than 20 years ago [15]. To determine whether memantine has clinical potential as an antiepileptic drug, it is necessary to study not only its anticonvulsant activity but also its behavioral effects. To compare memantine with a drug influencing NMDA receptors in another way, we also studied the effects of the subunit-specific antagonist ifenprodil, which binds to the NR2B subunit of NMDA receptors [16]. There are no data on the possible effects of these two drugs on motor performance and spontaneous behavior in immature animals. Therefore, we compared the action of these two drugs in motor tests selected from a battery published by Altman and Sudarshan [17] and on spontaneous locomotor and orienting activity in an open field test. Repeated exposure to an open field extends the spectrum of evaluated phenomena with habituation as simple nonassociative learning.

2. Methods

The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy of

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Sciences to be in agreement with Animal Protection Law of the Czech Republic (fully compatible with European Community Council Directives 86/609/EEC).

2.1. Anticonvulsant effects

Experiments were performed on Wistar albino rats 7, 12, 18, and 25 days old from the breeding of the Institute of Physiology. Only male animals were used in these experiments; they were pretreated intraperitoneally with memantine (Sigma, St. Louis, MO, USA) at doses of 2.5, 5, 10, 20, 30, and 40 mg/kg or ifenprodil (Tocris Cookson Ltd., UK) at doses of 10, 20, 40, and 60 mg/kg freshly dissolved in water so that the injection volume was 1 ml/kg (doses up to 20 mg/kg) or 2 ml/kg (higher doses). The doses were used in individual age groups according to actual results. Control animals received physiological saline 2 ml/kg. Individual age and dose groups consisted of 7–9 animals; control groups comprised larger numbers of rats ($\leqslant$16) because they were collected during three similar experimental series performed at the same time. Naïve animals were used for all experiments. Rats were injected with pentylenetetrazol (PTZ, Sigma, 100 mg/kg subcutaneously, freshly dissolved in distilled water) 20 min after memantine administration and/or 60 min after ifenprodil. Additional shorter intervals between ifenprodil and PTZ injections were used in 25-day-old animals. A 60 mg/kg dose of PTZ was administered to another group of 25-day-old rats in the memantine experiments; only three doses of memantine (20, 30, and 40 mg/kg) were used in this series. Rats were observed in isolation for 30 min after PTZ administration, and latency, pattern, and duration of seizures were recorded. In addition, all other behavioral phenomena and a lethal outcome were registered.

The time course of the anticonvulsant activity of the 20 mg/kg dose of either drug was studied in 12-day-old rats; the original intention—to compare this with the time course in 25-day-old animals—could be realized only with memantine because of the lack of effect of ifenprodil in this age group.

Incidence of seizures was statistically evaluated with Fisher's exact test. Seizure severity quantified on a 5-point scale (1 = isolated jerks, 2 = atypical minimal seizures, 3 = minimal seizures, 4 = generalized clonic seizures, 5 = generalized tonic–clonic seizures [18]), as well as latencies, was statistically evaluated by one-way ANOVA on ranks with subsequent pairwise comparison by means of Dunn's test (SigmaStat Systat). The level of statistical significance was always set at 5%.

2.2. Spontaneous behavior and motor performance

The experiments were performed in 12-, 18-, and 25-day-old Wistar albino rats. Rats in each age group belonged to at least five different litters and were balanced with respect to sex, except that for 25-day-old rats, only males were used. No difference was observed between male and female rat pups; therefore, their results are reported together. The effects of memantine and ifenprodil were evaluated in an open field test (n = 100 and 143, respectively) and in various motor performance tests (n = 94 and 99, respectively). Memantine was administered intraperitoneally in doses of 10, 20, and 40 mg/kg, and ifenprodil in doses of 5, 10, 20, and 40 mg/kg in the open field test and 10, 20, and 40 mg/kg in the motor performance tests. The 40 mg/kg dose was not used in 12-day-old rats in either part of the ifenprodil study. Control animals were again injected with 2 ml/kg saline. For either drug, each litter was divided into a control group and at least two groups treated with different doses. Individual age and dose groups consisted of 8–14 rats. All tests were conducted between 9:00 AM and 3:00 PM in a semi-soundproof room. Both open field and motor performance tests were conducted two times: 30 min and 4 h after injection.

2.3. Open field test

Spontaneous locomotor–exploratory behavior was monitored with a video recorder in the open field arena (48 × 48 × 30 cm). The floor of the arena was divided into 16 squares. Each rat was tested for 5 min in a session. The rat was placed into the left rear corner of the arena: the total number of squares crossed as an index of locomotor activity and the total number of rearings as an index of exploratory activity were recorded. In addition, the occurrence of stereotyped behavioral patterns was monitored. The experimental arena was carefully wiped after each animal exposure.

2.4. Motor tests

Four tests were employed: two tests for all age groups (negative geotaxis, bar holding) and two tests appropriate for age (surface righting test for 12- and 18-day-old rats and rotorod for 25-day-old rats). The animal's ability to pass or fail the task was evaluated within an arbitrary preset period. If the rat did not successfully complete the test within the allotted time, the score assigned was the limit.

2.4.1. Surface righting

The rat was placed in the supine position on a flat surface. Duration of righting to the upright position and coordinated turning was recorded. Each rat underwent three 60-s trials.

2.4.2. Negative geotaxis

The rat was placed on an inclined rough surface (30°) facing downward. The latency to turning the face upward was recorded. Each animal was tested for a maximum of 60 s.

2.4.3. Bar holding

A wooden bar 25 cm long and 1 cm in diameter was suspended 25 cm above a padded surface. The rat was allowed to grip the bar with its forepaws. Time spent on the bar was recorded within 120 s.

2.4.4. Rotorod

The apparatus consisted of a plywood horizontal rod (10 cm in diameter, 11 cm long) covered with sticking plaster. The rod was 30 cm above a cushioned floor. The rate of rotation was 5 revolutions/min. Rats were gently placed on the rotating rod with their heads against the direction of rotation. The duration of their stay on the rod was measured for 180 s at maximum. The test was performed twice in a close succession. The mean of the two times was taken as the score.

2.4.5. Statistics

Data were evaluated using two-way analysis of variance (ANOVA) with one between-group factor (treatment) and one within-subject factor (repeated sessions). Student–Newman–Keuls adjustment was used for post hoc analysis. Because the data from the motor performance tests did not always meet the criteria for parametric statistics, nonparametric Kruskal–Wallis analysis was performed; post hoc comparisons between control and drug-treated groups were carried out using Dunn's test. Because of the limited data on rearing in the open field, the proportion of animals exhibiting this pattern was analyzed by χ^2 test. Comparison of locomotor activity in the first and the second exposures to the open field was performed by means of a paired t test. The level of statistical significance was set at 5%.