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Anticonvulsant and behavioral effects of $GABA_B$ receptor positive modulator CGP7930 in immature rats

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ABSTRACT

Possible anticonvulsant action of $GABA_B$ receptor positive allosteric modulator CGP7930 was studied in cortical epileptic afterdischarges (ADs) in rat pups 12, 18, and 25 days old. Afterdischarges were induced by six series of stimulation of sensorimotor cortex, and CGP7930 (20 or 40 mg/kg i.p.) was administered after the first AD. In addition, the effects of CGP7930 on sensorimotor performance and behavior in open field and elevated plus maze were assessed.

CGP7930 decreased duration of ADs in 12-day-old but not in older rats. Motor phenomena (movements accompanying stimulation and clonic seizures) were not changed. CGP7930 only moderately affected sensorimotor performance, altered slightly spontaneous behavior in the open field, and did not influence behavior in the elevated plus maze in terms of an adaptive form of learning or anxiety-like behavior. Marked anticonvulsant action with subtle deficits in sensorimotor performance in 12-day-old rats suggests a possible use of CGP7930 as an age-specific anticonvulsant.

1. Introduction

Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmiter in the central nervous system, binds to at least two types of receptors, ionotropic $GABA_A$ and metabotropic glutamate $GABA_B$. Many antiepileptic drugs have $GABA_A$ receptors as the target [1]. In contrast, there are contradictory results concerning a role of $GABA_B$ receptor system in models of epileptic seizures. It is due to pre- as well as postsynaptic localization of these receptors. Presynaptic receptors may be autoreceptors decreasing release of GABA from presynaptic terminals and also heterosynaptic receptors on glutamatergic endings suppressing release of this excitatory transmitter [2]. Antagonists of $GABA_B$ receptors suppress genetical as well as pharmacological models of absence seizures but potentiate models of convulsive audiogenic seizures and they can induce convulsions in cortical and limbic structures in adult laboratory animals [3,4]. Because, in addition to $GABA_A$ receptors, (generally accepted as inhibitory since the end of the first week of postnatal life) the $GABA_B$ system represents an important inhibitory system at an early stage of brain development [5], we started to study the action of $GABA_B$ receptor agonists and antagonists in developing rats.

Our previous study showed that an antagonist of $GABA_B$ receptors, CGP 35348, increases duration of cortical epileptic afterdischarges (ADs) in immature rats [6]. Low-frequency rhythmic stimulation of sensorimotor cortical region elicits at least three different phenomena: (1) movements directly bound to individual stimuli, (2) epileptic afterdischarges characterized by spike-and-wave rhythm in the EEG (if intensity is high enough), and (3) clonic seizures of head and forelimb muscles [7,8]. Repeated stimulations with constant intensity resulted in progressive prolongation of ADs, especially marked in 12-day-old rats. This probably reflects an immaturity of mechanisms arresting seizures.

The two agonists studied, baclofen and SKF97541, exhibit a mixture of anticonvulsant and proconvulsant effects in the same age groups in this model [9]. The finding that the action of these two agonists is not identical – SKF97541 possesses more anticonvulsant and less proconvulsant activities than baclofen – led us to study the effect of $GABA_B$ receptor positive allosteric modulator CGP7930 [10]. The highest doses of CGP7930 (20 and 40 mg/kg) used in a study of PTZ-induced seizures exhibited anticonvulsant action against generalized seizures in rats 7, 12, 18, and 25 days old and in adults [11]. Positive allosteric modulators potentiate only active $GABA_B$ receptors, and therefore, their actions should be more specific and with milder unwanted effects [12,13] as was demonstrated in mice [14]. The allosteric modulator CGP7930 also has beneficial effects [13]. It is active against drug abuse — a decrease in craving was described in experimental animals (cocaine [15,16], nicotine [17]), and its moderate anxiolytic action as well as antidepressant profile were also found [18,14].

Moreover, we examined an effect of this drug on sensorimotor performance using a series of age-appropriate tests, spontaneous locomotor, and exploratory behavior in the open field (OF) and behavior in the elevated plus maze (EPM) similarly as in a previous study [19].

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2. Methods

2.1. Animals

Male rats of Wistar strain 12, 18, and 25 days old were used. To study the effects of CGP7930 on epileptic afterdischarges, each age and dose group consisted of 7–8 animals. The animals for behavioral studies were brought to the experimental room 1 h before testing. To assess the behavior in the OF, one control and three groups treated with different doses of CGP7930 were formed in each age group. The same animals were tested for motor performance. Different groups of 18- and 25-day-old animals were used to assess behavior in the EPM and sensorimotor performance on the rotarod. To prevent litter effects on statistical analysis, the animals were selected from different litters. Each age and dose group in the behavioral part of our study consisted of 10 animals.

The project was approved by the Animal Care and Use Committee of the Institute of Physiology to be in agreement with the Animal Protection Law of the Czech Republic and with European Community Council directive 86/609/EEC.

2.2. Drugs

CGP7930 (3,5-bis(1,1-dimethylethyl)-4-hydroxy-b,b-dimethylbenzenepropanol, Tocris Bioscience, UK) was dissolved in dimethylsulfoxide (DMSO) in a concentration of 5 mg/1 ml. Doses of 5-, 10-, 20-, and 40-mg/kg dose were used to test anticonvulsant effects. The same doses were used for behavioral testing, except for the dose of 40 mg/kg, because in a pilot experiment, we found that this dose suppressed locomotion. Control animals were injected with DMSO (8 ml/kg in electrophysiological experiments and 4 ml/kg in behavioral experiments, i.e., the amount corresponding to the highest dose of CGP7930, 40 and 20 mg/kg, respectively).

2.3. Elicitation of cortical epileptic afterdischarges

Cortical flat epidural stimulation and recording electrodes were implanted under ether anesthesia and fixed to the skull by means of fast curing dental acrylic. Surgery lasted less than 15 min, and then the animals were left to recover for 1 h. Electrical stimulation (15-s series of 1-ms biphasic pulses with 8-Hz frequency) started at an intensity of 3 mA. This intensity was sufficient to elicit ADs in two older groups, and higher intensities (average value was 4.2 ± 0.44 mA) had to be used in 12-day-old rats. Our previous experiments demonstrated threshold intensities for spike-and-wave type of ADs — 1.54 ± 0.18 mA in 12-, 0.93 ± 0.08 mA in 18-, and 0.98 ± 0.06 mA in 25-day-old rats, respectively [9]. Suprathreshold stimulation (approximately three times threshold) was repeated six times with 20-min intervals, and CGP7930 (5, 10, 20, or 40 mg/kg i.p.) or DMSO in a volume of 8 ml/kg was injected intraperitoneally 10 min after the end of the first AD. Electroencephalogram (amplified and digitalized at a rate of 500 Hz, Kaminskij Biomedical Systems, Prague) was recorded for 20 s before stimulation and at least 1 min after the end of AD. Motor phenomena were marked directly into EEG recording. Incidence, pattern, and duration of ADs and motor phenomena accompanying stimulation and ADs were evaluated. For quantification of motor phenomena, a modified Racine's scale was used [8,20]. During the experiment, the body temperature of the two younger age groups was maintained by means of a pad electrically heated to 34 °C (i.e., temperature in the nest).

2.4. Behavioral experiments

2.4.1. Open-field (OF) test

The animals were placed individually in the center of an arena ($48 \times 48 \times 30$ cm). The test was performed three times for 5 min at 20 min (session 1), 60 min (session 2), and 24 h (session 3) after drug/vehicle administration. The following behavioral variables were evaluated: locomotor activity expressed as the distance moved, exploratory behavior as the number of rearings (both with and without support together), and duration of self-grooming. Each animal was tested for motor performance after exposure to OF.

2.4.2. Sensorimotor tests

Four tests appropriate for the individual age groups were employed considering the time of appearance and maturation of some sensorimotor reflexes: negative geotaxis for 12- and 18-day-old rats, wire mesh ascending and rotarod for 18- and 25-day-old rats, and bar holding for all age groups. All the tests were performed three times, at 30, 60 min, and 24 h after CGP7930 or DMSO administration.

2.4.2.1. Negative geotaxis. The rats were individually placed on an inclined surface (30°), with the head facing downwards. The ability of pups to turn to 180° was measured. The maximal duration of this test was 60 s.

2.4.2.2. Wire mesh ascending. The rats were put at the lower end of the wire mesh (45×15 cm inclined at a 70° angle) placed at an edge of the desk. The time to reach a platform connected to the upper end of the mesh was measured with a limit of 120 s.

2.4.2.3. Bar holding. An animal was held by the nape, and its forepaws were allowed to touch a wooden bar (25 cm long, 1 cm in diameter suspended 25 cm above a soft surface). The time of fore- and hindlimb grasping was measured with a limit of 120 s.

2.4.2.4. Rotarod test. The animals were individually placed on a drum (diameter of 60 mm, width of each drum 85 mm) with a rough surface rotating at a speed of 5 rpm. Their heads were directed against the rotation, and the time to stay on the drum was measured with a limit of 120 s. The test was repeated three times in a close succession, and the average of the three values was taken as a result.

2.4.3. Elevated plus maze (EPM) test

Two open and two closed arms (30×10 cm, walls of the closed arms 30 cm high) connected by a central space (10×10 cm) were 50 cm above the floor. An animal was placed at the end of one open arm with the head directed to the periphery, and the transfer latency (the time it takes for the animal to move from the open arm to either one of the enclosed arms) was measured. A number of studies validated the utility of the EPM for the assessment of an adaptive form of spatial memory. Because during the initial exposure to the EPM an animal acquires phobic avoidance of the open arms and retains strong memory for this threat for a certain time, the transfer latency was significantly shortened when the repeated exposure to the EPM took place. After measurement of transfer latency, the rat was allowed to move freely in the maze for 5 min for the assessment of anxiety-like behavior. The percent of the time spent on open arms [(open arm time / total time) $\times$ 100] as an index of anxiety-like behavior and the number of entries into closed arms as an index of locomotion were calculated. The test was performed three times at 20 min (session 1), 60 min (session 2), and 24 h (session 3) after the drug administration. After completion of each session in the EMP, the animals were submitted to the rotarod test.

The behavior in the OF and the EPM was recorded by a video camera and evaluated off-line using the programs EthoVision and Observer (Noldus Information Technology). After each animal exposure, the OF and the EPM were wiped clean.

2.4.4. Statistics

Duration of ADs and intensity of motor phenomena in each age and dose group were evaluated with repeated measure ANOVA, and a