Effects of CGP7930 on Spontaneous Behavior, Anxiety and Learning in Immature Rats

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INTRODUCTION

Gamma-aminobutyric acid (GABA), the major inhibitory mediator in the central nervous system, binds to two types of receptors - ionotropic GABA-A and metabotropic GABA-B.

There are many data on GABA-A receptors [1] but behavioral effects of drugs influencing GABA-B receptors not so numerous. GABA-B system represents the main inhibitory system at early stage of brain development. Our studies on anticonvulsant action of these drugs in immature rats had to be extended to their behavioral effects. GABA-B receptor agonist baclofen is for a long time used in the treatment of spasticity[2]), therefore also effects on motor performance should be studied. We found marked anticonvulsant action of positive allosteric modulator of GABA-B receptors - CGP7930, therefore we started to study its other effects. CGP7930 - 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol, positive allosteric modulator of GABA B receptors increases the potency and efficacy of GABA at both native and recombinant GABA B receptors. This drug exhibits antidepressant, anxiolytic, and analgesic effect [3], and probably will find its use also in the treatment of drug addiction. Anticonvulsant drug with these positive side effects may find its place at least as the supportive therapy of epileptic patients.

Aim

This work was focused on the anxiolytic-like effect and on the possible negative effects on motor function and on spontaneous behavior in developing rats.

MATERIALS AND METHODS

Male Wistar rat pups were used - 12, 18 and 25 days old. The animals were maintained under 12/12h light/dark regime in a temperature (22±1°C) and humidity controlled rooms. All experiments were approved by the Animal Care and Use Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic. Three doses of CGP7930 were tested - 5, 10, and 20 mg/kg. Control siblings were treated with dimethylsulfoxide in a volume of 4 ml/kg (corresponding to the highest dose of CGP7930). Spontaneous behavior and possible anxiolytic-like effect were tested in the open field, motor abilities were examined using a battery of age-specific tests [4,5] - negative geotaxis, bar holding, wire mesh ascending test and rotarod test. Anxiolytic effect and influence on learning were tested in the elevated plus maze test. All these tests were repeated three times - 15, 60 min and 24 h after the injection (1st, 2nd and 3rd session).

1.) Motor Skills Tests

a) *Negative geotaxis:* rats were head down placed on an inclined rough surface (30°) and time to turn body to have head up was measured.
b) *Wire mesh ascending:* Rats were placed at the lowest part of wire mesh (45cm high and 15cm wide, inclined at a 70 degrees angle). Time to ascend to upper platform was measured.
c) *Bar holding:* Animals were put on the wooden bar (25cm long, 1cm in diameter, suspended 25cm above a padded soft surface) and they held with their forepaws. Time spent on the bar was measured up to a maximum of 120 s.
d) Rotarod test: The apparatus consisted of a plywood horizontal rod (10 cm in diameter, 11cm long) covered with sticking plaster in order to increase its roughness. The rod was placed 30 cm above a platform. It was programmed to rotate at 5 rpms. Rats were placed on the rotating rod with their heads against the direction of rotation. The duration of their stay on the rod was measured for 120s at maximum.

2.) Open Field

Spontaneous behavior and locomotor activity were monitored in the open-field arena (48x48x30cm). Each rat was tested for five minutes 15, 60 min and 24h after intraperitoneal injection. The arena was carefully wiped after each exposure. Behavior was recorded on a camera and distance passed, frequency and duration of various types of spontaneous behavior were calculated with

computer programs EthoVision XT and Observer XT (Noldus Information Technology).

3.) Elevated Plus Maze

The maze includes two open arms (30x10cm) and two closed arms (30x10x30cm) connected by central space localized 50 cm above the floor. The rat was placed at the end of an open arm – head direction from the center of the maze. Test was again repeated three times – 15, 60 min and 24 h after the injection. Latency to the entrance of the closed arm and time spent in open arms were measured. Each animal was tested for ten minutes.

Statistics

Two way repeated measures ANOVA with factor dose and session was used for statistical evaluation of results. Subsequent pairwise comparisons were performed with Student-Newman-Keul's test. The level of significance was set at $p<0.05$.

RESULTS

Main results demonstrated that CGP7930 does not significantly affect locomotion measured as a distance moved in the open field test. Experimental animals were calm and did not show high frequency of behavior and exploration. Motor skills were not significantly different in comparison with controls, but there were some differences between the first two sessions and the third session next day in 18day-old-rat. In 25 day-old-rat there is time spent on bar holding test in the group with dose 20 mg/kg CGP7930 significantly decreased in the first session. An anxiolytic-like effect was outlined.

DISCUSSION

Decreased locomotion might be caused by sedative effect of the drug or lower motivation as well as myorelaxation generated after injection. The differences between first two and third session might be due to the age factor - experimental animals are one day older and this could play serious role in the motor tests. Another possible explanation is that the decrease in motor skills is caused by myorelaxation, which is gone the next day.

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