



**Short communication**

## Effects of GABA<sub>B</sub> receptor ligands in rodent tests of anxiety-like behavior

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### Abstract:

GABAergic hypothesis of anxiety was introduced many years ago, however, a limited number of supporting data were accumulated so far and the role of GABA<sub>B</sub> receptors in behavioral processes related to the anxiety disorders has not been resolved.

In the present study, we examined anxiolytic activity of CGP 36742, a potent and selective GABA<sub>B</sub> receptor antagonist, in rodent tests/models. We have demonstrated that CGP 36742 (30 mg/kg) is active in several animal tests detecting anxiolytic activity (the elevated plus-maze, conflict drinking test and four-plate test). Moreover, we examined the effects of another antagonist – CGP 51176 and agonist – CGP 44532 of GABA<sub>B</sub> receptor in the four-plate test in mice. CGP 51176 (5 or 8 mg/kg) was inactive, while CGP 44532 (0.125 mg/kg) exhibited anxiogenic-like effect. These preclinical data further implicate GABA<sub>B</sub> receptor function in anxiety, and support the GABAergic hypothesis of this disorder.

### Key words:

CGP 36742, CGP 51176, CGP 44532, GABA<sub>B</sub> receptor, anxiety

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### Introduction

Anxiety is a normal emotion when it is adequate to the environmental situation. Inadequate or pathological anxiety is a well-recognized and common condition, which causes considerable distress to individuals, families and society in general [6, 24].

Anxiety disorders include generalized anxiety disorder, obsessive-compulsive disorder, panic disorder,

post-traumatic stress disorder and social and specific phobias [6, 7]. Pharmacological treatment of anxiety disorders involves drugs targeting either the  $\gamma$ -aminobutyric acid (GABA) system using benzodiazepines or 5-hydroxytryptamine (5-HT, serotonin) systems using 5-HT<sub>1A</sub> receptor agonists and selective 5-HT reuptake inhibitors (SSRIs). Unfortunately, both groups of drugs have drawbacks: benzodiazepines have many unwanted side-effects (tolerance, risk of dependence, sedation, cognitive impairments, ataxia), while the

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onset of action of SSRIs or 5-HT receptor ligands is slow [7]. Therefore, it is important to develop new anxiolytic agents. Some preliminary data indicate that substances influencing the GABA<sub>B</sub> receptors may represent a novel class of anxiolytics with a safer unwanted side-effect profile than benzodiazepines [7, 22]. But although GABA-mediated neurotransmission has long been known to play a crucial role in anxiety, data on the specific role of GABA<sub>B</sub> receptors are limited and variable [7].

In the present studies, we investigated the behavioral effects of acute treatment with the selective GABA<sub>B</sub> receptor antagonist, CGP 36742, in animal tests of anxiety: the elevated plus-maze test, conflict drinking test (Vogel test) and four-plates test. Moreover, in the four-plate test, we examined the activity of the other GABA<sub>B</sub> receptor antagonist, CGP 51176 and GABA<sub>B</sub> receptor agonist, CGP 44532.

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## Materials and Methods

### Animals and housing

The experiments were performed on male Wistar rats (200–250 g) and male albino Swiss mice (22–26 g). The animals were kept under a natural day-night cycle at room temperature of 19–21°C, with free access to food and water. Each experimental group consisted of 6–10 animals. Experiments were carried out between 9:00 a.m. and 2:00 p.m. by an observer blind to the treatment. All experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences and Collegium Medicum, Jagiellonian University in Kraków.

### Drugs

The following drugs were used: CGP 36742 (3-amino-propyl-n-butyl-phosphinic acid, Novartis, Basel, Switzerland), CGP 51176 (3-amino-2(R)-hydroxypropyl-cyclohexylmethyl-phosphinic acid, Novartis, Basel, Switzerland), CGP 44532 ((3-amino-2(S)-hydroxypropyl)-methyl-phosphinic acid, Novartis, Basel, Switzerland), diazepam (Valium Roche). The drugs were dissolved in saline and injected *ip* in a volume of 2 ml/kg or 10 ml/kg to rats or mice, respectively. CGP 36742,

CGP 51176, CGP 44532 were given 30 min and diazepam (as a reference standard compound) 60 min before behavioral tests. The doses of compounds have been selected based on previous behavioral data [19].

### Elevated plus-maze test

The construction and the testing procedure of the elevated plus maze were based on a method described by Pellow and File [20]. Each rat was placed in the center of the plus-maze, facing one of the closed arms immediately after a 5 min adaptation in a wooden box (60 × 60 × 35 cm). During a 5 min test period, two experimenters, who were sitting in the same room approximately 1 m from the end of the open arms, recorded the number of entries into the closed or the open arms, as well as the time spent in each type of arms. The entry with all four feet into one arm was defined as an arm entry. At the end of each trial the maze was wiped clean.

### Conflict drinking test (Vogel test)

A modification of the method of Vogel et al. was used [26]. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a Plexiglas box (27 × 27 × 50 cm), equipped with a grid floor of stainless steel bars and with a drinking bottle containing tap water. After the initial adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for further 10-min adaptation period during which they had free access to the drinking bottle. Afterwards, they were allowed a 30-min free drinking session in their home cage. After another 24 h water deprivation period, the rats were placed again in the test chamber and were allowed to drink for 30 s. Immediately afterwards, drinking attempts were punished with an electric shock (0.5 mA). The impulses were released every 2 s (timed from the moment when a preceding shock was delivered) between the grid floor and the spout of the drinking bottle. Each shock lasted for 1 s, and if a rat was drinking when an impulse was released, it received a shock. The number of shocks accepted throughout a 5-min experimental session was recorded.

In the shock threshold test, rats were placed individually in the box and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise by increasing manually the current (from 0.1 to 0.5 mA) delivered through the grid floor

until a rat showed an avoidance reaction. Each shock lasted 1 s.

In the free drinking test, an animal was allowed to drink from the water spout. The total amount of water (ml) consumed during 5 min was recorded for each animal.

#### Four-plate test in mice

A single mouse was placed gently onto the plate, and each animal was allowed to explore for 15 s. Afterwards, each time a mouse passed from one plate to another, the experimenter electrified the whole floor for 0.5 s, which evoked a visible flight reaction of the animal. If the animal continued running, it received no new shock for the following 3 s. The number of punished crossings was counted for 60 s [3].

#### Locomotor activity in mice

Locomotor activity was measured using photoresistor actometers (circular cages, 25 cm in diameter, two light sources, and two photoresistors). The animals were placed individually in an actometer for 6 min. Activity was measured at 3-min intervals to characterize dynamics of changes. The number of light beams crossed by an animal was recorded as the measure of horizontal locomotor activity.

#### Data analysis

The data were evaluated by a one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test or Student's *t*-test.  $p < 0.05$  was considered significant.

## Results

#### Elevated plus-maze test in rats

The total number of entries and total time spent (open plus closed arms) observed in control rats during the five-minute test session were 8.5 and 250.7 s, respectively, in the present set of experiments and were taken as 100%. In control rats 24% of the entries were made into the open arms [ANOVA:  $F(2, 15) = 4.827$ ,  $p < 0.05$ ] and 14.8% of the total time was spent in the

**Tab. 1.** The effect of CGP 36742 and diazepam on percent of time spent in open arms and percent of open arms entries in the elevated plus-maze test in rats

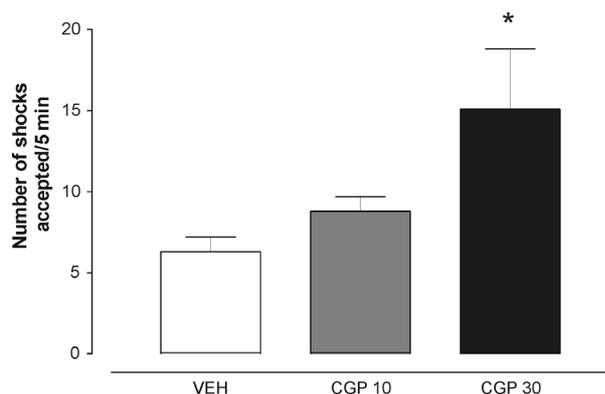
Compound	Dose mg/kg	Percent of time spent in open arms	Percent of open arms entries
Vehicle	–	14.8 ± 0.5	24.0 ± 2.9
CGP 36742	10	38.0 ± 8.7	37.9 ± 4.0
	30	51.5 ± 12.8*	55.9 ± 11.6*
Diazepam	5	70.6 ± 9.7**	62.8 ± 9.0**

CGP 36742 was administrated 30 and diazepam 60 min before the test. All animals received saline injection at appropriate time. Values are expressed as the means ± SEM of 6–7 rats per group. \*  $p < 0.05$ , \*\*  $p < 0.003$  versus vehicle-treated control group

open arms [ANOVA:  $F(2, 15) = 4.311$ ,  $p < 0.05$ ] (Tab. 1). CGP 36742 administrated at dose of 10 mg/kg did not change the number of entries into and time spent in the open arms. At a dose of 30 mg/kg, CGP 36742 significantly increased the percentage of the time spent in the open arms (by up to 52%) and the percentage of entries into the open arms (by up to 56%), but did not change the total number of entries nor the total time spent in the arms (either type). The reference compound, diazepam administrated at a dose of 5 mg/kg significantly increased the percentage of the time spent in the open arms [by up to 71%,  $t(11) = 5.287$ ,  $p < 0.0003$ ] as well as the percentage of entries into the open arms [by up to 63%,  $t(11) = 3.826$ ,  $p < 0.003$ ] (Tab. 1). Diazepam at a dose of 5 mg/kg significantly reduced (by 51%) the total number of entries (data not shown).

#### Conflict drinking test (Vogel test) in rats

CGP 36742 at a dose of 30 mg/kg, but not at a dose of 10 mg/kg, significantly increased by 140% the number of shocks accepted during the experimental session in the Vogel test [Fig. 1, ANOVA:  $F(2, 19) = 4.376$ ,  $p = 0.0273$ ]. The possibility that the efficacy of the effective dose of CGP 36742 was related to reduced perception of the stimulus, or to an increased thirst drive was excluded since CGP 36742 tested at the effective dose in the conflict drinking test changed neither the threshold current ( $0.4 \pm 0.1$  mA) nor water intake ( $12.2 \pm 0.6$  ml) compared to the vehicle treatment ( $0.4 \pm 0.1$  mA,  $11.3 \pm 0.4$  ml) (data not shown).



**Fig. 1.** Effect of CGP 36742 administration in the conflict drinking test in rats. CGP 36742 was administered at 30 min before the test. Each column represents the mean  $\pm$  SEM of 7–8 rats per group. \*  $p < 0.05$  versus vehicle-treated control group

**Tab. 2.** Effect of CGP 36742, CGP 51176, CGP 44532 and diazepam on the number of punished crossing episodes in the four-plate test in mice

Compound	Dose mg/kg	Number of shocks accepted/60 s
<b>A</b>		
Vehicle	–	3.2 $\pm$ 0.2
CGP 36742	10	3.0 $\pm$ 0.4
	30	4.9 $\pm$ 0.6*
Diazepam	2	5.4 $\pm$ 0.3**
<b>B</b>		
Vehicle	–	3.3 $\pm$ 0.3
CGP 51176	5	3.3 $\pm$ 0.3
	8	2.8 $\pm$ 0.3
CGP 44532	0.125	2.0 $\pm$ 0.3*
	0.25	2.7 $\pm$ 0.3

CGP 36742, CGP 51176, CGP 44532 were administered at 30 min and diazepam at 60 min before the test. All animals received saline injection at appropriate time. Values are expressed as the means  $\pm$  SEM of 6–8 mice per group. \*  $p < 0.05$ , \*\*  $p < 0.0001$  vs. vehicle-treated control group

#### Four-plate test in mice

CGP 36742 at a dose of 30 mg/kg significantly increased (by 53%) the number of punished crossings in the four-plate test [Tab. 2A, ANOVA:  $F(2, 27) = 5.839$ ,

$p < 0.01$ ]. Lower dose of the compound (10 mg/kg) did not affect the number of punished crossings in that test. The reference compound, diazepam at a dose of 2 mg/kg increased the number of crossings by 69% [Tab. 2A,  $t(17) = 6.218$ ,  $p < 0.0001$ ]. The second GABA<sub>B</sub> receptor antagonist CGP 51176 at doses of 5 and 8 mg/kg had no effect on the number of punished crossings [ANOVA:  $F(2, 21) = 1.33$ ,  $p < 0.285$ ]. However, CGP 44532, a GABA<sub>B</sub> receptor agonist at a dose of 0.125 mg/kg significantly decreased (by 39%) the number of punished crossings. The higher dose of this compound was inactive in the test [Tab. 2B, ANOVA:  $F(2, 20) = 5.75$ ,  $p < 0.01$ ].

These effects were not due to any confounding effects of the compounds on locomotor activity as acute administration of examined GABA<sub>B</sub> receptor ligands was devoid of any influence on locomotor activity (data not shown).

## Discussion

Clinical and preclinical evidence strongly implicates GABAergic dysfunction in anxiety [16]. However, although GABA<sub>B</sub> receptors were first proposed to play a role in psychiatric disorders such as depression and anxiety over 20 years ago [21], an evidence for specific role for these receptors is unclear.

Baclofen, the first GABA<sub>B</sub> receptor agonist, despite its drawbacks (narrow window of efficacy) has been an invaluable pharmacological tool in elucidating the role of GABA<sub>B</sub> receptors in several disorders, e.g. in depression and anxiety disorders [5, 21]. Its anxiolytic-like effects have been demonstrated in a number of animal models of anxiety and in clinical studies. It reduced separation-induced calling by mouse pups [18], enhanced punished drinking in rats [15, 25] and produced an anxiolytic-like response to novelty in T-maze [23], moreover, baclofen reversed the anxiogenic response induced by withdrawal from chronic diazepam or alcohol treatment [10, 11]. In clinical studies, baclofen reversed the anxiety associated with alcohol withdrawal [1] and posttraumatic stress [9]. Further, the novel positive GABA<sub>B</sub> receptor modulators, GS 39783 and CGP 7930 were active in the light-dark box, elevated zero maze test, elevated plus-maze test in rats and in stress-induced hyperthermia in mice [12, 22]. However, there are some obser-

vations showing baclofen's lack of effects in the elevated plus-maze test in mice [8] and rats [27] and in the Vogel conflict test in rats [2].

Controversial results have also been published on anxiolytic activity of GABA<sub>B</sub> receptor antagonists. One of them, CGP 35348 was reported to be active in the elevated plus-maze test in rats after central administration [27] but was inactive in an ethological version of the elevated plus-maze test in mice described in another publication [8]. The other ligand, SCH 50911 was active in the elevated zero maze [12]. Furthermore, it has been reported that GABA<sub>B1</sub> deficient mice were more anxious in the light-dark box and staircase test and had a panic-like response in the elevated zero maze test [17]. On the other hand, Mombereau et al. [17] in the same investigations showed that chronic pharmacological antagonism of GABA<sub>B</sub> receptors failed to alter anxiety-related behavior in the light-dark box.

In general, foregoing data support the concept that rather agonists and positive modulators of GABA<sub>B</sub> receptors may be regarded as potential anxiolytic drugs.

In the present study, CGP 36742, a new selective antagonist [14], was investigated in behavioral test commonly used to predict a potential anxiolytic-like activity: plus-maze test in rats, Vogel conflict drinking test in rats and four-plate test in mice. We have observed an anxiolytic-like activity of this compound in all the animal models used. The second GABA<sub>B</sub> receptor antagonist CGP 51176 [14] tested in the four-plate test in mice, unlike CGP 36742, has not shown any influence on the animals' behavior. This discrepancy in the activity of both tested antagonists may be caused by differences in their affinity for the GABA<sub>B</sub> receptors (IC<sub>50</sub> for GABA<sub>B</sub> receptors: CGP 36742 = 38 μM, CGP 51176 = 6 μM) [13, 14]. Moreover, CGP 44532, a GABA<sub>B</sub> receptors agonist [13], produced anxiogenic activity in the four-plate test in mice. Generally, the data are in line with the hypothesis that the anxiolytic effects of GABA<sub>B</sub> antagonists may be mediated by autoreceptor blockade-induced release of endogenous GABA, which activates postsynaptic GABA<sub>A</sub> receptors [27].

Summarizing, the results of the present study do not support the view that agonists rather than antagonists of GABA<sub>B</sub> receptors display anti-anxiety activity. One possible explanation for the variable data on the role of GABA<sub>B</sub> receptor ligands in anxiety refers to the localization of GABA<sub>B</sub> receptors on nerve ter-

minals. Postsynaptic GABA<sub>B</sub> receptors exerting inhibitory responses or activation of presynaptic GABA<sub>B</sub> receptors, acting as heteroreceptors or autoreceptors, may cause both excitation or inhibition [4, 16]. To fully elucidate which of these effects is necessary for anxiolytic effect, we need more experiments with novel selective agonists and antagonists of pre- and postsynaptic GABA<sub>B</sub> receptors.

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