

Comparative pharmacological activity of optical isomers of phenibut

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Abstract

Phenibut (3-phenyl-4-aminobutyric acid) is a GABA (γ -aminobutyric acid)-mimetic psychotropic drug which is clinically used in its racemic form. The aim of the present study was to compare the effects of racemic phenibut and its optical isomers in pharmacological tests and GABA_B receptor binding studies. In pharmacological tests of locomotor activity, antidepressant and pain effects, S-phenibut was inactive in doses up to 500 mg/kg. In contrast, R-phenibut turned out to be two times more potent than racemic phenibut in most of the tests. In the forced swimming test, at a dose of 100 mg/kg only R-phenibut significantly decreased immobility time. Both R-phenibut and racemic phenibut showed analgesic activity in the tail-flick test with R-phenibut being slightly more active. An GABA_B receptor-selective antagonist (3-aminopropyl)(diethoxymethyl)phosphinic acid (CGP35348) inhibited the antidepressant and antinociceptive effects of R-phenibut, as well as locomotor depressing activity of R-phenibut in open field test *in vivo*. The radioligand binding experiments using a selective GABA_B receptor antagonist [³H]CGP54626 revealed that affinity constants for racemic phenibut, R-phenibut and reference GABA-mimetic baclofen were 177 ± 2 , 92 ± 3 , 6.0 ± 1 μ M, respectively. We conclude that the pharmacological activity of racemic phenibut relies on R-phenibut and this correlates to the binding affinity of enantiomers of phenibut to the GABA_B receptor. © 2008 Elsevier B.V. All rights reserved.

Keywords: Phenibut; Optical isomer; Pharmacological activity; GABA_B receptors

1. Introduction

Phenibut (3-phenyl-4-aminobutyric acid) is a psychotropic drug that was introduced into clinical practice in Russia several decades ago (Lapin, 2001). Structurally it is a γ -aminobutyric acid (GABA)-mimetic and is thought to afford better penetration through the blood–brain barrier than GABA (Khaunina and Maslova, 1968). Phenibut possesses anxiolytic and nootropic activity, and it is used as a mood elevator and tranquilizer (Lapin, 2001; Sytinsky and Soldatenkov, 1978). In addition, due to its high tranquilizing and cognition enhancing activities it was included in the medical kit for the space flights of Soyuz-19/Salyut-4 (Neumyvakin et al., 1978). Structurally, phenibut is similar to baclofen (3-*para*-chlorophenyl-4-aminobutyric acid), another clinically used GABA receptor agonist that acts on metabotropic GABA_B receptors (Bowers, 2006). Baclofen differs from

phenibut by the presence of a chlorine atom in benzene ring. Both baclofen and phenibut are used clinically in their racemic forms even though they could be separated into R- and S-enantiomers (Fig. 1). The published information concerning pharmacological mechanisms of R- and S-phenibut and relative efficacy of both enantiomers remains obscure. Moreover, the receptor binding data obtained for R-baclofen is used to describe the possible activities of R-phenibut (Lapin, 2001).

It has been shown that biological activity resides in R-enantiomers of phenibut and baclofen, respectively (Allan et al., 1990; Olpe et al., 1978). In a receptor binding assay in rat brain membranes it was shown that R-baclofen has higher affinity for GABA_B receptors as racemic baclofen (Bowers et al., 1985). The receptor binding properties of racemic phenibut have been studied in rat cerebellar membranes using [³H]-R-baclofen as a labeled compound (Allan et al., 1990). R-phenibut was about 100-times more active as S-phenibut in this assay, but, interestingly, S-phenibut also displaced labeled baclofen from binding sites in rat cerebellar membrane preparations (Allan

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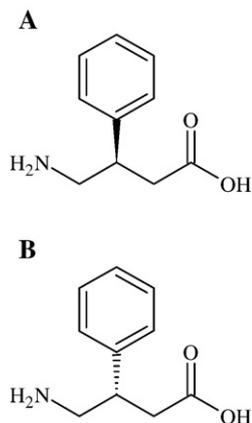


Fig. 1. Structures of R-phenibut (A) and S-phenibut (B).

et al., 1990). In addition, the same study examined the ability of R- and S-phenibut to depress the transmission of electrical signals in slices of the hippocampal region of the rat brain, where it was shown that only R-phenibut afforded a statistically significant activity (Allan et al., 1990).

The biological *in vivo* activities of enantiomers of phenibut have been studied in general pharmacological tests in mice, where it was shown that only the R-isomer was active (Khaunina, 1971). However, in immobilization stress experiments in rats it was observed that in some cases both R- and S-enantiomers possess stress-protective activity (Ahapkina, 2005). Therefore, we hypothesized that R-phenibut and S-phenibut could differ in their binding affinity to GABA receptors and, as a result, both isomers could demonstrate different pharmacological activities. The pharmacological activities of racemic phenibut and its optical isomers in different experimental tests and possible correlations to direct GABA_B receptor binding activity has never been thoroughly studied and compared before. Therefore, we tested the binding affinity of isomers of R- and S-phenibut to GABA_B receptors in rat brain membranes by using a selective GABA_B receptor antagonist [³H]CGP54626. Furthermore, we investigated the activity of racemic phenibut and both its optical enantiomers in open field, forced swimming (Porsolt), and nociception tests in mice *in vivo*. In addition, the influence of a centrally active blocker of GABA_B receptors CGP35348 (Ople et al., 1990) on the pharmacological activity of R-phenibut was also tested.

2. Materials and methods

2.1. Chemicals

Racemic phenibut was obtained from Olainfarm, Latvia. Racemic baclofen was from Polpharma, Poland. The R- and S-enantiomers of phenibut (98–99% ee) were prepared according to published procedure in Latvian Institute of Organic Synthesis (Veinberg et al., 2006). [³H]-CGP54626 ([S-(R*,R*)]-[3-[[1-(3,4-Dichlorophenyl)ethyl]amino]-2-hydroxy-propyl] ([3,4-³H]-cyclohexylmethyl) phosphinic acid; 50 Ci/mmol) was obtained from American Radiolabeled Chemicals, Inc., USA. (3-Amino-

propyl)(diethoxymethyl)phosphinic acid (CGP35348) was obtained from Tocris Bioscience, UK.

2.2. Animals

Male ICR and CBA (Porsolt test) mice and Wistar rats (Laboratory Animal Breeding Facility, Riga Stradins University, Latvia) weighing 23–25 g and 250–300 g, respectively, were housed under standard conditions (21–23 °C, 12 h light–dark cycle) with unlimited access to standard food (Lactamin AB, Sweden) and water. All experimental procedures were carried out in accordance with guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by Ethics Council of Animal Protection at the Veterinary and Food Service, Riga, Latvia.

2.3. Open-field test

The test apparatus was an octagonal arena (36 cm in diameter) with a black floor divided by white lines into eight triangle-shaped sections. The animals were gently placed in the center of the field and behavioral parameters were counted manually by one rater which was unaware of the treatments given. Testing consisted of three successive 4 min sessions that started at 30, 60 and 120 min after intraperitoneal (i.p.) administration of test drugs at doses of 10, 50 and 100 mg/kg. The number of horizontal (passage of horizontal lines with all four paws), vertical (rearing), exploration (hole inspection) activities were recorded. For antagonism studies, the dose of 100 mg/kg (i.p.) CGP35348 was given 10 min prior to 50 and 100 mg/kg R-phenibut or saline administration. The test session was performed 30 min after R-phenibut administration.

2.4. Forced swimming test

The test was performed essentially as described by Porsolt et al. (1977). Mice were individually placed in a vertical glass container (26 cm high, 10 cm in diameter), containing 19 cm of water maintained at 22 °C–25 °C. The total duration of immobility was recorded during the last 4 min of the 6-min test period. The immobility time was recorded using the EthoVision video tracking system (version 3.1., Noldus, Netherland). A mouse was considered immobile whenever it floated passively in the water and only made movements necessary to keep its head above the water line.

The animals received i.p. injection of racemic phenibut and S-phenibut at doses of 100 and 200 mg/kg and R-phenibut at doses of 10, 50 and 100 mg/kg 30 min prior to experiment. For antagonism studies, mice received i.p. injection of CGP34358 at dose of 100 mg/kg 10 min prior to R-phenibut administration (100 mg/kg).

2.5. Antinociception tests

2.5.1. Tail-flick test

The spinal tail-flick response to noxious thermal stimuli was assessed by a tail-flick apparatus (Model DS20, Ugo Basile,

Italy). Briefly, the mouse's tail was placed in a groove, which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Light then passed through the slit and activated the photocell which, in turn, stopped the recording timer. The intensity of the beam was adjusted to produce a mean control reaction time between 5 and 7 s. The cut-off time was fixed at 15 s in order to avoid any damage to the tail. After determination of baseline latencies, mice received drugs, and the reaction latencies were determined at 15, 30 and 60 min after i.p. injection of test compounds at doses of 50, 100 and 200 mg/kg. For GABA_B antagonism studies, mice were pretreated with CGP35348 (at dose 100 mg/kg, i.p.) 10 min prior to R-phenibut administration. R-phenibut was injected at doses of 50 and 100 mg/kg, and the test session was started 15 and 30 min later.

2.5.2. Hot-plate test

Mice were placed into a 19 cm wide glass cylinder on a heated (54 °C) metal plate (Model DS35, Ugo Basile, Italy). The latency to lick one of the hind paws or to jump off the plate was determined. Mice were removed from the hot-plate immediately after the response. The cut-off time was set to 30 s to avoid tissue damage. CGP35348 (at dose of 100 mg/kg, i.p.) or saline were administered 10 min prior to injection of R-phenibut at i.p. doses of 50 and 100 mg/kg or saline. The animals were tested 15 and 30 min after injection of R-phenibut or saline.

2.6. Conflict drinking test

The test was performed according to the modified method of Vogel et al. (1971). Briefly, 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 made of stainless steel bars and a drinking bottle with tap water. After the adaptation period, the animals were deprived of water for 24 h, and were then placed in the test chamber for another 10-min adaptation period during which they had a 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 again placed in the test chamber and were allowed to drink for 30 s. Immediately afterwards drinking was punished with electric shock (0.5 mA, 1 s). The electric impulses between the grid floor and the spout of the drinking bottle were released every 2 s (timed from the moment when a preceding shock was delivered). If rat was drinking when an impulse was released, it received a shock. The number of shocks obtained during a 5 min experimental session was recorded. The rats received drugs i.p. at doses of 25, 50 and 100 mg/kg on the test day 30 min prior to experiment.

2.7. General central nervous system (CNS) tests

The effects of drugs were evaluated at 30, 60, 120 and 180 min after i.p. administration at doses of 50, 100, 250 and 500 mg/kg.

A rota-rod test (Dunham and Miya, 1957) was used to measure the motor coordination (Model 7600, Ugo Basile,

Italy). One day before the experiment, the animals were trained on the apparatus, and the animals that failed to remain on the rotating rod for at least 90 s were excluded from the further testing. On the experiment day, the animals were placed on a rota-rod (16 rpm) and the number of animals falling off the rota-rod within the 180 s session was recorded.

The effect of drugs on motor performance was tested also in the chimney test according to Nowakowska et al. (2007). In this test, mice had to climb backwards up a Pyrex glass tube (30 cm length, 3 cm inner diameter). Mice successfully reaching the 20 cm mark within 30 s were selected for further testing.

The effect of drugs on muscle strength was examined in the traction test. Hence, the forepaws of a mouse were placed on a horizontal firmly fixed stick. The untreated mice grasped the stick with both forepaws and, when allowed to hang free, placed at least one hind foot on the stick within 5 s. Inability to perform that was scored as a failure of traction.

The rectal temperature of animals was measured using a thermometer (Thermalert TH-5, USA).

2.8. Brain membrane preparation and GABA_B receptor binding experiments

The modified procedures of Bischoff et al. (1999) and Asay and Boyd (2006) were used for the membrane preparation. Rats were sacrificed by decapitation and forebrain tissues were rapidly excised and homogenized in 1:25 w/v of an ice-cold buffer containing 320 mM sucrose, 1 mM KH₂PO₄ and 1 mM MgCl₂ (pH 7.5), using a motor-driven Teflon glass homogenizer. The homogenates were spun at 1000×g for 15 min at 4 °C and supernatants collected and then centrifuged at 18,000×g for 15 min at 4 °C. The pellet was re-suspended in 5 ml of ice-cold, distilled, deionized water, left on ice for 30 min and then centrifuged at 39,000×g for 15 min at 4 °C. Finally, the pellet was re-suspended in 25 volumes of Krebs–Henseleit (KH) buffer (120 mM NaCl, 6 mM glucose, 20 mM Tris, 4.7 mM KCl, 1.8 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, pH 7.4) and frozen at –80 °C for at least 42 h before use. On the day of the assay, the membranes were thawed at room temperature and kept on ice. Membranes were then washed 3 times with 25 volumes of ice-cold KH buffer by centrifugation at 18,000×g for 15 min at 4 °C. The pellet was re-suspended in 10 volumes of KH buffer for use in the assays. In GABA_B receptor binding assays, rat brain membranes (0.16 mg of protein per assay) were incubated with the GABA_B receptor-selective radioligand [³H]-CGP54626 (approximately 2 nM) and competing drugs, in 200 μL of KH buffer (pH 7.5) for 1.5 h at room temperature (Asay and Boyd, 2006). Non-specific binding for each assay was determined in the presence of 10 mM baclofen. The termination of binding assays was performed by filtering and washing on Whatman GF/C filters. All assays were performed in duplicate.

2.9. Statistical analysis

All results are expressed as mean±S.E.M. The data were analyzed by means of analysis of variance (ANOVA).

Whenever ANOVA was significant, further multiple comparisons were made using Tukey as the post hoc test. Effective dose 50 (ED₅₀) values were obtained by probit analysis (Finney, 1971). *P*-values less than 0.05 were considered to be significant. Concentration response curves were analyzed using commercially available software (Prism 4.0, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Open-field test

As can be seen in Fig. 2A, B, and C, 30 min after the administration of racemic phenibut and its R-enantiomer, we observed a significant and dose dependent decrease in the horizontal, vertical and exploratory activity of treated mice.

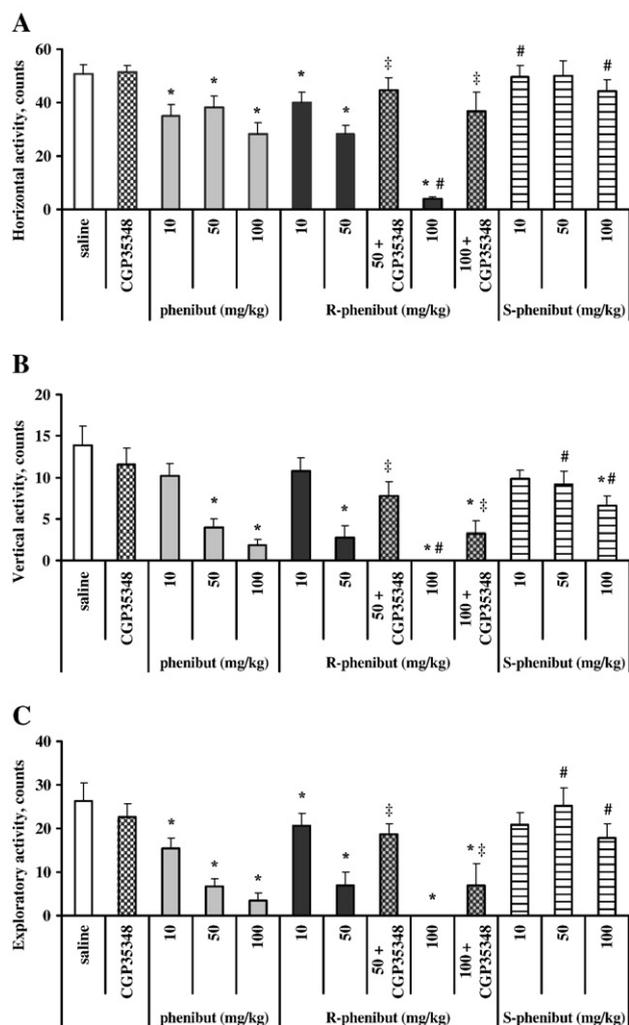


Fig. 2. Effects of racemic phenibut, R-phenibut and S-phenibut on locomotion and exploratory behavior in open field test in mice. (A) horizontal activity; (B) vertical activity; (C) exploratory activity. The activities were observed 30 min after i.p. administration of 10, 50 and 100 mg/kg of compounds. CGP35348 was administered i.p. at a dose of 100 mg/kg 10 min prior to drug treatment. Each column represents the mean \pm S.E.M. of 8 animals. **P* < 0.05 vs. saline treated group, #*P* < 0.05 vs. respective dose of racemic phenibut and ‡*P* < 0.05 vs. respective dose of R-phenibut.

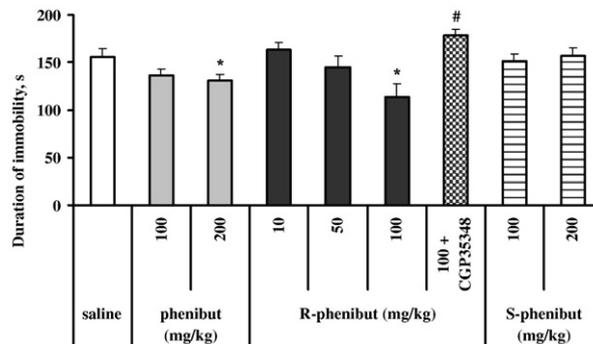


Fig. 3. Effect of racemic phenibut, R-phenibut and S-phenibut on duration of immobility time in forced swimming test in mice treated with compounds 30 min prior to test. Each column represents the mean \pm S.E.M. of 10 animals. **P* < 0.05 vs. saline treated group.

Moreover, at a dose of 100 mg/kg R-phenibut almost completely blocked all activities registered in the open field test (Fig. 2A, B, and C). The administration of racemic phenibut resulted in somewhat lower activity. Interestingly, the administration of 10 and 50 mg/kg of both racemic and R-phenibut induced similar effect, but at the dose of 100 mg/kg the locomotor activity in R-phenibut treated animals was much more depressed (Fig. 2A, B, and C). The only statistically significant effect of S-phenibut was observed at a dose of 100 mg/kg for vertical activity (Fig. 2B). Racemic phenibut and R-phenibut at doses of 50 and 100 mg/kg also decreased the number of vertical and exploratory activities 60 min after their administration (data not shown). Similar effects were observed in the case of racemic phenibut and R-phenibut 120 min after their administration at a dose of 100 mg/kg (data not shown). GABA_B receptor-selective antagonist CGP35348 at dose of 100 mg/kg did not influence horizontal, vertical and exploratory activity of mice (Fig. 2A, B, and C). The pre-treatment by CGP35348 statistically significantly blocked the locomotor inhibitory activity of R-phenibut at both doses of 50 and 100 mg/kg (Fig. 2A, B, and C).

3.2. Forced swimming test

The antidepressant effect of compounds was tested 30 min after i.p. administration of racemic phenibut and S-phenibut at doses of 100 and 200 mg/kg and R-phenibut at doses of 10, 50 and 100 mg/kg. The data presented in Fig. 3 show that the animals treated with R-phenibut at dose of 100 mg/kg showed antidepressant activity similar to that of racemic phenibut at two times higher dose of 200 mg/kg. S-phenibut did not exert any antidepressant effects at this dose and the duration of immobility was the same as in the case of untreated animals. GABA_B selective antagonist CGP35348 completely inhibited the antidepressant activity of R-phenibut at dose of 100 mg/kg (Fig. 3).

3.3. Antinociception tests

The data presented in Fig. 4A show that the administration of racemic phenibut at doses of 50 and 100 mg/kg did not induce

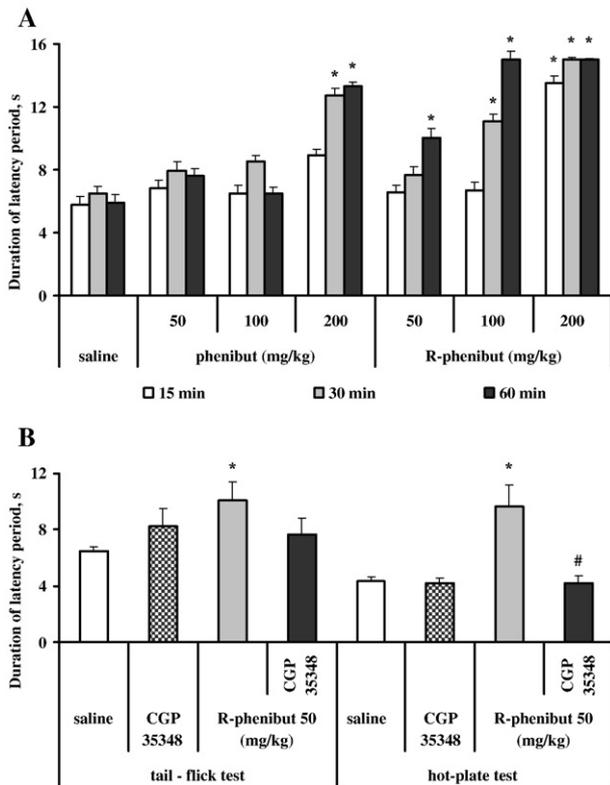


Fig. 4. Effect of racemic phenibut and R-phenibut in the antinociception tests in mice. (A) Tail-flick test. Mice received i.p. racemic phenibut and R-phenibut at dose of 50, 100 and 200 mg/kg. Antinociception was recorded at 15, 30 and 60 min after the i.p. administration of the compounds. (B) Effect of CGP35348 on activity of R-phenibut in tail-flick and hot plate tests. Mice received i.p. CGP35348 at dose of 100 mg/kg or saline 10 min prior to R-phenibut at dose of 50 mg/kg. The nociception was tested 30 min after treatment by R-phenibut. * $P < 0.05$ vs. control group, # $P < 0.05$ vs. the respective dose of R-phenibut.

any analgesic effects. At the highest dose of racemic phenibut (200 mg/kg), the statistically significant effect was observed at all time points checked (Fig. 4). Furthermore, 60 min after the administration of R-phenibut the analgesic effect was registered for all doses tested (50, 100 and 200 mg/kg) (Fig. 4). At a dose of 200 mg/kg, R-phenibut demonstrated analgesic activity even 15 min after its administration, and the duration of the latency period was 2-times longer than that of untreated controls (Fig. 4). S-phenibut did not exert any analgesic activity in dose intervals between 50–200 mg/kg (data not shown). As can be seen in Fig. 4B, CGP35348 at dose of 100 mg/kg inhibited the

Table 1

Activity of racemic phenibut, R-phenibut and S-phenibut in rota-rod, traction, chimney and rectal temperature tests. Compounds were administered i.p. at doses of 50, 100, 250 and 500 mg/kg. The effects were observed 30, 60, 120 and 180 min after drug administration. ED_{50} value was calculated by probit analysis

Tests	Phenibut (mg/kg, ED_{50})	R-phenibut (mg/kg, ED_{50})	S-phenibut (mg/kg, ED_{50})
Rota-rod test	536 ± 139	123 ± 12 ^a	> 500
Traction test	312 ± 62	129 ± 16 ^a	> 500 ^a
Chimney test	338 ± 160	157 ± 69 ^a	> 500 ^a
Rectal temperature	312 ± 99	117 ± 24 ^a	> 500 ^a

^a $P < 0.05$ vs. racemic phenibut group.

antinociceptive action of R-phenibut at dose of 50 mg/kg in both hot-plate and tail-flick tests. In hot-plate test, the pre-treatment with antagonist completely blocked the activity of R-phenibut (Fig. 4B). However, in tail-flick test the inhibitory activity of antagonist was not statistically significant with regard to R-phenibut treatment group (Fig. 4B).

3.4. Conflict drinking test

In conflict drinking test, racemic phenibut and its optical isomers were tested 30 min after i.p. administration at the doses of 25, 50 and 100 mg/kg. During 5 min experimental session, control group rats accepted 5.4 ± 1.2 shocks. The number of shocks accepted by drug-treated rats did not differ significantly from control group values, on average being for racemic phenibut 3.9 ± 1.7 ; for R-phenibut 2.6 ± 1.1 and S-phenibut 4.6 ± 1.2 counts.

3.5. General CNS tests

In the traction and chimney tests, the inhibitory activity on muscle function for racemic phenibut was observed at about 2-fold higher doses than in the case of R-phenibut (Table 1). In the rota-rod test the racemic phenibut unexpectedly turned out to be almost inactive ($ED_{50} = 536 \pm 139$ mg/kg), but the activity of R-phenibut was observed at similar doses ($ED_{50} = 123 \pm 12$ mg/kg) as in the traction test (Table 1). Also, the ED_{50} value for racemic phenibut in test of rectal temperature was similar to that for the appearance of muscle relaxant activity. In contrary, R-phenibut exerted a body temperature lowering effect at 2-fold lower doses (Table 1). Pre-treatment with S-phenibut did not bring about any activity in doses up till a dosage of 500 mg/kg.

3.6. $GABA_B$ receptor binding activity

In order to investigate whether enantiomers of phenibut could bind to $GABA_B$ receptors, we performed a radioligand displacement experiment in rat brain membrane fractions using

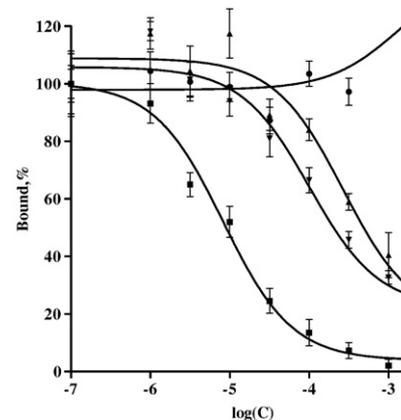


Fig. 5. Competition curves of baclofen (■), racemic phenibut (▲), R-phenibut (▼) and S-phenibut (●) in rat brain membranes obtained by using a $GABA_B$ receptor-selective ligand [3H]CGP54626 ($n = 3$). Concentration response curves were analyzed using GraphPad software.

a selective GABA_B receptor labeling compound [³H]-CGP54626. Baclofen was used as a known GABA_B receptor binding reference compound. Baclofen, racemic phenibut and R-phenibut demonstrated an affinity for GABA_B receptors, with the calculated K_i constants being 6±1, 177±2 and 92±3 μM, respectively (Fig. 5). In contrast, S-phenibut was not able to displace the labeled compound from brain membranes (Fig. 5).

4. Discussion

Phenibut is chemically related to baclofen, a known GABA_B receptor agonist, and should predictably exert similar biological activity. In order to characterize the pharmacological activity of enantiomers of phenibut, we used several experimental set-ups, in which the activity of baclofen and racemic phenibut was previously demonstrated. Obtained results confirm and broaden previous findings suggesting that the pharmacological activity of racemic phenibut resides in R-phenibut (Lapin, 2001). Thus, in general CNS tests, the effect of R-phenibut was found at about 2-fold lower doses than that of racemic phenibut, which is a 50:50 mixture of R-phenibut and pharmacologically inactive S-phenibut. In addition, we measured body temperature, because hypothermia is an indicator of the activity of baclofen and could have neuroprotective effects during hypoxia (Katz et al., 2004). Also in this test R-phenibut was the most active, but S-phenibut did not influence the body temperature of treated animals (Table 1).

We also measured the influence of enantiomers of phenibut on locomotor activity in the open field test and used the forced swimming test to determine the effect of the drug on depressive state. The fact that R-phenibut at the dose of 100 mg/kg both decreased the locomotor activity in open field test and decreased the immobility time in forced swimming test gives additional experimental evidence concerning antidepressant activity of phenibut. In almost all cases R-phenibut was the most active substance, and S-phenibut was found to be practically inactive (Figs. 2 and 3). In addition, also in nociception tests S-phenibut did not exert any analgesic activity while R-phenibut was more active than racemic phenibut (Fig. 4A and B). Thus, it could be concluded that the presence of S-phenibut in racemic phenibut only dilutes its pharmacological activity in a concentration-dependent manner. Therefore R-phenibut could be considered as a better drug candidate for clinics, since R-phenibut possess pharmacological effect at a lower dosage.

Even though the pharmacological activity of phenibut has been associated with its GABA-mimetic properties we did not find in the literature comparative data concerning the binding of R- and S-enantiomers of phenibut to the GABA_B receptor. The GABA receptor-related properties of racemic phenibut have been studied in several experimental set-ups without clearly distinguishing between enantiomers of phenibut and GABA receptor subtypes. Thus, racemic phenibut effectively displaced ³H-GABA in Ca²⁺-dependent GABA receptors binding in the presence of 50 μM (+) bicuculline, and it was hypothesized that phenibut might act via bicuculline-insensitive GABA receptors (Riago et al., 1982). Later it was suggested that racemic phenibut diminishes the intracellular concentration of cAMP via

GABA_B receptor activation and decreases functional activity of voltage-dependent Ca²⁺-ionic channels and Ca²⁺-activated outward K⁺-currents. Moreover, GABA receptors linked to benzodiazepine receptors have also been suggested as a component of motor depressing activity of racemic phenibut (Allikmets and Rago, 1983).

The aim of the present study was to compare the GABA_B receptor binding affinity of enantiomers of phenibut by using a specific GABA_B receptor labeling ligand for the estimation of binding affinities. Our data show unambiguously that R-phenibut directly binds to the GABA_B receptor and that it is about 30 times less active as baclofen in this assay (Fig. 5). Moreover, the calculated affinity constant of racemic phenibut was 2 times higher than that of R-phenibut. S-phenibut could not displace the labeled compound from brain membranes and, therefore, is inactive as a GABA_B receptor ligand. Thus, we can conclude that the binding affinities of racemic phenibut and R-phenibut correlate with their pharmacological activities described in the present study, and that S-phenibut has no activity in test systems where the key mechanism of action should be GABA_B receptor activity. In clinics, baclofen is a recognized therapy for severe spasticity in patients with multiple sclerosis (Ridley, 2006). However, the activity of phenibut in this field has never been seriously considered. In contrary, the anxiolytic effect and nootropic of phenibut are the most widely exploited clinical properties of the drug (Lapin, 2001), while baclofen is shown to suppresses cognitive behavior in animals (McNamara and Skelton, 1996). It should be noted that in the present study the effects of phenibut on exploratory activity in the open field test was observed already at a dose of 10 mg/kg, while the coordination inhibitory effect on muscle function was detected at 12-fold higher doses (Table 1). This finding might suggest that phenibut might have advantages over baclofen in treating disorders in which sedation and muscle relaxation are undesired. With regard to the tranquilizing activity of phenibut, we found that the enantiomers of phenibut have no diazepam-like activity in conflict drinking tests in rats. Since a GABA_B receptor-selective antagonist CGP35348 inhibited the antidepressant and antinociceptive effects of R-phenibut, as well as locomotor depressing activity of R-phenibut in open field test in vivo, we conclude that R-phenibut is a representative of GABA_B receptor modulating drugs.

Despite evidence from preclinical studies, the therapeutic benefits of GABA_B receptor ligands have yet to be fully realized (Bowery, 2006). In a recent study it was shown that baclofen decreased metabolic activity in brain tissues, while mild agonist action stimulated metabolism (Nasrallah et al., 2007). Since phenibut binds to GABA_B receptors with much weaker activity than baclofen, its pharmacological profile looks more close to that of second-generation GABA_B receptor modulators, which in tests of motor ability (rota-rod and locomotor activity) are devoid of sedative activity and cognitive impairment compared with baclofen and the anxiolytic agent chlordiazepoxide (Cryan et al., 2004; Cryan and Kaupmann, 2005).

In conclusion, the antidepressant, antinociceptive and locomotor depressing activity of racemic phenibut relies on

R-phenibut and correlates to the binding affinity of enantiomers of phenibut to GABA_B receptors.

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