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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 [^{3}H]CGP54626 revealed that affinity constants for racemic phenibut, R-phenibut and reference GABA-mimetic baclofen were 177±2, 92±3, 6.0±1 μM, 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.

Keywords: Phenibut; Optical isomer; Pharmacological activity; GABAB 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 (Bowery, 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 (Bowery et al., 1985). The receptor binding properties of racemic phenibut have been studied in rat cerebellar membranes using [^{3}H]-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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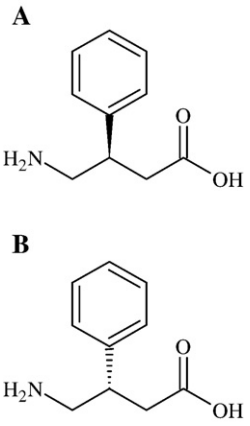

Fig. 1. Structures of R-phenibut (A) and S-phenibut (B).

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 [^{3}H]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). [^{3}H]-CGP54626 ([S-(R*,R*)]-[3-[[1-(3,4-Dichlorophenyl)ethyl]amino]-2-hydroxy-propyl] ([3,4-^{3}H]-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,