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Original research article

Optical isomers of phenibut inhibit [H^3]-Gabapentin binding *in vitro* and show activity in animal models of chronic pain

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ABSTRACT

Background: We report that R- and S-phenibut (β-phenyl-γ-aminobutyric acid) – derivatives of GABA – bind with an affinity of *c.a.* 90 μM to the gabapentin binding site in a competitive assay, a value comparable to that for previously claimed targets for this enantioermic molecule. This finding implied potential activity in neuropathic pain, this being one of the clinically validated indications for gabapentin.
Methods: The effect of phenibut on tactile allodynia was tested in a chronic constriction nerve injury (CCI) neuropathic pain model and against hypersensitivity following inflammation induced by inoculation using complete Freund's adjuvant (CFA) model.
Results: Indeed, a significant inhibitory effect on tactile allodynia was detected in rats in both employed chronic pain models with stronger and clearly dose dependent effect with R isomer.
Conclusions: The results confirm activity in chronic pain models predicted from affinity for the gabapentin site and suggests, at least partially, that α2δ-subunits of presynaptic voltage-gated calcium channels are involved in mediating this effect.
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Introduction

Phenibut (β-phenyl-γ-aminobutyric acid) – a derivative of GABA with improved blood–brain barrier penetration – was identified by Soviet scientists in the 1960s as a nootropic and anxiolytic drug [1–3]. In fact, it has a broad pharmacodynamic profile including anxiolytic effects, tranquilizing and anticonvulsant activity, and nootropic effects. It has been reported in the Russian literature to enhance physical and mental capacity, and to improve memory [3]. Phenibut belongs to a group of drugs combining the properties of tranquilizers and nootropics (atypical tranquillizers with nootropic range of activity), which has been termed "tranquilonootropics" [3].

The mechanism of action of phenibut has not been fully elucidated but it seems to act as an agonist or partial agonist at GABA-B receptors [4] and to a lesser extent on GABA-A receptors [3]. Sub-chronically dosed phenibut (100 mg/kg twice daily) caused an increase in the number of GABA-A and benzodiazepine binding sites without changes in K_D [5]. Phenibut also stimulates dopamine receptors [3] and produces acceleration of the intraneuronal synthesis and catabolism of dopamine [6]. In addition, phenibut stimulates serotoninergic processes in mice [7]. At the same time it has been reported that a single administration of phenibut (25 mg/kg, *ip*) did not significantly influence the levels of GABA, serotonin, and dopamine in various brain structures in Wistar rats, but produced a moderate decrease in the level of norepinephrine in the hippocampus and increased the content of the dopamine metabolite (3,4-dioxyphenylacetic acid) as well as reduced levels of the amino acid taurine in striatum [8].

Phenibut exists in the S(+)- and the R(−)-enantiomeric forms, which differ with regard to their neurochemical and behavioural profile. For example, R-phenibut was more potent (~100-fold) than S-phenibut in displacing [^{3}H]-R-baclofen from binding sites in rat cerebellar membranes [9]. A radioligand displacement experiment in rat brain membrane fractions using a selective GABA-B receptor labelling compound [^{3}H]CGP54626 showed 2 times higher activity of R-phenibut than racemic phenibut (Ki 92 ± 3 and 177 ± 2 μM, respectively) and no activity of S-phenibut. In this assay baclofen was about 15 times more active (Ki 6 ± 1) than R-phenibut [10]. As far as actions on GABA-B receptor is concerned, the activity also seems to be connected with the R enantiomer [9] and this is in line with most behavioural experiments [2].

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Structurally, apart from GABA and β-phenylethylamine, phenibut shows similarity to baclofen (β-p-chloro-phenyl-γ-aminobutyric acid) differing only by one chloride atom. However, it has been reported that phenibut and baclofen interact with different receptor populations in the guinea-pig isolated ileum, which may explain the differing therapeutic actions of these compounds [11].

Our initial screening of this compound on 85 targets revealed phenibut interactions with one gabapentin binding site. Gabapentin [1-(amino-methyl)cyclohexaneacetic acid] is a structural analogue of GABA with strong anti-hyperalgesic properties [12] most likely through interactions with the α2δ-subunit of presynaptic, voltage-gated calcium channels [13]. At the systemic level, it acts by reducing lesion-induced hyper excitability of posterior horn neurons involved in central sensitization of pain pathways [14], one of the mechanisms suggested to be crucial for chronification of pain [15]. In this regard, the purpose of the present study was to determine efficacy of the R- and S-enantiomers of phenibut in well-established rodent models of persistent pain where central sensitization is involved: *i.e.* peripheral neuropathy (chronic constriction nerve injury, CCI) and inflammatory pain (Complete Freund's adjuvant, CFA). The activity of phenibut in the later model has, to our knowledge, never been reported.

Materials and methods

Receptor binding

Receptor binding was performed in Wistar rats' brain cortex membranes according to the method of Gee [13] using 0.02 μM [^{3}H]-Gabapentin as a ligand. In short, incubation time was 30 min at 25 °C in 10 mM HEPES buffer (pH 7.4). Non-specific binding was 24% as determined using 100 μM gabapentin. Under these conditions, gabapentin had a Ki of 0.38 μM and B_{max} = 6.8 pmol/mg protein.

Behavioral experiments

Animals

Male Wistar rats (State Breeding Farm "Rappolovo", St. Petersburg, Russia) used in the study were allowed to acclimatize for 1 week before the study started. Animals were kept in groups of five under light controlled conditions (on 08:00–20:00 h), temperature 20–22 °C and humidity 50–70% respectively. During the experimental phase animals were housed individually in plastic T3 cages (Velaz, Czech Republic) with food (standard rodent lab chow, recipe ПК 120-1, "Laboratorsnab", Moscow, Russia) and tap filtered water available *ad libitum*.

Substances

The test drugs – R-phenibut and S-phenibut (synthetized by Merz Pharmaceuticals, Frankfurt, Germany) for injections (*ip*, 1 ml/kg) were dissolved in sterile water directly before administration.

Experimental procedure chronic constriction nerve injury model (CCI)

The sciatic nerve ligation procedure was originally described by Bennett and Xie [16]. In short, under halothane anaesthesia two incisions were made – one on each thigh, parallel to the femoral bone and approximately 1.5 cm long. The common sciatic nerves were exposed by blunt dissection through the *biceps femoris* on both sides. One paw was designated as "sham operated" while the other paw was designated as "ligated." The side of the "ligated" paw was counterbalanced. On the "ligated" paw side, proximal to the sciatic trifurcation four ligatures (4-0 silk) were tied loosely and spaced about 1 mm apart. After surgery animals were kept in a warm air environment (24 °C) for approximately 3–4 h.

14 days after surgery rats with mechanical hypersensitivity of the ipsilateral hind paw were randomly sorted into treatment groups. The vehicle or drug was administered over 3 successive days 30 min after a baseline test. Tactile sensitivity was retested 30, 60, 120, 180 and 240 min later.

Tactile sensitivity was evaluated according to the method of Dixon [17] *i.e.* the paw withdrawal thresholds were determined using an "up-and-down" von Frey hair procedure. Paws were touched with one of a series of 8 von Frey hairs (Stoelting, IL, USA) with logarithmically incremental stiffness (0.692, 1.202, 2.041, 3.630, 5.495, 8.511, 15.136, 28.840 g). For each rat, the withdrawal thresholds (WT) were always evaluated first on the left paw followed by the same procedure on the right paw. The tip of the hair was presented perpendicular to the mid-plantar surface avoiding the less sensitive footpads. Sufficient force was applied to cause slight buckling against the paw, and maintained for approximately 6 s. A positive response was recorded either if the paw was sharply withdrawn or if flinching was seen immediately upon the removal of the hair. Testing was initiated with the 3.63 g hair. Stimuli were presented consecutively either in ascending or descending order. In the absence of a response (negative response), the filament of the next greater force was applied.

Experimental procedure – complete Freund's adjuvant (CFA)

At the beginning of the experiment, all animals were screened for baseline tactile reactivity for both paws. The rats with normal nociceptive thresholds in both tests were subjected to inoculation with 0.04 ml of CFA (MP Biomedicals, Inc., France) containing of inactivated *Mycobacterium tuberculosis* into the centre of plantar surface of the left/right hind paw using a 30-gauge needle. 3–4 days after injection animals with tactile allodynia were randomly assigned into the treatment groups.

Testing of compounds was performed in rats which demonstrated low withdrawal threshold (less than 10 g) during two successive (Days 3–5 after CFA inoculation) tests. Evaluation of pain sensitivity was performed as described above for CCI model.

Statistical analysis

Data were presented as the mean ± SEM and analyzed using SPSS (version 11.5.1, SPSS Inc., USA) statistical software packages. Results were considered significant at $p < 0.05$. Data were analyzed using ANOVA which, if significant, was followed *post hoc* by the Dunnett's test.

Results

Receptor binding

R- and S-phenibut inhibited [^{3}H]-Gabapentin binding to rat cortical membranes with respective IC_{50} of 87.1 μM and 91.0 μM (Ki 60 μM). Hill coefficients were 0.83 and 0.82. Under the same conditions gabapentin was much more effective with an IC_{50} of 0.09 μM and Hill 0.63.

Pain sensitivity assay

After treatment with the highest dose of R-phenibut (100 mg/kg) on the first day of administration, all rats adopted a characteristic posture. Animals assumed a lying spread position (probably accompanied by muscular hypo tonus, with closed eyes and open lips). The rats were drowsy but were able to execute movement after stimulation. In the following days of the experiment, such postures were no longer observed. This

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