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Positive allosteric modulation of $GABA_B$ receptors ameliorates sensorimotor gating in rodent models

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Abstract

Background—Converging evidence points to the involvement of γ-amino-butyric acid B receptors ($GABA_BRs$) in the regulation of information processing. We previously showed that $GABA_BR$ agonists exhibit antipsychotic-like properties in rodent models of sensorimotor gating deficits, as measured by the prepulse inhibition (PPI) of the acoustic startle reflex. The therapeutic potential of these agents, however, is limited by their neuromuscular side effects; thus, in the present study we analyzed whether *rac*-BHFF, a potent $GABA_BR$ positive allosteric modulator (PAM), could counter spontaneous and pharmacologically induced PPI deficits across various rodent models.

Methods—We tested the antipsychotic effects of *rac*-BHFF on the PPI deficits caused by the *N*-methyl-D-aspartate glutamate receptor antagonist dizocilpine, in Sprague-Dawley rats and C57BL/6 mice. Furthermore, we verified whether *rac*-BHFF ameliorated the spontaneous PPI impairments in DBA/2J mice.

Results—*rac*-BHFF dose-dependently countered the PPI deficits across all three models, in a fashion akin to the $GABA_BR$ agonist baclofen and the atypical antipsychotic clozapine; in contrast with these compounds, however, *rac*-BHFF did not affect startle magnitude.

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Conflicts of interest: Pari Malherbe and Andrew W Thomas are employees of Hoffmann-La Roche.

Conclusions—The present data further support the implication of $GABA_B$Rs in the modulation of sensorimotor gating, and point to their PAMs as a novel promising tool for antipsychotic treatment, with fewer side effects than $GABA_B$R agonists.

Keywords

$GABA_B$ receptors; prepulse inhibition of the startle; sensorimotor gating; schizophrenia; NMDA receptors; DBA/2J mice

Introduction

Ample evidence shows that γ-amino-butyric acid (GABA), the main inhibitory neurotransmitter in the CNS, is implicated in schizophrenia pathogenesis [1-3]. In particular, several clinical investigations have documented deficits in the expression of the metabotropic $GABA_B$ receptors ($GABA_B$Rs) in the cortex and hippocampus of schizophrenia patients [4-6]. The mechanisms supporting the involvement of $GABA_B$Rs in psychotic disorders remain elusive; however, recent findings suggest that the dysfunction of this receptors in the cortex leads to alterations of glutamate signaling and excitatory/inhibitory imbalances [7-8], which contribute to the aberrant information processing and cognitive deficits in schizophrenia [9-10].

In keeping with this background, our group and others showed that the prototypical $GABA_B$R agonist baclofen (BAC) countered the disruption of prepulse inhibition (PPI) of the acoustic startle reflex produced by the blockade of N-methyl-D-aspartate glutamate receptors (NMDARs) in rats [11-12] and mice [13]. This endophenotype is widely regarded as a heuristic index of sensorimotor gating, the cognitive function that enables pre-attentional information filtering and governs the detection of perceptual salience [14]; notably, PPI deficits are featured in schizophrenia and related neuropsychiatric disorders [15-16].

In subsequent studies, we found that BAC rescued the marked deficits in sensorimotor gating present in DBA/2J mice [17], a strain that features antipsychotic-sensitive PPI deficits [18].

These findings collectively highlight $GABA_B$R as a highly promising target for antipsychotic treatment. Indeed, Daskalakis and George [19] hypothesized that $GABA_B$R activation may be the mechanism underlying the unique ability of the antipsychotic agent clozapine (CLO) in reducing the severity of negative symptoms in schizophrenia. While BAC monotherapy does not elicit significant antipsychotic effects [20], preliminary studies have documented its therapeutic effectiveness as an adjunctive treatment [21-22]. The therapeutic employment of BAC in schizophrenia is limited by several factors, including its poor ability to cross the blood-brain barrier, its short latency of action [23], as well as its potentially severe side effects, including muscle flaccidity, sedation, loss of reflexes, and, at higher dosages, bradycardia, respiratory depression, hypothermia and coma [24]. A new class of $GABA_B$R positive allosteric modulators (PAMs) has been recently developed [25] to harness the therapeutic potential of $GABA_B$R activation without eliciting the side effects of BAC. The mechanism of these compounds is based on the enhancement of the effects of

GABA on $GABA_BRs$ [26-27]. One of the most potent drugs in this family, *rac*-BHFF [(*R,S*)-5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one] [25], has been shown to produce behavioral effects without altering motor coordination and nervous reflexes, and with a much better safety index than $GABA_BR$ agonists [25; 28-31]. Here we show that *rac*-BHFF elicited antipsychotic-like effects on the pharmacologically induced and spontaneous gating deficits in rodent models, as assessed through the paradigm of the prepulse inhibition (PPI) of the acoustic startle reflex; although these effects were comparable to those of BAC and CLO, *rac*-BHFF did not induce the same alterations of startle reflex associated with these drugs.

Materials and methods

Animals

We used behaviorally-naïve male Sprague-Dawley rats weighing between 250-300 g, and male DBA/2J and C57BL/6J between 18-24 g. Animals were housed 4/cage in a room maintained at a temperature of 22°C and kept under an artificial 12/12-h light/dark cycle. Animals were given *ad libitum* access to food and water and handled for 5 min daily to minimize experimental stress. All experimental procedures were approved by the ethical committee of the University of Cagliari and carried out in strict accordance with the guidelines for experimental animals care [European Economic Community (86/609; DL 27/01/92, number 110)].

Drugs

rac-BHFF (Hoffmann-La Roche, Basel, Switzerland) was suspended in a mixture containing Cremophor EL, 1,2-propanediol and distilled water (4:1:30 ratio) and administered intragastrically (per os, PO) at an injection volume of 10 ml/kg. The NMDAR antagonist dizocilpine (DIZ; Sigma-Aldrich, Milan, Italy) was dissolved in 0.9% saline and administered subcutaneously (SC). BAC (Tocris Cookson, Bristol, UK) was dissolved in saline and administered intraperitoneally (IP). CLO (Sigma-Aldrich) was dissolved in a single drop of 1 N HCl and diluted with saline; the pH was adjusted to 7 with $NaHCO_3$. Parenteral injections were administered in injection volumes of 1 ml/kg for rats and 10ml/kg for mice.

Experimental procedures

Acoustic startle reflex and PPI were measured in 4 sound-attenuated chambers (Med Associates, St Albans, USA) with fan ventilation. Each chamber consisted of a Plexiglas cylinder (9 cm diameter for rats and 3.2 cm diameter for mice) mounted on a piezoelectric accelerometric platform and connected to an analogue-digital converter. Background noise and acoustic bursts were conveyed by two separate speakers, properly spaced from the cylinder so as to produce a variation of sound within 1 dB across it. Both speakers and startle cylinders were connected to a main PC computer, which detected and analyzed all chamber variables by means of specific software. Before every testing session, acoustic stimuli and mechanical responses were calibrated via specific devices supplied by Med Associates.

The experimental procedure was based on the protocols described in Frau *et al* [32]. Briefly, three days before the experiment, animals went through a brief baseline startle session. Animals were exposed to 70 dB background white noise for a 5 min acclimation, followed by presentation to a randomized sequence of twelve 40 ms acoustic pulses of 115 dB, interposed with three trials in which an 82 dB prepulse preceded the 115 dB pulse by 100 ms. Subsequently, treatment groups were established so that average startle responses and % PPI (calculated as: 100-[(mean startle amplitude for prepulse+pulse trials/mean startle amplitude for pulse alone trials) × 100]) were equivalent across groups. On the testing day, each animal was placed in the cylinder for a 5-min acclimation to 70 dB background white noise, which continued for the remainder of the entire session. The session consisted of three consecutive blocks of trials. Unlike the first and the third block, during which animals were presented with only five pulse-alone trials of 115 dB, the second block displayed a pseudorandom sequence of 50 trials, including 12 pulse-alone trials, 30 trials of pulse preceded by 74, 78 or 86 dB prepulses (ten for each level of prepulse loudness) and eight no stimulus trials, where only background noise was delivered. Intertrial intervals (ITI) were selected randomly between 10 and 15 s. The duration of pulses and prepulses were 40 and 20 ms, respectively. Prepulse-pulse delay amounted to 100 ms.

Data analysis

Normality and homoscedasticity of data were verified by Kolmogorov-Smirnov and Bartlett's tests. Data were compared across groups by one-way or two-way ANOVAs, as appropriate. As no interaction between prepulse levels and treatment were found in the statistical analysis, %PPI values were collapsed across prepulse intensity to represent average %PPI. *Post-hoc* analyses were performed using Tukey's test with Spjøtvoll-Stoline correction. Significance threshold was set at 0.05.

Results

Effects of rac-BHFF on the PPI deficits induced by DIZ in Sprague Dawley rats

In the first experiment, we investigated whether *rac*-BHFF pretreatment could prevent the PPI disruption induced by the NMDAR antagonist DIZ in Sprague Dawley rats. Animals were pretreated with either *rac*-BHFF (25-50 mg/kg, PO) or vehicle (control) and sixty minutes later were given an injection of either DIZ (0.1 mg/kg, SC) or saline (control). Animals were subjected to PPI testing five min after the last treatment.

No significant differences in startle amplitude were found between pretreatment-treatment groups (Fig. 1A) [Interaction: $F_{2,47}$=0.10; NS]. Conversely, we found a significant pretreatment × treatment interaction (Fig. 1B) [Interaction: $F_{2,47}$=4.62, P<0.05] between groups. *Post-hoc* analyses revealed that the highest dose of *rac*-BHFF significantly reversed DIZ-induced PPI deficits (P<0.05; Tukey's test).

In contrast, BAC (5 mg/kg, IP) produced a significant reduction in startle amplitude [Main effect: $F_{1,37}$=9.44; P<0.01] (Fig.1A); furthermore, it significantly prevented the PPI impairments caused by DIZ [Interaction: $F_{1,37}$=4.89; P<0.05; P<0.05 for vehicle-DIZ vs BAC-DIZ comparisons] (Fig. 1B). As expected, CLO (5 mg/kg, IP) elicited similar effects,

with a general reduction of startle magnitude [Main effect: $F_{1,37}$=7.17; P<0.05] (Fig.1A) and a significant reversal of DIZ-mediated PPI deficits [Interaction: $F_{1,37}$=5.27; P<0.05; P<0.05 for vehicle-DIZ vs CLO-DIZ comparisons] (Fig. 1B).

Effects of rac-BHFF on the PPI deficits induced by DIZ in C57BL/6J mice

Next, we studied whether *rac*-BHFF (6.25-12.5 mg/kg, PO) exhibited antipsychotic-like effects in C57BL/6J mice treated with DIZ (0.3mg/kg, IP). In parallel with our results on rats, no differences were found between groups for startle amplitude (Fig. 2A) [Interaction: $F_{2,59}$ = 1.99, NS]; however, we detected a marked pretreatment × treatment interaction (Fig. 2B) [$F_{2,52}$=5.32, P<0.01]. Specifically, the PPI-disruptive effects of DIZ (P<0.01) were prevented by *rac*-BHFF treatment at a dose of 12.5mg/kg (P<0.001; Tukey's test). As previously shown [17], BAC (5 mg/kg, IP) did not affect startle amplitude in C57BL/6J mice (Fig.2A); notably, the PPI analysis indicated main effects for both pre-treatment (BAC) [$F_{1,41}$=7.00; P<0.05] and treatment (DIZ) [$F_{1,41}$=8.13; P<0.01], but no significant interactions [$F_{1,41}$=2.32; NS] (Fig. 2B). Conversely, CLO (5 mg/kg, IP) reduced startle magnitude [Main effect: $F_{1,41}$=7.84; P<0.01] (Fig.2A) and significantly countered the PPI disruption induced by DIZ [Interaction: $F_{1,41}$=4.81; P<0.05; P<0.05 for vehicle-DIZ vs CLO-DIZ comparisons] (Fig. 2B).

Effects of rac-BHFF on the spontaneous PPI deficit displayed by DBA/2J strain of mice

In the last experiment we evaluated the intrinsic effects of *rac*-BHFF on the spontaneous low PPI baseline displayed by DBA/2J mice. Animals were treated with VEH or rac-BHFF (6.25-12.5 mg/kg, PO) and subjected to PPI sessions sixty minutes later. The $GABA_BR$ PAM did not elicit any changes in startle amplitude at any dose (Fig. 3A) [$F_{2,35}$=0.99, NS]. In contrast, low PPI in DBA mice was countered by *rac*-BHFF [$F_{2,35}$=4.63 P<0.05)], specifically at the dose of 12.5 mg/kg (P<0.05; Tukey's test) (Fig. 3B). BAC and CLO failed to affect startle amplitude, but significantly countered DIZ-induced PPI deficits [BAC: $F_{1,20}$=10.28; P<0.01; CLO: $F_{1,20}$=12.36; P<0.01] (Fig. 3B).

Discussion

The results of this study showed that *rac*-BHFF, a potent $GABA_BR$ PAM, dose-dependently reversed DIZ-induced PPI deficits in both SD rats and C57BL/6J mice, and rescued the PPI impairments displayed by DBA/2J mice. Overall, these effects were akin to those elicited by the $GABA_BR$ agonist BAC and the atypical antipsychotic CLO, and substantially confirm previous findings by our group and others [11-13; 18] on the therapeutic potential of $GABA_BR$ activators on sensorimotor gating deficits induced by NMDAR blockade. Notably, in contrast with BAC and CLO, *rac*-BHFF did not significantly reduce the magnitude of startle reflex, irrespective of the animal model and dose. It is also worth mentioning that the same doses of *rac*-BHFF that elicited antipsychotic-like effects in our models also failed to affect locomotor responses or other spontaneous behavioral manifestations in the home cage [unpublished observations]. Taken together, our findings complement previous preclinical data on the beneficial effects of $GABA_BR$ PAMs [33-34] and highlight this class of compound as a novel putative avenue for antipsychotic therapy with fewer side effects than $GABA_BR$ antagonists.

One of the key problems in the potential employment of BAC as an add-on treatment lies in the exacerbation of locomotor impairments and sedative effects caused by dopamine D_2 receptor antagonism; indeed, in preliminary studies we observed that the association of BAC and antipsychotic drugs, such as CLO and haloperidol, was not suitable for behavioral studies in rodents, in view of the serious impairments in neuromuscular coordination produced by such combinations. From this perspective, $GABA_BR$ PAMs may afford a safer and tolerable alternative as antipsychotic adjunctive therapies for schizophrenia or related disorders. Future clinical and preclinical studies are warranted to evaluate this interesting perspective and validate the potential usefulness of *rac*-BHFF and similar agents in the treatment of psychoses.

The PPI of the acoustic response refers to the reduction in the response amplitude to a sudden and intense startling stimulus [pulse], when it is immediately preceded by a weaker non-startling pre-stimulus [35]. This phenomenon is widely regarded as a dependable index of sensorimotor gating integrity, and is typically impaired in schizophrenia [17]. In rodents, DIZ and other NMDAR antagonists produce marked PPI deficits, which are sensitive to CLO and other atypical, but not typical, antipsychotics [36-38]. The effects of NMDAR antagonists on sensorimotor gating are in line with the well-known psychotomimetic effects of these drugs [39] and other alterations of informational processing [40].

Although the mechanisms by which DIZ and other NMDAR blockers impair PPI remain unclear, several studies point to a key role of the prefrontal cortex (PFC) and hippocampal regions in these phenomena [12; 41]. These areas are characterized by a large density of pre- and postsynaptic $GABA_BRs$, which finely regulate basal glutamatergic and dopaminergic functions [42]. Accordingly, disturbances in $GABA_BR$ expression or function may affect informational salience by altering the inhibitory/excitatory balance of several neurotransmitter systems in corticolimbic regions.

It has been reported that MK-801 and PCP stimulate cortical glutamate release in PFC and hippocampus [43]. In this scenario, rac-BHFF could counteract the disinhibition of neuronal activity produced by exaggerated NMDAR stimulation in these areas or, alternatively, modulate distinct forebrain pathways under the control of non-NMDA glutamatergic receptors, such as AMPA and Kainate [44]. Alternatively, rac-BHFF may counter DIZ-mediated PPI-deficits by acting on the pallidotegmental nucleus (PTn). This predominantly GABAergic area exhibits high levels of $GABA_BRs$ and acts as an interface between the brainstem and forebrain regions implicated in PPI regulation regions [45]. Accordingly, DIZ has been recently shown to induce PPI deficits through alterations of the giant neurons of this region; notably, $GABA_BR$ activation reversed these impairments by stabilizing the hyperactivation of these nuclei [13].

rac-BHFF also significantly ameliorated the PPI deficits on DBA/2J mice, a strain characterized by spontaneous gating impairments sensitive to antipsychotics [19], as well as other phenotypes reminiscent of schizophrenia symptoms, such as poor exploration as well as high aggression and social avoidance [46-49].

The antipsychotic-like actions of rac-BHFF in this murine strain may be related to their reduced expression of $GABA_B$Rs and NMDARs across the cortex and hippocampus, in comparison with C57BL/6J mice [18; 50].

Several limitations in the present study should be acknowledged. First, our analysis was limited to the behavioral analysis of startle reflex and PPI, but did not include the testing of other paradigms with great relevance to the negative and cognitive symptoms of schizophrenia-spectrum disorders, such as object recognition and social interaction test [51-52]. Second, we did not evaluate the effects of *rac*-BHFF in animal models with high translational validity with respect to schizophrenia, such as DISC1- and neuregulin1-deficient mice, or rodents subjected to chronic administration of DIZ or other NMDAR antagonists, which may have better simulated the neurobiological impairments associated with schizophrenia [53-58]. Third, our research did not encompass testing of $GABA_B$ negative allosteric modulators or antagonists, which may be essential for a full definition of the role of these receptors in schizophrenia-related endophenotypes.

Although further investigations are needed to address these limitations, our findings extend and support for the role of $GABA_B$Rs in the pathophysiology of psychiatric disorders associated with sensorimotor gating disturbances, and point to PAMs of these targets as interesting therapeutic tools to treat cognitive deficits and negative symptoms in schizophrenia.

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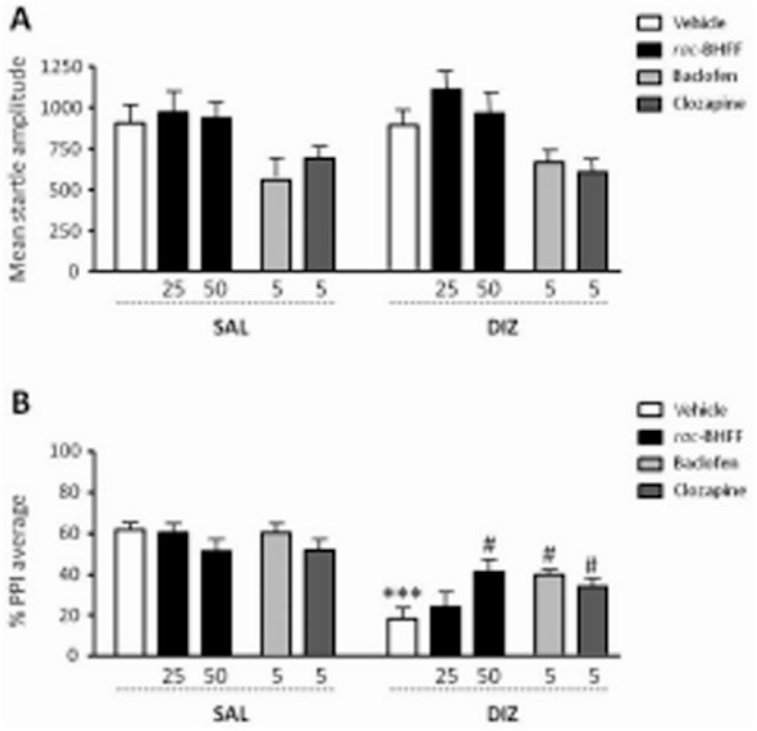

Figure 1.

Effects of *rac*-BHFF on the mean startle amplitude (A) and prepulse inhibition deficits (B) induced by dizocilpine (DIZ, 0.1 mg/kg, SC) in Sprague Dawley rats, compared to baclofen and clozapine. All doses are given in milligrams per kilogram and are indicated below the horizontal axis. Values represent mean ± SEM for each treatment. Percent prepulse inhibition (PPI) values were collapsed across all three prepulse intensities (4, 8, and 16 dB above 70 dB background noise). For all experimental groups, n=8-12. SAL, saline. ***, $P<0.001$, compared to VEH+SAL group; $^{\#}$, $P<0.05$, compared to VEH+DIZ group. For further details, see text.

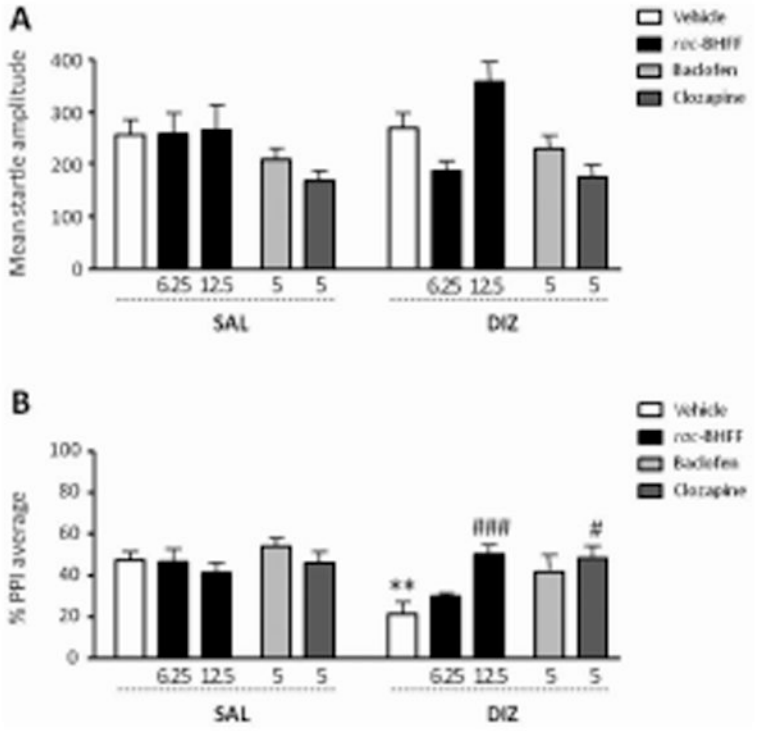

Figure 2.

Effects of *rac*-BHFF on the mean startle amplitude (A) and spontaneously low prepulse inhibition deficits (B) induced by dizocilpine (DIZ, 0.3 mg/kg, IP) in C57BL/6J mice, compared to baclofen and clozapine. All doses are given in milligrams per kilogram and are indicated below the horizontal axis. Values represent mean ± SEM for each treatment. Percent prepulse inhibition (PPI) values were collapsed across all three prepulse intensities (4, 8, and 16 dB above 70 dB background noise). For all experimental groups, n=8-12. SAL, saline. **, $P<0.01$, compared to VEH+SAL group; $^{###}$, $P<0.001$, $^{#}$, $P<0.05$, compared to VEH+DIZ group. For further details, see text.

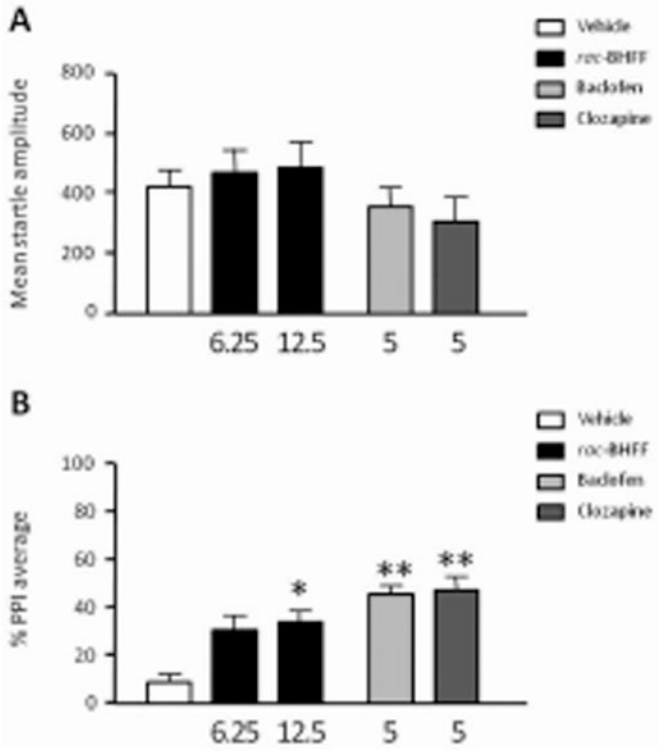

Figure 3.

Effects of *rac*-BHFF on the mean startle amplitude (A) and spontaneous prepulse inhibition deficits (B) displayed by DBA/2J mice, compared to baclofen and clozapine. All doses are given in milligrams per kilogram and are indicated below the horizontal axis. Values represent mean ± SEM for each treatment. Percent prepulse inhibition (PPI) values were collapsed across all three prepulse intensities (4, 8, and 16 dB above 70 dB background noise). For all experimental groups, n=8-10. **, P<0.01, compared to VEH group. For further details, see text.