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$GABA_B$ receptor activation exacerbates spontaneous spike-and-wave discharges in DBA/2J mice

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ABSTRACT

Rich evidence has highlighted that stimulation of γ-amino-butyric acid $(GABA)_B$ receptors increases the occurrence of spike-and-wave discharges (SWDs), the electroencephalographic (EEG) landmark of absence epilepsy (AE). Recent findings suggest that the outcomes of $GABA_B$ activation *in vivo* are contingent on the chemical characteristics of the agonist. In particular, the endogenous ligand γ-hydroxybutyrate (GHB) and its precursor γ-butyro-lactone (GBL) have been shown to elicit different effects than the prototypical $GABA_B$ agonist baclofen. In view of these premises, the present study was aimed at the characterization of the effects of baclofen (0.5–10 mg/kg, i.p.) and GBL (5–100 mg/kg, i.p.) on the spontaneous SWDs and locomotor activity of DBA/2J mice.

While both baclofen and GBL dose-dependently increased SWDs episodes, high doses of the latter (100 mg/kg, i.p.) reduced the occurrence of these phenomena and increased the number of isolated spikes. Interestingly, both compounds elicited a dose-dependent reduction of locomotor activity, in comparison with their vehicle-treated controls. The $GABA_B$ selective antagonist, SCH50911 (50 mg/kg, i.p.), reversed the changes in SWD occurrence and locomotion induced by baclofen and GBL, but failed to elicit intrinsic effects on either paradigm. These results indicate that $GABA_B$ receptor signaling might exert differential effects on SWDs in DBA/2J mice.

1. Introduction

Absence epilepsy (AE) is an idiopathic, non-convulsive epilepsy characterized by brief, sudden interruptions of consciousness and minor automatisms.[1] Such episodes, generally benign and self-limited, are concomitant with distinct electrocorticographic (ECoG) alterations, consisting in bilateral synchronous bursts of spike–wave discharges (SWDs) at typical frequency around 3–4 Hz.[2] Although the molecular bases of AE are largely elusive, the wealth of evidence supports the involvement of γ-aminobutyric acid (GABA) transmission in their pathophysiology. In particular, several studies suggest that $GABA_B$ receptor activation may exacerbate AE in several animal models.[3] Recent studies have suggested that abnormalities in $GABA_B$ receptor signaling may account for several phenotypical alterations exhibited by DBA/2J mice,[4,5] a strain exhibiting EEG patterns reminiscent of those observed in AE.[5,6] Capitalizing on this background, we addressed the present study to investigate the role of $GABA_B$ receptors in the expression of SWDs in DBA/2J mice.

Recent lines of evidence suggest that the *in vivo* outcomes of $GABA_B$ activation are contingent on the chemical characteristics of the agonist. In particular, the endogenous ligand γ-hydroxybutyrate (GHB) and its precursor γ-butyro-lactone (GBL) have been shown to elicit $GABA_B$-dependent behavioral effects which differ from those mediated by the prototypical agonists of this receptor,[7–9] such as baclofen.

Although both GHB and baclofen have been shown to induce, in both humans and laboratory animals, EEG abnormalities similar to those associated with AE,[10,11] the specific impact of each agonist has not been compared to date. This premise prompted us to test the EEG response of DBA2/J mice to baclofen and GBL, in comparison with the locomotor alterations induced by both drugs. The latter compound was preferred to GHB on account of its

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numerous experimental advantages: first, it exhibits a greater ability to cross the blood–brain barrier[12]; second, it is inherently inactive and rapidly converted to GHB after parenteral administration[13]; finally, it shows a better dissociation between EEG abnormalities and alterations of thermoregulation than its metabolite.[14]

To understand whether the differences between baclofen and GBL may be actually mediated by $GABA_B$ receptors, the effects of the two compounds were also studied in presence of a $GABA_B$ selective antagonist, SCH50911.

2. Materials and methods

2.1. Animals

Juvenile (4–5 weeks old) male DBA/2J mice (Harlan, Como, Italy) ($n = 131$; weight: 25–30 g each) were housed four per cage under a 12 h light/dark cycle (light on at 8:00 AM), in conditions of constant temperature (21 ± 2 °C) and humidity (60%), with food and water *ad libitum*. All experimental procedures were approved by the local ethical committee and conducted in conformity with the University of Cagliari guidelines. The experimental preparation followed the methods previously described.[5] Briefly, mice were anesthetized and placed in a stereotaxic apparatus (David Kopf, mod. 900). Each skull was exposed and perforated in four points, located above both sensorimotor cortices (FPr and FPl), 0.5 mm anterior to the bregma (Cz), and over the cerebellum (G2). A four-pin male socket was positioned into the holes, secured to the skull with epoxy resin and covered with acrylic cement to improve retention.

2.2. Drugs

The following drugs were used in this study: $GABA_B$ receptor agonists baclofen and γ-butyro-lactone (GBL), as well as $GABA_B$ antagonist SCH50911 (Tocris Cookson, UK). All drugs were dissolved in saline 0.9% and administered intraperitoneally (i.p.) in an injection volume of 10 ml/kg. The dose ranges of baclofen and GBL were selected so as to have similar efficacy, based on the comparison of their ED_{50} values[8] and on preliminary observations by our group.

2.3. EEG recordings

EEG recordings were acquired on a portable EEG polygraph (BQS 98 System Micromed, Mogliano Veneto, Italy), and the Electrode impedance was maintained at <5 kΩ. Digital EEG signals were filtered with elliptical filter banks to obtain the optimal resolution of broadband parameters. Given that synchronization analysis requires a zero-phase filtering distortion, data were further processed by forward–backward filtering.[15] The off-line SWDs analysis was accomplished separately by trained researchers blinded to this experimental phase of the study. In addition to visual inspection, SWDs morphology was assessed by means of a customized algorithm aimed at detecting significant variations occurring under a pre-settled threshold, based on the analysis of the fractal dimension of the EEG signal.[16]

DBA/2J mice exhibited an EEG pattern with 12–18 Hz low-medium voltage background activity mixed with 6–12 Hz high-voltage SWDs events. To maintain normality and homoscedasticity criteria in the sampled population, experiments were performed on the basis of SWD event frequency in the baseline EEG recording by selecting animals displaying between 6 and 25 SWDs/30 min (with a median value of 13 in the overall tested population) while mice showing very low or very high number of SWDs (more than two standard deviations above or below group mean values) were excluded from the study. Each treatment group consisted of 5–7 animals, and each animal was injected with only one dose throughout the study. Recordings started between 8 and 10:30 AM, and were analyzed in two blocks, respectively before and after drug administration. The first block, lasted 60 min (two 30-min intervals) and was used to monitor the baseline conditions of each animal. In this phase of EEG recording, DBA/2J mice exhibited characteristic spontaneous short-lasting spiking activity bursts (4–12 s duration and 250–550 μV amplitude), superimposed to baseline activity of 50–120 μV EEG rhythms.

The second block lasted 90–150 min (three to five 30-min intervals) and was used to assess the effects of the treatment. SWD analysis was based on their number (calculated as the average number of events occurring in a 30-min interval) and mean duration for pre- and post-injection blocks were calculated.

Episodes of drowsiness and sleep were discarded, as previous studies in other rodent models of AE showed relevant SWDs variations during sleep.[17]

2.4. Locomotor activity

We tested the impact of $GABA_B$ ligands on the locomotor activity in a different group of DBA/2J mice. The motility cages (Omnitech Digiscan Animal Activity Monitor, Columbus, OH, USA) featured 2 sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart above the cage floor. After a 30-min acclimatization period in the apparatus, each animal was injected and its locomotor activity was studied for further 90 min. Locomotion was measured with the horizontal activity counts in 10-min intervals.

2.5. Statistical analyses

Number and mean duration of SWDs were analyzed by 2-factor ANOVAs, with treatment dose as an independent factor and blocks (pre- and post-injection) as repeated measures. Locomotor activity was analyzed by 2-factor ANOVAs, with dose as independent factor and time (consecutive 10-min intervals). Post hoc comparisons were performed with Tukey's test. Alpha was set at $p < 0.05$.

3. Results

3.1. Effects of baclofen on SWD

In the first experiment (Fig. 1), the effects of baclofen (0.5–10 mg/kg, i.p.; $n = 5$–7/group) on SWD number and mean duration were tested and compared with both vehicle-treated mice and their individual baseline values. Baclofen was found to exert a significant main effect on SWDs occurrence [$F(1,29) = 38.44$, $p < 0.001$]. The analysis of dose × block interactions also revealed a significant effect [$F(5,29) = 10.63$, $p < 0.001$]. Post hoc comparisons further established that the doses of 2.5, 5 and 10 produced a significant increase in comparison to their baseline and to vehicle-treated subjects. Conversely, baclofen did not produce any significant change in SWD duration (Fig. 1d) at any dose tested. No difference in baseline SWD number or duration was found.

3.2. Effects of baclofen on locomotor activity

As changes in the number of SWDs may reflect behavioral alterations (such as profound sedation), we evaluated the behavioral impact of baclofen (2.5–10 mg/kg, i.p.) on the motor activity of a different group of DBA/2J mice ($n = 5$/group). The evaluation of the effects of baclofen (2.5–10 mg/kg, i.p.) on locomotor activity revealed a main dose effect [$F(3,16) = 5.91$, $p < 0.01$], which was found to reflect a significant difference between the animals treated with vehicle and those injected with the 10 mg/kg dose of the $GABA_B$