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Carbamazepine aggravates absence seizures in two dedicated mouse models

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ABSTRACT

Background: The aim of this study was to evaluate the effect of carbamazepine (CBZ) upon chemically induced absence seizures and in a genetic absence seizures model in the mouse.
Methods: The γ-butyrolactone (GBL)-induced acute absence seizures and the stargazer spontaneous absence seizures mice models were used to characterize the aggravation of absence seizures induced by oral CBZ treatment. The effect of CBZ upon GABA inward-currents in Ltk cells expressing human recombinant α1β2γ2, α2β2γ2, α3β2γ2 and α5β2γ2 $GABA_A$ receptors was evaluated by means of patch clamp.
Results: GBL administration induced motor impairment in NMRI mice. High dose CBZ (25 mg/kg body weight) had no effect on motor performance but exacerbated the behavioral incoordination observed for GBL. Also, coadministration of a high dose CBZ and GBL impaired spontaneous locomotion. Moreover, CBZ was investigated after oral administration to evaluate the potential to aggravate GBL-induced acute spike-and-wave discharges (SWD) in the electroencephalogram. High dose CBZ significantly aggravated SWD induced by GBL. Likewise, in the stargazer mouse model of genetic spontaneous absence seizures, CBZ significantly aggravated SWD frequency and duration. Pre-treatment with the T-type Ca^{2+} channel blocker ethosuximide (200 mg/kg body weight) prevented the CBZ aggravation of SWD induced by GBL and in the stargazer mouse. CBZ increased in a concentration dependent manner sub-maximal α1β2γ2 and α3β2γ2 GABA currents.
Conclusion: CBZ aggravates absence seizures as assessed in two dedicated mouse models of absence seizures. Facilitation of sub-maximal α1β2γ2, and α3β2γ2 GABA currents by CBZ may play a role in CBZ-induced GABA-mediated aggravation of absence seizures.

Introduction

Typical generalized absence seizures are distinctively different from any other seizure type and pharmacologically unique. Absence seizures are characterized behaviorally by a paroxysmal loss of consciousness of abrupt and sudden onset and offset that is associated with bursts of bilaterally synchronous spike-and-wave discharges (SWD) in the electroencephalogram (EEG) [1]. Absence epilepsy is characterized by a sudden interruption of both physical and mental activity without major loss of postural tone [2]. Typical absence seizures are generated as a result of complex interactions between the thalamus and the cerebral cortex [3–7]. This thalamocortical circuitry is under the control of several specific inhibitory and excitatory systems arising from the forebrain and brainstem. Corticothalamic rhythms are believed to be involved in the generation of SWD that are the characteristic EEG signs of absence seizures. The involvement of the thalamocortical circuits, particularly the contribution of the ventrobasal thalamus and the reticular thalamic nucleus, in the propagation of absence seizures has been established in several species [7,8]. The reticular thalamic nucleus is made up mainly of γ-aminobutyric acid (GABA)-containing neurons that project to the thalamic relay nuclei [7].

Ethosuximide (ESM) has been used extensively for absence seizures and is a valuable pharmacological tool in studies of absence epilepsy [7]. The spontaneous pacemaker oscillatory activity of thalamocortical circuitry involves low threshold T-type Ca^{2+} currents

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in the thalamus [9]. ESM is presumed to reduce these low threshold T-type Ca^{2+} currents in thalamic neurons and likely to be responsible for its efficacy in primary generalized absence epilepsy [7,9].

Aggravation of seizures by antiepileptic drugs is a serious problem in clinical practice. Drugs enhancing GABA function in the brain exacerbate both experimental and clinical absence seizures [10,11]. Carbamazepine (CBZ) has been reported to exacerbate absence seizures in patients afflicted with generalized epilepsies characterized by bursts of diffuse and bilaterally synchronous spike-and-wave EEG activity [12,13]. The possible mechanism underlying the aggravation of absence seizures by CBZ involves a $GABA_A$ receptor-mediated action within the ventrobasal thalamus region [14].

Here we used two mouse models to further evaluate aggravation of absence seizures by CBZ. The first model was the γ-butyrolactone (GBL)-induced acute absence seizures in the mouse [15]. GHB (γ-hydroxybutyric acid) is a GABA metabolite that occurs naturally in the mammalian brain. When administered intraperitoneally (*ip*) it can induce generalized absence seizures [15]. GBL is the prodrug of GHB and is biologically inactive in the brain. However, an active lactonase in the serum and liver convert GBL to GHB. Thus, when GBL is administered *ip* it results in the rapid onset of bilaterally synchronous SWD that correlate with an almost immediate appearance of GHB in the brain [11,15–19]. GBL-induced absence seizures in the mouse are well documented in the literature and the occurrence of 3–6 Hz SWD recorded in the EEG are accompanied by generalized absence seizure behavior, such as staring, and suppression of locomotor activity [15–19]. This acute model of absence epilepsy is self-limiting and resolves within a defined period of administration of GBL [20]. In the mouse, the effects of GBL can be prevented by the administration of ESM or $GABA_B$ antagonists, but not by valproate [19,21]. The second model used was the spontaneous absence seizures in the stargazer mutant mouse [22]. The stargazer (stg) mutant mouse harbors a transposon insertion in the second intron of the calcium channel c2 subunit (cacng2) locus [23,24]. Mice homozygous for the spontaneous mutation stargazer ($Cacng2^{stg}$) reveal frequent, prolonged, generalized cortical SWD with behavioral arrest [22]. Typical paroxysmal spike burst occurs at a frequency of 6–7 spikes/s with a mean discharge duration of 6 s and a mean rate of discharge activity of 125/h. The abnormal brain wave patterns are similar to those seen in humans afflicted with absence epilepsy [22]. ESM has been shown to have an antiabsence effect in the stargazer mouse [21].

The aim of this study was to evaluate the effect of CBZ upon chemically-induced absence seizures and in a genetic absence seizures model in the mouse. Moreover, the effect of CBZ upon GABA inward-currents in Ltk cells expressing human recombinant α1β2γ2, α2β2γ2, α3β2γ2 and α5β2γ2 GABAA receptors was evaluated by means of patch clamp.

Materials and methods

Animals and general procedures

Male NMRI mice (7-week-old) were obtained from Harlan Laboratories (Barcelona, Spain). Seven-week-old male mice homozygous for the spontaneous mutation stargazer (strain name: B6C3Fe *a/a*-$Cacng2^{stg}$/J, stock number: 001756, generation N50) were obtained from The Jackson Laboratory (Bar Harbor, USA). Animals were delivered to the laboratory three weeks before experiments during which time they were acclimatized to vivarium conditions under controlled environmental conditions (12 h light/dark cycle [7.00 h/19.00 h], temperature 22 ± 3 °C, relative humidity 30–80%) with free access to food (Rodent Maintenance Diet 2014, Harlan Teklad, Harlan Laboratories) and tap water. All animal procedures conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the Portuguese law on animal welfare (Decreto-Lei 113/2013). The number of animals used was the minimum possible in compliance with current regulations and scientific integrity.

CBZ was synthesized in the Laboratory of Chemistry of BIAL-Portela & C[a], S.A. GBL was obtained from Merck (Darmstadt, Germany). All other chemicals and materials were from Sigma–Aldrich (St. Louis, USA), unless noted otherwise. GBL and ESM were dissolved in physiological saline, which served as vehicle. CBZ was dispersed in 0.2% hydroxypropylmethylcellulose (HPMC) in distilled water, which served as vehicle. For GBL, doses were prepared V/V and for ESM and CBZ doses were prepared by separate weighings (W/V). Preparations were made freshly for each day of administration and precautions were taken to preserve the homogeneity of suspensions during the period of administration. The administration volume was 10 ml/kg body weight for all administrations. GBL [16–19,21] and ESM [19,21] doses were selected from the literature. CBZ doses correspond to the reported ED_{50} (10 mg/kg) and ED_{75} (25 mg/kg) in the mouse maximal electroshock seizure (MES) test [25]. Percentage of impairment was calculated as (180 − latency to fall)/180 × 100 and percentage of variation was calculated as (experimental value × 100/vehicle mean) − 100.

Rotarod test

Motor coordination assessment was performed as described before [26]. NMRI mice (n = 7–10 per group) were placed on an accelerating rotarod (Ugo Basile, Gemonio, Italy) one day before test trial on a rod rotating at a speed of 15 rpm for a period of 180 s (habituation trial). If mice fell off during this period they were replaced on the rod. On test trial, mice were placed on a rod rotating at a speed of 15 rpm. The time that a mouse maintains its balance on the rotating drum was recorded (cut-off = 180 s). The trial was ended when the mouse either falls off the rod, jumps off the rod, or performs a passive rotation (the mouse grasps the surface of the rod and rides around the rod instead of walking on it). Task was performed one hour after CBZ oral (*po*) administration and 20 min post-GBL *ip* administration, as previously described [16].

Activity meter test

Locomotor activity test was performed as described elsewhere [27]. Briefly, NMRI mice (n = 10 per group) were placed in a 40 cm × 40 cm open field arena (San Diego Instruments, San Diego, USA) and the total ambulation and the number of rears were registered. CBZ was administered *po* 40 min before GBL administration. Test was performed for a period of 30 min starting automatically five minutes after GBL *ip* administration, as reported earlier [18].

Telemetric electroencephalogram (EEG) trace monitoring in the mouse

Telemetry EEG method follows that described by Weiergräber et al. [28]. Ten-week-old NMRI mice (n = 6–8 per group) were anesthetized with 75 mg/kg *ip* ketamine (Merial, Lyon, France) and 1 mg/kg *ip* medetomidine (Orion, Espoo, Finland). Eight 10-week-old stargazer mutant mice were anesthetized with 240 mg/kg *ip* avertin (tribromoethanol). A midline 2-cm skin incision on the head and neck was made and the subcutaneous tissue was bluntly separated. A TA11ETA-F10 telemetry device (Data Sciences International, New Brighton, USA) was implanted into a subcutaneous dorsal pocket and leads were coiled into a loop and anchored to surrounding tissue. Two burr holes (0.7 mm in diameter) were positioned with an electric high-speed drill (Foredom Electric, Bethel, USA). Electrodes were shortly bent at the tip and placed