Behavioural Brain Research 225 (2011) 373–376

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Vigabatrin has antiepileptogenic and antidepressant effects in an animal model of epilepsy and depression comorbidity

Emilio Russo[a,*], Rita Citraro[a], Francesca Scicchitano[a], Agostina Urzino[a], Rosario Marra[b], Vincenzo Rispoli[c], Giovambattista De Sarro[a]

[a] Chair of Pharmacology, Department of Experimental and Clinical Medicine, School of Medicine, University of Catanzaro, Italy
[b] National Council of Research, Institute of Neurological Science, Catanzaro, Italy
[c] Department of Pharmacobiological Sciences, School of Pharmacy, University of Catanzaro, Italy

ARTICLE INFO

Article history:
Received 22 June 2011
Received in revised form 12 July 2011
Accepted 17 July 2011
Available online 22 July 2011

Keywords:
Anticonvulsant
Absence
Epileptogenesis
SWD
Forced swimming test

ABSTRACT

To evaluate the effects of Vigabatrin (VGB) treatment, on both absence seizure and depressive-like behaviour development in the WAG/Rij rat model of absence seizures. Early long-term treatment with VGB could alter the development of absence pathology, by significantly reducing seizure generation and synchronization in contrast to its pro-absence effects observed after acute or subchronic administration. We have demonstrated the antidepressant effects of a sub-chronic treatment with VGB in both wistar and WAG/Rij rats. In contrast, following an early long-term treatment, VGB antidepressant effects were only observable in WAG/Rij rats. In conclusion, VGB has antiepileptogenic and antidepressant properties in the WAG/Rij rat model despite its pro-absence effects suggesting that epilepsy and depression, in this animal model, are directly related and that seizure development inhibition also reduces the development of depressive behaviour.

Epilepsy is a heterogeneous syndrome characterized by recurrently and repeatedly occurring seizures. Although able to inhibit the epileptic seizures, the currently available antiepileptic drugs (AEDs) have no effects on the process of epilepsy development, known as epileptogenesis [1]. Furthermore, since psychiatric comorbidity such as depressive disorders in epileptic patients has been suggested to be directly related from a neurobiological point of view [2] and that there are only few data regarding the study of behavioural comorbidity in animal models and a lack of information regarding drug use implication, the study of behavioural disturbances has a great potential value [3–5].

We have previously reported that an early long-term drug chronic oral treatment (from 1.5 to 5 months) of WAG/Rij rats, a validated animal model of absence epilepsy, with some AEDs, i.e. ethosuximide, levetiracetam, suppressed the development of absence seizures [5,6]; furthermore, such treatment are also able to influence depressive-like behaviour in this strain [5]. GABA is the major inhibitory neurotransmitter in the brain and has an important role in epileptogenesis and depression [1,7]. Loss of GABAergic neurones [8] and $GABA_A$ receptor mediated inhibition [9] are associated with chronic epileptogenicity following status epilepticus (SE). Some studies demonstrated that the expression of various $GABA_A$ receptor subunits is altered before the development of spontaneous seizures [10–12]. Vigabatrin (VGB) is an AED that specifically increases whole-brain GABA levels through irreversible inhibition of the GABA catabolic enzyme GABA-transaminase [13]. VGB has been demonstrated to induce high and long-lasting enhancement of intra-(synaptosomal) and extracellular concentration of GABA [14,15]. VGB has a different efficacy with specific types of seizures. Clinical data show that VGB is more effective against partial epilepsy than against generalized epilepsy [16]. In animal models, the anticonvulsive potency of VGB varies greatly from low effectiveness in the models of convulsive generalized epilepsy (maximal electroshock, audiogenic seizures) [17,18] to significant protective effect in the kindling model of partial epilepsy [14,19].

It has been demonstrated that VGB tends to aggravate certain seizure types, such as myoclonic jerks and absences [20], suggesting a quantitative GABAergic involvement in the mechanism(s) underlying the starting and stopping of an ongoing spike and wave discharge (SWD) in absence epilepsy. When considering depressive disorders, numerous studies suggest a deficit of the GABAergic system both in humans and animal models [21]; on the other hand, VGB is often associated with an increased risk of depressive disorder development in epileptic patients [22], however, a short term antidepressant activity of VGB in the forced swimming test model of depression was previously demonstrated [23]. Based on the

* Corresponding author at: Department of Experimental and Clinical Medicine, School of Medicine, University of Catanzaro, Via T. Campanella, 115, 88100 Catanzaro, Italy. Tel.: +39 0961 712395; fax: +39 0961 774424.
E-mail address: erusso@unicz.it (E. Russo).

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.07.030

prominent role of the GABAergic system in epilepsy, epileptogenesis and depression, in the present work, we examined the effects of VGB long-term or sub-chronic treatment, on both absence seizure development and onset of depressive-like behaviour in WAG/Rij rats using a forced swimming test (FST).

Male WAG/Rij rats and age-matched Wistar rats were used. Rat progenitors were purchased from Charles River Laboratories s.r.l. (Calco, Lecco, Italia) at a body weight of ~60 g (4 weeks old). Following arrival, animals were housed three or four per cage and kept under controlled environmental conditions (60 ± 5% humidity; 22 ± 2 °C; 12/12 h reversed light/dark cycle; lights on at 20.00 h). Dams of all strains were selected as previously reported [5] and housed 2 per cage, whereas, all offspring after weaning (P21) were housed three or four per cage. Animals were allowed free access to standard laboratory chow and water until the time of experiments. Procedures involving animals and their care were conducted in conformity with the international and national law and policies (European Communities Council Directive of 24th November 1986, 86/609EEC). All efforts were made to minimize animal suffering and to reduce the number of animal used.

Vigabatrin (VGB; Sigma-Aldrich Co. Ltd, Poole, U.K.) was administered orally at a dose of ~100 mg/kg/day dissolving 500 mg in 600 ml of drinking water. Dosage was calculated on the basis of the knowledge that rats drink on average 10–12 ml/100 g/day [5] and was chosen because of the lack of adverse events in previous reports [24].

Two different groups of rat were randomly created for an early long-term treatment and a sub-chronic treatment (5 days), respectively. *Early long-term treatment protocol*: rats ($N = 10$) started treatment at P30 and were kept on VGB for an additional 17 weeks, up to the age of ~5 months; treatment was then stopped and animals were normally housed up to the age of 6 months when they were tested (see below). *Sub-chronic treatment*: in this group, animals ($N = 10$) of both strains were orally administered for only 5 consecutive days at the age of 6 months and then tested 12 h after drug administration cessation. Control animals ($N = 10$) were kept under standard animal house conditions during the same time window of the corresponding treated group. During this period, animals were weighed weekly every Monday between 9:00 and 11:00 a.m.

Surgery and EEG recordings: All WAG/Rij rats at the age of ~6 months were chronically implanted, under chloral hydrate anaesthesia (400 mg/kg i.p.; Carlo Erba, Milan, Italy), using a Kopf stereotaxic instrument, with five cortical electrodes for EEG recordings [25]. Stainless-steel screw electrodes were implanted on the dura mater over the cortex [25]: two in the frontal region (AP = 2; $L = \pm 2.5$) and two in the parietal region (AP = −6; $L = \pm 2.5$). The ground electrode was placed over the cerebellum. All animals were allowed at least 1 week of recovery and handled twice a day. In order to habituate the animals to the recording conditions, the rats were connected to the recording cables, for at least 3 days before the experiments. The animals were attached to a multichannel amplifier (Stellate Harmonie Electroencephalograph; Montreal, Quebec, Canada) by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movements for the animals. All EEG signals were amplified and conditioned by analog filters (filtering: below 1 Hz and above 30 Hz at 6 dB/octave) and subjected to an analog-to-digital conversion with a sampling rate of 300 Hz. The quantification of absence seizures was based on the number and the duration of EEG spike wave discharges (SWDs) [25]. Animals underwent three recording periods, starting at 9.00 am, for 3 consecutive days. Every recording session lasted 3 h without injection of any drug for every group.

The forced swimming test (FST) has been previously used for measuring the immobility time and assess depressive-like behaviour in rodents including WAG/Rij rats [5,26]. We have followed our previously described protocol [5]. Briefly, in the initial 15-min habituation session (excluded from the data analysis), animals were individually forced to swim in a plastic cylinder (47 cm in height and 38 cm in inside diameter) containing 38 cm of water (25 ± 1 °C). The 5-min test session began 24 h later. Test sessions were videorecorded by means of a digital camera (Medi@com Sport Cam Plus M-MDVS; resolution: 640 × 480 – 30 fps; Mediacom, Milan, Italy) fixed above the cylinders; the obtained AVI files were later analysed by three independent observers, two of them blind to the treatment protocol. The duration of immobilization, including passive swimming, was measured. The criterion for passive swimming was floating vertically in the water while making only those movements necessary to keep the head above the water. After the FST, animals were removed and dried with a towel before being placed in their home cages.

All statistical procedures were performed using SPSS 15.0.0 software (SPSS Inc., Chicago, Illinois, USA). EEG recordings were subdivided into 30 min epochs, and the duration and number of SWDs were treated separately for every epoch. Such values were averaged and data obtained were expressed as mean ± S.E.M. Treated animals were compared by one-way analysis of variance (ANOVA), the treatment being the only variable, followed by a *post hoc* Bonferroni test.

The mean immobility time (IT) of Wistar rats at 6 months of age was considered equal to 100% and every other time obtained in the test was reported with respect to this value and expressed as a percentage of it. Immobility times were compared by one-way ANOVA followed by Bonferroni's *post hoc* test with the treatment being the only variable. The data are expressed as means ± S.E.M. All tests used were two-sided and $P < 0.05$ was considered significant. Any behavioural responses, excluding immobility, were recorded, but not statistically analysed for each animal.

The EEG recordings' analysis of control WAG/Rij rats, at 6 months of age, showed that in this group the mean number of SWDs (nSWDs) for a 30 min epoch was 11.31 seizures with a mean total duration (dSWDs) of 70.54 s and a mean single duration (sSWD) of 5.65 s (Fig. 1). The 5 days sub-chronic treatment with VGB in 6 month old WAG/Rij rats significantly ($P \leq 0.05$) increased every SWDs parameter considered. nSWDs, dSWDs and sSWD, were respectively increased of 65.52%, 86.83% and 17.69% (see Fig. 1) which is in agreement with previous reports [24].

Early long-term treatment with VGB at a dose of ~100 mg/kg/day was able to reduce the development of absence seizures, significantly ($P \leq 0.05$) reducing every SWDs parameter in adult WAG/Rij rats. nSWDs, dSWDs and sSWD, were respectively reduced of 51.81%, 41.52% and 28.85% (see Fig. 1).

In sub-chronic experiments (5 days of treatment), VGB was able to significantly ($P \leq 0.05$) decrease immobility times (IT) in both rat strains (Fig. 1) in comparison to their respective control rats. The video analysis of the forced swimming test (FST) of early long-term treated WAG/Rij rats revealed that they had reduced IT when compared to their controls whereas, VGB early long term treatment in wistar rats had no effects (Fig. 1). Animal growth was not different within groups.

Previous reports [5,6] demonstrated that an early long-term treatment, with selected AEDs, before seizure onset, can significantly modify the development of absence epilepsy, altering the electroencephalographic phenotype of the disease in WAG/Rij rats also influencing the development of depressive-like behaviour in this particular strain. Here, we showed that early long-term treatment with VGB, starting before seizure onset (~1 month of age), could alter the development of absence pathology, by significantly reducing seizure generation and synchronization in contrast to its pro-absence effects observed after acute or subchronic administration according to our results and previous reports [24]. Furthermore, we have demonstrated the anti-depressant effects