Seizure 52 (2017) 35–40

Contents lists available at ScienceDirect

Seizure

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

Full Length Article

Behavioral effects of antiepileptic drugs in rats: Are the effects on mood and behavior detectable in open-field test?

Eva Zimcikova[a,*], Julius Simko[b], Iva Karesova[c], Jan Kremlacek[d], Jana Malakova[c]

[a] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Kralove, Charles University, Heyrovskeho 1203, 50005 Hradec Kralove, Czech Republic
[b] Department of Neurology, Faculty of Medicine and University Hospital, Charles University, Sokolska 581, 50005 Hradec Kralove, Czech Republic
[c] Institute of Clinical Biochemistry and Diagnostics, Faculty of Medicine and University Hospital in Hradec Kralove, Charles University, Sokolska 581, 50005 Hradec Kralove, Czech Republic
[d] Institute of Pathological Physiology, Faculty of Medicine in Hradec Kralove, Charles University, Sokolska 581, 50005 Hradec Kralove, Czech Republic

ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form 11 September 2017
Accepted 19 September 2017
Available online xxx

Keywords:
Antiepileptic drugs
Open-field test
Behavior
Anxiety

ABSTRACT

Purpose: Behavioral side effects of antiepileptic drugs (AEDs) are common including both positive and negative effects on mood, anxiety, depression, and psychosis. We aimed to evaluate behavioral patterns in rats after administration of lamotrigine, levetiracetam, phenytoin, topiramate, carbamazepine, gabapentin, pregabalin, and zonisamide.
Methods: The open-field test was performed and locomotion, rearing, grooming, central latency and defecation were recorded over a 5 min interval for each rat (8 rats in each group receiving AED and 16 controls). Kruskal-Wallis nonparametric test or ANOVA were used to assess differences among the groups.
Results: The experimental groups did not differ in latency to enter the center compartment, neither in the decline of locomotor activity in the 1st and the 5th minute of the observation, nor in number of rears. Significant differences among groups were observed in the total number of lines crossed, grooming, as well in the number of fecal pellets. Locomotor activity was significantly increased in lamotrigine, if compared with gabapentin and pregabalin (ANOVA; $p < 0.05$). Rats exposed to topiramate displayed a significantly increased number of grooming (when compared to pregabalin: $p < 0.01$). Defecation (the number of fecal pellets) significantly increased in the gabapentin and carbamazepine group.
Conclusion: There are significant differences between AEDs in terms of their behavioral profile. It is of great importance to evaluate these effects in clinical practice to bring more clear insight into these positive or negative side effects of AEDs.

1. Introduction

The effects of antiepileptic drugs (AED) on mood and behavior have not been fully understood so far. It is difficult to distinguish which psychopathological manifestations are induced by drug therapy, or perhaps are due to other factors affecting the patient. Psychiatric disorders in epilepsy are frequent and in many cases of multifactorial origin; AEDs constitute only one of the possible causes [1]. It has been suggested that seizures and psychiatric disorders may share an underlying brain abnormality, or may be causally related. Furthermore, the reason of comorbid psychiatric disorders observed in many patients with epilepsy may be iatrogenic [2]. In fact, both positive and negative behavioral effects have been associated with various AEDs [3].

The open-field test (OFT) provides a popular animal model of anxiety-like behavior and permits the evaluation of drug related effects on different aspects of animal behavior. The first exposure to the OFT can be used to assess the effects of various drugs on the levels of anxiety as well as on non-specific effects of drugs on the locomotor activity. Upon repeated exposure to the OFT, animals quickly habituate to the OF and their locomotion tend to decrease [4]. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but these are also measures of exploration and anxiety. The high frequency of such behaviors indicates increased locomotion and exploration, and

* Corresponding author.
E-mail addresses: eva.zimcikova@faf.cuni.cz, eva.tlusta@faf.cuni.cz (E. Zimcikova).

http://dx.doi.org/10.1016/j.seizure.2017.09.015
1059-1311/

refers to a lower level of anxiety. Generally, rearing behavior is taken as an index of rodent emotionality (increased frequency of rears being associated with exploratory behavior). The number of central square entries and the duration of time spent in the central square, as well as latency to enter the central square are regarded as indicating measures of exploratory behavior and anxiety. Rodents spontaneously prefer staying in peripheries to activities in the center. Increased time spent in the central part or decreased latency to enter the central part are indications of anxiolysis [5]. Grooming behavior is a displacement response and is expected to be displayed in any novel environment. Grooming can serve to allay anxiety in stress situation. The role of grooming as a behavioral marker of depression has been much less studied, compared to the data on grooming responses to anxiety [6]. Defecation (number of fecal boli) is used as a measure of anxiety as well [7,8].

We aimed to describe behavioral patterns in rats after lamotrigine (LTG), levetiracetam (LEV), phenytoin (PHT), topiramate (TPM), carbamazepine (CBZ), gabapentin (GBP), pregabalin (PGB), zonisamide (ZNS) administration and to compare the behavioral effects of AEDs associated with depressive symptoms (TPM and ZNS) to AEDs with neutral or positive effects on mood, anxiety and depression (LTG, LEV, PHT, CBZ, GBP, PGB).

2. Methods

2.1. Animals

All animals received humane care in accordance with the guidelines set by the institutional Animal Use and Care Committee of Charles University, Prague, Faculty of Medicine in Hradec Kralove, Czech Republic. The protocol of the experiment was approved by the same committee. The experiments used eighty 24-week-old male albino Wistar rats (Biotest s.r.o., Konarovice, Czech Republic) and ended at the age of 36 weeks. The animals were hosted in groups of 4 in plastic cages (size: $60 \times 38 \times 20$). During the experimental period the animals were maintained at controlled conventional conditions (12 h light and 12 h dark, temperature 22 ± 2◦C, air humidity 30—70%). Tap water and standard laboratory diet (SLD; VELAS, a.s., Lysa nad Labem, Czech Republic) or SLD enriched with AED were given ad libitum (besides the test period). The weight of rats was monitored once a week.

Rats weighing 256.3 g ± 17.7 SD (median: 258.5 g; SEM: 1.98) at the beginning of the experiment were divided into nine groups and received either SLD (16 controls) or SLD enriched with one of the following antiepileptic drugs (8 rats in each group receiving AED): lamotrigine (35 mg/25 g of the SLD; Lamotrigine, Glenmark), topiramate (55 mg/25 g of the SLD; Topiramat, Glenmark), levetiracetam (101 mg/25 g of the SLD; Levetiracetam, UCB Pharma), phenytoin (80 mg/25 g of the SLD; Phenytoinum, G.L. Pharma GmbH), carbamazepine (28 mg/25 g of the SLD; Carbamazepinum, G.L. Pharma GmbH), gabapentin (28 mg/25 g of the SLD; Gabapentinum, Gedeon Richter Plc.), pregabalin (12 mg/25 g of the SLD; Pregabalinum, Pfizer Manufacturing Deutschland GmbH), zonisamide (19 mg/25 g of the SLD; Zonisamid, Eisai Ltd.). The amount of SLD given was weighted at the beginning and the rest of SLD that have been left in the cage was weighted at the end to assess how much of SLD the animals ate. The mean amount of SLD eaten by one rat was: in lamotrigine group 38.5 g; topiramate 30.25 g; levetiracetam 28.55 g; phenytoin 28.41 g; carbamazepine 22.71 g; gabapentine 24.07 g; pregabaline 22.42 g; zonisamide 21.29 g. The real AED dose eaten by one rat was: 55.9 mg of lamotrigine; 66.6 mg of topiramate; 115.3 mg of levetiracetam; 90.9 mg of phenytoin; 25.4 mg of carbamazepine; 27.0 mg of gabapentine; 10.8 mg of pregabaline; 16.2 mg of zonisamide.

The open-field test was performed at the age of 36 weeks.

At the end of the experiment, the 36-weeks old animals were sacrificed via blood withdrawal from the abdominal aorta under ether anaesthesia, and the serum obtained from the samples was aliquoted and stored at −80◦C for subsequent biochemical analyses. LTG, LEV, GBP, PGB, ZNS concentrations in the samples were determined using a modified high-performance liquid chromatography method with ultraviolet [9,10] or mass spectrometry (MS) detection [11,12]. Topiramate concentrations were determined using modified gas chromatography with MS detection [13]. Cobas Integra 400 Plus (based on Fluorescence Polarization Immunoassay) was used to measure PHT and CBZ concentrations using commercial kits provided by Roche.

2.2. Open-field test

The open field apparatus was constructed of wooden box and measured 60×60 cm with 60 cm walls. The entire apparatus was painted black except for the floor in white. The lines divided the floor into sixteen evenly spaced squares (15×15 cm). The central part consisted of 4 squares in the center of the apparatus. In the novel test situation, each animal was placed in the bottom right corner of the test apparatus and videotaped for 5 min using a video camera located 100 cm away from the arena. During each interval between the phases of experiments the arena was cleaned with cotton soaked in 70% alcohol. All videotapes were analyzed by experimenter. For each rat the following behaviors were recorded:

1. Locomotion: The number of lines crossed by the rat over 5–min interval
2. Rearing: The frequency of rats standing on their hind limbs
3. Self-grooming: rapid cleaning movements of the forelegs towards the face and/or the body. Both complete and non-complete grooming interrupted at some point along the body were counted together. (A typical complete grooming bout starts with the rat scratching its face, progressively moving down along the body and terminating with the tip of the tail.)
4. Fecal pellets: The number of fecal pellets excreted by each individual rat
5. Central latency – the time delay to enter the central part of the apparatus (i.e. the four squares in the center of the apparatus). An entry into the central part was scored when the rat placed two front paws and the head in the respective area. Failure to enter the central part resulted in a latency score of 5 min for each measure.

Locomotor activity in the first and the fifth minute of observation was compared to determine if reactions to the environment decreased.

2.3. Statistical analysis

Statistical analysis was performed using R software version 3.2 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria), with “nortest” package. Kruskal-Wallis nonparametric test assessed differences among the groups in case data were found to have non-normal distribution in Anderson-Darling test, and ANOVA for normally distributed data. To compare variables with a significant inter-group variability the Wilcoxon rank-sum test or *t*-test were used for data with non-normal or normal distribution, respectively. The returned *p*-values were adjusted using the false discovery rate for multiple comparisons. The results were presented as median and the 25th and 75th percentiles. The level of statistical significance was preset to $p < 0.05$.