ACUTE VIGABATRIN-PHENOBARBITONE-INTERACTION ON EXPLORATORY BEHAVIOUR OF RATS

Fathi M. Sherif Abudalla S. El-Hwuegi Eva Kumlien

Summary

Vigabatrin (gamma-vinyl GABA) is an irreversible inhibitor of the enzyme GABA-transaminase (GABA-T) which is responsible for the catabolism of the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the brain. Vigabatrin causes a several fold increase in the levels of brain GABA. The current study investigated further the effects of acute treatment with vigabatrin (100 mg/kg, i.p.) & phenobarbitone sodium (20 mg/kg, i.p.), alone and in combination, in two rat behavioural models of exploratory activity: the elevated plus-maze model of anxiety and the open field test of locomotor activity. A single injection of vigabatrin or phenobarbitone alone, produced anxiolytic effects in the elevated plus-maze test and increased locomotor activity in the open field test. In contrast, after the concomitant administration of both drugs, the anxiolytic effects were no longer produced in the elevated plus-maze. The increased locomotor activity was also diminished in both tests of exploratory behaviour. These results shed light on the GABA hypothesis of anxiety, insofar as the increased availability of GABA, resulting from either GABA-T inhibition (vigabatrin) or facilitation of GABA-mediated chloride channels (phenobarbitone), seems to result in an increased emotional reactivity which, however, subsequently disappears during combined treatment.

Keywords

Anxiety, elevated plus-maze test, exploratory behaviour, GABA-T, interaction, phenobarbitone sodium, open-field test, vigabatrin.

Introduction

In the mammalian central nervous system (CNS), gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter[1]. Modulation of GABA-ergic activity can influence CNS events in a number of ways. Thus a reduction in GABA availability has been implicated in a variety of neurological and psychiatric disorders[2] including epilepsy[3], anxiety and depression[4]. In the brain, GABA is metabolised by a transamination reaction catalysed by the enzyme GABA-transaminase (E.C. 2.6.1.19; GABA-T) (for review, see Sherif, ~). Thus, agents capable of enhancing GABA-ergic inhibition via the $GABA_A$ receptor have therapeutic uses as anxiolytics and anti-convulsants[6]. Vigabatrin (gamma-vinyl GABA) is a selective irreversible inhibitor of GABA-T[7], causing a rapid dose-dependent increase in brain GABA levels[8]. Effective central inhibition of GABA-T was demonstrated following peripheral administration of vigabatrin[7,9]. Clinically, vigabatrin is demonstrated to have a consistent anticonvulsant effect in patients with chronic drug-resistant epilepsy[10].

Barbiturates belong to another class of drugs that are still used in the management of grand-mal epilepsy[11]. Until the discovery of benzodiazepines in 1960s, barbiturates were the drugs of choice in the treatment of anxiety[12]. However, some barbiturates (phenobarbitone) have specific anticonvulsant and anxiolytic effects, which do not seem to be a reflection of non-specific central nervous system depression, and these have a continuing clinical use [12]. There is a substantial evidence of a specific interaction of barbiturates and the GABA system. Thus, barbiturates bind onto a specific recognition site on the $GABA_A$

receptor chloride complex[13]. Stimulation of the barbiturate receptors leads to an increase in the binding of GABA to its receptor with a consequent increase in the inhibitory effect[12].

Previously, Sayin *et al.*[14], Sherif and Oreland[15] and Sherif *et al.*[16] reported that vigabatrin has an anxiolytic-like effect in the elevated plus-maze model of anxiety in rats. The elevated plus-maze model is based upon an unconditioned aversion to heights and open spaces[17], and was validated for rodents[18,19]. The validation of this test has also been reported in Pellow and File[20], showing that it can detect the activity of non-benzodiazepines anxiolytics, and of several putative anxiogenic compounds. In a recent communication, pre-treatment with vigabatrin significantly potentiated the sleeping time of barbiturates in rats[21]. Therefore, the aim of this study was to further explore the interaction between vigabatrin and phenobarbitone on rat exploratory behaviour using the elevated plus-maze test and open-field behaviour. Doses of the compounds chosen for the present investigation have been selected on the basis of the previous Studies[16,21].

Method

Adult male albino Wistar rats weighing 302.0±22.9 gram (Mean ± S.D.) at the beginning of the experiments were used in this study. They were inbred at the Central Animal Laboratory at the Medical University, Tripoli, Libya. The animals were housed in a standard laboratory cages (55 x 33 x 20 cm), in a group of four per cage. They were maintained under a 12 h reversed light cycle (lights off 7.00 a.m.) in a temperature-controlled environment (22 ± 2°C). Rats were housed in these conditions for one week before behavioural testing. Food (pellets containing essential nutrients) and tap-water were freely allowed. Measurements of exploratory behaviour have been conducted during the dark phase of the light cycle in a dimly illuminated laboratory between 10.00 a.m. and 1.00 p.m.

Vigabatrin (gamma-vinyl GABA) has been obtained from Astra-Pharmacia Pharmaceutical Company, Uppsala, Sweden, as a gift and phenobarbitone sodium was purchased from (BDH Chemicals Ltd Poole England).

The elevated plus-maze apparatus was made of wood, consisted of two opposite open arms (50 x 10 cm) without side walls, and two opposite enclosed arms of the same size, with side walls and end wall (40 cm in height) The arms were connected from the central platform (10 x 10 cm), forming the shape of a plus sign, and each arm was divided by lines into three equal squares[15]. The maze was elevated to a height of 50 cm from the ground.

The open-field apparatus was a simple square arena (100 x 100 x 40 cm). The arena has been divided into 16 equal squares, thus allowing the observer to record: number of squares visited and number of rearings, during a 4 minute time period[15].

The rats were randomly assigned into four groups as follows: the control group received only physiological saline (i.p., $n = 8$), the phenobarbitone group received phenobarbitone sodium in a dose of 20 mg/kg (i.p., $n = 8$), 30 minutes prior to testing, and the vigabatrin group received 100 mg/kg vigabatrin (i.p., $n = 8$), 3 hours prior to the testing, and the fourth group ($n = 8$), was treated with 20 mg/kg phenobarbitone sodium, 2.5 hours after vigabatrin pre-treatment (1~0 mg/kg, i.p.). Thirty minutes. later, the activity was recorded in the elevated plus-maze and open field apparatus.

For measuring anxiolytic effects of the drugs in the elevated plus-maze, the animal was gently taken from the home cage and placed in the center of the plus-maze apparatus, with its head facing the closed arm, and observed for a period of 4 min. As traditionally employed, the key indices of anxiety in this test are the proportion of open arm entries and proportion of time spent on the open arms[19]. The following parameters were recorded: time spent, number of squares crossed and number of entries into open and closed arms. The behavioural parameter was considered on a certain arm when the animal had placed all of its four feet on that arm.

For measuring the locomotor activity in the open-field test, the rat was gently placed (after the plus-maze testing) in the center of the apparatus and observed for a period of 4 minutes. The apparatus can differentiate the vertical locomotor activity - rearing activity from the horizontal locomotor activity - ambulatory activity (number of squares visited with all of its four feet on the square).

Statistical analysis

Analysis of variance (one-way ANOVA) and subsequent *post-hoc* analysis with the Fisher's PLSD test were performed in order to detect any difference between means of the control and individual groups.

Results

In Table 1, analysis of the data by ANOVA revealed significant differences between the groups of rats, treated with phenobarbitone and vigabatrin alone, and in combination, in time spent on ($F = 7.96$; $P < 0.0001$) and number of entries into ($F = 3.45$, $P < 0.05$) open arms of the elevated plus-maze model of anxiety. Further analysis with the Fisher test indicated that treatment with a single dose of phenobarbitone sodium (20 mg/kg) produces a significant increase in the time spent on the open arms ($P < 0.005$) and a significant decrease in the time spent on the closed arms ($P < 0.01$) of the maze in comparison with control rats (Table 1). The single dose of vigabatrin (100 mg/kg) produced effects similar to that of phenobarbitone in increasing the time spent on open arms ($P < 0.01$) and in decreasing the time spent on closed arms ($P < 0.005$) of the maze. Similar observations have also been found in the number of open arm entries (Table 1). However, pre-treatment with vigabatrin completely abolished the effect of phenobarbitone on the open and closed arms of the plus-maze. Thus, rats receiving both treatments showed no difference from the control group with regards to the time spent and number of entries into open and closed arms of the elevated plus-maze test (Table 1). The analysis of the data with ANOVA has also revealed significant changes in the percentage of time spent on open arms per total time ($F = 7.51$: $P < 0.005$) and in the number of open arm entries per total arm entries ($F = 4.33$; $P < 0.01$) between the groups (Table 1). Further analysis with Fisher test indicated that the percentage of time spent on the open arms per total arms is significantly increased in the groups of rats treated with phenobarbitone ($P < 0.005$) and vigabatrin ($P < 0.01$) alone, however, with no change in the rats receiving both treatments. With regard to the percentage of open arm entries per total entries, similar increases were observed after phenobarbitone ($P < 0.005$) and after vigabatrin ($P < 0.005$) treatments, however, without a change in rats which received both treatments in comparison with control rats.

The effects of the treatment with phenobarbitone and vigabatrin alone, and in combination, on line crossings on the arms of the maze are also shown in Table 1. Thus, significant changes in the line crossings on the open ($F = 3.62$; $P < 0.05$) and closed ($F = 3.42$; $P < 0.05$) arms of the elevated plus-maze test were found after the treatments. Further statistical analysis indicated a significant increase of these measures after treatment with phenobarbitone or vigabatrin in comparison with control group. However, pre-treatment with vigabatrin has abolished the effect of phenobarbitone (Table 1). The locomotor activities of rats, as calculated from the plus-maze data, for groups of phenobarbitone and vigabatrin alone and in combination are also given. Thus, a significant difference in the total number of arm entries ($F = 3.44$, $P < 0.05$) was observed. Treatment with phenobarbitone and vigabatrin alone significantly increased the total number of arm entries ($P \sim 0.01$ and $P < 0.01$, respectively), however, rats which received combined treatment did not show any significant difference from the control rats.

Group	Control	Vigabatrin	Phenobarbitone	Vig. + Phenobarb.
Time spent on				
open arms	11.3 ± 6.16	29.88 ± 5.76**	39.38 ± 5.52***	10.25 ± 4.42
closed arms	200.87 ± 12.87	113.88 ± 12.57***	145.75 ± 14.84**	192.63 ± 15.82
Lines crossed on				
open arms	1.50 ± 0.96	4.5 ± 0.43**	6.38 ± 1.5**	2.38 ± 1.02
closed arms	9.63 ± 2.61	15.88 ± 1.19*	19.50 ± 4.36*	9.13 ± 2.52
No. of entries into				
open arms	1.63 ± 0.82	4.25 ± 0.53**	5.13 ± 0.74***	2.25 ± 1.03
closed arms	3.63 ± 0.82	5.25 ± 0.82	6.38 ± 1.73	3.25 ± 0.82
Total no. of arm entries				
	5.0 ± 1.65	9.5 ± 0.98**	12.3 ± 2.72**	5.5 ± 1.68
% time spent on open arms/total time				
	5.9 ± 3.6	21.7 ± 4.0**	23.0 ± 3.5***	5.9 ± 2.8
% open arm entries/total arm entries				
	20.0 ± 6.2	44.2 ± 2.8***	45.6 ± 4.2***	27.3 ± 9.2

Data shown are means ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.005 significantly different from the control.
No statistically significant differences were found between the Vig. + Phenobarb.-treated rats and control rats.

Table 1: Effects of treatment with Phenobarbitone Sodium (20mg/kg, i.p.) and Vigabatrin (100mg/kg, i.p.) alone, and in combination on rat behaviour in the elevated plus-maze model of anxiety.

In Figures 1 and 2, an analysis of the data with ANOVA has indicated significance differences between the treated groups in the number of squares visited (horizontal activity, $F = 4.12$, $P < 0.01$) and number of rearings (vertical activity, $F = 7.45$, $P < 0.005$) in the open-field test. Phenobarbitone alone (20 mg/kg) was able to significantly increase the horizontal ($P < 0.01$) and vertical ($P < 0.005$) activities in comparison with control rats and rats treated only with vigabatrin (100 mg/kg). There were, however, no significance differences between the control rats and rats treated with vigabatrin, with regard to the horizontal and vertical activities (Figures. 1 and 2). Pre-treatment of rats with vigabatrin has completely abolished the stimulatory effect of phenobarbitone on the locomotor activity in the open field behaviour.

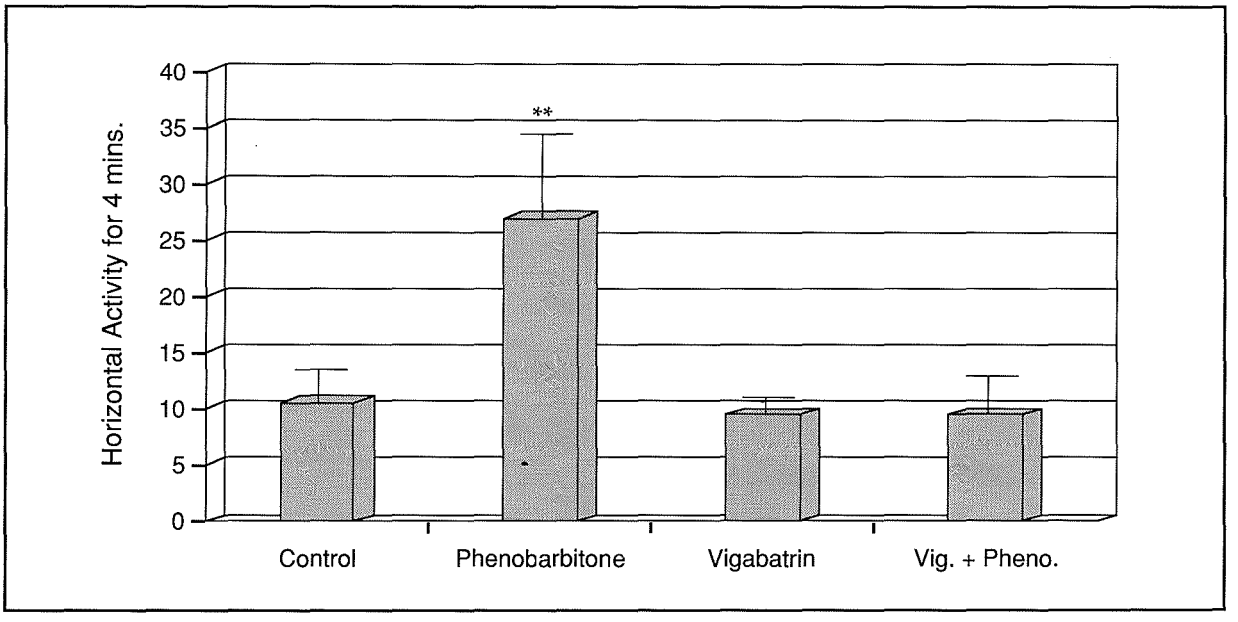

Figure 1: Effects of administration of phenobarbitone sodium (20mg/kg, i.p.) and vigabatrin (100mg/kg, i.p.) alone, and in combination on rat horizontal activity in the open-field test. Data are means ± S.E.M., **P < 0.01 significantly different from the control group by Fisher's PLSD test.

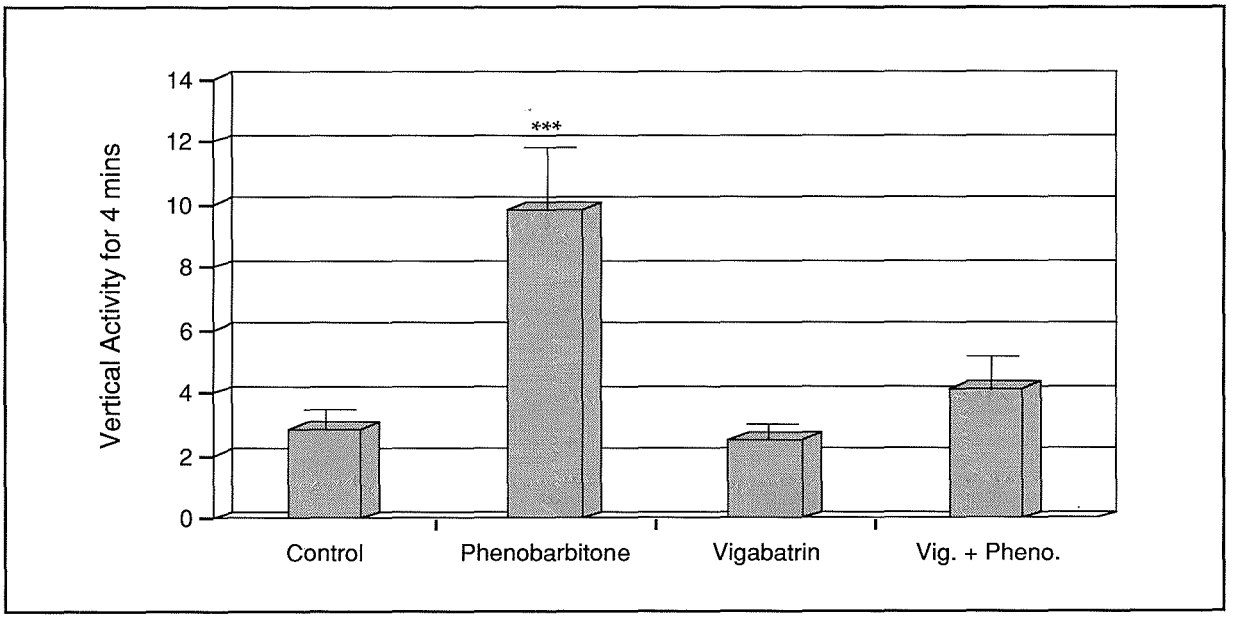

Figure 2: Effects of administration of phenobarbitone sodium (20mg/kg, i.p.) and vigabatrin (100mg/kg, i.p.) alone, and in combination on rat vertical activity in the open-field test. Data are means ± S.E.M., ***P < 0.005 significantly different from the control group by Fisher's PLSD test.

Discussion

Our present results are consistent with a number of reports[12,14,15,16] showing that vigabatrin and phenobarbitone alone produced a release of the suppressed behaviour induced by the aversive stimuli of the open arms in the elevated plus-maze model of anxiety, as indicated by the increase in time spent and in the number of entries into open arms of the maze. Recently, the elevated plus-maze test and open-field behaviour have extensively been used to investigate the exploratory behaviour in rodents[22]. The role of GABA in anxiety has been suggested by findings that anxiolytics agents such as the benzodiazepines and barbiturates bind to specific recognition sites on the GABA-chloride complex (see Introduction). Because barbiturates and GABA-T inhibitors act postsynaptically to enhance GABA-ergic neurotransmission, cross generalization between these classes can be taken as evidence that GABA-ergic mechanisms are involved in the transduction of their anxiolytic and exploratory behaviour. However, the major finding presented in this study was that pre-treatment of rats with vigabatrin abolished the anxiolytic action of phenobarbitone sodium in the elevated plus-maze model of anxiety (Table I) and the increased locomotor activity in the open-field test (Figs 1 and 2). The anxiolytic effect of vigabatrin has also disappeared in phenobarbitone-treated rats. This behavioural result suggests that another neurotransmitter system may be involved or GABA has a dual effect against anxiety and exploratory behaviour. At low levels of GABA-ergic activity (under the effect of either vigabatrin or phenobarbitone) GABA would inhibit the anxiogenic pathways, while at high levels of the activity (under the effect of combined treatment), GABA inhibits anxiogenic and anxiolytic pathways in the brain resulting in masking of the anxiolytic effects. Previously, indirect role of GABA in anxiety by inhibiting the anxiogenic noradrenergic pathway to the *locus coeruleus* and serotonergic neurons in the dorsal raphe has also been suggested[23]. It is possible, therefore, that the dual effect of GABA reported in this study might have come from a non-specific effect of GABA.

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The Authors

This article is an original work presented by Dr. Fathi Mohamed Sherif B. Pharm., PhD. (Sweden). Dr. Sherif is the Chairman of the Department of Pharmacology, Faculty of Pharmacy, Al-Fateh Medical University, Tripoli, Libya. Dr. Abudalla Saleem El-Hwuegi is Professor of Pharmacology at the same department. Dr. Eva Kumlien M.D., PhD. is a neurologist at the Department of Neurology, Uppsala University Hospital, Uppsala, Sweden.