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Anxiolytic-Like Effect of the GABA-Transaminase Inhibitor Vigabatrin (Gamma-Vinyl GABA) on Rat Exploratory Activity

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SHERIF, F., J. HARRO, A. EL-HWUEGI AND L. ORELAND. *Anxiolytic-like effect of the GABA-transaminase inhibitor vigabatrin (gamma-vinyl GABA) on rat exploratory activity.* PHARMACOL BIOCHEM BEHAV **49**(4) 801-805, 1994.—Vigabatrin (gamma-vinyl GABA, GVG) is an irreversible inhibitor of GABA-transaminase (GABA-T). This study addressed the question of whether or not the inhibition of GABA-T has an anxiolytic effect in rats. Diazepam (1.5 mg/kg) and GVG (50 and 500 mg/kg) increased the tendency of rats to explore in the elevated plus-maze test, whereas the effect of general locomotor activity was diminished. The sedative effect of GVG (500 mg/kg) was more pronounced 6 h than 2 h after IP administration. The present findings suggest that even a partial inhibition of GABA-T results in a reduction of anxiety measures in a novel environment.

Gamma-vinyl GABA Vigabatrin GABA-transaminase Elevated plus-maze Open field Exploratory activity Anxiety

GAMMA-AMINOBUTYRIC ACID (GABA) is the major inhibitory amino acid neurotransmitter in the mammalian brain [see (20) for review]. GABA is synthesised in the presynaptic nerve terminals by decarboxylation of glutamic acid, the process being catalyzed by glutamic acid decarboxylase (GAD), and released into the synaptic cleft where it activates the postsynaptic GABA receptors. GABA is then taken up by presynaptic nerve terminals and glia. Degradation of GABA is catabolized by GABA-transaminase (GABA-T) into glutamic acid and succinic semialdehyde (13).

There is strong evidence implicating GABA in numerous pharmacological effects of the benzodiazepines anxiolytics. Thus, benzodiazepines supposedly exert their anxiolytic effect by potentiating GABAergic transmission through their binding at their recognition site on the $GABA_A$ receptors (5). Accordingly, elevation of GABA levels in the brain should also produce anxiolysis. However, behavioural studies have not shown consistent anxiolytic-like effects of GABA receptor agonists in conditioned conflict procedures [see (4) and references therein]. More recently, emphasis has been put on the behavioural tests of anxiety using spontaneous behaviour. In the social interaction test and the elevated plus-maze test, several $GABA_A$ receptor agonists showed an anxiolytic profile (4). Furthermore, aminooxyacetic acid (AOAA) and valproic acid, inhibitors of GABA-T, displayed similar anxiolytic-like properties. Thus, it seems that even endogenous GABA can exert an anxiolytic action, further concentrated when its catabolism is slowed down. Unfortunately, neither AOAA nor valproate are pharmacologically pure GABA-T inhibitors (14,25).

Vigabatrin (gamma-vinyl GABA, GVG) is a highly selective catalytic irreversible suicide inhibitor of the enzyme GABA-T [see (19,21,22) for reviews]. Because GVG is a structural analogue of GABA, the enzyme accepts this agent as a

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substrate. GVG is converted into a reactive intermediate, which binds covalently to the active site of the enzyme, resulting in irreversible inhibition. This in turn causes a dose-dependent increase in GABA, confirming GABA-T inhibition in animal brain tissue (12). Because a deficiency in the GABAergic system has been implicated in epilepsy (15,26), manipulation of the GABA system has been extensively explored as a possible treatment for epilepsy. GVG has been shown to have an anticonvulsant effect in various experimental epilepsy models, such as audiogenic, strychnine-induced seizures, and photogenically induced seizures in rodents (16,24). In a number of controlled clinical studies, a significant reduction in seizure has also been shown (1,2).

Recently, the effect of vigabatrin on rat exploratory activity was examined (23). Vigabatrin was found to produce anxiolytic-like effects in the elevated plus-maze test at a rather high dose of 1 g/kg, whereas it was sedative at a lower dose in two other tests based on exploratory activity. In this article, we have further characterized the effect of vigabatrin on the rat behaviour in exploratory activity tests.

METHOD

Animals

Adult male Wistar rats (inbred at the central animal laboratory at the Medical University, Tripoli) weighing 238.1 ± 2.3 g were used in the experiments. The animals were housed in groups of four at 24 ± 2°C in a room with a reversed 12 L : 12 D schedule (lights off at 0600 h). Rats were habituated to the housing conditions for 2 weeks before testing. Rats were allowed access to tap water and food (pellets containing essential nutrients) ad lib. All experiments were carried out during the dark phase between 0900 and 1500 h in a dim light during June, 1993.

Drugs

Vigabatrin (GVG, Merrell Dow, USA) was dissolved in saline. Diazepam (Hoffmann-La Roche, Switzerland) was suspended in a vehicle of 0.9% saline with few drops of Tween 85 (Sigma), yielding an approximately 1% solution of Tween, which was also used as a vehicle.

Apparatus

The elevated plus-maze was made of wood, with two opposite open arms, 50 × 10 cm, and two opposite closed arms of the same size but enclosed by 40-cm-high side walls and end wall. The arms were connected by a central platform and thus the maze formed a plus-sign. Each open arm was divided into three equal squares. The maze was elevated to a height of 50 cm from the ground.

The open field was a square arena 100 × 100 cm with 40-cm-high side walls. The arena was divided into 16 equal squares.

Spontaneous locomotor activity was measured in a plastic square open field arena (43 × 43 × 30 cm, similar size compared to the home cage) by an automatic device (Opto-Varimex-3, Columbus Inst. Int. Corp., Columbus, OH).

Procedure

Rats were randomly assigned into groups as follows: the control groups received only saline IP (n = 24) and GVG groups (each n = 8) were injected with 50, 100, 250, and 500 mg/kg, IP, respectively, and tested 2 h (or 6 h) following injection. Another group of rats (n = 8) was given 1.5 mg/kg diazepam IP and tested 30 min after injection.

The elevated plus-maze test was conducted as described by Handley and Mithani (7) with some modifications (9). A rat was taken from the home cage and was placed at the central square of the plus-maze with its head facing a closed arm. During 4 min, the following measures were recorded: number of entries into open and closed arms, time spent on open and closed arms, and number of squares crossed in the open arms. An arm entry was counted when the animal had placed all of its four paws on it.

In the open field test, an animal was placed at the centre of the apparatus for 4 min. Parameters registered were the number of squares visited (with all four feet on one square) and the number of rearings.

For testing the locomotor activity in Opto-Varimex-3, each rat was placed alone at the centre of the test cage and its activity was scored over a 10-min period with a multichannel counter. The variables recorded for each animal were distance travelled (ambulation), number of individual movements, time spent in ambulation, and time spent in rearing.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to evaluate the data. Post hoc comparisons by Fisher's PLSD test were used to compare group means with the control. Data are expressed as the mean ± SEM.

RESULTS

The effects of treatment with GVG and diazepam, respectively, on the behaviour of rats in the elevated plus-maze are shown in Table 1. Drug treatment had an overall effect on the time spent in the open arms ($F = 3.83, p < 0.01$), number of crossed squares in the open arms ($F = 3.36, p < 0.01$), number of entries into the open arms ($F = 5.20, p < 0.001$), the total number of entries ($F = 5.77, p < 0.001$), and the ratio open/total arm entries ($F = 2.89, p < 0.05$). Post hoc Fisher's PLSD test revealed that diazepam (1.5 mg/kg) treatment significantly increased all the measures except the total number of entries. GVG at the dose of 50 mg/kg induced a significant increase in all measures of behaviour. Also, at 500 mg/kg, GVG elicited a significant increase in the time spent and in the number of squares crossed in the open arms of the plus-maze. In the control group, significant positive correlation between the number of open and closed arm entries was observed ($r = 0.73, p < 0.001$). However, this correlation did not persist for all drug treatment groups (Table 1).

The open/total arm entries ratio is presented is Table 1 in two ways: first, as calculated using the open/total arm entry ratios of individual animals, and second, as a ratio between group means of open and total arm entries. As it can be readily seen, in some groups, a considerable difference exists between the values obtained. As an example, in the GVG 500-mg/kg group, the apparent percentage of open arm entries out of the number of total arm entries is 34%; however, in fact it was 56%. If an animal does not make any open arm entries, the number of entries into closed arms does not influence the ratio value. Therefore, because the GVG 500-mg/kg group consisted of rats that either made many open arm entries or looked sedated and made no open arm entries and only one closed arm entry, the ratios calculated in the conventional way (ratios of individual animals) differ from the actual situation.

The effect of GVG 500 mg/kg 2 and 6 h after the treatment

TABLE 1

EFFECT OF GVG AND DIAZEPAM ON THE BEHAVIOUR OF RATS IN THE ELEVATED PLUS-MAZE

	Time Spent on Open Arms	Crossed Squares on Open Arms	Number of Entries Into the Open Arms	Total Number of Entries	A	B	C
Control	13 ± 3.2	2.4 ± 0.6	1.6 ± 0.3	5.9 ± 0.8	21 ± 4	27	0.73*
Diazepam	47 ± 12.0†	5.3 ± 1.3†	3.9 ± 0.9†	7.4 ± 1.3	46 ± 8‡	53	0.64
GVG-50	60 ± 11.6*	5.6 ± 0.7‡	5.5 ± 0.7§	11.6 ± 1.0§	47 ± 4‡	47	0.02
GVG-100	15 ± 8.0	1.9 ± 1.3	1.6 ± 1.1	4.6 ± 1.3	20 ± 9	35	0.40
GVG-250	26 ± 12.5	1.6 ± 0.7	1.6 ± 0.7	4.4 ± 1.2	23 ± 9	36	0.80†
GVG-500	44 ± 21.2†	5.3 ± 1.3†	2.0 ± 0.9	3.6 ± 0.9	34 ± 14	56	0.10

Values are expressed as mean ± SEM. Statistical analyses used were as follows: one-way ANOVA for over-all difference between groups and Fisher's PLSD test for the difference between individual groups.

A is a percentage entries of open entries per total entries by individual measuring. B is a percentage entries of open entries per total entries by mean measuring. C is a correlation between the number of open and closed arm entries.

*†‡§Compared to the control group: *$p < 0.001$, †$p < 0.05$, ‡$p < 0.01$, §$p < 0.0001$.

on the activity of rats in the open field test is shown in Fig. 1. ANOVA indicated an overall effect of GVG treatment that was significant for the number of squares visited ($F = 3.69$, $p < 0.05$), but missed significance for the number of rearings ($F = 3.02$, $p = 0.06$). Thus, GVG (500 mg/kg) significantly decreased the number of square visits in the open field 6 h after IP administration.

Locomotor activity of the rats after GVG (50 mg/kg) and diazepam (1.5 mg/kg) treatment as scored by Opto-Varimex-3 over a 10-min period is shown in Fig. 2. Significant differences between the groups were found in the scores of ambulation distance ($F = 5.60$, $p < 0.01$), ambulation time ($F = 11.88$, $p < 0.001$), and vertical movements ($F = 5.28$, $p < 0.05$). No significant difference was found in the number of individual movements. Both GVG (50 mg/kg) and diazepam (1.5 mg/kg) treatments significantly reduced the scores of ambulation distance and time, whereas only diazepam treatment caused a significant decrease in the vertical activity.

DISCUSSION

The levels of the major inhibitory neurotransmitter GABA in all parts of the brain have been found to be increased several-fold (2–20-fold) after inhibition of the enzyme GABA-T, which is responsible for the degradation of GABA (11). In this study, we have shown that a low dose (50 mg/kg) of GVG exerts behavioural changes similar to diazepam.

Of the three behavioural paradigms used, the elevated plus-maze has been validated for testing the anxiolytic/anxiogenic effects of drugs (18). Routinely, the open/total arm entries ratio is used to assess the effect of a drug on anxiety level, whereas the number of total arm entries serves as a control of general locomotor activity. However, several authors have suggested that it is inappropriate to use the number of total arm entries as a measure independent of anxiety (8,17), because this approach presupposes the invalidated assumption that an increase in the number of open arm entries after anxiolytic treatment is brought about along with a concomitant decrease in the number of closed arm entries. Indeed, a positive correlation between the validated anxiety measures and the total number of arm entries in the plus-maze has convincingly been shown (10). In the present study, we have also shown that a positive correlation exists between the number of open and closed arm entries. Therefore, other behavioural tests must be used in parallel to show that an increase in the plus-maze activity is not due to increase in general locomotor

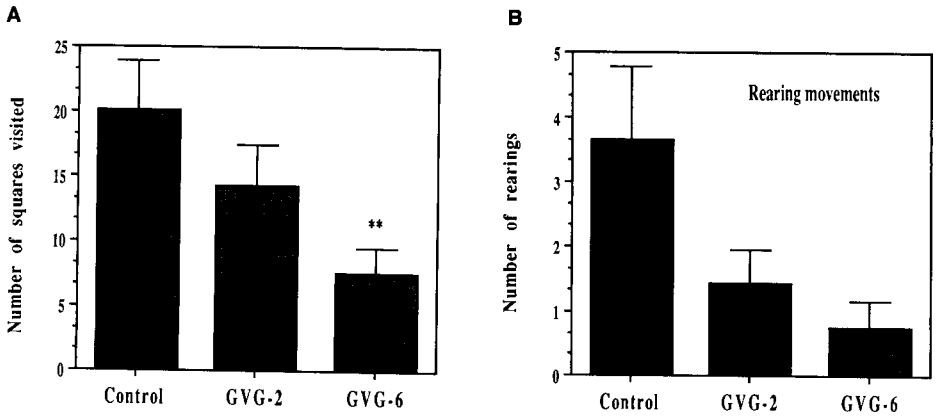

FIG. 1. (a) Effect of GVG (500 mg/kg) administration on the number of squares visited in the open field 2 and 6 h after treatment. **Significantly different from control, $p < 0.01$. (b) Effect of GVG (500 mg/kg) administration on the number of rearings in the open field 2 and 6 h after treatment. No significant difference was found.

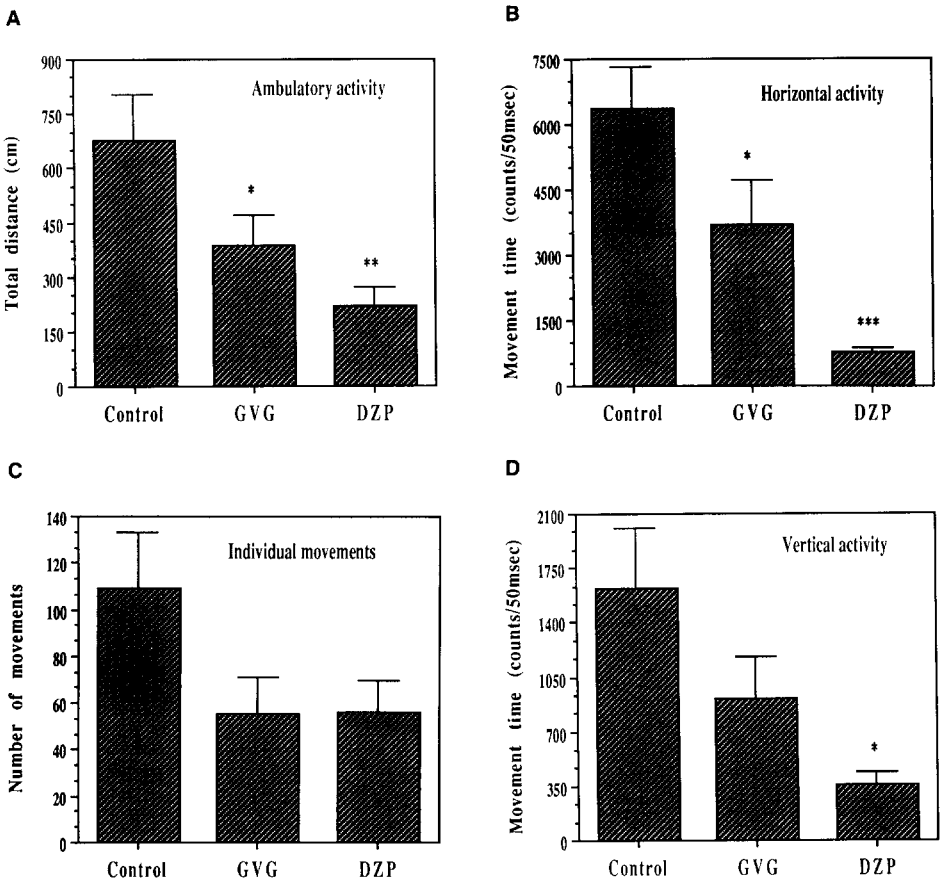

FIG. 2. (a) Effect of treatment with 50 mg/kg GVG and 1.5 mg/kg diazepam on the ambulation distance. Total distance (cm) travelled estimated for 10 min. *Significantly different from control, $p < 0.05$, and **significantly different from control, $p < 0.01$. (b) Effect of treatment with 50 mg/kg GVG and 1.5 mg/kg diazepam on the ambulation time. Movement time (counts per 50 ms) estimated for 10 min. *Significantly different from control, $p < 0.05$, and ***significantly different from control, $p < 0.001$. (c) Effect of treatment with 50 mg/kg GVG and 1.5 mg/kg diazepam on the number of individual movements. No significant difference was found. (d) Effect of treatment with 50 mg/kg GVG and 1.5 mg/kg diazepam on the vertical activity. Vertical activity (counts per 50 ms) estimated for 10 min. *Significantly different from control, $p < 0.05$.

activity. This is best provided in a paradigm that causes minimal fear and has a longer duration than a plus-maze experiment. The automatic recording of animal behaviour in Opto-Varimex-3 in a test field similar to the home cage during 10 min demonstrated that when the environment is relatively neutral and does not promote exploratory drive, GVG (50 mg/kg) actually has a sedative effect.

A separate experiment comparing the effect of a large dose of GVG (500 mg/kg) on the rat open field activity 2 and 6 h after administration shows that 2 h after drug treatment, its effect is not yet completely manifested (Fig. 1). In spite of this, as was shown in Table 1, even the smallest dose of GVG tested (50 mg/kg) had an anxiolytic-like effect on the activity of the rats in the elevated plus-maze test 2 h after administration. Because the accumulation of GABA develops slowly after GVG treatment, reaching peak values 24 h after drug administration (6,12), it seems that only a relatively small increase in brain GABA levels is necessary for an anxiolytic-like effect of GVG. According to Bolton et al. (3), 50 mg/kg of GVG produces less than 20% of GABA-T inhibition 6 h after IP administration. Thus, neophobia in rats can be reduced by relatively minor changes in GABA metabolism.

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