Scientia Pharmazeutica (Sci. Pharm.) **69**, 203–210 (2001)

Antinociceptive Activity of Vigabatrin in Mice After Prolonged Treatment: Possible Development of Tolerance

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Abstract

We have evaluated vigabatrin (γ-vinyl-γ-aminobutyric acid), an irreversible inhibitor of γ-aminobutyric acid (GABA)-transaminase responsible for GABA degradation, for its effects on nociceptive response, changes in spontaneous locomotor activity and body temperature in mice after a prolonged treatment regimen. The mice received vigabatrin 0.26% w/v chronically in drinking water for 7, 14 and 21 days. Changes in locomotion, body temperature and nociception were recorded after 7, 14 and 21 days respectively in different groups. Also, possible withdrawal symptoms were determined up to three days after 7, 14 or 21 days of treatment. In another experiment the animals were given acutely, 250 mg/kg of vigabatrin by oral gavage and the changes in response to the said parameters were assessed 90 min after treatment. Acute treatment increased the latency in the hot-plate reaction time and a highly significant decline in locomotion and body temperature. In contrast to the acute treatment studies, there were essentially no effects of vigabatrin on nociception, locomotor activity or body temperature either on the last day of each treatment or upon withdrawal for the next consecutive three days.

We conclude that the changes in nociception, locomotion and body temperature after acute treatment with vigabatrin are due to neuromediator interactions and a possible direct effect of GABA accumulation. After prolonged treatment tolerance to the pharmacological effects of vigabatrin develop that was evident by no change in nociception, locomotion and body temperature. This may be attributed to the possible failure in the maintenance of GABA pools resulting in a reduction in enhanced GABA release mediated by vigabatrin in acute treatment. Further studies of mechanisms by which vigabatrin tolerance develops to these pharmacological responses are warranted.

Keywords: Vigabatrin, prolonged treatment, tolerance, antinociception, thermoregulation, withdrawal.

Introduction

Vigabatrin is an enzyme-activated irreversible inhibitor of γ-aminobutyric acid -transaminase (GABA-T; EC, 2.6.1.19),[1] the enzyme responsible for degrading of the neurotransmitter GABA. It has anticonvulsant activity in both experimental models and epileptic cases.[2-3] Treatment of experimental animals with vigabatrin increases *in vivo* GABA release from certain areas *i.e.* cerebral cortex, spinal cord, retina and other tissues[1, 4-8] and from mouse embryonic cultured neurons *in vitro*.[9] Also dramatic decreases in frequency in seizures in refractory epileptics are associated with the alterations in central nervous system GABA metabolism.

The evidence for this increase in GABA in human epileptics is not direct, but the rise in GABA concentrations in the CSF is presumed to reflect an increase in the central release of GABA.[10-12] Shin and co-workers[13] used 1500 mg/kg i.p. vigabatrin in rats and showed a lengthening of after discharge

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duration at 8 hours after administration, with a significant reduction of motor duration and after discharge duration up to 3 days. Similar results were obtained by Löscher and colleagues.[14]

Literature on bioavailabilty studies suggests that vigabatrin can raise brain GABA concentrations dose-dependently in animals, including both rodents and primates[2,15-17] and humans.[10,18] These increases varied individually ranging from 2 to 5-fold. Böhlen *et al.*[18] demonstrated that GABA concentrations in rat brain and/or CSF are significantly correlated after systemic administration of GABA-T inhibitors. Jung *et al.*[19,20] and others[10] showed an increase in CSF-GABA after an oral dosage of 1, 2 or 6g of vigabatrin for 3 days and the mean increase in GABA was dose-related, being 68, 108 and 201 pmol/ml respectively after 24 hours of last dosage.

Evidence in the literature has suggested that some neuropsychological conditions including analgesia, mania, Parkinsonian tremor and rigidity, Huntington's chorea, epilepsy and schizophrenia[2,21-23] could be successfully treated with agents that cause an increase in endogenous GABA-pools in CNS. Vigabatrin interferes with the GABA and produces antinociception in the hot-plate test maximum at 4-6 hours of its administration.[24] Induction of antinociception between these periods was correlated to an increase in GABA levels after acute treatment and had already been reported.[19,22] In one study[25] dealing with chronic treatment, tolerance to antiseizure action of vigabatrin in epileptic gerbils had been described. This problem was further explored by Neal and Shah,[6] who demonstrated that chronic treatment with vigabatrin results in a significant decrease in glutamic acid decarboxylase (GAD) activity in cerebral cortex and cord. This tolerance may arise from increased feedback inhibition of GAD with a consequent reduction of presynaptic GABA-pools.[6]

Keeping in view the marked rise in brain GABA concentrations after acute administration[19,20] and its effects in relation to nociception, locomotion and body temperature and possible development of tolerance in a prolonged treatment as seen in its antiseizure action, it was thought of worth to investigate the changes in: a) nociceptive response, locomotor activity and body temperature with vigabatrin given on a chronic basis, and, b) "prolonged" effects of vigabatrin after chronic treatment's withdrawal in mice for nociception, locomotion and thermoregulation.

Experimental

Animal stocks

Swiss albino mice (SWR) weighing 22-27g, bred at Experimental Animal Care Center, College of Pharmacy, King Saud University Riyadh, Saudi Arabia were used. The animal were housed in groups to acclimatize to the laboratory conditions for three days before the start of experiment as for diet, water, temperature (22 ±1°C), relative humidity and light cycle (7:0 a.m. - 7:0 p.m.). Food and water were made available *ad libitum*.

Drug administration procedures

Vigabatrin (Marion Merrell Dow Ltd. Uxbridge, Middlesex, UK) was administered in the drinking water, as reported previously by Raza *et al.*[26] Mice were given vigabatrin solution with daily increments in concentration, in a logarithmic ratio of 0.11. This procedure enables relatively large increases in concentration to be achieved very quickly.

Different treatment groups of mice were given a 0.12% w/v solution on day 1, increased to 0.15%, 0.20% and 0.26% w/v on days 2, 3 and 4 respectively. This was considered that vigabatrin consumption could be increased in this fashion without impairing fluid intake until a concentration in

excess of 0.26% w/v is achieved. Thus in a 21 days study period, 0.26% w/v vigabatrin was given as a drinking water for days 5-21 and was found to be a quick and reliable way to achieve a high intake of drug in the drinking fluid. The daily intake of the animal was recorded and the dose was calculated in the range of 259.0 ± 7.2 (Mean ± SEM) mg/kg/day (Table 1), of the body weight which has already been reported in the literature.[27]

Table 1: Summary of intake of vigabatrin in the drinking water.

Days	Water intake in control group ml/kg/day	Concentration of vigabatrin (%)	Fluid intake in treatment group ml/kg/day	Vigabatrin intake in treatment group mg/kg/day
1	131	0.12	143	175
2	121	0.15	116	145.3
3	138	0.20	93	165.6
4	125	0.26	106	276.5
5-21	108.6 ± 0.88	0.26	99.6 ± 2.76	259.0 ± 7.2

Readings are the mean of eight animal observations in each group.

Test procedures

Three sets consisting of two groups each (8 animals in each group, one control and one treatment) were used to see the changes in responses of mice for nociception, locomotion and body temperature after 7, 14 and 21 days respectively. All the control groups received tap water instead of drug. In the treatment groups the drinking fluid (vigabatrin 0.26%) was substituted with water at the end of each treatment period and the impact of withdrawal on the said parameters was measured after 24, 48 and 72 hours (consecutive three days) of the last treatment.

In another experiment one group of mice was treated with vigabatrin acutely (0.3ml of 21mg/ml/25g animal ≅ 250 mg/kg; P.O.). In comparison a control group was given orally the same volume of tap water. Ninety min after treatments the animals were tested for changes in nociception, locomotion and body temperature.

The nociceptive response of mice, at the end of each treatment period and withdrawal study points was determined by using a hot-plate (Columbus Instruments International Corp., Ohio, USA). The instrument was thermostatically controlled to a temperature of 55±0.5°C fitted with a stopwatch accurate to $^1/_{10}$ of a second. Latency to forepaw lick or jumping off the hotplate was recorded for each animal. Treatment cases where the animal failed to respond the test was stopped maximum at 40 seconds to prevent tissue injury.

The activity of mice was recorded by using activity meters (Columbus Instruments International Corp). The activity cage was equipped with horizontal sensitive bars. The number of horizontal movements made by the animal inside the cage on the sensitive bars were recorded by the processing unit attached to activity cage automatically. Mice in pairs of two were placed inside the activity cage and movements were recorded (counts/2min) for consecutive two minutes at each time point.

Rectal temperature of mice was measured by using a rectal probe (model YSI 400) connected to a digital thermometer (Applex, Pb0331, Panlab, France) which was sensitive to 0.1°C temperature changes giving a stable reading in 15 seconds. All the measurements were made at a controlled room temperature of 22 ± 1°C.

Statistical analysis

Statistical comparisons were made by using analysis of variance (ANOVA) with significance

defined as $P<0.05$. In post-hoc analysis the difference in mean response of the treatment groups was compared statistically with its control group by using Student's *t*-test and limits of error are expressed as standard errors (±SEM).

Investigations and results

Acute vigabatrin treatment of mice resulted in significant increase in hot-plate latency ($P<0.05$) and a significant decline in locomotion ($P<0.01$) and body temperature ($P<0.001$) (Table 2).

Table 2: Effect of acute vigabatrin treatment on the nociception, locomotion and body temperature of mice.

Group No.	Treatment Dose	Mean ± SEM					
		Hot-plate reaction Time (sec)		Locomotion (counts/2min)		Body temperature (°C)	
		Pre-Test	Post-treatment	Pre-test	Post-treatment	Pre-test	Post-treatment
1	Control (tap water)	9.8 ± 0.94	10.6 ± 1.23	322 ± 13.6	291 ± 8.5	36.8 ± 0.15	36.6 ± 0.13
2	Vigabatrin (250 mg/kg)	11.0 ± 1.36	16.6 ± 1.69*	299 ± 31.4	221 ± 16.0**	36.6 ± 0.84	35.1 ± 0.12***

Eight mice were used in each group. Treatments were significantly different at $P<0.05$ (ANOVA). In post-hoc analysis observations in the treatment groups were compared to the control at its respective time points for a particular response. *$P<0.05$; **$P<0.01$; ***$P<0.001$ (Student's t-test). Pretest and post-treatment observations were compared in the same group. $P>0.05$.

Vigabatrin in the drinking water for 7 days did not result in any change in nociception, locomotor activity or body temperature at the end of treatment. There was essentially no effect of vigabatrin with this regimen on its withdrawal for 24, 48 and 72 hours. A comparison of pretest values and on 7th day had no significant differences in all the parameters observed (Table 3).

Table 3: Effect of vigabatrin treatment for 7 days and after withdrawal on the nociception, locomotion and body temperature of mice.

Parameter	**Treatment**	**Pretest**	**Test**	**Withdrawal**		
		Day Zero	**7 days**	**24 hours**	**48 hours**	**72 hours**
Hot-plate latency (seconds)	Control	9.21± 1.26	9.29 ± 1.32	15.14 ± 2.22	12.57 ± 2.14	12.43 ± 0.79
	Vigabatrin	10.15± 1.37	11.43 ± 1.34	14.43 ± 1.76	15.00 ± 2.45	12.14 ± 0.96
Locomotor activity (Counts/2 min)	Control	302.05± 32.1	285.5 ± 30.50	289.75 ± 16.45	241.00 ± 15.02	202.00 ± 6.47
	Vigabatrin	286.25± 27.5	232.25 ± 30.50	231.25 ± 29.64	254.25 ± 11.52	233.25 ± 25.69
Body temperature (°C)	Control	36.82± 0.12	36.74 ± 0.16	36.73 ± 0.16	36.54 ± 0.15	36.39 ± 0.18
	Vigabatrin	36.77± 0.15	36.60 ± 0.18	36.56 ± 0.13	36.53 ± 0.18	36.53 ± 0.20

Each reading is mean ± SEM of eight observations in a group. Treatments were significantly different at $P<0.05$ (ANOVA). In post-hoc analysis treatment and withdrawal mice were compared with the respective control group. $P>0.05$ (Student's t-test). Pretest and post-treatment observations were compared in the same group. $P>0.05$.

Vigabatrin in the drinking water for 14 days did not induce any antinociception either on the last day of treatment or the whole period of withdrawal observation. The same regimen induced a significant ($P<0.05$) decline in locomotor activity at the end of treatment and second day of withdrawal. There were essentially minimal effects on body temperature of mice with this treatment or its withdrawal. A comparison of the pretest and post-treatment results showed a significant decline in locomotion in both control and vigabatrin groups after 14 days (Table 4).

Table 4: Effect of vigabatrin treatment for 14 days and after withdrawal on the nociception, locomotion and body temperature of mice.

Parameter	Treatment	Pretest	Test	Withdrawal		
		Day Zero	14 days	24 hours	48 hours	72 hours
Hot-plate latency (seconds)	Control	9.05± 1.17	10.14 ± 1.77	8.43 ± 1.04	8.57 ± 0.65	8.71 ± 1.23
	Vigabatrin	9.27± 0.99	7.86 ± 0.86	9.14 ± 0.96	7.43 ± 1.67	10.00 ± 2.16
Locomotor activity (Counts/2 min)	Control	288.30± 14.50	199.25 ± 16.68[a]	207.75 ± 27.45	202.75 ± 19.46	184.75 ± 15.48
	Vigabatrin	291.84± 17.7	132.00 ± 21.12*[a]	203.25 ± 15.46	260.75 ± 7.18*	216.75 ± 11.26
Body temperature (°C)	Control	36.8± 0.06	36.66 ± 0.08	36.34 ± 0.22	36.51 ± 0.24	36.64 ± 0.13
	Vigabatrin	36.67± 0.12	36.69 ± 0.10	36.29 ± 0.15	36.61 ± 0.07	36.84 ± 0.06

Each reading is mean ± SEM of eight observations in a group. Treatments were significantly different at $P<0.05$ (ANOVA). In post-hoc analysis treatment and withdrawal mice were compared with the respective control group. *$P<0.05$ (Student's t-test). Pretest and post-treatment observations were compared in the same group. [a]$P<0.05$.

Vigabatrin given in the drinking fluid for 21 days did not induce any significant change in nociceptive response, locomotor activity and body temperature when compared to control values. However, this resistance to any change was persistent after withdrawal for 1, 2 and 3 days. But the variation in response was significant in the hot-plate latency in the control group after 21 days. On the other hand a significant decline in locomotion was observed after 21 days in both control and vigabatrin treatment group when compared to pretest values on day zero (Table 5).

Discussion

The chronic treatment of mice with vigabatrin failed to induce any change in nociceptive response at any of the points tested and even after withdrawal. Similarly these results were further supported by no change in body temperature with minor fluctuations, at any interval tested and at withdrawal. However, a significant decrease in spontaneous locomotor activity was observed both in 14- and 21 days observation period when compared to respective control groups. Literature reports have suggested that spontaneous locomotor activity during exploration decreases across aging.[28] Similarly, age dependet alterations in thermal and mechanical nociceptive threshold are also evident.[29]

There is reasonable evidence that vigabatrin increases GABA level and exerts its anticonvulsant action indirectly acting as a GABA-mimetic and increases GABA release at the nerve terminals in

cerebral cortex[4,5] and mouse embryonic cultured neurons.[9] As such our results on acute treatment study are in agreement with the previous reports.[10,24,30] After prolonged treatment of mice in the present study, the drug's failure to induce any such change in nociception, body temperature and locomotion could be attributed to the development of tolerance.

To our knowledge, there have not been any reports of tolerance to these effects of vigabatrin. However in one study, daily administration of vigabatrin at a dose that effectively provided 60% protection initially resulted in a marked tolerance at 17 days being 13% protection in epileptic gerbil.[25] The evidence is growing in favor of its tolerance. Neal and Shah[6] demonstrated a large increase in GABA release in different areas of the CNS after an acute injection of vigabatrin. This vigabatrin facilitated GABA release was strikingly reduced during chronic vigabatrin treatment. It has also been documented that prolonged treatment with vigabatrin increased GABA levels in the tissue and may effect the balance in the cellular distribution of GABA pools. This redistribution of GABA pools has already been documented.[7] So, these high levels of GABA might outweigh the nerve ending decreased GABA release by prolonged treatment and hence a failure in GABA related responses.

Table 5: Effect of vigabatrin treatment for 21 days and after withdrawal on the nociception, locomotion and body temperature of mice.

Parameter	Treatment	Pretest	Test	Withdrawal		
		Day Zero	21 days	24 hours	48 hours	72 hours
Hot plate latency (seconds)	Control	10.01± 1.26	15.43 ± 1.39[a]	17.00 ± 2.52	17.57 ± 2.90	13.50 ± 3.66
	Vigabatrin	9.87± 1.15	10.86 ± 1.79	21.42 ± 3.03	16.57 ± 3.34	14.14 ± 3.07
Locomotor activity (Counts/2 min)	Control	321± 17.4	270.50 ± 13.58[a]	264.00 ± 19.92	218.75 ± 26.93	235.00 ± 37.90
	Vigabatrin	308± 26.2	233.75 ± 18.01[a]	252.75 ± 11.92	225.50 ± 6.61	192.75 ± 13.52
Body temperature (°C)	Control	36.45± 0.18	36.24 ± 0.20	37.18 ± 0.12	37.20 ± 0.09	36.77 ± 0.08
	Vigabatrin	36.55± 0.16	36.61 ± 0.13	36.93 ± 0.11	37.31 ± 0.07	36.82 ± 0.11

Each reading is mean ± SEM of eight observations in a group. Treatments were significantly different at $P<0.05$ (ANOVA). In post-hoc analysis treatment and withdrawal mice were compared with the respective control group. $P>0.05$ (Student's t-test). Pretest and post-treatment observations were compared in the same group. [a]$P<0.05$.

Another explanation for the development of tolerance (failure to see GABA-ergic responses) is that vigabatrin chronic treatment failed to maintain any increase in GABA stores that was enhanced in early phase of treatment. This may be attributed to a reduction in GABA synthesis during this treatment. It is evident that during treatment with GABA-T inhibitors, the high levels of GABA are attained that reduces the GAD activity in rat, mice and gerbils.[31,32] Feedback inhibition of GABA synthesis might be another explanation for a decrease in GABA levels or its release after vigabatrin chronic treatment.

GABA released into the synaptic cleft is inactivated by uptake systems in the nerve endings and glial cells. The GABA taken up by glia is quickly degraded and does not accumulate there. However,

when GABA-T is withdrawn, the glia can accumulate substantial amounts of GABA and that under the influence of vigabatrin cellular GABA-pools are redistributed.[7] Neal and Shah[6] have also shown that prolonged treatment with vigabatrin may result in glial accumulation of GABA, which outweighs the decrease in nerve ending GABA. This may be due to an increase in tissue GABA contents accompanying a decrease in high-K evoked GABA release from glial cells.[33]

In conclusion our results clearly show that prolonged use of vigabatrin does not induce any change in nociception, locomotion or body temperature of mice with this regimen. It was felt that during prolonged treatment tolerance to its pharmacological effects on locomotor activity, antinociception and body temperature changes develop that was confirmed in this study which failed to alter the response to the said parameters. It is hoped that future research in this line may offer better understanding of underlying mechanisms and thus its better use in different epileptic conditions and other pathological states.

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Received November 15th, 2000
Accepted April 18th, 2001