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Thermoregulatory and In-vivo Anti-inflammatory Effects of Vigabatrin In Rat and Mice

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Abstract

Effects of acute administration of vigabatrin (VGB) that has significant GABA-mimetic properties were studied for its antiinflammatory, antigranuloma effects in rats and thermoregulatory actions in mice. Treatment of rats with VGB (125, 250 and 500 mg/kg, i.p doses) caused a significant and persistent inhibition in the carrageenan induced paw edema. Inhibitory effect at high dose (500 mg/kg, which was about 10-fold of the maximal effective dose 50 mg/kg in humans) was 40-, 41- and 39% of the control at 2-, 3- and 4 hours after the treatment. In cotton-pellet-granuloma study, only the high dose was significantly ($P<0.05$) effective and inhibition in granuloma was 17 and 28% of the control at 250 and 500 mg/kg doses, respectively. In another model, leukocyte migration to the inflamed peritoneal cavity was used as a parameter in rats. In this model, VGB (500 mg/kg, i.p) induced a significant ($P<0.05$) reduction in leukocyte migration to the inflamed peritoneal cavity when administered 30 min before carrageenan. This was comparable to indomethacin (10 mg/kg) that also caused a significant ($P<0.05$) reduction in leukocyte migration. The inhibition in the leukocyte migration was 66 and 61% with VGB and indomethacin, respectively. In thermoregulation studies, the rectal temperature of normothermic mice declined dose dependently. In another part of this study all the doses of VGB induced a significant reduction in body temperature at 45 min following drug administration in yeast-induced hyperpyrexic mice. The hypothermic response diminished after 90 min, 3 hours and 6 hours of treatment at 125, 250 and 500 mg/kg doses respectively and none of the dose showed any change in rectal temperature at 24-hour study point.

The results of the present study indicate that vigabatrin has the potential to induce anti-edema, antigranuloma and leukocyte anti-migratory effects in inflamed peritoneal cavity and reduce the rectal temperature in normothermic as well as hyperthermia-induced mice with acute regimen. These effects are thought to be the result of GABA accumulation, its interaction with PG biosynthesis and other neuromediators.

Keywords: Vigabatrin, inflammation, thermoregulation, granuloma, leukocyte migration.

Introduction

Drugs such as γ-vinyl GABA (vigabatrin, VGB), γ-acetylenic GABA or aminooxyacetic acid increase brain and cerebrospinal fluid concentrations of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, by binding irreversibly to GABA-transaminase (GABA-T).[1-5] Extensive clinical use of VGB has shown that it is potentially effective against different seizure types, especially against complex partial seizures in epileptic cases[6,7] and in experimental models for the treatment of secondary generalized epilepsies and partial epilepsies.[8-12] It has a proven efficacy in adjuvant therapy in patients with refractory epilepsy[13-20]

During the last few years, many investigators have explored the correlation of VGB therapy and central GABA systems in epilepsy, analgesia, mania and certain other disease conditions. It is well known that activation of GABA-ergic system leads to gradual, but significant, changes in the biochemical and functional activity of other neurotransmitter pathways. These multiple actions

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enhance the side-effect potential of GABA-mimetics. However, the multiplicity of action broadens the spectrum of therapeutic activity. Literature has revealed that GABA is involved in temperature regulation,[21] epilepsy,[22-24] mania,[25] analgesia,[26-28] Huntington's chorea and Parkinson's disease,[29] anxiety, sedation and anesthesia[30] and muscle spasticity.[31] It is also evident that GABA-ergic agents have some side effects and, as a consequence, measurable quantities of GABA were found in the peripheral organs and fluids suggesting that this inhibitory neurotransmitter may regulate some functions outside the central nervous system (CNS).[32-34] Other studies have suggested that GABA-ergic mechanism might be connected with some states in experimental animals and humans.[35,36] Evidence is also suggestive of the extensive interconnecting neurons which link the GABA-ergic with the monominergic and serotonergic systems, raising the possibility that analgesic, thermoregulatory and some other effects of GABA could be mediated, at least partially, by the interaction of these systems.

Keeping in view the rise in GABA levels in CNS, peripheral organs and body fluids after treatment with GABA-ergic agents and extensive interconnection of neuromediators and their interactions, the present study was designed to investigate VGB's effects on experimental acute inflammation, granuloma formation and leukocyte migration in rats. Also, the effects of VGB on thermoregulation in normothermic and yeast-induced hyperpyrexic mice were investigated.

Material and Methods

Animals

Swiss albino mice (SWR) weighing 20-25 g and Wistar albino rats, 8-10 weeks old, weighing 175-200 g (bread at Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh) were used. These animals were housed in groups to acclimatize to the laboratory conditions for three days before the start of the experiment as for diet, water, temperature (22±1°C), relative humidity and light cycle (7:0 am to 7:0 p.m.). Food and water were made freely accessible.

Drugs

Vigabatrin (γ-vinyl GABA, VGB) (Marion Merrell Dow Ltd. Middlesex, England), carrageenan sodium and Brewer's yeast (BDH Chemicals Limited, Poole, UK), sodium salicylate (E. Merck, AG, Darmstadt, Germany), indomethacin (Conforted®, Dumex Ltd., Denmark) were used. All the other reagents and chemicals used in this work were of analytical reagent grade. The drug solutions were prepared daily, freshly and the drugs were dissolved in distilled water. The doses of the VGB selected were 125, 250 or 500 mg/kg, which exceed the maximal effective dose in humans by about 2.5, 5 and 10 folds respectively.

Protocols and administration procedures

Carrageenan-induced paw edema in rat

A total of 30 rats divided in 5 groups were injected with 0.1 ml of 1% freshly prepared aqueous suspension of carrageenan (sodium salt) into the hind paw of each rat to produce acute inflammation as described by Winter *et al.*[37] Treatment groups were as follows: Group 1 served as control; Group 2 was injected intraperitoneally (i.p) with indomethacin (25 mg/kg); Groups 3, 4 and 5 were injected with VGB 125, 250 or 500 mg/kg (i.p) respectively, 30 min before carrageenan injection. Changes in paw volume were measured by using a plethysmometer (Appelex, Bagneux, France) that was sensitive to 0.01ml volume changes at 60, 120, 180 and 240min intervals posttreatment. Pretreatment readings were also noted. Percent inhibition in inflammation expressed as inhibition of the increase in paw volume was calculated according to the following formula[38]

$$\text{Percent inhibition} = \left[1-\left({}^{a-x}/_{b-y}\right)\right] \times 100$$

Where x and a are the mean paw volume of the rats before and after injection of carrageenan, respectively, in the treatment group; and y and b are the mean paw volume of the rats before and after carrageenan injection respectively in the control group.

Cotton pellet granuloma in rat

The technique used by Goldstein *et al.*[39] was employed with few modifications. Sterilized cotton pellets were made, weighed individually (11.6 ± 0.20 mg; Mean ± S.D.), introduced subcutaneously (s.c) into the groin region in each rat and stitched to avoid dropout of pellets. A total of 25 rats were divided in five groups randomly and following treatments was given. Three groups were treated with VGB (125, 250 or 500 mg/kg i.p). Another group was administered with indomethacin (25 mg/kg, i.p), whereas the fifth group was injected with normal saline and served as control. Treatment was given once daily for four consecutive days by i.p route. A day after the last treatment, the rats were killed by diethylether anesthesia, stitches were cut and wet cotton pellets (impregnated with exudate) were removed. Extraneous material was isolated if any and pellets were dried overnight at 60°C to a constant weight in an oven at controlled temperature. The increase in cotton pellet weight was considered as granuloma tissue deposit. Results are expressed as % inhibition in tissue granuloma deposit calculated by using the expression described above. Where "*a-x*" is the difference in mean weights of cotton pellets after granuloma deposition in the treatment group and "*b-y*" is the difference in mean weights of cotton pellets after granuloma deposition in the control group.

Inflammation in peritoneal cavity and leukocyte migration in rat

The technique described by Baird *et al.*[40] was employed with few modifications to assess leukocyte migration in response to carrageenan-induced inflammation in peritoneal cavity. Rats were injected i.p with 0.3 ml of 1% carrageenan (sterile aqueous suspension) under light ether anesthesia and randomly assigned to treatment groups. Three groups of five rats each were given the following pre-treatments: Group 1, saline 1 ml/kg; Group 2, indomethacin 10 mg/kg and Group 3, VGB 500 mg/kg. All the treatments were given 30 min before the carrageenan injection and four hours later 5 ml of saline containing heparin (20 i.u/ml) were injected into peritoneal cavity of the rats under anesthesia. The abdomen was massaged gently and then opened along the midline. The fluid was withdrawn and total number of leukocytes was counted under a microscope using haemocytometer (Neubauer Improved, Assistent, Germany). In another part of the experiment, glass slides were prepared from the same peritoneal fluid and stained by Leishman's stain. Differential counts of 200 cells per preparation were made and percentage of polymorphonuclear neutrophils (PMN) out of total count (200) was determined to express the results.

Thermoregulation testing in mice

Two different models for the regulation of body temperature in mice were used. In the first model, normothermic mice were used to determine the hypothermic response. Three different groups of mice were injected i.p with aqueous solution of VGB (125, 250 and 500 mg/kg). Another group received normal saline and served as control.

In the second model, mice were administered s.c with a 20% aqueous suspension of brewer's yeast (20 ml/kg) to induce hyperpyrexia.[41] Eighteen hours after yeast injection, rectal temperature was recorded to assess the hyperthermic response. Only, the animals having a rectal temperature rise of +0.6 to 1.0°C were used to have a uniformity of the response. The animals were fasted for the entire duration of the experiment with free access to water. Body temperature was recorded by using a rectal probe (Model YSI 400) connected to a digital thermometer (Appelex, Pb 0331, Panlab, France) which was sensitive to 0.1°C temperature change. All the measurements were made at controlled room temperature (22±1°C). Rectal temperature recorded 30 min prior to drug administration was used as predrug control. Three different groups of hyperthermic mice (7 each) were administered with VGB (125, 250 and 500 mg/kg i.p). Sodium salicylate (300mg/kg i.p) and normal saline (10ml/kg i.p) groups were also used for comparison.

Statistics

Results are expressed as mean ± standard errors of means (otherwise indicated). The mean observation in a treatment group was compared statistically with its control group by using Student's *t*-test.

Results

Effect on carrageenan-induced edema in the rat paw

Indomethacin (25 mg/kg) caused a significant inhibition in paw edema at all the points examined within four hours (inclusive). The treatment with the low dose (125 mg/kg) of VGB did not show any effect on paw edema, while the inhibition caused by intermediate and high dose was quite significant at all the observation points till 240 min. However, the inhibition caused by high dose (500 mg/kg) was persistent at 2, 3 and 4 hour observation time being 40, 41 and 39% of the control group (Table 1).

Table 1: Effect of vigabatrin on carrageenan-induced paw edema in rats.

Group No.	Treatment/dose (mg/kg i.p.)	Paw volume (ml) Post-treatment (Mean ± SEM)				
		0 min	60 min	120 min	180 min	240 min
1	Control (Saline 1 ml/kg)	0.89±0.04	1.08±0.04	1.26±0.03	1.60±0.03	1.69±0.07
2	Indomethacin (100)	0.81±0.06	0.95±0.04* (26)	1.05±0.04** (35)	1.13±0.03*** (55)	1.17±0.03*** (55)
3	Vigabatrin (125)	0.81±0.01	1.05±0.02 (-)	1.22±0.04* (-)	1.46±0.09 (8)	1.57±0.11 (5)
4	Vigabatrin (250)	0.69±0.03	0.91±0.04* (-)	1.16±0.02* (-)	1.34±0.06** (8)	1.32±0.06** (21)
5	Vigabatrin (500)	0.91±0.05	1.09±0.03 (5)	1.13±0.04* (40)	1.33±0.05** (41)	1.40±0.06** (39)

Six animals were used in each group. Groups 2, 3, 4 and 5 were statistically compared with group 1 at its respective time points. *P<0.05; **P<0.01; ***P<0.001 (Student's *t*-test). Readings in parenthesis show the percent inhibitions.

Effect on cotton pellet granuloma formation in rat

Treatment of rats implanted with cotton pellets by vigabatrin (500mg/kg i.p.) for 4 days caused a significant inhibition (28%, $p<0.05$) in granuloma formation. The lowest dose was found ineffective whereas this drug at 250 mg/kg induced a slight (17%) but non-significant inhibition. Indomethacin, a potent antiinflammatory and antigranuloma drug (25 mg/kg/day) caused a highly significant (51%, $p<0.01$) reduction in the granuloma formation (Table 2).

Table 2: Effect of vigabatrin on cotton pellet granuloma formation in rats.

Group No.	Treatment/dose (mg/kg i.p.)	Granuloma deposition (mg) Mean±SEM	Percent inhibition (%)
1	Control (saline 1 ml/kg)	25.78±2.50	-
2	Indomethacin (25)	12.52±1.61**	51
3	Vigabatrin (125)	26.05±2.50	-
4	Vigabatrin (250)	21.26±1.19	17
5	Vigabatrin (500)	18.64±1.88*	28

Readings are mean ± SEM of 5 animal observations. Groups 2, 3, 4 and 5 were statistically compared with group 1 at its respective time points. *P<0.05; **P<0.01; (Student's *t*-test).

Effect on inflamed peritoneal cavity and leukocyte migration in rat

Four hours after i.p injection, carrageenan (3 mg/cavity) induced a significant ($p<0.05$) increase in total leukocyte count in the peritoneal exudate. A slight, but not significant, rise in neutrophil percentage was also evidenced. Indomethacin (10 mg/kg, i.p.) and VGB (500mg/kg i.p.) induced a significant reduction ($p<0.05$) in leukocyte migration (66% and 61%, respectively) to the inflamed peritoneal cavity when administered 30 min before carrageenan. In the same experiment, it was

noted that carrageenan treatment did not induce a significant rise in PMN percentage when compared to control. It was also noted that with the same dose regimen indomethacin and VGB had no significant decline in PMN percentage in the peritoneal exudate when compared to carrageenan alone (Table 3).

Table 3: Effect of vigabatrin on carrageenan induced leukocyte migration into the inflamed peritoneal cavity in rats.

Group No.	Treatment/Dose (mg/kg; i.p.)	Total leukocytes ($x10^3$ cells/mm^3)	PMN (%)	Leukocyte migration inhibition, (%)
1	Control Saline 10 ml/kg	39.90±7.61	66.90±3.39	0
2	Indomethacin (10)	13.39±6.64*	56.14±4.24	61
3	Vigabatrin (500)	15.38±4.28*	62.10±5.3	66

Readings are Mean ± SEM of 5 observations. Groups 2 and 3 were statistically compared to group 1. *P<0.05 (Student's *t*-test).

Changes in rectal temperature of mice

The lower two doses of VGB were found ineffective to reduce the rectal temperature in mice except at 90 min where 250 mg/kg caused a significant decline in temperature. The same effect was observed in the normothermic rats only with the high dose at 3-hour time point. The rest of the experiment was conducted on the mice because of the ease in handling the mice. In the same experiment treatment of mice with VGB 500 mg/kg significantly reduced body temperature at early observation period that was highly significant ($p<0.001$) at 90 min of drug administration that returned to normal within 6-12 hours and remained insignificant thereafter (Table 4).

Table 4: Effect of vigabatrin on the body temperature of normothermic mice in a circadian rhythm.

Group No.	Treatment/Dose mg/kg; i.p.	Rectal temperature °C Pre- and Post-treatment							
		Pretest 0 min	15 min	45 min	90 min	3 hr	6 hr	12 hr	24 hr
1	Control 0.9% NaCl	36.7± 0.17	36.6± 0.14	36.2± 0.33	36.3± 0.18	35.6± 0.12	35.3± 0.20	36.3± 0.35	36.0± 0.38
2	Vigabatrin (125)	36.6± 0.12	36.4± 0.09	36.5± 0.14	35.9± 0.20	35.8± 0.21	35.5± 0.29	35.8± 0.29	35.3± 0.13
3	Vigabatrin (250)	36.9± 0.09	36.5± 0.13	36.0± 0.30	35.6± 0.23*	35.3± 0.18	35.7± 0.26	35.6± 0.35	35.4± 0.52
4	Vigabatrin (500)	36.9± 0.13	36.0± 0.15*	35.5± 0.24*	34.6± 0.30***	33.9± 0.38**	34.1± 0.46*	34.8± 0.72	35.5± 0.38

Groups 2, 3 and 4 were statistically compared to group 1 at its respective time points. Data are Mean ± SEM of seven observations. *P<0.05; **P<0.01; ***P<0.001 (Student's *t*-test).

In another series of experiments with hyperthermic mice, sodium salicylate (300mg/kg i.p.) declined the rectal temperature highly significantly ($p<0.001$) from 45 min up to 3hr of observation period. In this experiment the antipyretic response to vigabatrin was dose-dependent. All the doses induced moderately significant decline in rectal temperature at 45min following drug administration. However, this antipyretic response diminished after 90min, 3hours and 6hours of treatment at 125, 250 and 500 mg/kg of vigabatrin, respectively. Furthermore, the response produced by high dose remained persistent and declined between 6-12 hour observation period; and none of the dose showed any change in rectal temperature at 24 hour observation period (Table 5).

Discussion

The cascade of events, mediated by certain autocoids culminating in inflammation and different factors which contribute to initiate, or limit the inflammatory response are fairly known.[42] Literature

survey has revealed that inflammatory response to carrageenan is a localized process and the swelling caused by it is due to bradykinin formation without the involvement of antigen.[43] It is also known to involve the phasic progression in edema.[43-45] The consequences of this inflammation are: the release of histamine and 5-HT followed by the kinins and finally leading to the stimulation of PGs being the mediators of inflammation. This process is not sudden and brief but continues over a relatively long interval (usually hours). Our data demonstrate that indomethacin, suppressed the inflammation induced by intraplantar carrageenan injection at the early phases and was evident by slightly significant to highly significant inhibition in edema during 1- to 4- hours, respectively. El-Mahdy *et al.*[46] using indomethacin (12 mg/kg, i.p.) produced 82% inhibition of carrageenan-induced paw edema, 3 hours after carrageenan injection. The high dose (500 mg/kg) of VGB was also effective in suppression of edema (40-, 41- and 39% in 2, 3 and 4 hours, respectively) in the same period of observation being highly significant ($p<0.01$). Literature has suggested the possible modulatory effect of central neurotransmitters in peripheral inflammation.[46-49] In one study Bhattacharya *et al.*[50] demonstrated that GABA-ergic neurotransmitter system had a modulatory anti-inflammatory effect on carrageenan-induced paw edema. In another experiment, the central administration of GABA attenuated the peripheral edema.[47]

Table 5: Effect of vigabatrin on the body temperature of hyperthermic mice in a circadian rhythm.

Group No.	Treatment/Dose mg/kg; i.p.	Rectal temperature °C Pre- and Post-treatment							
		Pre-test	15 min	45 min	90 min	3 hr	6 hr	12 hr	24 hr
1	Control	38.1±	37.8±	38.0±	38.0±	37.8±	37.4±	36.5±	36.7±
	0.9% NaCl	0.19	0.28	0.22	0.23	0.27	0.24	0.52	0.82
2	Sodium salicylate	37.9±	36.7±	36.2±	36.1±	36.1±	37.6±	36.9±	35.2±
	(300)	0.20	0.25*	0.14***	0.19***	0.13***	0.17	0.40	1.64
3	Vigabatrin (125)	37.5±	37.2±	37.2±	37.2±	37.1±	36.8±	36.9±	35.9±
		0.22	0.19	0.11**	0.21*	0.18	0.21	0.32	0.98
4	Vigabatrin (250)	38.1±	37.7±	37.0±	37.1±	37.2±	36.9±	35.9±	36.8±
		0.09	0.14	0.16**	0.11**	0.11*	0.11	0.59	0.31
5	Vigabatrin (500)	38.2±	37.4±	36.5±	36.5±	35.6±	34.9±	34.3±	35.9±
		0.10	0.31	0.44**	0.31**	0.47**	0.72**	0.96	0.41

Groups 2, 3, 4 and 5 were statistically compared to group 1 at its respective time points. Data are Mean ± SEM of seven observations. *P<0.05; **P<0.01; ***P<0.001 (Student's *t*-test).

Most of the studies concerning the mechanism of action cite the fact that VGB causes a large increase in GABA from three areas of CNS *i.e.* cerebral cortex; spinal cord; and retina[51,52] and other tissues[53,54] and GABA is known to interact with the central cholinergic, catecholaminergic; and serotonergic (5-HT) systems.[55,56] Rolf and Voges[57] showed that in presynaptic neurons VGB did not have any effect on serotonergic activity. However, VGB caused a competitive inhibition of 5-HT uptake to about 160% of the control in human platelets in an *in vivo* system. Castro-Lopes and co-workers[58] have suggested that after subcutaneous carrageenan injection, dorsal horn GABA is regulated by the increase of noxious inflow conveyed by unmyelinated c fibers from the inflamed tissue and could be a possible cause for the elevation of GABA (local) to elicit an inhibitory effect on inflammation and proliferation.

The results of bioassay of VGB in cotton pellet granuloma test also revealed its anti-inflammatory activity in the proliferative phase and response was significant ($p<0.05$) but only at higher dose that caused an inhibition in granuloma formation by 28%. In comparison to paw edema, the inhibition was not very significant and the responses in paw edema were more marked. Indomethacin, as an inhibitor of proliferation[59,60] at the same dose that used for paw edema inhibition, caused highly significant antigranuloma effects.

In inflamed peritoneal cavity leukocyte migration model, it was observed that carrageenan induced significant increase in leukocyte migration to peritoneal cavity. Although the results on overall leukocyte count are in agreement with many earlier studies,[61,62] but the increase in percentage of neutrophils was not significant in the present work. Most of the NSAIDs like aspirin and indomethacin have been shown to inhibit cyclooxygenase selectively. It is suggested that the drugs, which inhibit PG synthesis either by PG synthetase (cyclooxygenase) or 5-lipooxygenase are more potent anti-inflammatory agents.[63,64,65] It is well known that lipooxygenase product (leukotrienes and HETEs) of arachidonic acid and PGs are thought to be inflammation mediator.[66]

In our study, indomethacin significantly reduced leukocyte migration to the inflamed area and a slight, but not significant, decrease in neutrophil percentage was observed. Although the results with NSAIDs on leukocyte migration are at variance, Vazquez *et al.*[67] found that indomethacin decreased both carrageenan-induced edema in rat paw and neutrophil migration in the rat peritoneal cavity stimulated by carrageenan. Our results confirm these findings. Yamashita *et al.*[68] using glycogen (i.p.) in mice, claimed that indomethacin reduced the accumulation of white cells in peritoneal exudate. Blackham *et al.*[69] using indomethacin at doses which exceeded those required for inhibition of PGE_2, reduced PMN migration in inflamed area (in rats and mice). On the other hand, Souza *et al.*[70] claimed that indomethacin did not inhibit the neutrophil migration induced by Clostridium Toxin B in rabbit and hamster. Rocha *et al*[71] and many others claimed similar findings. In this work, VGB (500 mg/kg) showed inhibitory effects on leukocyte migration and neutrophil percentage, almost similar to that induced by indomethacin (10 mg/kg). However, the effects of VGB on cyclooxygenase pathway and PMN migration are not known.

An effort was made to study effects of VGB on PGs (known to be involved in nociception, inflammation and temperature regulation). Different concentrations of VGB failed to inhibit contractions, induced by arachidonic acid and PGE_2 in the isolated guinea pig ileum. However, it inhibited arterial prostacycline as seen by inhibition of its anti-aggregatory effect on ADP-induced platelet aggregation (unpublished data, not shown). Romstedt and Huzoor-Akbar[72] found that flurazepam (one of the benzodiazepines, which are known to release GABA and potentiate its actions) inhibited human platelet activation by inhibiting the release of arachidonic acid, its conversion into PGs and by blocking the action of PGs on platelets. These findings suggested that, GABA have the potential to inhibit PG synthesis and/or antagonize PGE_2 responses.

The present study also revealed that VGB reduced the rectal temperature in mice. In normothermic mice, the high dose (500 mg/kg) only, showed a significant reduction in rectal temperature till 6 hours. After that the body temperature recovered to normal between 6- to 12-hours. The regulation of body temperature is controlled by hypothalamic chemical neuromediators like dopamine, serotonin and acetylcholine.[73] Also, the hypothermia was more prominent in the hyperthermic model. These findings are in agreement with the observations of Zarrindast and Dibayan.[21] and suggest that this fall in body temperature was due to the enhancement of GABA in the brain.[2,74,75] These observations also corroborated the role of GABA in this action as confirmed by the work of Ghosh and Poddar[76] who indirectly suggested that an enhancement of GABA accumulation might induce a decrease in body temperature. In another experiment for the determination of interaction of different neuromediators in thermoregulation, Ghosh and Poddar[77] demonstrated that there is an involvement of serotonergic regulation in the opioidergic-cholinergic interaction via GABA system.

These findings are in agreement with Sancibrian *et al.*[78] who has demonstrated the failure of naloxone to reverse the hypothermic response induced by GABA or muscimol in restrained rats. On the other hand, methysergide (a non-selective 5-HT receptor antagonist) at a dose that antagonized the hypothermic effect of 5-HTP, a precursor of 5-HT, also antagonized the hypothermic effect induced by VGB (500mg/kg). This suggested serotonergic involvement (as claimed by others such as Morishima and Shibano[79]), in VGB-induced hypothermia. These results indicating GABA

involvement in thermoregulation agreed with the findings of Serrano *et al.*[80] and Minano *et al.*[81] who suggested that the hypothermia induced in rats by i.p. GABA might be mediated by serotonin.

It can be concluded from the previous data, that VGB-induced anti-hyperthermic and anti-inflammatory actions may be mediated through its inhibitory effect on PGs. However, VGB-induced hypothermia in normothermic mice seemed to be reversed by the inhibition of PG synthesis, as claimed by Sancibrian *et al.*[78] who found that indomethacin (5mg/kg) antagonized GABA and muscimol hypothermia in restrained rats. They suggested the involvement of PGs in GABA and muscimol effects. Similar results were obtained by De Bernardis *et al.*[82] who found that the blockade of PG synthesis by indomethacin significantly decreased the gastroprotective action of GABA, Na and Mg valproate on ethanol-induced gastric haemorrage in rats. From Sancibrian *et al.*[78] and De Bernardis *et al.*[82] work, it could be suggested that, VGB-induced hypothermia in mice may be mediated through PGs. The mechanism of the effect of VGB on body temperature could be summarized as follows: the anti-hyperthermic effect of VGB may be through PG inhibition, whereas VGB-induced hypothermia may be mediated by PG activation.

The results of the present study indicate that vigabatrin has the potential to induce anti-edema, antigranuloma and leukocyte anti-migratory effects in inflamed peritoneal cavity and reduce the rectal temperature in normothermic as well as hyperthermia-induced mice with acute regimen. These effects are thought to be the result of GABA accumulation, its interaction with PG biosynthesis and other neuromediators.

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