Effect of lamotrigine alone or in combination with conventional antiepileptic drugs on locomotor activity in mice

J. J. Luszczki

ABSTRACT

Objective: To evaluate the acute adverse effects with respect to exploratory and spontaneous locomotor activity of mice produced by lamotrigine (LTG) alone or in combination with conventional antiepileptic drugs (AEDs) such as valproate, diphenylhydantoin, carbamazepine and phenobarbital.

Material and Methods: Adult male mice were given an i.p. injection of LTG alone or in combination with conventional AEDs. When the maximum anticonvulsant activity of AEDs was at its peak, the exploratory and spontaneous locomotor activity of animals was electronically monitored. Three parameters (ambulatory activity, rearing activity and total distance traveled by animals) as indicators of the locomotor activity of the animals tested were electronically evaluated by means of the locomotor monitor system.

Results: The combination of LTG and valproate was devoid of any adverse effects on the locomotor activity. In contrast, the combination of LTG and carbamazepine drastically reduced the exploratory and spontaneous locomotor activity of animals in all analyzed parameters. Likewise, diphenylhydantoin combined with LTG also displayed the hypolocomotor effect as compared to the control animals. Phenobarbital either alone or in combination with LTG reduced the rearing activity.

Conclusion: Combining LTG with valproate is advantageous because of a lack of any hypolocomotor effects, whilst the combination of LTG and carbamazepine leads to a drastic reduction in locomotor activity in animals. LTG combined with diphenylhydantoin produces a substantial decrease in the activity in mice, whereas phenobarbital alone exclusively reduces the rearing activity of the animals tested.

KEY WORDS: Anticonvulsant, exploratory behavior, spontaneous locomotor activity

Introduction

Polytherapy in epilepsy is a preferred treatment regimen in patients with intractable seizures.\(^1\) In such cases, the rationale for combining some antiepileptic drugs (AEDs) is usually based upon the presumptions concerning two aspects of efficacious treatment. The first aspect is directly related to the anticonvulsant activity of the combination drugs, whilst the second one takes into consideration the side-effect profile of coadministered AEDs.\(^2,3\)

In order to evaluate the side-effect profile of AEDs in combinations, all available (theoretically favorable) combinations should be examined on animals. It is widely accepted that in animal models, some neurotoxic effects produced by the AEDs in combinations can be easily determined, which would provide sufficient background for their further clinical use in patients.\(^4,5\) To precisely assess the neurotoxic profile of AEDs in combinations, several behavioral tests can be applied as follows: rotarod, chimney test and locomotor activity test in rodents.\(^6\) Quite recently, the electronically measured locomotor activity test in rodents, evaluating the spontaneous (natural) activity of freely moving animals without any extorted movements, has been accepted as an eligible and sophisticated model to study the potential risk of neurotoxic effects produced by drugs.\(^7,8\) Therefore, it can be considered as an experimental (animal) model useful for assessing the neurotoxic effects produced by AEDs alone or in combinations in terms of spontaneous and exploratory activity of animals.
We have not come across any study on the effects of AEDs on the exploratory and spontaneous behavior of mice. Therefore, this experiment was especially designed to investigate the behavioral effects of some combinations between LTG (a newer AED, clinically used in patients with generalized tonic-clonic convulsions, and partial seizures with or without secondary generalization) and conventional AEDs. Moreover, the assessment of any behavioral change in terms of exploratory and spontaneous locomotor activity in mice after LTG injection, was performed. The alternations in the locomotor activity of animals after acute exposure to LTG alone or in combination with conventional AEDs were investigated for adequately assessing the locomotor profile of AEDs in combinations.

Material and Methods

The experiments were performed on Swiss albino male mice, purchased from a licensed dealer. The animals were kept under standardized laboratory conditions with free access to food (chow pellets) and tap water and a natural light-dark cycle for 7 days prior to the experiments. The experimental groups consisted of 8-10 animals each, weighing 20-26 g. All experiments were carried out between 09.00 am and 03.00 pm. The animals were tested in strict accordance with the current European Community and Polish legislation on animal experimentation. The experimental procedures listed were approved by the Local Ethics Committee at the Medical University of Lublin (License No. 191/2001/207/01).

The following AEDs were used in this study: Lamotrigine (LTG, Glaxo Wellcome, Kent, UK), valproate magnesium (VPA - kindly donated by IGN-Polfa S.A. Rzeszów, Poland), carbamazepine (CBZ - a gift from Polfa, Starogard, Poland), diphenylhydantoin (DPH, Polfa, Warsaw, Poland) and phenobarbital (PB, Polfa, Cracow, Poland). LTG, CBZ, PB and DPH were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, USA). Each monitor consisted of a 41 x 41 x 32 cm Plexiglas activity box partitioned with an acrylic cross into four (20 x 20 x 32 cm) quadrants. Mice were tested in the opposite quadrants of each individual cage and recording of their locomotor activity started throughout two consecutive 15 min-intervals. In order to diminish the response of the animals to the mild stress produced by handling and injection, the mice were placed in the respective monitor quadrants, at least 15 min (not immediately) after the i.p. injection of AEDs or vehicle. Since the anesthetic and/or analgesic drugs may interfere with free plasma or brain concentrations of LTG or conventional AEDs, the animals did not receive such drugs before locomotor activity testing. The first measure of animals’ activity is the rate of habituation to a novel environment. Thus, during prolonged exposure to a new environment, animals typically spend progressively less time in movement and exploration. So, the second measure is considered as the rate of spontaneous activity of mice. The test procedure was conducted automatically without the presence of the experimenter in the test room. LTG alone or in combination with conventional AEDs, (at the doses previously obtained from the isobolographic analysis of interactions in the maximal electroshock-induced seizures for the fixed-ratio of 1:1 combinations) were examined for locomotor activity in mice. Because of the existence of differences between locomotor patterns of control animals, the respective controls (as reference values) were evaluated on each day of the experiments, and compared with the respective AED values.

Baseline values were means for vehicle (1% solution of Tween 80) for each examined parameter. Because of robust between-control group differences in baseline values, effects of each drug (administered alone or in combinations) were analyzed independently and compared with the respective control (as the reference group). Statistical analysis was performed with one-way ANOVA. Post-hoc test was conducted to determine which drug effects differed significantly from control values. All differences tested post hoc were considered significant at \( *P<0.05 \), \( **P<0.01 \) and \( ***P<0.001 \) following Bonferroni's correction for multiple tests.

Results

Locomotor activity following vehicle and acute LTG adminis-
Figures 1A - 1B: Influence of LTG alone or in combination with conventional AEDs upon the ambulatory activity of animals in the locomotor activity test.

The white columns represent the ambulatory activity scores of the animals tested (as means ± SEM) evaluated during the first period of time (0-15 min) – habituation to a novel environment. The grey columns represent the means±SEM of ambulatory activity scores of the animals tested, evaluated during the second period of time (15-30 min). Statistical analysis performed with the use of ANOVA followed by post-hoc Bonferroni’s test revealed a significant reduction of ambulatory activity scores as compared to the control (CTRL) values at *P<0.05, **P<0.01 and ***P<0.001, respectively.

Figures 2A - 2B: Influence of LTG alone or in combination with conventional AEDs upon the rearing activity of animals in the locomotor activity test.

The white columns represent the rearing activity scores of the animals tested (as means ± SEM) evaluated during the first period of time (0-15 min), whereas the grey ones represent the means of rearing activity scores of the animals tested (SEM as error bars) evaluated during the second period of time (15-30 min). The animals’ activity was statistically compared with control animals by ANOVA with Bonferroni’s a posteriori test. ** - significant reduction of the respective rearing activity scores at P<0.01 vs. the control (CTRL) group. *** - significance at P<0.001 vs. the CTRL-group.

Inhalation (baseline control activity):

LTG injected separately at the doses ranging between 1.4-3.4 mg/kg did not affect any locomotor activity parameters as compared to the respective controls (Figures 1, 2, and 3).

Locomotor activity following acute administration of conventional AEDs in combinations with LTG:

1) Ambulatory Activity - AA

LTG at the dose of 3.4 mg/kg combined with CBZ (8.1 mg/kg) drastically reduced AA scores of the mice from 2208 to 1149 (by 2-fold) evaluated during the first period of time [F(6, 49) = 23.990; P<0.001], and from 1154 to 437 (by 2.5-fold) [F(6, 49) = 17.435; P<0.001] evaluated during the second period of time measured (Figure 1A). Similar effects were observed for CBZ (8.1 mg/kg) administered alone, which significantly reduced AA scores of mice tested in the first 15 min-interval from 2208 to 1713 [F(6, 49) = 23.990; P<0.05] and in the second 15 min-interval from 1154 to 734 [F(6, 49) = 17.435; P<0.01] of locomotor activity (Figure 1A). Moreover, the combination of LTG (2.1 mg/kg) with DPH (4.7 mg/kg) considerably diminished AA scores of animals from 1632 to 802 (by 2-fold) during the second period of time of spontaneous activity in mice [F(6, 49) = 5.597; P<0.001], being without any effects on this parameter during the habituation to a novel environment (Figure 1B).

2) Rearing Activity - RA

LTG at the dose of 3.4 mg/kg combined with CBZ (8.1 mg/kg) drastically reduced RA scores in mice, evaluated in the first from 385 to 225 (by 1.7-fold) [F(6, 49) = 5.359; P<0.001] and the second period of time from 212 to 48 (by 4.4-times) [F(6, 49) = 13.205; P<0.001] (Figure 2A). Furthermore, CBZ (8.1 mg/kg) injected alone evoked significant reduction in RA scores of animals tested in both periods of time—— from 385 to 257 (by 1.5-fold) [F(6, 49) = 5.359; p<0.01], and from 212 to 117 (by 1.8-fold) [F(6, 49) = 13.205; P<0.01], respectively (Figure 2A). Surprisingly, a substantial reduction in RA scores was
Discussion

Results presented herein indicate evidently that CBZ at the dose of 8.1 mg/kg significantly reduced per se AA, RA and TD traveled by mice in both 15 min-periods of locomotor activity measurement. The first period of time as the rate of habituation to a novel environment is considered as exploratory locomotor activity, whereas the second 15 min-interval is a measurement to a novel environment. The first period of time as the rate of habituation to a novel environment. The grey columns represent the means of the total distance traveled by the mice (SEM) evaluated during the second period of time (15-30 min). Statistical analysis of data, performed with the use of ANOVA followed by a post-hoc Bonferroni multiple comparison test, revealed a significant reduction of the total distance traveled by animals, as compared to the control (CTRL) animals at *P<0.05, **P<0.01 and ***P<0.001, respectively.

3) Total Distance - TD

Statistical analysis of the data from TD traveled by animals revealed that CBZ (6.1 mg/kg) injected alone or in combination with LTG (3.4 mg/kg) significantly reduced TD traveled in both periods of time from 833 to 379 (by 2.2-fold) [F(6, 49) = 24.975; P<0.001], and within the second interval (testing session) – by 1.6- and 1.9-fold [F(6, 49) = 24.975; P<0.001], respectively (Figure 2B). Also, the combination of DPH (4.7 mg/kg) with LTG (2.1 mg/kg) considerably diminished RA scores of mice in the locomotor activity test, during the first – from 459 to 266 (by 1.7-fold) [F(6, 49) = 26.723; P<0.001] and the second time interval – from 265 to 94 (by 2.8-fold) [F(6, 49) = 26.723; P<0.001], respectively (Figure 2B).

Figures 3A - 3B: Influence of LTG alone or in combination with conventional AEDs upon the total distance traveled of animals in the locomotor activity test. The white columns represent the means of the total distance (in cm) traveled by the animals (SEM as error bars) evaluated during the first period of time (0-15 min) – habituation to a novel environment. The grey columns represent the means of the total distance traveled by the mice (SEM) evaluated during the second period of time (15-30 min). Statistical analysis of data, performed with the use of ANOVA followed by a post-hoc Bonferroni multiple comparison test, revealed a significant reduction of the total distance traveled by animals, as compared to the control (CTRL) animals at *P<0.05, **P<0.01 and ***P<0.001, respectively.

observed for PB (4.7 mg/kg) injected alone or combined with LTG 1.4 mg/kg in both 15 min-intervals tested, i.e., during the habituation – by 1.8- and 1.5-fold [F(6, 49) = 26.723; P<0.001], and within the second interval (testing session) – by 1.6- and 1.9-fold [F(6, 49) = 24.975; P<0.001], respectively (Figure 2B). Also, the combination of DPH (4.7 mg/kg) with LTG (2.1 mg/kg) considerably diminished RA scores of mice in the locomotor activity test, during the first – from 459 to 266 (by 1.7-fold) [F(6, 49) = 26.723; P<0.001] and the second time interval – from 265 to 94 (by 2.8-fold) [F(6, 49) = 24.975; P<0.001], respectively (Figure 2B).

Discussion

Results presented herein indicate evidently that CBZ at the dose of 8.1 mg/kg significantly reduced per se AA, RA and TD traveled by mice in both 15 min-periods of locomotor activity measurement. The first period of time as the rate of habituation to a novel environment is considered as exploratory locomotor activity, whereas the second 15 min-interval is a measure of spontaneous locomotor activity of the animals tested. The present data also indicate that the combination of LTG (3.4 mg/kg) with CBZ (8.1 mg/kg) drastically reduced locomotor activity of mice in both 15 min-intervals examined. Similar effects were seen with DPH (4.7 mg/kg) combined with LTG (2.1 mg/kg). The former combination evidently decreased the scores of all evaluated parameters in both periods of time. In contrast, DPH injected at a single dose of 4.7 mg/kg did not affect any locomotor activity parameters of the animals challenged with the test. Bearing in mind the fact that the main mechanism of action of LTG is closely mediated by sodium channel blockade, it became evident that other sodium channel blockers (such like CBZ or DPH) could aggravate the individual side-effects when combined with LTG. In the early 1990s, there has appeared a hypothesis of additivity, based on presumptions that the effects produced by AEDs with similar mechanism(s) of action, exert an additive interaction (i.e. simple summation of effects between two AEDs co-administered). In the present study, it was clearly shown that by combining two sodium channel blockers one can obtain a considerable reduction of locomotor behaviors of the animals tested, which could result from the potentiation rather than the summation of the hypolocomotor effects produced by these AEDs in combinations. It is substantial to note that LTG administered alone, at the doses ranging between 1.4-3.4 mg/kg, did not affect any spontaneous locomotor activity, being without effects on TD traveled, AA or RA of the mice.

It has to be highlighted that drug doses for LTG alone and conventional AEDs in the locomotor activity test were based upon the previous study concerning the isobolographic analysis of interactions between LTG and conventional AEDs in maximal electroshock-induced seizures in mice. The main goal of the present study was to estimate some locomotor effects exerted by AEDs in combinations, at fully active anticonvulsant doses. This is why, the drug doses were injected at the fixed-ratio drug dose combinations obtained from the maximal electroshock-test, and not from the chimney test, performed for evaluating potential neurotoxic adverse effects of AEDs as regards the impairment of motor coordination in mice.

Furthermore, it was observed that PB (4.7 mg/kg) injected alone or in combination with LTG (1.4 mg/kg) drastically reduced RA scores of the animals tested in both periods of time. 

References:

10. Indian J Pharmacol | October 2004 | Vol 36 | Issue 5 | 306-311
having no impact on AA scores. Since none of the analyzed locomotor activity parameters were altered by LTG, the observed reduction in locomotor behaviors seems to be exclusively evoked by PB. Although, the neurological bases of animal and human behaviors are not yet fully understood, spontaneously emitted behaviors, like motor activity patterns, exploration and stereotypy have been used extensively in pharmacological studies. Based upon the examination of the baseline values for TD, AA and RA scores in PB-treated animals, for the first time, a “floor effect” evoked by PB was observed. This effect is generally induced by drugs which disturb muscle strength and balance in animals. It should be noted that RA is considered as the more sensitive parameter of spontaneous motor activity than AA and TD traveled, because rearing arguably involves more muscle strength and balance than movement in the horizontal plane. In case of PB injected alone or combined with LTG, it was shown that: a) RA scores were essentially absent (drastically reduced) in both periods of time examined; b) AA and TD traveled by animals were not appreciably lower in comparison with control animals. Thus, the PB-induced “floor effect” was elicited in the present study. Only VPA (95 mg/kg) injected alone or combined with LTG (1.8 mg/kg) possessed a favorable locomotor profile, exerting no influence on the locomotor activity of the animals tested. So, this AED combination may offer the safest profile with respect to the locomotor activity.

It has to be stressed that the drugs in combinations were injected at different time intervals in order to reach the peak of a maximum effect of both AEDs in vivo. Therefore, LTG was injected 45 min, DPH 105 min, PB 45 min, and CBZ or VPA 15 min prior to the locomotor activity evaluation. In the present study, a maximum effect of both AEDs was considered as the point of measure, in which AEDs exerted maximal neurotoxic effects on locomotor activity. In actual clinical practice, the AEDs in combinations are given concomitantly without consideration of a maximal effect of each AED administered. However, the knowledge of any possible adverse (neurotoxic) effects evoked by AEDs administered at anticonvulsant doses should be evaluated in experimental studies, when the drugs are present in vivo at maximally effective doses. This is why the AEDs were administered at different times to test their maximal effects. The readers should be aware of the fact that with locomotor activity monitoring, only the measure of locomotor functions of animals followed by injection of AEDs is provided, without any additional information concerning the other side-effects produced by the studied drugs in combinations, e.g., the weight gain, rash and tremor.

Another important presumption has to be presented herein. It is widely accepted that after chronic administration of AEDs, whether animals or humans, there is adaptation to the permanently administered doses of the drugs. In such a situation, the impairment of locomotor function may never appear or may be observed mainly if the cumulative doses of administered AEDs transgressed their level of toxicity. In such cases, tolerance to adverse effects develops along with the duration of drug treatment, especially when AEDs are administered chronically. Nonetheless, in clinical practice tolerance may independently appear for anticonvulsant or neurotoxic effects evoked by the combinations of AEDs. This is why LTG and conventional AEDs (i.e., CBZ, DPH, VPA and PB) were given to animals only once (singularly), at the doses corresponding to the ED₉₀ of mixtures at the fixed-ratio of 1:1 against electroconvulsions. The mice, after receiving the drugs at doses protecting 50% of animals against electroconvulsions, were challenged with the locomotor activity test for evaluating acute effects of mixtures of LTG with conventional AEDs. The evaluation of the acute effects of the anticonvulsant doses of AEDs on the locomotor activity was the main goal of this study.

Generally, our results are consistent with some clinical observations, concerning the application of the examined AEDs in combinations. It has been observed that LTG combined with VPA exerted a favorable interaction in clinical practice, whereas LTG combined with CBZ was not advantageous in patients with intractable seizures. It has to be stressed that the combination of LTG and CBZ was also unfavorable in the maximal electroshock-induced seizures in mice, showing sub-additive (antagonistic) interaction, and exerted lower antiseizure activity in patients than either LTG or CBZ given as monotherapy. On the contrary, LTG combined with VPA in isobolography showed a beneficial interaction, which has also been clinically effective in patients with drug-resistant epilepsy.

Summing up, LTG combined with sodium channel blockers like CBZ or DPH, potentiated the hypolocomotor activity of AEDs. In contrast, LTG combined with VPA was without a significant impact on locomotor activity of the animals tested, injected with fully active anticonvulsant drug-dose combinations. The combination of LTG with PB displayed a selective locomotor dysfunction, as PB injected alone considerably reduced the rearing activity in the animals tested.

Acknowledgement

The generous gifts of valproate magnesium from ICN – Polfa S.A. (Rzeszów, Poland) and carbamazepine from Polfa (Stargard, Poland) are greatly appreciated. The author is grateful to Professor Dr. hab. Stanisław J. Czuczwar for his valuable comments and suggestions after reading the manuscript. Dr. Jarogniew J. Luszczki is a recipient of the Fellowship for Young Researchers from the Foundation for Polish Science. This study was supported by a grant (6PO3F 026 20) from the State Committee for Scientific Research, Warsaw, Poland.

References

10. Luszczki JJ, Czuczwar M, Kis J, Krysa J, Pasztelan I, Swiader M, Czuczwar SJ. Interactions of lamotrigine with topiramate and first-generation antiepileptic