ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LX, N 2, 113 SECTIO D 2005

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# *Influence of gabapentin and its combination with phenobarbital on the ambulatory activity in mice*

Gabapentin (GBP), a cyclic analogue of gamma-aminobutyric acid (GABA) was primarily designed as a GABA agonist, but in neurochemical studies the drug had no affinity to $GABA_A$ receptor complex. In clinical practice, the adjunctive and monotherapeutic use of GBP resulted in a significant improvement in patients either with focal or secondary generalized partial seizures (2). In experimental models of epilepsy, the drug was shown to protect the animals from pentylenetetrazole-induced tonic, but not clonic convulsions (4). Although GBP was virtually ineffective in the maximal electroshock test, the drug enhanced the anticonvulsant activity of conventional and some newer antiepileptic drugs (AEDs) in this test (1). In contrast, GBP was shown to aggravate spike-and-wave discharges (3).

The aim of this study was to evaluate the effect of GBP and phenobarbital (PB) administered alone or in combination on the exploratory and spontaneous locomotor activity of mice. To-date, the effect of GBP and PB in combination on the locomotor behaviors of experimental animals has never been assessed. The alterations in locomotor functioning of animals following a single exposure to GBP alone or combined with PB would allow us to determine the adverse-effect profile of these AEDs.

## MATERIAL AND METHODS

Animals. The experiments were carried out on male Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experimental temperature was 21 ± 1°C and mice were on a natural light-dark cycle. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (consisting of 8–12 animals). Each mouse was used only once. All experimental procedures were approved by Local Ethics Committee at the Medical University of Lublin.

Drugs. The following drugs were used in this study: GBP (Parke-Davis, Germany) and PB (Polfa Warsaw, Poland). PB was dissolved in distilled water, while GBP was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water. Both drugs were administered intraperitoneally (i.p.) in a volume of 0.1 ml/g body weight, 60 min prior to the test.

Locomotor activity. Locomotor activity of animals was assessed by means of a Digiscan Animal Activity Monitor system (Omnitech Electronics, Columbus, OH, USA). Three activity chambers were located in a quiet room. Each activity chamber contained 16 photobeams positioned 5 cm apart: 8 on the x-axis and 8 on the y-axis. Photocells located on the wall, directly opposite each photobeam, were activated when the animal interrupted the beam. By recording which beams were interrupted, the

distance the animal traveled (cm) during the testing was determined to provide a measure of horizontal (ambulatory) activity. Each chamber was partitioned with acrylic cross into 20 x 20 cm quadrants. Mice were tested in one quadrant of each unit (i.e., two mice per chamber). The photocells of each activity chamber were connected to a Digiscan Analyzer, which allowed the gathering of data on PC computer. In our study, horizontal (ambulatory) activity of animals, total distance traveled (in cm), number of horizontal movements and movement time (in s) were measured.

Experimental design. The first locomotor activity measure was performed to habituate the animals to testing conditions and, particularly, to the activity chamber. This test began immediately after i.p. injection of vehicle. The next day, the mice were evaluated in the same experimental conditions, except for the administration of the AEDs, instead of a vehicle injection. GBP (66.9 mg/kg) and PB (6.69 mg/kg) were administered to animals, at doses corresponding to their median effective dose for the mixture of both component drugs, evaluated in the maximal electroshock-induced seizure test. The AEDs were given to animals at times scheduled for the maximal electroconvulsive test, according to Świąder et al. (5). Each mouse, immediately after injections, was placed into the activity chamber. Measurement was made twice for 15 min. First measure is considered as an exploratory activity of animals, while the second is defined as a measure of spontaneous activity of mice.

Statistics. Results from the locomotor activity were statistically analyzed by Kruskal-Wallis nonparametric ANOVA test followed by Bonferroni post-hoc test.

## RESULTS

### EXPLORATORY LOCOMOTOR ACTIVITY TESTING

PB (6.69 mg/kg) markedly increased all locomotor activity parameters investigated during the first period of the exploratory behavior in mice. The drug significantly increased the total distance traveled by animals from 646.8 to 1511 cm ($P<0.01$; Table 1). Similarly, the number of movements within the 15-min of observational period was significantly increased from 128.7 to 188.8 ($P<0.05$; Table 1). Also, the movement time was considerably lengthened from 73 to 174 s ($P<0.01$; Table 1). Considering all locomotor parameters, the total activity score for animals administered with PB (6.69 mg/kg) was considerably greater than that for the control animals (Table 2). In contrast, neither GBP (66.9 mg/kg) injected alone, nor combined with PB (6.69 mg/kg) affected the locomotor parameters during the exploratory locomotor activity testing (Table 1).

Table 1. Effect of gabapentin and phenobarbital on the exploratory locomotor activity in mice

| AMBULATORY (HORIZONTAL) ACTIVITY | | | | |
|---|---|---|---|---|
| Locomotor parameters | Control | PB (6.69) | GBP (66.9) | PB (6.69) + GBP (66.9) |
| Total activity score | 1545.67 ± 108.52 | 2567.00 ± 201.75** | 1451.17 ± 141.35 | 1745.50 ± 125.27 |
| Total distance (cm) | 646.83 ± 93.42 | 1511.00 ± 168.19** | 724.17 ± 40.90 | 954.50 ± 98.64 |
| Number of movements | 128.67 ± 14.42 | 188.83 ± 14.80* | 136.00 ± 12.87 | 104.17 ± 7.50 |
| Movement time (s) | 73.08 ± 9.94 | 174.12 ± 19.37** | 98.70 ± 12.35 | 104.40 ± 11.09 |

Values are presented as means ± SEM of at least 12 determinations. Statistical evaluation of data was performed with Kruskal-Wallis nonparametric ANOVA test. *$P<0.05$ and **$P<0.01$ vs. control

SPONTANEOUS LOCOMOTOR ACTIVITY TESTING

PB (6.69 mg/kg) considerably increased the total distance traveled by animals within the second 15-min period of testing. The drug significantly increased the distance traveled by animals from 305 (control) to 530 cm (PB-treated group; $P<0.05$; Table 2). In contrast, the combination of PB (6.69 mg/kg) with GBP (66.9 mg/kg) drastically reduced the total distance traveled by animals from 305 (control) to 29.8 cm ($P<0.001$; Table 2). Likewise, PB administered alone increased the number of movements of animals within the second 15-min of observational period (from 66.7 to 121.8, at $P<0.05$; Table 2). The combination of PB with GBP drastically reduced the number of movements of animals from 66.7 to 19 (at $P<0.01$; Table 2). The analysis of the movement time revealed that the animals administered with PB spent much more time moving in comparison with the animals administered with the combination of both AEDs (PB 6.69 mg/kg + GBP 66.9 mg/kg) (Table 2). Overall, the total activity score for animals injected with PB alone was significantly higher (at $P<0.05$) than that of animals administered with the combination of both AEDs (Table 2).

Table 2. Effect of gabapentin and phenobarbital on the spontaneous locomotor activity in mice

| AMBULATORY (HORIZONTAL) ACTIVITY | | | | |
|---|---|---|---|---|
| Locomotor parameters | Control | PB (6.69) | GBP (66.9) | PB (6.69) + GBP (66.9) |
| Total activity score | 1046.83 ± 141.34 | 1400.83 ± 69.31* | 760.33 ± 65.77 | 246.67 ± 10.14** |
| Total distance (cm) | 305.00 ± 73.24 | 530.00 ± 69.39* | 270.00 ± 28.29 | 29.83 ± 3.36*** |
| Number of movements | 66.67 ± 17.53 | 121.83 ± 14.12* | 55.67 ± 2.82 | 19.00 ± 3.14** |
| Movement time (s) | 36.08 ± 10.50 | 69.67 ± 11.94* | 46.32 ± 3.32 | 4.80 ± 0.71** |

Statistical evaluation of data was performed with Kruskal-Wallis nonparametric ANOVA test. Results are presented as means ± SEM. *$P<0.05$; **$P<0.01$ and ***$P<0.001$ vs. control group

## DISCUSSION

Here we show that PB administered alone at a constant dose of 6.69 mg/kg increased both, the exploratory and spontaneous locomotor activities of animals tested. In contrast, neither the exploratory nor spontaneous locomotor activities of animals were significantly altered following the injection of gabapentin alone. It was surprising that PB combined with GBP markedly reduced spontaneous locomotor activity of animals within the second 15-min period of observation, whilst the drug administered alone considerably increased both, exploratory and spontaneous locomotor activities of animals. Another remarkable change in animals' behavior should be discussed herein. The combination of both AEDs did not alter the exploratory locomotor activity of animals within the first 15-min period of observation, whereas, it drastically reduced the spontaneous behavior of the same animals within the second 15-min of observational period.

## CONCLUSIONS

1. The changes observed in animal behaviors for the combination of PB with GBP strongly votes against their combination in further clinical practice, albeit GBP has been shown to interact synergistically with PB in the maximal electroshock in mice (1).

2. Lack of any significant changes in the ambulatory locomotor activity of animals administered with GBP alone, during the exploratory and spontaneous activities testing, is of some clinical importance, worthy of further consideration.

Acknowledgements. This study was supported by a grant (KBN P05A 033 19) from the State Committee for Scientific Research, Warsaw, Poland.

## REFERENCES

1. Borowicz K. et al.: Effect of gabapentin on the anticonvulsant activity of antiepileptic drugs against electroconvulsions in mice: an isobolographic analysis. Epilepsia, 43 (9), 956, 2002.
2. Czuczwar S. J., Patsalos P. N.: The new generation of GABA enhancers. CNS Drugs, 15, 339, 2001.
3. Dalby N. O., Nielsen E. B.: Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin. Epilepsy Res., 28, 63, 1997.
4. Mares P., Haugvicova R.: Anticonvulsant action of gabapentin during postnatal development in rats. Epilepsia, 38 (8), 893, 1997.
5. Świąder M. et al.: Interaction of topiramate with conventional antiepileptic drugs in mice. Eur. J. Pharmacol., 399 (1), 35, 2000.

## SUMMARY

The effects of gabapentin and phenobarbital alone or in combination on the exploratory and spontaneous locomotor activities, with respect to ambulatory (vertical) locomotion of mice, were evaluated in this study. Phenobarbital administered alone increased both, the exploratory and spontaneous activities of animals tested, whereas the administration of gabapentin has no significant impact on these parameters in animals. In contrast, phenobarbital combined with gabapentin exclusively reduced the spontaneous locomotor activity of animals, but not the exploratory activity, which was unchanged by the combination of both AEDs.

Wpływ gabapentyny oraz jej połączenia z fenobarbitalem na motoryczną aktywność u myszy

Praca ocenia wpływ gabapentyny i fenobarbitalu, podawanych osobno jak i w kombinacji, na poznawczą i spontaniczną aktywność ruchową w zakresie ruchliwości poziomej u myszy. Fenobarbital zwiększał obie aktywności motoryczne myszy, podczas gdy gabapentyna nie miała żadnego istotnego wpływu na te parametry u zwierząt. Kombinacja fenobarbitalu z gabapentyną wyjątkowo zmniejszała ruchliwość spontaniczną, ale nie wpływała na ruchliwość poznawczą, która pozostała niezmieniona.