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Topiramate combined with carbamazepine reduces the exploratory and spontaneous locomotor activities in mice

The last decade of the XXth century was abundant with clinical approvals of newer second-generation antiepileptic drugs (AEDs), considered as the efficacious medications in some specific forms of epilepsy. Topiramate (TPM) is one of newer AEDs, possessing multiple diverse mechanisms of action, of which, the inhibition of voltage-sensitive Na^+ channels (7); potentiation of gamma-aminobutyric acid (GABA)-mediated inhibitory neurotransmission through binding to a novel site on the $GABA_A$-receptor complex (10); blockade of excitatory neurotransmission through a negative modulatory effect on Ca^{2+}-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) and kainate subtypes of glutamate receptors (5); inhibition of neuronal L-type high-voltage-activated Ca^{2+} channels (11); and inhibition of the carbonic anhydrase isoenzymes CA II and CA IV, may account largely for its broad-spectrum anticonvulsant activity (2). In experimental models of epilepsy, the drug, which is active against maximal electroshock (MES)-induced seizures (4), considerably reduces seizure activity and afterdischarge duration in amygdala-kindled rat (8), and protects the animals from pentylenetetrazole-induced seizures (1). Moreover, in genetically epilepsy-prone rats, TPM reduces tonic seizures and decreases spike-wave discharges (3).

The aim of this study was to evaluate the effects of TPM, administered alone and in combination with carbamazepine (CBZ), on the exploratory and spontaneous locomotor activities in mice. Previously, it was reported that TPM (administered at a constant dose of 5 mg/kg) potentiated the anticonvulsant activity of carbamazepine (CBZ) in the MES-test in mice (6). In the present study, we studied the effects of TPM (5 mg/kg), CBZ (6.6 mg/kg) and their combination on the exploratory and spontaneous locomotor activity of mice. To-date, the effect of TPM and CBZ in combination on the locomotor behaviors in mice has never been assessed. The alterations in locomotor functioning of animals following a single exposure to TPM alone or combined with CBZ would allow us to determine the adverse-effect profile of these AEDs.

MATERIAL AND METHODS

A n i m a l s. 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 and tap water. Temperature in the experimental room was 22 ± 1°C and the 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 animals). Each mouse was used only once. All experimental procedures were approved by Local Ethics Committee of Lublin.

Drugs. The following AEDs were used: TPM (Topamax; Cilag AG, Schaffhausen, Switzerland) and CBZ (Amizepin; Polfa Warsaw, Poland). The AEDs were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (i.p.) in a volume of 0.1 ml/kg body weight. CBZ was given – 30 min. and TPM – 120 min prior to the test, as presented elsewhere (6).

Locomotor activity monitoring. Locomotor activity of animals was assessed with a Digiscan Animal Activity Monitor System (Omnitech Electronics, Columbus, OH, USA). Each monitor consisted of a 41 x 41 x 32 cm Plexiglas open field box with a grid of infrared beams mounted horizontally every 2.5 cm and vertically every 4.5 cm. Photocells located on the wall directly opposite each photo-beam were activated when the animal interrupted the beam. Each box was partitioned with acrylic cross into four (20 x 20 x 32 cm) quadrants. Mice were tested in the opposite quadrants of each unit (i.e. two mice per box). The photocells of each activity box were connected to the Digiscan analyzer that transmitted the number of beam breaks (activity data) to a computer. During operation, the pattern of beam interruptions was recorded and analyzed by IBM-PC compatible computer. Interruption of any beam was recorded as an activity score. All activity data were collected during two consecutive 15-min periods. Cumulative counts were compiled and downloaded every 15 min into the data collection software, which organized these counts into different motor indices. Three representative motor indices were analyzed as follows: 1) total distance traveled in cm – measuring the amount of forward activity of animals; 2) ambulatory activity – measuring the total number of beam interruptions that occurred in the horizontal sensors during a given sample period; and 3) rearing activity – measuring the total number of vertical photo-beam interruptions within the sample period.

Experimental design. In this experiment the mice were not habituated to the test apparatus; therefore, the test procedure consisted of two independent, consecutive measures. After injection of vehicle or the respective AED doses, the mice were placed in the centre of individual cages and recording of their locomotor activities started throughout two consecutive 15 min-intervals. 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 movements and exploration. So, the second measure is considered as the rate of spontaneous activity of mice.

Statistics. Data from the locomotor activity test were statistically evaluated with the analysis of variance (ANOVA) followed by Bonferroni's post-hoc test for multiple comparisons.

RESULTS

EXPLORATORY LOCOMOTOR ACTIVITY TESTING

TPM (5 mg/kg) combined with CBZ (6.6 mg/kg) markedly reduced the ambulatory activity scores of the mice from 1926 to 1018 ($p<0.001$) (Fig. 1A). In contrast, neither TPM (5 mg/kg) nor CBZ (6.6 mg/kg) administered singly altered significantly this parameter in mice (Fig. 1A). Moreover, TPM combined with CBZ sharply reduced rearing activity scores in mice from 969 to 398 ($p<0.01$) (Fig. 1B). Again, TPM (5 mg/kg) and CBZ (6.6 mg/kg) administered alone did not affect rearing activity scores in animals tested (Fig. 1B). Statistical analysis of data revealed that TPM (5 mg/kg) co-injected with CBZ (6.6 mg/kg) significantly shortened the total distance traveled by animals from 347 to 84 cm ($p<0.001$) (Fig. 1C). In contrast, TPM (5 mg/kg) and CZB (6.6 mg/kg) administered alone had no impact on total distance traveled by the animals (Fig. 1C).

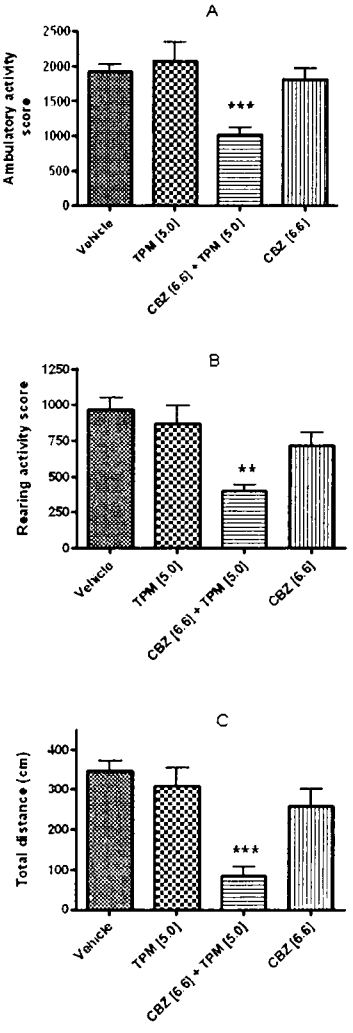

Fig. 1. Influence of topiramate, carbamazepine and its combination on the exploratory locomotor activity in mice. Columns represent the means (± SEM as the error bars) of the ambulatory activity (A), rearing activity (B), and total distance traveled by animals (C) evaluated during the first period of time (0-15 min) – habituation to a novel environment. Means were calculated from at least of 8 determinations. Statistical evaluation of data was performed with one-way ANOVA followed by Bonferroni's post-hoc test. **$p<0.01$ and ***$p<0.001$ vs. vehicle-treated animals

SPONTANEOUS LOCOMOTOR ACTIVITY TESTING

TPM combined with CBZ drastically reduced the ambulatory activity scores in mice from 1197 to 754 [$p<0.001$] (Fig. 2A). In contrast, the ambulatory activity of animals injected either with TPM (5 mg/kg) or CBZ (6.6 mg/kg) was not significantly changed (Fig. 2A). Furthermore, TPM combined with CBZ considerably reduced rearing activity scores in mice from 521 to 229 [$p<0.01$] (Fig. 2B). In

contrast, both AEDs administered separately did not alter this parameter in animals tested (Fig. 2B). Additionally, it was found that TPM (5 mg/kg) in combination with CBZ (6.6 mg/kg) significantly diminished the total distance traveled by animals from 198 to 97 cm [$p<0.05$] (Fig. 2). Inversely, neither TPM nor CBZ administered alone significantly shortened the total distance traveled by animals (Fig. 2C).

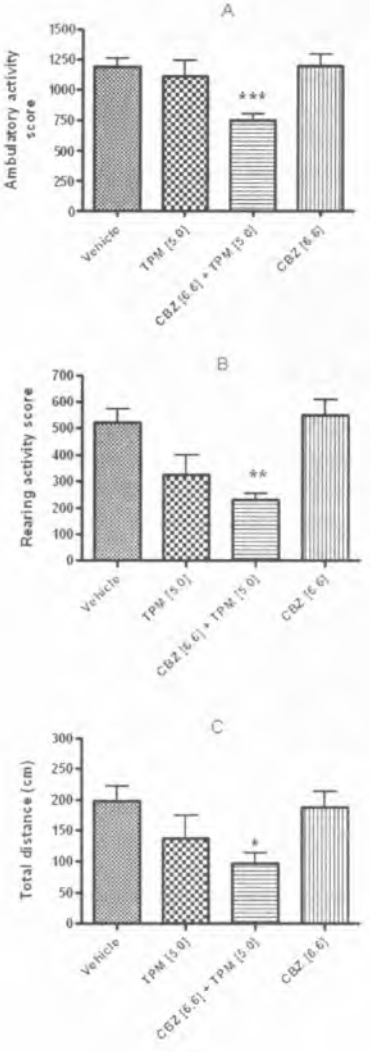

Fig. 2. Effect of topiramate alone or combined with carbamazepine on the spontaneous locomotor activity in mice. Columns represent the means (± SEM as the error bars) of the ambulatory activity (A), rearing activity (B), and total distance traveled by animals (C) evaluated during the second period of time (16–30 min) – to spontaneous locomotion in animals. Means were calculated from at least of 8 determinations. Statistical evaluation of data was performed with one-way ANOVA followed by Bonferroni's post-hoc test. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs. vehicle-treated animals

DISCUSSION

Our findings indicate clearly that TPM combined with CBZ markedly reduced the exploratory and spontaneous locomotor activities in mice with relation to all the examined parameters. In contrast, TPM given singly, at a dose of 5 mg/kg, did not affect ambulatory and rearing activities nor did it have any impact on the total distance traveled by the mice. Moreover, neither the exploratory nor spontaneous locomotor activities of animals were altered after the injection of CBZ alone.

Previously, it was documented that TPM (5 mg/kg) enhanced the anticonvulsant activity of CBZ, valproate, phenobarbital and diphenylhydantoin (6). Moreover, TPM administered alone or combined with AEDs (at doses providing a 50% protection against MES) resulted in no adverse effects, as measured in the chimney test (motor coordination) or passive avoidance task (long-term memory). It is noteworthy that the investigated combination between TPM and CBZ was pharmacokinetic in nature, since it was found that TPM considerably elevated the free plasma level of CBZ (6). The observed reduction in exploratory and spontaneous locomotor activities following the i.p. administration of both AEDs, when compared to control (vehicle-treated animals), provides evidence that the combination may not be useful in the clinical setting.

It should be highlighted that the electronically-monitored locomotor activity test in rodents seems to be a good screening allowing preselection of drugs affecting CNS and changing locomotor functioning in experimental animals.

CONCLUSIONS

1. The changes observed in animals' behavior for the combination of TPM with CBZ strongly votes against their combination in further clinical practice, albeit TPM has been shown to interact synergistically with CBZ in the maximal electroshock in mice.

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

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SUMMARY

This study was aimed at determining the acute effect of topiramate (TPM – a newer antiepileptic drug) co-administered with carbamazepine (CBZ) on the exploratory and spontaneous activities in mice. Locomotor activity was monitored electronically using a Digiscan system with relation to the ambulatory and rearing activities as well as the total distance traveled by animals within two 15-min. periods. Results indicated that TPM (5 mg/kg) and CBZ (6.6 mg/kg) administered alone did not alter the exploratory and spontaneous locomotor activities with respect to all parameters studied in mice. In contrast, the drug combination of CBZ (6.6 mg/kg) with TPM (5 mg/kg) considerably reduced both, spontaneous and exploratory locomotor activities in animals. As the combination of TPM with CBZ reduced locomotion in experimental animals, the utmost caution is required during clinical application of these drugs in combination.

Topiramat podawany z karbamazepiną redukuje poznawczą i spontaniczną aktywność ruchową u myszy

Zamierzeniem pracy była ocena wpływu topiramatu (TPM – nowego leku przeciwpadaczkowego) podawanego łącznie z karbamazepiną (CBZ) na poznawczą i spontaniczną aktywność ruchową u myszy. Aktywność ruchową monitorowano elektronicznie przy użyciu systemu Digiscan, oceniając ruchliwość poziomą, pionową oraz całkowity dystans pokonany przez zwierzęta w ciągu 2 okresów po 15 min. Wyniki wykazują, że TPM (5 mg/kg) i CBZ (6.6 mg/kg) podawane osobno nie zmieniały poznawczej i spontanicznej aktywności ruchowej w zakresie wszystkich badanych parametrów u myszy. Przeciwnie, kombinacja CBZ (6.6 mg/kg) z TPM (5 mg/kg) znacząco zmniejszała poznawczą i spontaniczną aktywność ruchową u zwierząt. Wnioskując, skoro kombinacja TPM z CBZ zmniejszała aktywność motoryczną u zwierząt doświadczalnych, zaleca się znaczną ostrożność podczas łącznego podawania obu leków w warunkach klinicznych.