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The antinociceptive effects of topiramate evaluated in writhing test in mice

Ocena antynocyceptywnych efektów topiramatu w teście przeciągania u myszy

INTRODUCTION

Topiramate (TPM) is a newer generation antiepileptic drug used in the treatment of partial-onset and primary generalized tonic-clonic seizure, and also Lennox-Gastaut syndrome in patients as young as 2 years in adjunctive therapy [12]. Chemically, it is a monosaccharide – D-fructose derivative with a sulfamate functionality which distinguishes it from other antiepileptic drugs (AEDs) [21].

TPM reveals multiple mechanism of action which includes: 1) voltage-dependent sodium channel blockade and modulation 2) enhancement of GABA receptor–mediated inhibition (however, it is noteworthy that TPM does not bind the benzodiazepine place on the GABA receptor complex), 3) antagonism of excitatory transmission represented by antagonistic effect at AMPA (but not NMDA) receptor sites [16,21] 4) some isozymes of carbonic anhydrase inhibition (especially CA-II and CA-IV) and 5) negative modulation of voltage-gated calcium ion channels. Modulation of K^+ conductance and proteins regulating neurotransmitter release from synaptic terminals has also been proposed as potential mechanism of action of TPM [22].

A variety of pharmacodynamic properties of TPM suggests potential efficacy in conditions other than epilepsy. Additionally, TPM possesses neuronal stabilising properties which may be beneficial in the treatment of pain [28].

Recently, newer AEDs are used by choice in the treatment of non-epileptic pathologies e.g. pain conditions [2,7]. Some of them are first choice drugs used in the prophylaxis of headache and also belong to the group of adjunctive drugs for various types of neuropathic pain [13]. It is the result of their exceptional mechanism of action, better tolerability and better pharmacokinetic profiles compared to standard drugs used in neuropathic pain and migraines such as tricyclic antidepressants or conventional AEDs [20].

Large controlled studies have assessed the efficacy of TPM in migraine prevention. Therefore, it is accepted for migraine prophylaxis in numerous countries throughout the world [24]. Numerous studies have proven that TPM reduces intensity, duration and frequency of migraine appearance [20,22] and improves the quality of life in migraine patients [3,22]. Moreover, it has been suggested

that TPM reduces the risk of transforming episodic headache to chronic form of headache [22] and even helps in reversion of chronic migraine to episodic one [5]. Some other data point to therapeutic clinical benefits of TPM in the management of chronic migraine, basilar migraine and vestibular migraine, cluster headache [2] and what is worth emphasizing in pediatric migraine [2,8,12].

TPM influences the central and peripheral nervous system, which can lead to changes in pain producing processes. This phenomenon allowed presuming efficacy of TPM in neuropathic pain i.e. diabetic neuropathy, trigeminal neuralgia [4,6,11]. Unfortunately, the discrepancy between the results of number of studies has been observed [7,20,28] and some authors suggest that TPM may not be effective in the treatment of neuropathic pain. Some pilot studies have also pointed to efficacy of TPM in several psychiatric conditions, including alcohol dependence, binge-eating disorder, bulimia nervosa, posttraumatic stress disorder [24] and also in difficult-to-treat conditions associated with medication overuse [22].

As it has been mentioned above, many AEDs are widely investigated by scientists for their potential analgesic properties. Literature data concerning effectiveness of TPM in pain is incomplete and controversial. Therefore, the aim of present paper is to study the antinociceptive activity of TPM, both given alone and in combination with other substances with confirmed analgesic properties in the writhing test in mice.

MATERIALS AND METHODS

Animals. Experiments were carried out on male Albino Swiss mice (18–30 g). Animals were kept in 8–10 to a cage at room temperature of 20 ± 1 ºC and 12 h light/dark cycle. Standard food (Murigran pellets, Bacutil, Motycz, Poland) and water were available *ad libitum* with exception of experiments when food was taken away. All experiments were performed between 9:00 a.m. and 3:00 p.m. Animals were acclimatized to the experimental room for about 2 h before testing, were used only once and sacrificed immediately after the test with a lethal dose of gaseous carbon dioxide (CO_2).

All behavioral experiments were carried out according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC), and approved by the Local Ethics Committee.

Drugs. The following drugs were used: topiramate (Topamax, Janssen-Cilag, Belgium), naloxone hydrochloride (Sigma, USA), morphine hydrochloride (Polfa, Poland), ethanol (Polmos, Poland), diazepam (Relanium, Polfa, Poland), ketamine (Ketanest, Parke-Davis, Germany).

The writhing test. The nociceptive responses in mice were investigated in the writhing test according to Koster et al. [15]. Each mouse received one intraperitoneal (*ip*) injection of 10 mg/kg of 0.6% acetic acid solution to evoke writhing. The mice were placed singly in a glass cylinder (35 cm high, 25 cm in diameter) and the number of abdominal constrictions (writhing episodes) was counted during a 10 min period, starting 5 min after the acetic acid administration. TPM was injected subcutaneously (*sc*), as a suspension in 0.5 % tylose solution, 60 min before the test. The other drugs (excluding ethanol, which was given intragastrically (*po*), 15 min before acetic acid) were injected *sc*: 10 min (naloxone and diazepam), 20 min (morphine) and 30 min (ketamine) before the acid. The absolute mean values of writhing episodes in control groups ranged from 16.80 ± 3.323 to 30 ± 5.018 (mean ± SEM) and were shown as 100%. The equivalent volume of vehicle (tylose) and 0.6% acetic acid solution was administered to the control groups.

Statistical analysis. The obtained data have been analyzed by using One-way ANOVA analysis of variance. Post hoc comparisons were carried out by Tukey-Kramer test. P values < 0.05 and lower have been considered as statistically significant.

RESULTS

TPM administered at the doses of 25, 50 and 100 mg/kg (*sc*) (Fig.1) significantly ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively) decreased the number of writhing episodes when compared to vehicle. The slight, nonsignificant effect was observed after the administration of TPM at the dose of 12.5 mg/kg which was accepted as the threshold dose (Fig.1). Our unpublished data have shown that the strongest effect of TPM (25 mg/kg, *sc*) reveals 60 min after the administration, therefore the writhing test was conducted 60 min after injection of the anticonvulsant.

Naloxone (5 mg/kg, *sc*) was able to reverse ($p < 0.05$) antinociceptive effects of TPM (25 mg/kg) (Fig.2).

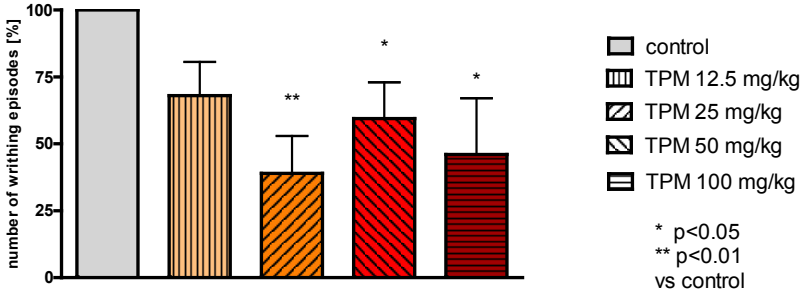

Fig. 1. The antinociceptive activity of various doses of topiramate (TPM) assessed in the writhing test in mice. Each bar represents the mean ± SEM for a group of 8–10 mice. The data are expressed as per cent of control group. * $p < 0.05$, ** $p < 0.01$ vs control group (one-way ANOVA test)

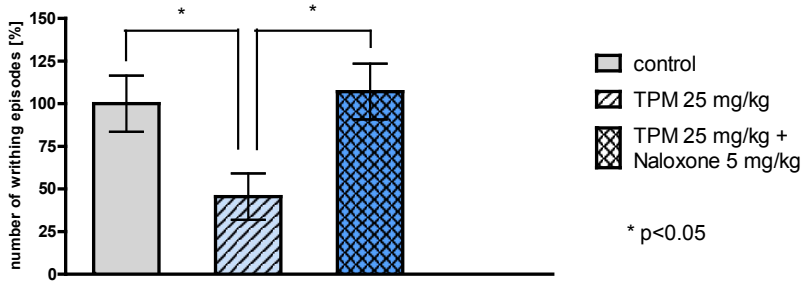

Fig. 2. The influence of naloxone on antinociceptive activity of topiramate (TPM) assessed in the writhing test in mice. The results are expressed as means ± SEM for a group of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 %. * $p < 0.05$ (one-way ANOVA test)

Co-administration of TPM (12.5 mg/kg) and ethanol (1 g/kg) (both administered at the threshold doses) has resulted in antinociception observed as a reduction in writhing response. This effect was significant ($p<0.05$) in comparison to TPM group but not ethanol group (Fig.3).

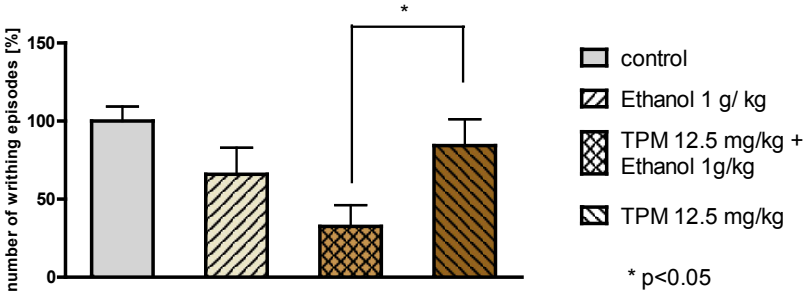

Fig. 3. The influence of topiramate (TPM) on antinociceptive activity of ethanol (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 %. * $p < 0.05$ (one-way ANOVA test)

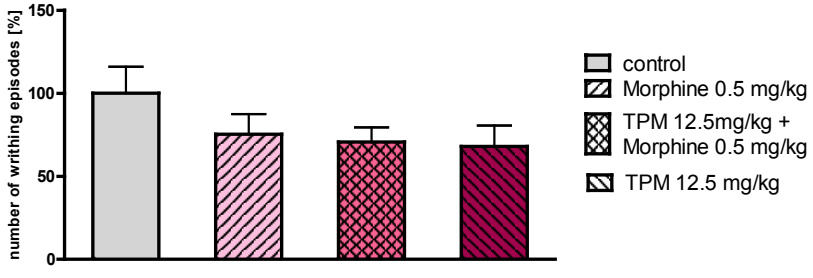

Fig. 4. The influence of topiramate (TPM) on antinociceptive activity of morphine (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 % (one-way ANOVA test)

The lack of co-operation was noted when TPM (12.5 mg/kg) was associated with one of the drugs: morphine (0.5 mg/kg) (Fig.4), diazepam (1.25 mg/kg) (Fig.5) or ketamine (10 mg/kg) (Fig 6). All substances given alone at the subthreshold doses did not decrease or slightly decreased the number of writhing episodes in mice (respectively Fig.4, 5 and 6).

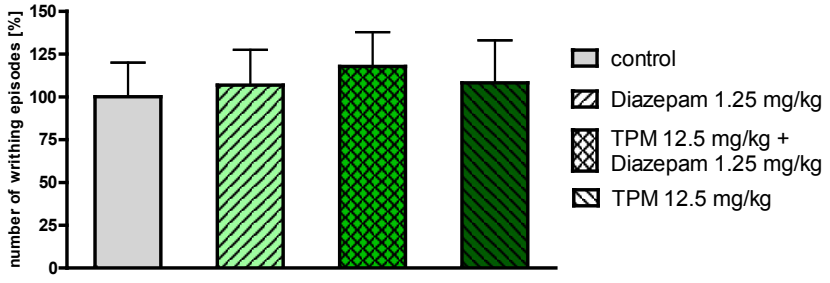

Fig. 5. The influence of topiramate (TPM) on antinociceptive activity of diazepam (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 % (one-way ANOVA test)

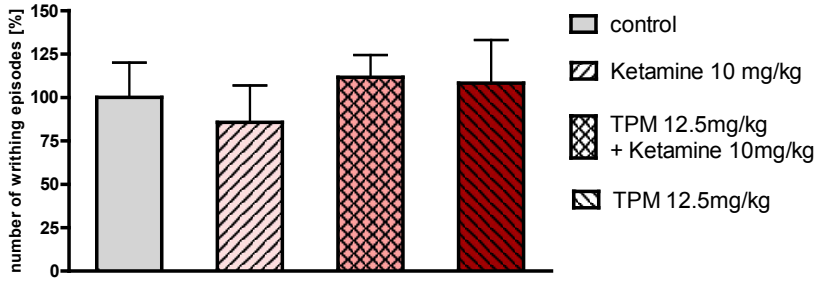

Fig. 6. The influence of topiramate (TPM) on antinociceptive activity of ketamine (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 % (one-way ANOVA test)

DISCUSSION

Many of the putative mechanisms of action that have caused the newer AEDs effective anti-seizure medications might also allow them to alleviate the pain [20]. Based on the hypothesis that epilepsy shares together with migraine and neuropathic pain several pathogenetic mechanisms, some AEDs are used in the prevention of migraine and other types of pain [1,20]. Imbalance between excitatory glutamate-mediated transmission and GABA-mediated inhibition in specific brain areas and lower threshold for the induction of long-term changes in neuronal excitability (sensitization and kindling) both in epilepsy and migraine, have been postulated [1]. Moreover, abnormal activation of voltage-operated ionic channels has been implicated in these two pathological conditions. Also cortical spreading depression has been found to be involved in both pathophysiology of epilepsy and generation of migraine aura [1,22].

The presumable mechanism of action of AEDs in migraine is probably due to decreasing brain excitability, as well as increasing the threshold for activation in the brainstem areas important for initiating migraine [2]. It is essential ability, especially that migraine and epilepsy are comorbid conditions: epilepsy occurs more commonly in patients with migraine than in general population, and *vice versa*, migraine in epileptics [1].

The analgesic properties of TPM have been analyzed in various models of pain in animals what has shed some light on mechanisms involved in observed activity.

Some studies have pointed to TPM's analgesic action in various models of neuropathic pain. Among others, its antiallodynic effect in both Chung and Seltzer models of neuropathic pain was observed in rats [30].

The efficacy of TPM in acute pain tests has also been evaluated. Lopes et al. [18] have reported an effectiveness of TPM in formalin and hot plate tests. They have found that TPM's analgesic action was reversed by naloxone (2 mg/kg, s.c.), which would suggest that the opioid system may participate in the observed effects, whereas the activation of ATP-dependent potassium channels or serotoninergic system via 5HT2A and 5HT3 receptors is not involved in mechanism of antinociceptive effects of TPM [18]. However, other authors have not shown the activity of TPM in formalin test in rats [23]. This discrepancy may be explained by the use of a higher dose of anticonvulsant by Lopes et al. [18], compared to smaller doses and different animal species used in other studies.

In the present study it has been decided to choose writhing test which is a model of visceral pain. In this test both peripheral and central analgesia effects are estimated. Many investigators have used it and recommended as a simple and very sensitive screening method. Furthermore, a good correlation between the potencies of analgesics in writhing test in animals and their clinical potencies has been noted [17,29].

The writhing test has already been used to evaluate analgesic properties of TPM by Stepanović et al. [25]. However, different route of administration (*sc*) and higher doses of TPM were used in our experiments. Moreover, the antinociceptive action of TPM in combination with morphine, ethanol, ketamine and diazepam has been additionally analyzed in our study.

In the present study, it has been shown that TPM, given at the doses of 25, 50 and 100 mg/kg (*sc*), was able to induce antinociceptive action in writhing test in mice, and maximal intensity of observed effect has been noticed 60 min after administration of the drug. The described activity is not dose-dependent but rather limited, because of the similarity of antinociceptive power of three effective doses. The power of TPM-induced antinociception is comparable to that of 1 mg/kg of morphine observed in the writhing test in mice [19]. These results are partially in agreement with these of Stepanović et al. [25] in which even lower dose of TPM (10 mg/kg) than these used in our study had antinociceptive activity. This discrepancy may be also the result of different route of TPM's administration – *sc* and *po*. Moreover, antinociceptive activity of TPM was not the result of potent motor disturbing effect or sedation because it did not alter motor performance even at high doses (400–1500 mg/kg, *po*), for up to 2 h of observation in mice [25].

The antinociceptive effect of TPM (25 mg/kg) was significantly attenuated by naloxone (5 mg/kg) which is consistent with findings presented by Lopes et al. [18]. It should be noted that naloxone given alone at this dose did not alter the response of mice to chemical nociceptive stimuli in the writhing

test [26]. Naloxone is μ-, κ- and δ-opioid receptor competitive antagonist with especially high affinity for μ-opioid receptor [14]. Activation of the μ- receptor by an agonist causes, among others, strong analgesia. δ-opioid receptor is also thought to play a role in analgesia [14]. Analgesic effects of such opioid agonists as morphine are antagonized by naloxone. Ability of naloxone to diminish the antinociception of TPM seems to suggest connection with the endogenous opioid system. However, it is essential to note that this dose of naloxone may affect not only opioid receptors. On the other hand, the observed lack of synergism of TPM with morphine in writhing test puts into question above suggestion and seems to eliminate at least μ-opioid receptors as a target of TPM's antinociceptive action. Thus, the noticed differences may be a result of the affinity to δ-opioid receptors presented by naloxone and the lack of such affinity in a case of morphine.

Ethanol exerts strong effect, especially on the central nervous system, through disturbing the balance between excitatory and inhibitory influences in the brain. Although it does not have its own receptor [27], general literature data points to the $GABA_A$ receptor as an important target for the activity of ethanol [10]. The antinociceptive effects of ethanol are well known. The ethanol-produced antinociception and the developement of tolerance to this effect as a result of its chronic administration in mice and rats were confirmed by Fidecka et al. [9]. In connection with the influence of ethanol on δ-opioid receptors, it is supposed that the opioid system participates in its antinociceptive effects [9]. The antinociceptive effects of threshold dose of TPM were significantly enhanced by ethanol which could be a result of their common influence on GABA and/or hyperexcitable aminoacids systems. Moreover, in light of the mentioned above partial conclusions, it may also point to participation of the opioid system via δ-opioid receptors in observed effects.

The interactions between TPM and ketamine or diazepam have not been shown. Therefore, the participation of GABA and hyperexcitable aminoacids systems seems to be debatable and further studies are required to precisely determine the role of both these systems in TPM's mechanism of action.

At the end, it should be underlined that anticonvulsant and analgesic effects of AEDs do not necessarily correlate and therefore the anticonvulsant and analgesic activity may be due to separate, unrelated mechanisms [23].

In conclusion, our studies confirm the effectiveness of TPM in writhing test in mice and seem to suggest that the opioid system, at least partially, participates in the analgesic mechanism of action of the drug. However, the mechanism of the analgesic action of TPM has not been yet fully delineated and further investigations in this area are needed.

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SUMMARY

In the present study the antinociceptive activity of topiramate (TPM), new antiepileptic drug with multiple mechanism of action, in writhing test in mice was evaluated. It has been shown that TPM, given at the doses of 25, 50 and 100 mg/kg, was able to induce antinociception.

The diminution of TPM's antinociception by naloxone and the lack of interactions between TPM and morphine, suggest that δ-opioid receptors are engaged in observed antinociceptive effects of TPM. Moreover, the antinociceptive effects of threshold dose of TPM were significantly enhanced by ethanol which could be a result of their common influence on GABA and/or hyperexcitable aminoacids systems and may also point to participation of δ-opioid receptors. The interactions between TPM and ketamine or diazepam have not been shown. Therefore, the role of GABA and hyperexcitable aminoacids systems in mechanism of action of TPM seems to be debatable. In conclusion, our studies confirm the effectiveness of TPM in writhing test in mice and seem to suggest that opioid system, at least partially, participates in analgesic mechanism of action of the drug. However, further investigations in this area are needed.

Keywords: topiramate, antinociception, writhing test, mice.

STRESZCZENIE

W prezentowanej pracy oceniano antynocyceptywną aktywność topiramatu (TPM), leku przeciwpadaczkowego nowej generacji o złożonym mechanizmie działania, w teście przeciągania u myszy. Wykazano, iż TPM podawany w dawkach 25, 50 i 100 mg/kg wykazywał właściwości antynocyceptywne. Zmniejszenie aktywności antynocyceptywnej TPM przez nalokson, jak również brak interakcji pomiędzy TPM a morfiną, sugeruje, że receptory δ-opioidowe są zaangażowane w obserwowane efekty TPM. Dodatkowo antynocyceptywne działanie progowej dawki TPM było istotnie nasilane przez etanol, co może świadczyć o wpływie obydwu substancji na układ GABA-ergiczny i/lub aminokwasów pobudzających, jak również może wskazywać na udział w obserwowanych receptorów δ-opioidowych. Jakkolwiek, brak interakcji pomiędzy TPM a ketaminą lub diazepamem wydaje się podważać rolę układu GABA-ergicznego i układu aminokwasów pobudzających w mechanizmie działania TPM.

Podsumowując, nasze badania potwierdzają skuteczność TPM w teście przeciągania u myszy i wydają się sugerować przynajmniej częściowy udział układu opioidowego w przeciwbólowym mechanizmie działania tego leku. Niemniej jednak, kolejne badania w tym kierunku są wymagane

Słowa kluczowe: Topiramat, antynocycepcja, test przeciągania, myszy