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ARAŞTIRMA / RESEARCH

Effects of acute topiramate administration on post-traumatic stress disorder in rats

Akut topiramat uygulamasının sıçanlardaki travma sonrası stres bozukluğu üzerine etkileri

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Abstract

Purpose: The aim of this study was to investigate the effects of acute systemic topiramate administration on anxiety index and freezing time, plasma estrogen and progesterone levels, and salivary gland immunoglobulin A in a post-traumatic stress disorder rat model.

Materials and Methods: A total of eighteen female Wistar rats used in the study were exposed to predatory odor stress. One week later, saline was administered to the control group and 15 μM and 30 μM topiramate to the treatment groups, after which the animals were exposed to the trauma reminder and their behavior was monitored in the elevated plus maze. At the end of the experiment, blood samples were taken, animals were sacrificed, salivary glands were removed immediately after.

Results: Topiramate suppressed anxiety index and freezing time in rats with post-traumatic stress disorder at both 15 μM and 30 μM doses compared to the control group. A positive correlation was observed between plasma estrogen level and anxiety index in the control group, and topiramate suppressed this correlation in a dose-dependent manner. Topiramate did not change the plasma progesterone level, but suppressed the salivary gland immunoglobulin A level at the low dose.

Conclusion: These findings obtained in our study indicate that topiramate may be effective in the treatment of post-traumatic stress disorder.

Keywords:. Anxiety index, estrogen, freezing time, progesterone, salivary immunoglobulin A

Öz

Amaç: Bu çalışmada, travma sonrası stres bozukluğu sıçan modelinde akut sistemik topiramat uygulamasının anksiyete indeksi ve donakalma zamanı, plazma östrojen ve progesteron düzeyleri ile tükürük bezi immünoglobulin A üzerine etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmada kullanılan toplam on sekiz dişi Wistar sıçan yırtıcı koku stresine maruz bırakıldı. Bir hafta sonra kontrol grubuna serum fizyolojik, tedavi gruplarına 15 μM ve 30 μM topiramat uygulandı, ardından hayvanlar travma hatırlatıcısına maruz bırakıldı ve davranışları yükseltilmiş artı labirentinde izlendi. Deneyin sonunda kan örnekleri alındı, sakrifiye edilen hayvanların tükürük bezleri çıkarıldı.

Bulgular: Topiramat travma sonrası stres bozukluğu olan sıçanlarda hem 15 μM hem de 30 μM dozlarda kontrol grubuna kıyasla kaygı indeksini ve donakalma zamanını baskıladı. Kontrol grubunda plazma östrojen düzeyi ile kaygı indeksi arasında pozitif korelasyon gözlendi, topiramat bu korelasyonu doza bağımlı olarak baskıladı. Topiramat plazma progesteron seviyesini değiştirmedi, fakat tükürük bezi immünoglobulin A seviyesini düşük dozda baskıladı.

Sonuç: Çalışmamızda elde edilen bu bulgular topiramatın travma sonrası stres bozukluğu tedavisinde etkili olabileceğine işaret etmektedir.

Anahtar kelimeler: Kaygı indeksi, östrojen, donakalma zamanı, progesteron, tükürük immünoglobulin A

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a disorder that occurs as a result of the exposure to a traumatic event and manifests with symptoms such as fear, helplessness, avoidance, anxiety, hypervigilance, and sleep disturbance when the patient faces with the situations that remind this previous event[1]. In PTSD studies, changes have been observed in the brain regions such as medial prefrontal cortex (mPFC), hippocampus and amygdala, and in glutamatergic, serotonergic, gamma-aminobutyric acidergic (GABAergic), and noradrenergic neurotransmitter systems[2]. These changes are thought to lead to impairments in the ability to adapt and respond to future stressors[3]. In response to trauma, the hypothalamic-pituitary-adrenal (HPA) axis is also activated and contributes to the maintenance of homeostasis by modulating neurotransmitter and hormone levels[4]. In addition, post-traumatic changes occur in the levels of secretory-immunoglobulin A (s-IgA) of salivary gland secretions, tears and breast milk, which limits bacterial epithelial adhesion and penetration and thus exerts a protective effect on mucous membranes[5-7]. Some factors that increase susceptibility to PTSD have been identified. Examples of these are previous exposure to trauma, a family history of psychiatric illness or PTSD, and being a female[1]. In the current treatment of PTSD, although drugs such as serotonin reuptake inhibitors, antipsychotics, adrenergic receptor blockers, benzodiazepines, etc. are used in addition to psychotherapy, adequate response cannot be obtained[8]. A better understanding of the developmental mechanisms of PTSD will increase the effectiveness of the treatments.

Topiramate is a mixed-acting antiepileptic drug which was produced as an intermediate product during the process of diabetes drug development[9]. Antagonizing voltage-gated sodium and calcium channels, increasing GABAergic current mediated by $GABA_A$ receptors, suppressing glutamatergic activity by affecting α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, and inhibiting carbonic anhydrase 1-4 isoenzymes are still the known mechanisms of action[10,11]. Topiramate is effective in the epilepsy therapy as well as in migraine prophylaxis, weight loss and mood disorders[10,11].

It is thought that various anticonvulsants, including topiramate, may be effective in the treatment of PTSD, since traumatic events may cause limbic nuclei may become kindled or abnormally sensitized, leading to psychic and physical arousal and psychiatric disturbances[12]. In some studies with PTSD patients, topiramate has been shown to suppress nightmares and flashbacks, as well as the PTSD Checklist-Civil Version score[13]. Although there is insufficient evidence, some meta-analyses from randomized controlled clinical trials indicate that topiramate is effective in the treatment of PTSD[14,15]. Besides increasing GABAergic activity, suppression of glutamatergic activity via AMPA receptors is held responsible for these effects of topiramate[13,16]. Our hypothesis is that acut intraperitoneally (i.p.) topiramate administration may be effective on the anxiety index and freezing time, plasma estrogen, plasma progesterone, and salivary gland s-IgA levels in a predatory odor stress PTSD rat model. In the study, we demonstrated that topiramate suppressed anxiety index and freezing time, suppressed dose-dependently both positive correlation between anxiety index and estrogen level, and s-IgA level.

MATERIALS AND METHODS

Animals

In this study, a total of eighteen adult female Wistar rats (200-250g) were used in three groups as control (saline, n = 6), TPM 15 (topiramate 15 μM, n = 6) and TPM 30 (topiramate 30 μM, n=6). All experimental procedures in accordance with the 'Guide for the Care and Use of Laboratory Animals' were approved by the Local Ethics Committee for Animal Experiments of Tekirdag Namık Kemal University (TNKU DHEK, 2020-11-06, 09.30, T2020-535). The rats obtained from Experimental Animals Application and Research Center of Tekirdag Namık Kemal University (TNKU-DHUAM) were housed with a reversed 12 hours light/dark cycle at 21±3°C and 50±5% humidity. There was unlimited access to standard rat chow and water.

Experimental procedure

Following a one-week acclimatization period, on the 1st day of the experiment, the animals were sequentially exposed for 10 minutes to cat litter (predator odor stress) used by the same cat for two consecutive days and cleaned of pellets/stools[17]. After exposure, animals were given a one-week rest

period. During this period, only daily feed and water controls of the animals were made. On the 8th day of the experiment, the animals were treated according to the group. The animals in the TPM 15 and TPM 30 groups were given i.p. 15 μM and 30 μM topiramate (Sigma-T0575), respectively. The animals in the control group were administered the same volume of saline, which was used to dissolve topiramate. Ten minutes after the treatment, the animals were sequentially exposed to clean, unused cat litter for 10 minutes as a trauma reminder. To assess the anxiety state immediately after exposure to the trauma reminder, the animals were placed in an elevated plus maze (EPM) and a video record was performed for 5 minutes. Animals whose blood samples were taken to measure plasma estrogen and progesterone levels were sacrificed under high-dose thiopental (Sigma-T1019) anesthesia. For s-IgA level determination, salivary glands were removed and placed in a formaldehyde solution.

Elevated plus maze

An EPM 50 cm above the ground consists of four arms, two open (50 cm x 10 cm) and two closed (50 cm x 10 cm), surrounded by 40 cm high walls. Rats were placed in the center square of the EPM immediately after exposure to the trauma reminder, facing open arms. The EPM was cleaned with a 5% ethanol solution before measurements of the next animal were made.

After the experiment, the video recordings were read. First of all, the total duration of the freezing behavior, which is defined as the condition that the rats do not make any movement other than breathing, was calculated during the experiment. The condition in which all four paws were in the arm was considered an arm insertion. Anxiety index was calculated according to the formula below by evaluating the number of open arm entries, the total number of entries on both open and closed arms, and the time spent on the open arm (seconds (s))[18]:

$$\text{Anxiety index} = 1 - 1/2\ ((x/300s) + (y/z))$$

x: total time spent in open arms, y: number of open arm entries, z: total number of entries into all four arms.

Biochemical and histopathological examination

Biochemical and histopathological examinations were performed blindly by the same biochemist and pathologist, respectively. Blood samples from rats were centrifuged without delay, estrogen and progesterone levels in the obtained plasma were measured using the "ECLIA" electrochemiluminescence immunoassay and Roche/Hitachi cobas c 8000 analyzers. The salivary gland samples, which were removed immediately after the experiment and preserved in formaldehyde solution, were stained with immunoglobulin A antibodies using a BenchMark XT automatic machine and evaluated with an Olympus CX41 light microscope. Cells stained with immunoglobulin A were counted in each of three randomly selected 400 x fields, with results expressed as average per unit area.

Statistical analysis

With the G Power 3.0.10 software, the total sample size was calculated as fifteen, taking alpha error=0.05, power=0.80 and effect size medium (d=1.0). It was decided to use a total of eighteen rats in three groups by adding one animal to each group. Statistical analyses were performed using GraphPad Prism 8 software. The normality of data distribution was tested by the Kolmogorov-Smirnov test. One-way ANOVA post hoc Tukey's test was used for anxiety index, freezing time, plasma estrogen, plasma progesterone and s-IgA levels analysis. The correlation between the anxiety index and the plasma estrogen level was examined with the Spearman correlation test. Data were expressed as mean±standard error of mean (SEM). For all statistical calculations, significance was considered as $p<0.05$.

RESULTS

Anxiety index was found to be statistically significantly lower compared to the control group (0.87 ± 0.06) in the TPM 15 (0.42 ± 0.14) and TPM 30 (0.38 ± 0.1) groups treated with 15 μM and 30 μM topiramate, respectively ($p< 0.05$, Figure 1a). In TPM 15 (33.33 ± 6.02) and TPM 30 (10.83 ± 1.5) groups, freezing time was found to be statistically significantly lower than that in the control group (81.5 ± 5.33, $p < 0.01$, Fig. 1b), and also in the TPM 30 group compared to the TPM 15 group ($p < 0.05$, Figure 1b). No significant change was observed in plasma estrogen levels between control (15.17 ± 3.63), TPM 15 (9.09 ± 1.28) and TPM 30 (18.27 ± 4.03) groups (Figure 2).

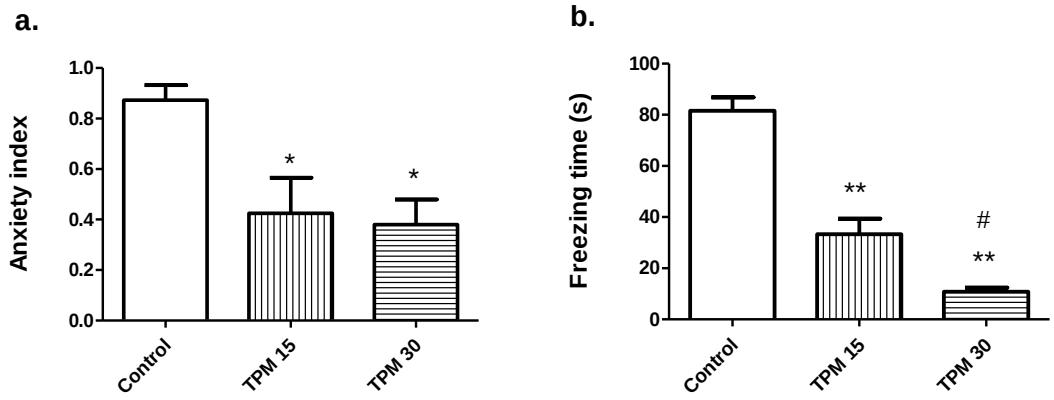

Figure 1. The effects of 15 μM (TPM 15, n = 6) and 30 μM (TPM 30, n = 6) topiramate administered i.p. on anxiety index (a) and freezing time (b).

The same volume of saline was applied to the control group (n = 6). Results were expressed with mean ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared to control group, $^{\#}p < 0.05$ compared to TPM 15 group.

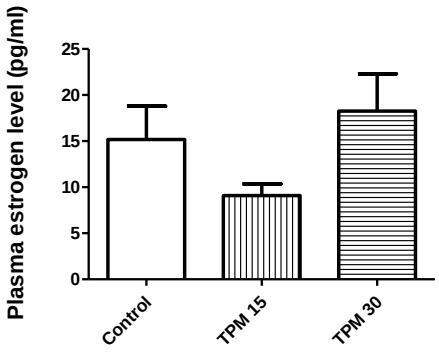

Figure 2. The effects of 15 μM (TPM 15, n = 6) and 30 μM (TPM 30, n = 6) topiramate administered i.p. on plasma estrogen level.

The same volume of saline was applied to the control group (n = 6). Results were expressed with mean ± SEM.

A statistically significant positive correlation was found between anxiety index and plasma estrogen level in the control group ($p = 0.017$, Spearman $r = 0.94$, Figure 3a). No significant change was observed in plasma progesterone levels between control (37.25 ± 5.52), TPM 15 (50.58 ± 7.32) and TPM 30 (50.05 ± 3.66) groups (Figure 4a). Topiramate applied to the TPM 15 group (3.83 ± 0.4) at a dose of 15 μM suppressed the salivary gland s-IgA level statistically significantly compared to both the control group (8.33 ± 0.56) and TPM 30 group (10.83 ± 1.5) administered topiramate at a dose of 30 μM ($p < 0.001$, Figure 4b). The effects of topiramate on the salivary gland s-IgA examined histopathologically compared to the control group, are presented in Figure 5.

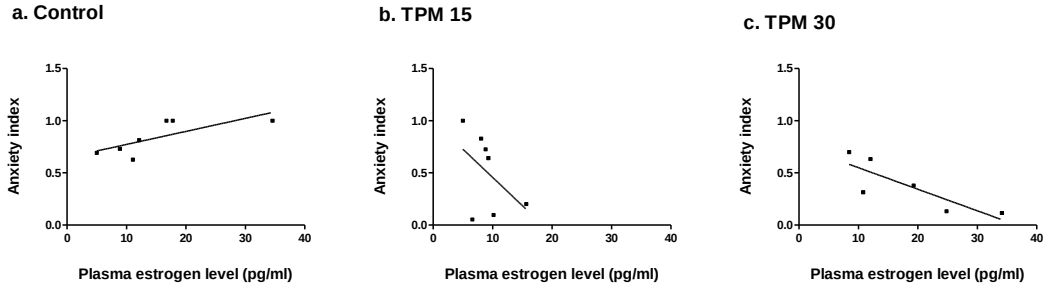

Figure 3. The effects of 15 µM (b, TPM 15, n = 6) and 30 µM (c, TPM 30, n = 6) topiramate administered i.p. on the correlation between anxiety index and plasma estrogen level.

The same volume of saline was applied to the control group (a, n = 6). Results were expressed with mean ± SEM.

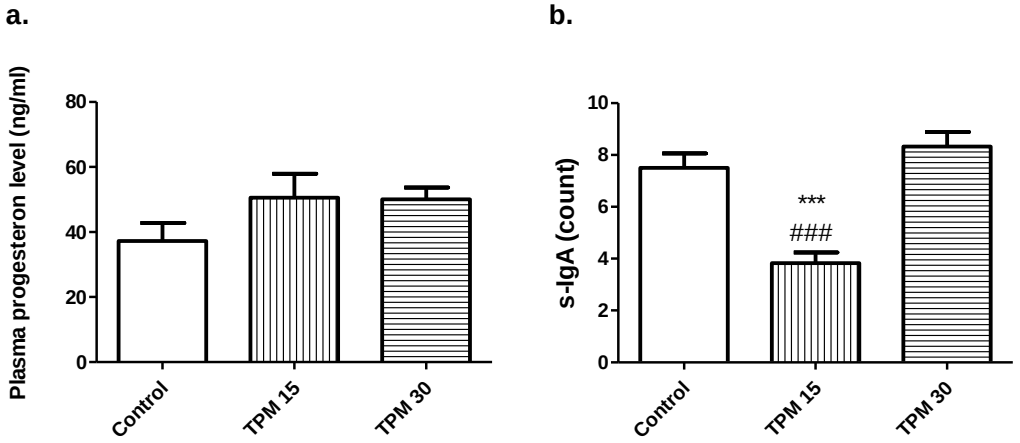

Figure 4. The effects of 15 µM (TPM 15, n = 6) and 30 µM (TPM 30, n = 6) topiramate administered i.p. on plasma progesterone (a) and s-IgA (b) levels.

The same volume of saline was applied to the control group (n = 6). Results were expressed with mean ± SEM. $^{***}p < 0.001$, compared to control group, $^{###}p < 0.001$ compared to TPM 30 group.

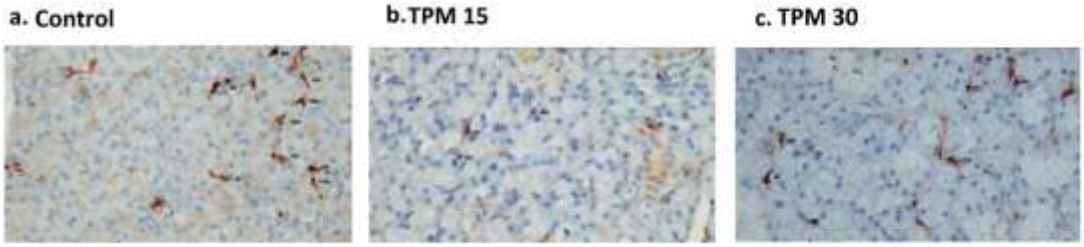

Figure 5. The effects of 15 µM (b, TPM 15, n=6) and 30 µM (c, TPM 30, n=6) topiramate administered i.p. on salivary gland s-IgA levels (IgA immunohistochemistry, original magnification x 400, ←: IgA positive cells).

DISCUSSION

The first finding we obtained in our study is that acute i.p. applied topiramate suppresses the anxiety index calculated according to the data obtained from the EPM. The basolateral (BLA) and central (CeA) nuclei of the amygdala attract more attention in anxiety disorders, including PTSD, which manifests with symptoms of anxiety, fear, undesirable memories, and hypervigilance[19]. BLA receives negative sensory information from the brain's sensory cortex and thalamus and transmits it to CeA via excitatory glutamatergic pathways, and also stimulates inhibitory GABAergic pathways between it and CeA[20]. Modulation of GABAergic pathways originating from CeA and extending to hypothalamus and brain stem leads to somatic manifestations of anxiety disorders[21]. In addition to the amygdala, the mPFC and anterior cingulate cortex also plays an important role in the regulation of anxiety[22]. Communication between these cortical areas and BLA occurs via excitatory glutamatergic pathways and in both directions[23].

Numerous studies have been conducted on the GABAergic system, the importance of which is known in PTSD. Local application of the $GABA_A$ agonist muscimol to the CeA and mPFC produced anxiolytic-like behavior, while microinjection of competitive (bicuculline) and non-competitive (picrotoxin) antagonists of the same receptor into the BLA resulted in anxiety-like behavior[24,25]. Local injections of $GABA_B$ agonist (baclofen) and antagonist (2OH-saclofen) into both BLA and CeA did not change anxiety[24]. In another study, the effects of the benzodiazepine receptor agonist midazolam, the inverse agonist, DMCM, and the competitive antagonist of the benzodiazepine binding site of the $GABA_A$ receptor, flumazenil, on anxiety were investigated. In this study, it was shown that midazolam reduces anxiety-like behavior, DMCM exhibits anxiety-like behavior, and flumazenil abolishes this DMCM-related effect[26]. Reducing the in vivo expression of the glutamic acid decarboxylase 67, the main catalyst of GABA production in the brain, resulted in reduced anxiolytic-like effect of benzodiazepine diazepam[27].

One of the important pathways that play a role in the pathophysiology of PTSD is the glutamatergic pathways. Local application of N-methyl-D-aspartate (NMDA) to the mPFC produced anxiogenic-like effects, while application of NMDA antagonists to the same region produced anxiolytic-like effects[28]. It has been shown that AMPA antagonist LY326325 reduces anxiety symptoms at low doses and increases it at high doses29,30. AMPA/kainate selective antagonist NBQX increased anxiogenic behavior in a dose-dependent manner29. Chemogenetic activation of excitatory neurons of the mPFC produced anxiolytic-like effect31. The fact that topiramate, which increases GABAergic current and suppresses glutamatergic transmission10,11, reduces the anxiety index in PTSD points to the possibility of its use in treatment.

The secondary finding in our study is that acute i.p. administered topiramate suppresses the freezing time, which is defined as the inability to perform any movement other than breathing, in EPM depending on the dose. Freezing behavior is used as a measure of fear frequently seen in PTSD[32]. In PTSD, patients experience fear similar to that in the first event when exposed to a trauma reminder, due to an impairment in fear extinction or an inability to extinguish a conditioned fear response[33]. In addition, there is a positive correlation between the severity of the trauma in the first event and the freezing time[34]. Studies show that freezing behavior is associated with serotonergic dysfunction in the hippocampus, one of the brain regions known to be important in PTSD[32,35]. Among the known mechanisms of action of topiramate, it has no direct effect on serotonergic pathways[10,11]. The reduction in freezing time after topiramate treatment in our study may be due to its indirect effect on serotonergic pathways by stimulating the $GABA_A$ receptor and increasing the GABAergic current and/or antagonizing the AMPA/kainate receptor and suppressing glutamatergic pathways. The greater suppression of freezing time after high-dose topiramate may be due to the indirect effect being more pronounced than the direct effect at higher concentrations.

Another finding in our study is that we observed a positive correlation between the anxiety index and plasma estrogen level, and topiramate treatment suppressed this correlation significantly at high doses. We preferred female Wistar rats in our study, since being female is among the factors that increase sensitivity in PTSD[1]. Studies have shown that estrogen has effects on anxiety and these effects are produced directly through its receptors widely distributed in the mPFC, hippocampus and

amygdala, and indirectly by modulating the serotonergic, dopaminergic, adrenergic and cholinergic systems and the HPA axis[36-38]. The relationship between estrogen level and anxiety has been found to be contradictory: low estrogen levels in some studies, while high estrogen levels in others have been associated with anxiety[39,40]. This finding in our study may be due to the indirect suppression of anxiety index by topiramate by increasing GABAergic current and/or decreasing glutamatergic activity in estrogen-affected pathways. As we mentioned above, the emergence of this finding regarding high doses of topiramate may be due to the fact that its indirect effect is more dominant at higher doses.

Another finding in our study is that topiramate did not affect the plasma progesterone level. In studies on anxiety disorders, the relationship between plasma progesterone level and anxiety was found to be inconsistent. In some studies, it has been reported that as the plasma progesterone level rises, anxiety also increases and neurosteroids such as allopregnanolone, which is produced from progesterone and exerts anxiolytic effect by stimulating $GABA_A$ receptors, decreases[41,42]. In another study, it was observed that progesterone administration caused anxiolytic behavior, the progestin receptor antagonist RU38486 did not reverse this effect, and it was blocked by picrotoxin and 5α-reductase inhibitor (N,N-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxamide)[43]. There is also a study showing that the progesterone level does not change in anxiety disorder[39]. The fact that topiramate did not change the plasma progesterone level in our study indicates that there is no decrease/blockade in the conversion of plasma progesterone to the anxiolytic neurosteroids.

In our study, we also observed that while topiramate did not affect s-IgA level at high dose, it suppressed it significantly at low dose. According to studies, s-IgA levels may increase or decrease in response to stress/trauma[5]. It is thought that acute stress is associated with increased s-IgA, while chronic stress is associated with decreased s-IgA[44,45]. Although some studies have shown that there may be a link between the level of s-IgA and the HPA axis that is activated in response to stress, the mechanisms by which this change occurs are not yet known[45,46]. In studies conducted with mothers and their children who were exposed to war, the s-IgA level was measured to be high and anxiety symptoms accompanied it[6,7]. In our study, we observed that high s-IgA level accompanied an increase in the anxiety index in the control group, which was the untreated PTSD group, topiramate suppressed both the IgA level and the anxiety index at low doses, and suppressed only anxiety at high doses. This may be due to the mixed effect of topiramate.

The limitation of our study is that the effects of topiramate were not examined at the receptor level. The strengths of our study are that the use of the predatory odor stress test to create a PTSD rat model, the experimental procedure was performed by the same medical pharmacologist, the biochemical and histopathological examinations were performed blindly by the same biochemist and pathologist, respectively.

PTSD, is an anxiety disorder resulting from changes in brain regions such as mPFC, hippocampus and amygdala, and glutamatergic, GABAergic, etc. systems due to previous trauma, and accompanied by changes in plasma estrogen and IgA levels. Current treatment remains inadequate, as the developmental mechanisms are not yet clarified. In our study, acute systemic administration of topiramate suppressed the anxiety index and freezing time. Topiramate suppressed the correlation between plasma estrogen level and anxiety in a dose-dependent manner. Topiramate suppressed salivary gland s-IgA levels at low dose, but this effect disappeared at high dose. These findings obtained in our study indicate that topiramate, a mixed-acting antiepileptic, may be among the treatment options for PTSD.

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