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Research Article
Nasal Oxytocin's Anticonvulsant Effects on Pentylenetetrazol-induced Convulsions in Rats

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Abstract

Background and Objective: Neurodegenerative proteins and pro-inflammatory mechanisms may cause epilepsy through excitatory-inhibitory imbalance. Drug resistance is common, therefore this disease requires affordable, accessible therapy. High-dose intraperitoneal oxytocin (OT) reduced seizures in experiments. Drug delivery is easy through the nasal mucosa. This study goal was to explain the anti-convulsant properties of nasal OT and its mechanism in rat seizures caused by pentylenetetrazol (PTZ). **Materials and Methods:** As, 36 male adult Sprague-Dawley rats were randomly assigned to 1 of 2 groups: Group A was utilized for electroencephalograms (EEG), whereas, group B was assigned to cognitive and behavioural evaluations. Of the thirty six rats, eighteen were used for behavioral investigations and the other eighteen were used for EEG recordings. Following the administration of OT, 35 mg kg^{-1} PTZ was used to capture the EEG. For 10 days, 40 g/kg/day of OT was administered orally, followed by the injection of PTZ about 70 mg kg^{-1} dose for behavioral investigations. In order to record the EEG, the reference electrode and the dura was implanted with electrodes above the left side of cortex of frontal section and cerebellum, respectively. The racine convulsion scale scores, percentage of spike in EEG, first myoclonic jerk beginning moment and levels of IL-1β, GAD-67, TNF-α and MDA in brain specimens were all compared across groups. **Results:** In comparison to the saline group, the first myoclonic jerk beginning time was noticeably faster in the group of OT ($p<0.05$). The convulsion scale used by Racine did not alter appreciably. In comparison to the saline group, the OT group had decreased spike percentages ($p<0.05$). The OT group's TNF-α, MDA and IL-1β levels were found to be considerably lower than those of the saline group ($p<0.01$, $p<0.001$ and $p<0.001$). In OT group's, GAD-67 increased considerably ($p<0.001$). **Conclusion:** Findings of this study show that OT has inhibitory actions against PTZ-triggered seizures as well as against the oxidative and inflammatory harm caused by PTZ.

Key words: Epilepsy, nasal oxytocin, pentylenetetrazol, seizure, rat model, neuroinflammation, oxidative stress

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Epilepsy is still one of the leading nervous system diseases in the world. It is thought that there are currently 70 million patients[1]. The mechanism of this disease is roughly the imbalance between the inhibitory and excitatory mechanisms. This instability is brought on by the buildup of neurodegeneration proteins including human tau and β-amyloid, the stimulation of proinflammatory events like neurogenesis, transforming growth factor β, interleukin 1β and activin receptor-like kinase, as well as alterations in ion channels[1-3]. Whatever the cause, epilepsy leads to mortality due to accidents that occur during seizures. Therefore, it is a preventable cause of mortality with treatment. Although the treatment is not very costly, it is difficult for patients to access treatment because epilepsy disease occurs mostly in the middle income countrys[1,2]. Therefore, accessible and inexpensive treatment methods are sought for this disease.

Neuropeptides are very important for the central nervous system to work in harmony. Peptides have very few drug-drug interactions and do not cause toxicological side effects. This is because they are metabolized by enzymes[4]. Although oxytocin is a 9 amino acid peptide, it has an effect on birth, breastfeeding and human behavior. The hypothalamus' paraventricular and supraoptic nuclei are where it is produced and is transported from there to the secretory center posterior pituitary for peripheral effects[4,5]. Experimentally, an anti-inflammatory effect has been observed with the application of OT and nitric oxide (NO) and NADPH oxidase gene expression have both been shown to be considerably decreased by OT[5,6]. In experimental studies, it was observed that the use of intraperitoneal (i.p.) high-dose OT stopped the seizures[7].

The nasal mucosa is an easily accessible area and provides a practical access route for the administration of drugs. There is no risk of gastrointestinal ingestion or toxicity in intranasal administration. Therefore, the therapeutic effect begins quickly[8,9]. It is easy to access and provides ease of use in emergency situations.

When administered, the selective gamma-aminobutyric acid A ($GABA_A$) receptor blocker pentylenetetrazole (PTZ) causes generalized tonic-clonic seizures that are dose-dependent[9].

In this investigation, EEG recordings was used to examine whether OT given orally on PTZ-triggered convulsions in rats has an anticonvulsant action.

MATERIALS AND METHODS

Study area: From January, 2021 to May, 2022, the research was conducted at the Experimental Animals Application and Research Center at Demiroglu Bilim University in Istanbul, Turkey.

Ethical approval: The local experimental animal research ethics committee gave its approval to the study (Demiroglu Science University, No: 05210203). The US Institute of Health's worldwide criteria were followed when conducting the experiments.

Experimental animal care: For this study, 48 male Sprague-Dawley rats deliberation 200-250 g each were used (24 rats for EEG recording and 24 rats for behavioral studies). The rats were bred for a morning-and-evening cycle of 12 o'clock Formun Altı (light from 7:00 a.m. to 7:00 p.m). They were fed in quiet rooms at temperatures close to 22-24°C, with known regular lab food and without water restriction.

Experimental procedures: A group for EEG analysis and an another group for activity evaluation had 36 rats in total. Groups A and B were assigned in a blind fashion. Rats were given general anesthesia before a stereotaxic drill was used to make a hole in the brainbone. For the reference EEG recording partner, electrodes (polyamide steel wires, 0.0001 m diameter and resistance 1/0.01 m) were made on the tissue layer in the left cortical area (0.0002 m lateral of the layer, 0.0015 m anterior to the bregma) and were installed in the capacitive formation (0.0015 m posterior, plane from the lambda)[3,10]. After that, the electrodes were attached with the dental acrylic procedure (A combination of several metals used to restore teeth may be found in dental acrylic).

Groups	Control groups	Salin+PTZ group	nOT+PTZ group
Group A (EEG experiment)	A1	A2	A3
Group B (behavioral experiment)	B1	B2	B3

The rats were anesthetized intraperitoneally (i.p) with ketalar (0.08 g kg^{-1}) and xylazine (0.004 g kg^{-1}) 0.035 g kg^{-1}. The Pentylenetetrazole use is suitable for evaluating brain activity with EEG. However, higher doses should be administered for behavioral changes. 70 mg kg^{-1} leads to suitable observable changes in activity but is not suitable for measuring brain activity. After the electrodes were placed, we waited 12 days for the fixation, after which 18 rats were separated into 3 different groups (n = 6): Groups A1, A2 and A3.

EEG experiment (Group A): Group A1 did not receive medication (control). Group A2 received just saline through the nasal route, whereas, Group A3 received 40 g/kg/day of OT (Oxytocin, 20 g mL^{-1}, Vetas) via the nasal route over the course of ten days. Ahead of injecting PTZ (35 mg kg^{-1}, i.p.), the medications were given. All groups-aside from group A1-35 mg kg^{-1} of PTZ was administered while having their EEGs recorded. Five minutes after PTZ management, a unique apparatus records all EEG graphics. As shown above, behavioral analysis recordings and techniques were designed[11].

The aforementioned procedures were followed for all EEG recordings and behavioral evaluations carried out like previously published by Erdogan *et al.*[12] and Borae *et al.*[13]. We amplified the signals 10,000 repetitions with filtration through 1 and 60 Hz to create 60 min EEG recordings[11,12]. The electroencephalography was captured using the BIOPAC MP150 (Biopac, Santa Barbara, US) and the percentage of spikes was calculated. Epileptiform activity or "percent spikes", is a measure of the proportion of 1 s boxes having at least one spike that was used by two clinical neurophysiologists to analyze EEG data[12].

Behavioral experiment (Group B): In accordance with the procedure outlined by Solmaz *et al.*[14], a group of 18 rats (Group B) was separated into 3 different groups (n = 6), designated as B1, B2 and B3. Group B1 was designed as the group of control and was not receive any agent. Saline was applied through the nasal route to Group B2 and 40 g/kg/day of oxytocin was administered via the nasal route to Group B3 over the course of 10 days. The PTZ (70 mg kg^{-1}, ip) injection was given 30 min after the treatments were given. The presence and type of seizures were assessed using the Racine convulsion scale scoring system (RCS)[11,15] and we also evaluated the first myoclonic jerk (FMJ) beginning time (TFMJ) (70 mg kg^{-1} PTZ only): The relevant seizure categories were noted: 6: Deadly convulsion, 5: Seizures of tonic-clonus with the absence of the righting reflexes, 4: Feeding while having violent tension convulsions, 3: Regularly occurring myoclonic jerks and motor arrest (For the purpose of determining the FMJ start time, this stage's timing was noted), 2: Exercise-related tightness combined with clear eyesight, 1: Pinnae and vibrissae jerking and 0: There isn't a seizure. As mentioned above, animals were also evaluated for FMJ[11]. The starting time was noted in secs. The time frame for seizure observation was constrained to 30 min since all animals exhibiting tonic-clonic seizures passed away[11].

Measurement of brain lipid peroxidation (MDA): According to a prior description by Bora *et al.*[13], malondialdehyde (MDA) concentrations as reactive thiobarbituric acid compounds determined the presence of lipid peroxidation in tissue samples (TBARS). Unfortunately, TBARS chemical agent and acetic acid were applied to the samples taken before being combined and heated for 60 min at 100°C. The specimens were run at 3000 r min^{-1} for 20 min after chilling in the ice, resulting in a maximum absorption scan at 535 nm. Tetraethoxypropane was used to compute MDA levels, which were then expressed as a nmol g^{-1} macromolecule as previously described by Quintana *et al.*[16].

Evaluation of brain protein levels in brain tissue samples: The entire amount of protein in brain specimens was quantified by the Bradford technique utilizing serum albumin from bovines as a standard.

Analysis the levels of IL-1β, TNF-α and GAD-67 in brain tissue samples: The brain is harvested and preserved under the ideal circumstances for biochemical investigation when kept at -20°C, similar to Solmaz *et al.*[14] in research on dexketoprofen. To prepare the whole brain specimens for tissue investigation, they were blended in a sonicator with 5 volumes of phosphate-buffered saline (pH level 7.4) and then for 15 min, centrifuged at 5000 g. The total macromolecular concentration in the brain's homogenous materials is then calculated using Bradford's methods after the supernatant has been collected.

The levels of IL-1β, TNF-α and GAD-67 (MyBioSource Co. Ltd.) in brain material homogenates were measured utilizing an Enzyme-Linked Immunosorbent Assay (ELISA) Kit. For absorbance measurements, every specimen was examined twice as per the guidelines provided by the manufacturer and via a microplate reader (Multiskan-go, Thermo Fisher, NH, USA).

Statistical analysis: The findings were provided as the Mean±Standard error (SEM). Version 15.0 of Windows SPSS is used for data distribution. To ascertain if a value range includes a standard distribution, the Shapiro-Wilk control is applied. By using differential analysis, the Kruskal-Wallis Test was identified and than the FMJ test was performed (ANOVA). To find differences between test groups, the Mann-Whitney U and *post hoc* Bonferroni Tests were implemented. It was agreed that a value of $p<0.05$ was significant statistically.

RESULTS

Assessment of groups based on spike percentage: We discovered that providing treatment of 40 µg/kg/day OT via nasal way during 10 days reduces significantly the convulsion stage more than PTZ+saline group as percentages change from 37.5-21.6% ($p<0.05$). By comparing the percentage of spikes to the B2 group, the seizure activity has significantly attenuated (Table 1 and Fig. 1).

Findings from behavioral experiments: In contrast to the PTZ+saline group, the findings of the behavioral investigation demonstrate that nasal application of OT has an anti-seizure impact in this rat model (Table 2).

We found that administration of 40 µg/kg/day OT via nasal way for 10 days was reduced significantly in the convulsion stage. From 4.1 (extremely severe as 6 points indicated deadly seizure activity), the average RCS score decreased to 3.6. On the other hand, 40 µg/kg/day OT administered via nasal way for 10 days delayed the TFMJ. While the average TFMJ of the untreated B2 group was 79.1s, the average TFMJ of the B3 group given OT was 128.5s ($p<0.05$) (Table 2).

Biochemical analysis: For oxidative stress measurement, we use MDA and compare PTZ (70 mg kg^{-1})+saline solution with the control group, MDA augments from 58.1-80.1 nmol g^{-1} which is statistically significant ($p<0.05$). The MDA levels considerably decreased in comparison to the PTZ+saline group after OT (40 g/kg/day) treatment and subsequent PTZ application ($p<0.01$). For understanding the anti-seizure mechanism and of course anti-inflammatory actions, we

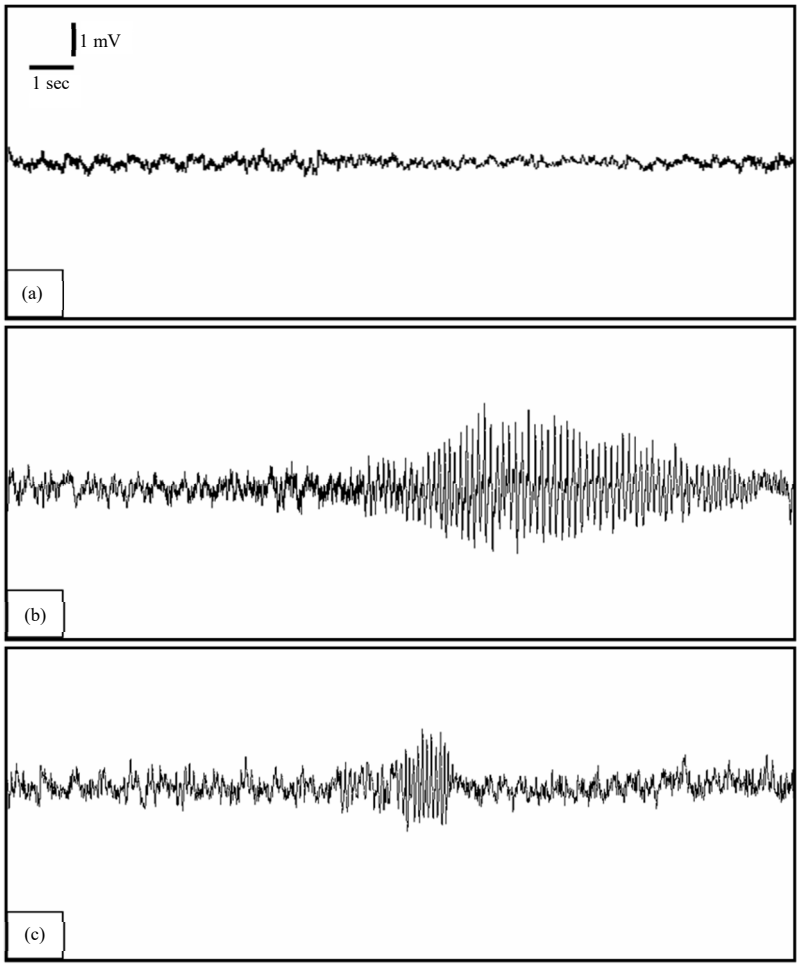

Fig. 1(a-c): Representative tracings from the EEG experiment, (a) (A1) (Control) (n = 6), (b) (A2) (PTZ and saline) (n = 6) and (c) (A3) (PTZ and 40 µg kg^{-1} nasal oxytocin) (n = 6)

As expected, there were virtually no spike waves seen in group A1. Dense spike wave activity was seen in group A2 due to the unopposed induction of seizures with 35 mg kg^{-1} of PTZ (i.p.), with a mean spike wave percentage score of 37.5%. There was significant abatement of seizure activity as quantified by spike wave percentages in group A3 with the addition of 40 µg kg^{-1} nasal oxytocin, A3 21.6% as compared to A2 ($p<0.05$)

Table 1: Evaluation of groups in terms of spike percentage results

Drugs Group	Spike percentage (%)	p-value*
A1-control (n = 6)	0	<0.05
A2- PTZ (35 mg kg^{-1}) and nasal saline (n = 6)	37.5±4.8	
A3- PTZ (35 mg kg^{-1}) and 40 µg kg^{-1} nasal oxytocin (n = 6)	21.6±3.3*	

*ANOVA test was used, Data were expressed as Mean±SEM, 40 µg kg^{-1} nasal oxytocin IP significantly reduced seizure activity on EEG as measured by spike-wave percentage compared with PTZ+saline group (*p<0.05) (different from PTZ and saline group)

Table 2: Behavioral experiment results

Drugs Group	Convulsion stage (Racine)	FMJ onset time (sec)	p-value*
B1-control (n = 6)	0	0	<0.01
B2-PTZ (70 mg kg^{-1}) and nasal saline (n = 6)	4.1±0.2	79.1±5.2	
B3-PTZ (70 mg kg^{-1}) and 40 µg kg^{-1} nasal oxytocin (n = 6)	3.6±0.3	128.5±6.9*	

*One way ANOVA test was used, Data were expressed as Mean±SEM, 40 µg kg^{-1} nasal oxytocin was not significantly reduced the Racine's convulsion score (RCS) compared to B2 Group, 40 µg kg^{-1} nasal oxytocin significantly reduced the time to first myoclonic jerk (TFMJ) compared to B2 group, *p<0.01 (different from PTZ and saline group)

Table 3: Evaluation of MDA, IL-1, TNF alpha and GAD results in behavioral experimental group

Parameter	Control (n = 6)	PTZ (70 mg kg^{-1}) and nasal saline (n = 6)	PTZ (70 mg kg^{-1}) and 40 µg kg^{-1} nasal oxytocin (n = 6)
Brain MDA level (nmol g^{-1})	58.1±3.9	80.1±6.9*	64.5±4.3$^{\#}$
Brain IL-1 beta (pg mg^{-1} protein)	1.9±0.2	3.1±0.1*	2.2±0.5$^{\#}$
Brain TNF-alfa (pg mg^{-1} protein)	96.5±6.2	165.8±7.3**	102.4±9.4$^{\#\#}$
Brain GAD-67 (pg mg^{-1} protein)	10.2±0.9	5.5±1.1**	9.3±1.7$^{\#\#}$

*One way ANOVA test was used, Data were expressed as Mean±SEM, Malondialdehyde (MDA) and Brain IL-1 levels were found to be significant when compared to the B2 group with control group (*p<0.05), likewise, the TNF-alfa and GAD-67 levels were statistically significantly higher than the control group (**p<0.001). In the group treated with 40 µg kg^{-1} nasal oxytocin, a statistically significant decrease in MDA and Brain IL-1 levels were observed compared to the B2 group ($^{\#}$p<0.01). However, a significant decrease was observed in TNF-alfa and GAD-67 levels compared to the B2 group ($^{\#\#}$p<0.001)

evaluate IL-1β, TNF-α and GAD 67. If we compare with the control group evaluate IL-1β and TNF-α significantly increase (1.9-3.1, 96.5-165.8) (p<0.05 and p<0.01) with the application of PTZ 70 mg kg^{-1} and decrease also significantly with 40 µg kg^{-1} OT applied group (3.1-2.2, 165.8-102.4) (p<0.01 and p<0.001). In contrast, GAD67, which evaluates GABA activity in the brain, decreased with PTZ 70 mg kg^{-1}+saline compared to the control group (10.2-5.5 pg mg^{-1}) (p<0.001) and increased in the group of PTZ 70 mg kg^{-1}+40 µg kg^{-1} OT compared to PTZ+saline group (5.5-9.3 pg mg^{-1}) (p<0.001). All findings regarding biochemical parameters are presented in Table 3.

DISCUSSION

Some studies show that intranasal oxytocin is a potential candidate agent in the medication of diseases such as borderline personality disorder, schizoaffective disorders and autism for which there is currently no cure. In addition, pain and appetite control began to be recommended for the stabilization of aging-related cognitive behaviors[16].

As a result of this study, it was reported that hippocampal administered OT prolongs the FMJ onset time and decreases generalized tonic-clonic seizures caused by PTZ[17]. Erbaş *et al.*[7], who investigated the effect of intraperitoneal OT on seizures in an experimental rat model, reported that an anti-convulsive effect occurred at doses of 80 and 160 ng kg^{-1} [7]. Again, in a study on rats, Wong *et al.*[18] administered OT both intranasally and intraventricularly. They showed that nanoparticle encapsulated OT (nNP-OT) given intranasally at a dose of 50 µg significantly aggravates and improves the neuronal system and social behavior against triggered seizures in the SCN1A-induced epilepsy mouse model. They suggested that nNP-OT administered at a dose of 50 µm reached higher concentrations in the cerebrospinal fluid compared to plasma, thus not causing systemic side effects. In this study, OT has been shown to be an effective anticonvulsive[18]. The routes of administration used for experimental animal studies are not practical and usable for use in humans. In this study, we demonstrated the anticonvulsive efficacy of nasally-administered OT. The nasal route is practically applicable to patients and easy to use. In their study investigating the presence of intranasally administered OT in brain tissue, Lee *et al.*[19] used an animal model labeled d5OT. Intranasally administered d5OT at doses of 40 IU and 80 IU was detected in CSF and brain tissue, orbitofrontal cortex, striatum, brain stem and thalamus. Since these regions are the brain regions located in the trigeminal and olfactory nerve orbits, they suggested that OT reaches the brain by following this route in intranasal administration. Thus, they suggested that OT given by the nasal route does not need the ability to pass the blood-brain barrier and may be faster and more effective than the intranasal route[19].

Intranasal administration allows rapid onset of therapeutic effects of the drug, reducing the time that drugs lose in initial elimination. It also significantly reduces side effects, is non-invasive, painless and can ultimately be easily administered by patients or doctors in an emergency. In the case of chronic drug use, it can be an alternative to parenteral or oral administration[8,20]. Numerous published studies demonstrate the nasal effectiveness of OT. Quintana *et al.*[21] reported in their study that a pupillary response was obtained with an 8 IU dose nasal OT and that nasal OT could reach the amygdala. Smith *et al.*[22] reported that OT levels were detected in the amygdala and hippocampus as a result of the animal study in which nasal OT was administered. In their study on human subjects, Striepens *et al.*[23] demonstrated the presence of OT levels in CSF and plasma after 24 IU of nasally administered OT.

The result showed that OT, which has a good transition to the central nervous system when administered nasally, shows its anticonvulsive effect through anti-inflammatory processes. We found that IL1-β and TNF-α levels, which increased after induction with PTZ, decreased in rats given nOT. During an epileptic seizure, inflammatory cytokines increase in the brain[3]. Many clinical experiments with TSPO positron emission tomography showed that seizures generated by temporal and frontal lobe epilepsy and focal cortical abnormalities are associated with increased mRNA levels of TNF-α, IL-6 and IL-1β cytokines and revealed control the levels of Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor-Beta 1 (TGF-β1)[18]. Microglia and astrocytes that have been stimulated generate the pro-inflammatory cytokine TNF-α. TNF-α up-regulates AMPA receptors. Increased AMPA receptors allow the excessive intake of calcium, causing neurotoxicity[24]. Roseti *et al.*[25] showed that in temporal lobe epilepsy, IL-1β inhibits approximately 30% of GABA-induced synaptic transmission and causes neuronal hyper-excitability, leading to seizure formation. The GABA is an inhibitory neurotransmitter. The enzyme glutamate decarboxylase (GAD) converts glutamate into γ-amino butyric acid (GABA). The GAD is found in the brain as the GAD67 isoenzyme[26,27]. An increase in the level of GAD67 means that the synthesis of GABA is increased and the inhibitory effect is dominant. In this study, GAD67 expression was significantly reduced in the PTZ+saline group in comparison to the control group ($p<0.001$). The GAD67 was considerably elevated in the OT group in comparison to the PTZ+saline group ($p<0.001$). This showed that nOT increases the inhibitory effect by increasing GABA synthesis. In addition, we think that decreased MDA levels also contribute to this inhibition. Likewise, lipid peroxidation products increase seizure susceptibility through peripheral inflammation, hippocampal microglial activation reliant on COX-2 and increased expression of TNF-α, IL-6 and IL-1β[2,28].

CONCLUSION

In this study, the anticonvulsant impact of oxytocin via its anti-inflammatory activity when administered intranasally was proved. With nasal oxytocin, seizures will be less severe and probably shorter, less induction of cytokines and less oxidative stress were expected. We think that studies with different doses in intranasal application will shed light on the subject.

SIGNIFICANCE STATEMENT

One of the most prominent diseases of the neurological system worldwide is epilepsy. The disequilibrium between both the excitatory and inhibitory mechanisms is basically the disease's pathophysiology. Epilepsy pharmaceutical therapy has not been effective or well-tolerated and anti-epileptic drugs remain to produce a broad range of side effects. Over 30% of epilepsy patients are unresponsive to treatment, regardless the expansion and variety of anti-epileptic drugs. Therefore, additional study is needed to understand the pathophysiology of epilepsy and how to treat it. In experimental investigations, it was noted that the administration of high-dose intraperitoneal oxytocin had effectively inhibited seizures. The nasal mucosa is a convenient location for administering medications since it is readily accessible. It is simple to use and accessible in emergency scenarios. Pentylenetetrazole (PTZ), a specific $GABA_A$ receptor blocker, induces dose-dependent generalized tonic-clonic seizures. In this work, we demonstrated that oxytocin's anti-inflammatory effects when given intranasally had an anticonvulsant effects. With intranasal oxytocin, seizures will likely be fewer and lesser drastic and less cytokine production and oxidative stress are anticipated. Studies using various dosages for intranasal administration, in our opinion, will clarify the issue.

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