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Oxytocin inhibits pentylentetrazol-induced seizures in the rat

Oytun Erbas[a], Mustafa Yılmaz[b,*], Huseyin Anil Korkmaz[c], Saylav Bora[a], Vedat Evren[a], Gonul Peker[a]

[a] Ege University Faculty of Medicine, Department of Physiology, Izmir, Turkey
[b] Mugla University Faculty of Medicine, Department of Neurology, Mugla, Turkey
[c] Dr. Behcet Uz Children Disease and Surgery Tranning and Research Hospital, Department of Pediatric Endocrinology, Izmir, Turkey

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ABSTRACT

We aimed to reveal the anti-convulsant effects of oxytocin (OT) in pentylenetetrazol (PTZ)-induced seizures in rats. Thirty rats were randomly divided into 5 equal groups. Using stereotaxy, we implanted electroencephalogram (EEG) electrodes in the left nucleus of the posterior thalamus. After 2 days, the first and second groups were used as the control and PTZ (35 mg/kg) groups, respectively. We administered 40, 80 and 160 nmol/kg OT + 35 mg/kg PTZ to the rats, constituting the third, fourth, and fifth groups, respectively, for 5 days. At the end of 5 days, we recorded EEGs via bipolar EEG electrodes. After 12 h, all groups except the first received 70 mg/kg PTZ and we determined the dose–response ratio. Racine's Convulsion Scale was used to evaluate seizures. The spike–wave complex percentage in the EEG was determined as 0% for the first group, 38.6% ± 7.2 for the second group, 36.4% ± 5.6 for the third group, 4.3% ± 1.8 for the fifth group and 4.1% ± 1.1 for the fifth group. The fourth and fifth groups had significantly decreased spike–wave complex percentages compared to the second group ($p < 0.0001$). OT may prevent PTZ-induced epilepsy on an EEG. OT may also be considered for use in the treatment of epilepsy in the future.

1. Introduction

Epilepsy is the second most common neurodegenerative disease and is characterized as recurrent spontaneous seizures arising from abnormal electrical activity in the brain [5]. Despite extensive experimental and clinical studies, the etiology of epilepsy cannot be described perfectly. The treatment of epilepsy with medication has not been effective and well-tolerated, and anti-epileptic drugs still have a wide range of side effects. Despite the increasing number and variety of anti-epileptic drugs, more than 30% of epilepsy cases are refractory to treatment [11]. Thus, more studies need to be developed in the field of pathophysiology or the treatment of epilepsy. Existing research findings have suggested that oxidative stress can disturb the balance of electrical activity in the brain and lead to seizures. Some drugs that have neuroprotective effects and scavenger reactive oxygen species (ROS), may have a beneficial effect on the treatment of epilepsy. Oxytocin (OT) is a neurohypophysial nonapeptide synthesized in the paraventricular and supraoptical nuclei of the hypothalamus and plays a role in lactation and parturition [18,31]. Recent studies have demonstrated that OT exerts anti-inflammatory effects in different inflammation models. The major biochemical pathway for ROS formation proceeds through O_2^- production, which is generated by NADPH oxidase. Administration of OT significantly decreased the gene expression of both NADPH oxidase and nitric oxide (NO) [16,19]. It was reported that OT has a powerful antioxidant effect on some tissues [29].

Pentylenetetrazol (PTZ), which is a selective blocker of the $GABA_A$ receptor, is a drug used as a chemical kindling and is characterized by dose-dependent subconvulsion and generalized tonic–clonic seizures following-injection [32]. The features of epileptic seizure are abruptness and reiteration [29]. Recent studies have demonstrated that PTZ-induced seizures result in electrographic, molecular and endocrine responses in the brain. It has been suggested that OT has an anti-convulsive effect in different rat models [3]. In contrast, OT might increase seizures [21].

We mainly aimed to investigate whether OT exhibited an anti-convulsant effect on PTZ-induced seizures using EEG recordings of rats. We hypothesize that different doses of OT will inhibit experimental, PTZ-induced epilepsy in rats, according to EEGs. Both OT and PTZ have been shown to affect glutamate [22,28]. Thus we prefer a PTZ-induced epilepsy model.

2. Materials and methods

All experiments, performed in the study, were carried out according to the rules in *the Guide for the Care and Use of Laboratory Animals*, as adopted by National Institutes of Health (U.S.) and

* Corresponding author at: Department of Neurology, Mugla University Medical School, 48000 Mugla, Turkey. Tel.: +90 252 211 4839; fax: +90 252 211 1345.
E-mail addresses: mustafayilmaz@mu.edu.tr, dr_mustafay@hotmail.com (M. Yılmaz).

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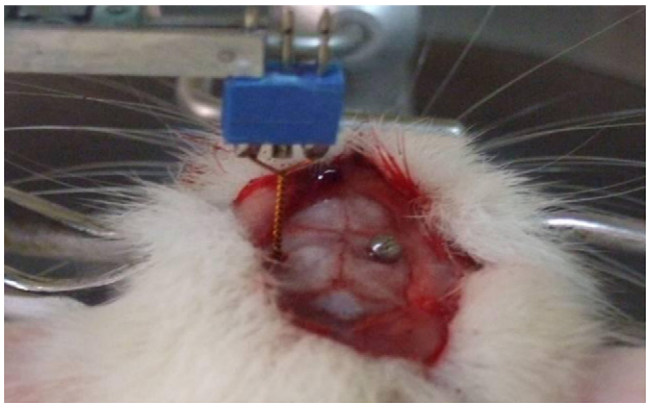

Fig. 1. EEG electrode placement in cranium by drilling holes.

received the Ege University Animal Ethics Committee's consent. Thirty male Sprague–Dawley rats, weighing 200–250 g were used. The rats were housed in quiet rooms with a 12–12 h light–dark cycle (light from 07.00 to 19.00) and a 22–24 °C ambient temperature, and were given standard laboratory food and tap water ad libitum. The rats were aged between 8 and 12 weeks. First, by opening a small hole with a drill under anesthesia then using the stereotaxic method taking bregma as reference (coordinates AP: −3.6 mm, L: +2.8 mm, DV: −5.0 mm), an exterior insulated bipolar EEG electrode (100 μm in diameter) was placed in the left thalamic nucleus [25]. The electrodes were fixed using a dental acrylic (numerous alloys are used in the making of dental restorations). Rats were anesthetized using ketamine (40 mg/kg) (Alfamine®, Ege Vet, Alfasan International B.V. Hollanda) and xylazine (4 mg/kg) (Alfazyne®, Ege Vet, Alfasan International B.V. Hollanda) intraperitoneally (ip). Selective reductions in $GABA_A$ receptor subunits in the thalamus may play a role in the pathophysiology of absent epilepsy, because all the EEG recordings were taken from the thalamic region. The placement of EEG electrodes by drilling holes in the cranium is shown in Fig. 1.Two days after the electrode was fixed, the rats were divided into 5 groups ($n = 6$). The first group was described the control. The second group was administered 35 mg/kg PTZ (Sigma Aldrich) and saline ip, the third group 35 mg/kg PTZ and 40 nmol/kg OT ip, the fourth group 35 mg/kg PTZ and 80 nmol/kg OT ip and the fifth group 35 mg/kg PTZ and 160 nmol/kg OT ip. The drugs were administered once-daily for 5 days.

EEGs were recorded by bipolar EEG electrodes after 5 days (Fig. 2). EEG recordings were taken in awake rats in a special container. An EEG recording was taken every 20 min. The signals were amplified 10,000 times and filtered with a range

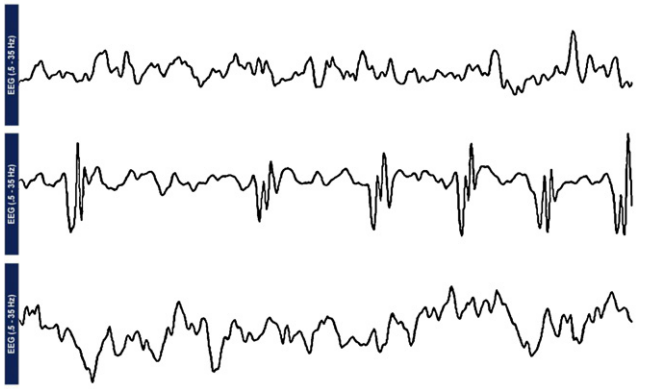

Fig. 2. EEG recording of each group: upper: first group (control), middle: second group (PTZ), lower: fifth group (PTZ ± 160 nmol/kg OT).

Table 1
Convulsion scale.

Groups	Convulsion stage
Normal and saline (control)	0
PTZ (70 mg/kg) and saline	5 ± 0
PTZ (70 mg/kg) and 40 nmol/kg OT	5 ± 0
PTZ (70 mg/kg) and 80 nmol/kg OT	3.14 ± 0.26*
PTZ (70 mg/kg) and 160 nmol/kg OT	3.0 ± 0.21**

The significance of * and ** is same as Table 2.

of 1–60 Hz. System records were taken using the Biopac MP30 amplifier system and evaluated according to PSA (power spectral density analysis) methods. During this process delta 1–3.9 Hz, theta 4–7.9 Hz, alpha 8–11.9 Hz, and beta 12–20 Hz waves in the EEG were accepted as the ratio of percentage in PSA methods. We affirmed the electrode location histologically following euthanization. After 12 h, all groups except the first received 70 mg/kg PTZ, and we determined the dose–response ratio. Racine's Convulsion Scale was used to evaluate the seizures as follows: 0 = no convulsion; 1 = twitching of vibrassae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myclonic jerks; 4 = tonic clonic seizure while the animal remained on its feed; 5 = tonic–clonic seizure with loss of the righting reflex; 6 = lethal seizure [13,14]. The observation period for PTZ-induced seizures was limited to 30 min. Immediately, rats were placed in a plexiglas cage and then observed the severity of seizures using a seven-point semiquantitative scale to measure time to onset after PTZ treatment.

2.1. Statistical analysis

Data analyses were performed using the SPSS for Windows, version 15.0.1 (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) was used in evaluation of the effects of OT on PTZ-induced seizures. EEG traces were analyzed via the aid of Biopac MP 150 analysis software. Power spectra were expressed as means. The paired *t*-test was used to compare the differences of powers at each frequency. *p*-Values of <0.05 were regarded as significant.

3. Results

Racine's Convulsion Scale is described in Table 1. The percentages of delta, theta, alpha, beta, spike–wave complex are shown in Table 2. EEG abnormalities in relation to the groups presented as follows. The percentages of delta and theta waves were significantly changed in the fourth and fifth compared to the PTZ (second) ($p < 0.001$). In contrast, the treatment of PTZ-induced seizures by 40 nmol/kg OT did not result in significant differences in waves compared to the PTZ group ($p > 0.05$). Likewise, the percentages of alpha and beta waves were more significantly changed in the second and third groups compared to the first (control) group ($p < 0.001$). The theta waves in the fourth and fifth groups were significantly increased compared to the PTZ group ($p < 0.001$). The spike–wave complex in the second and third groups was increased more significantly compared to the first (control) group ($p < 0.001$). However, the treatment of the third group is not significant compared to the PTZ group ($p > 0.05$). In addition, the comparison of both spike waves and other waves between the fourth and fifth group is not significant ($p > 0.05$). Comparing the convulsion scores among the groups indicated the severity of seizures after the injection of PTZ. The fourth and fifth groups showed similar effects and both were highly significant compared to the PTZ group ($p < 0.001$).