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Original Article

Effects of Intracerebroventricular Injection of the Steroidal and Non-Steroidal Anti-Inflammatory Drugs on the Seizures during the Estrous Cycle in Rat

Fazlelahi, Z[1], Kaboutari, J[2]*, Zendehdel, M[3], Panahi, N[1]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
3. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, 14155-6453, Tehran, Iran

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Corresponding Author: kaboutari-j@sku.ac.ir

Abstract

Because of the mutual relationship between neural inflammation and seizure, this study aimed to determine the effects of intracerebroventricular (ICV) injection of the steroidal and non-steroidal anti-inflammatory drugs on pentylenetetrazol (PTZ)-induced seizures during the estrous cycle in rats. A total of 105 adult female Wistar rats were selected and divided into seven groups, including the control (saline), ketorolac tris salt (7.5, 15, and 30 µg), and methylprednisolone acetate (0.15, 0.3, and 0.6 µg), each with four subgroups (proestrus, estrus, metestrus, and diestrus) and three replicates (n=5). After a week of acclimatization, the estrous phase determination and synchronization were performed. Acute epilepsy was inspired by the intraperitoneal injection of 80 mg/kg of PTZ 30 min after the ICV injection of ketorolac and methylprednisolone acetate. The initiation time of myoclonic seizures (ITMS), the initiation time of tonic-clonic seizures (ITTS), seizure duration (SD), and mortality rate (MR) were measured for 30 min. Data were shown as mean±SD and analyzed using One-way ANOVA followed by Tukey–Kramer multiple comparison post hoc test (P<0.05). According to the results, ketorolac (15 and 30 µg) and methylprednisolone acetate (0.3 and 0.6 µg) significantly increased the ITTS and ITMS but decreased SD during the estrous cycle, compared to the control (P<0.05). Moreover, MR and SD were significantly decreased by ketorolac (7.5, 15, and 30 µg) and methylprednisolone (0.3 and 0.6 µg), compared to the control during the estrous cycle (P<0.05). Therefore, it seems that both ketorolac and methylprednisolone possess dose-dependent anticonvulsant effects that may decrease neural inflammation.

Keywords: Intracerebroventricular, Methylprednisolone acetate, Ketorolac tris salt, Rat, Seizure

1. Introduction

Catamenial epilepsy is a brain disorder associated with the estrous cycle in some epileptic women of focal or general types, which is defined as a two-fold increase in the tendency to convulsions during, immediately before, or at the end of the estrous cycle and occurs in about 30% of women with epilepsy (1, 2). Fluctuations in the ovarian hormones during the estrous cycle and electrolyte imbalance are considered the main causative factors of catamenial epilepsy (3). According to the theories proposed in 1950, progestogen produces anticonvulsant and estradiol pro-convulsant effects; therefore, steroid hormones can modulate the onset and propagation of seizures. In rodents, a correlation was observed between serum estradiol/progestogen levels and the incidence of seizures in ovulatory cycles, and the highest seizure activity was correlated with increased estrogen levels (4). Pentylenetetrazol (PTZ) has been widely used experimentally for seizure induction via inhibiting the

$GABA_A$ receptors (4). It is known that sex hormones' effect on seizure can be mediated through neurosteroids in the central nervous system (CNS). Progesterone neuroactive metabolites, such as allopregnanolone and pregnanolone, can affect GABAergic receptors as the most abundant inhibitory receptors in the CNS (2). Despite several years of research and different theories on the etiology of epilepsy, its complicated cellular and molecular mechanisms are not fully understood. The relationship between seizures and cerebral edema is one of the interesting challenges in the treatment of seizures. There is a mutual relationship between neuroinflammation and the occurrence of seizures. Seizures can initiate the production and release of pre-inflammatory mediators and the occurrence of neurogenic inflammation. Cerebral edema is effective in determining the seizure threshold in prone areas of the brain and thus plays a critical role in the speeding up and reversibility of seizures. Seizures and the resulting cell damage can cause inflammation, which can speed up seizures and cause neural cell damage. Non-steroidal anti-inflammatory drugs (NSAIDs) increase the anticonvulsive effect of valproic acid (5, 6). They downregulate P-glycoprotein expression and brain activity and exhibit anticonvulsant activity by regulating prostaglandins synthesis (7). They inhibit cyclooxygenase (COX)-1 or COX-2 isozymes (8). Despite the role of sex hormones in catamenial epilepsy, as well as the physiological function of the SAIDs/glucocorticoids and NSAIDs on the reproductive system, there is no report on the role of the central methylprednisolone acetate and ketorolac tris salt injection on catamenial epilepsy. Therefore, we investigated the antiepileptic effects of intracerebroventricular (ICV) injection of the SAIDs and NSAIDs on seizures during the estrous cycle in rats.

2. Materials and Methods

2.1. Animals

In total, 105 adult female Wistar rats (180±20 g) were prepared and kept under standard conditions, according to the European Community Guidelines for Laboratory Animals (an ambient temperature of 23±1°C, a 12-h dark/light cycle, and relative humidity of 55-56%) in standard cages with ad libitum access to chow pellets and clean water. After one week of acclimatization, the animals were randomly divided into seven groups (n=5) each with three replicates to investigate the possible antiepileptic effects of methylprednisolone acetate and ketorolac tris salt during various stages of the estrous cycle. Before the study, sexual puberty was approved using vaginal smears, and those animals with two regular estrous cycles were selected for the experiments, and then the estrous synchronization was performed (9).

2.2. Synchronization of the Estrous Cycle

Before performing the experiments, vaginal smears were obtained daily to determine the stage of the estrous cycle based on the dominant cell type. Large round nucleated cells indicated proestrus, masses of the cornfield squamous epithelial cells indicated estrous, round nucleated epithelial cells with leukocyte infiltration showed metestrus, and the predominance of leukocytes was determined as diestrus (9, 10).

2.3. Surgery Procedure

Following the one-week adaptation period, animals were anesthetized with an intraperitoneal (i.p) injection of ketamine (80 mg/kg, JHP Pharmaceuticals, USA) and xylazine (10 mg/kg, Dechra Pharmaceuticals PLC, England) and then placed in a stereotaxic device (Stoelting, Wood Lane, IL, USA). The skull was leveled off around the bregma after incising the scalp. A stainless-steel guide cannula (23-gauge, 12 mm length) was performed as (Ap=-0.8 mm, DV=-3.3 mm, ML=+1.6 mm) and placed in the right lateral cerebral ventricular (11). The cannula was then fixed to the skull. A -12.5 mm stylet was placed in the cannula to keep it from blocking before drug administration. Before initiating the experiments, animals were allowed a one-week recovery.

2.4. Study Procedure

This study investigated the antiepileptic effects of ketorolac tris salt and methylprednisolone acetate on

PTZ-induced epilepsy during the estrous cycle in rats. A total of 105 adult female Wistar rats were randomly divided into seven groups, including the control (saline), ketorolac tris salt (7.5, 15, and 30 µg), methylprednisolone acetate (0.15, 0.3, and 0.6 µg) (Table 1), each with three replicates and four subgroups, including proestrus, estrus, metestrus, and diestrus. Each group received the ICV administration of saline, as well as different doses of ketorolac and methylprednisolone. After the seizure induction by the i.p administration of PTZ (80 mg/kg), animals' behavior was monitored for 30 min to assess seizure duration (SD), the mortality rate (MR), the initiation time of myoclonic seizures (ITMS), and the initiation time of tonic-clonic seizures **(**ITTS) (12). All experiments were conducted from 9 to 12 a.m. to avoid the impact of circadian rhythm on seizure susceptibility (12).

2.5. Cannula Verification

At the end of the experiments, the placement of the cannula in the lateral ventricle was determined by the ICV administration of 10 µL methylene blue. After that, animals were euthanized with a high dose of pentobarbital and decapitated. The placement of the cannula's tip and the dye's diffusion into the lateral ventricle were visually monitored (13).

2.6. Statistical Analysis

Data were expressed as mean±SD, and the analysis was performed using a One-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison post hoc tests (P<0.05).

Table 1. Treatment procedure in Experiment 1

Group Estrous Cycle	First injection	Second injection*
Control	Normal saline	PTZ (80 mg/kg)
Ketorolac tris salt (7.5 µg)	Ketorolac tris salt (7.5 µg)	PTZ (80 mg/kg)
Ketorolac tris salt (15 µg)	Ketorolac tris salt (15 µg)	PTZ (80 mg/kg)
Ketorolac tris salt (30 µg)	Ketorolac tris salt (30 µg)	PTZ (80 mg/kg)
Methylprednisolone acetate (0.15 µg)	Methylprednisolone acetate (0.15 µg)	PTZ (80 mg/kg)
Methylprednisolone acetate (0.3 µg)	Methylprednisolone acetate (0.3 µg)	PTZ (80 mg/kg)
Methylprednisolone acetate (0.6 µg)	Methylprednisolone acetate (0.6 µg)	PTZ (80 mg/kg)

*30 min after the first injection; PTZ, Pentylenetetrazol

3. Results

The antiepileptic effects of ketorolac tris salt (7.5, 15, and 30 µg) on the ITMS and ITTS during various phases of the estrous cycle are shown in figures 1 and 2. As can be seen, 15 and 30 µg of ketorolac tris salt increased the onset of ITMS and ITTS in all phases of the estrous cycle, in comparison with the control's dose dependently (P<0.05), while there was no significant difference between the 7.5 µg dose and the control (P>0.05).

According to figures 3 and 4, methylprednisolone acetate (0.3 and 0.6 µg) significantly increased the onset of ITMS and ITTS in all phases of estrous, in comparison with the control (P<0.05). However, there was no significant difference between the 0.15 µg dose and the control (P>0.05).

The effects of ketorolac tris salt (7.5, 15, and 30 µg) and methylprednisolone acetate (0.3 and 0.6 µg) on SD in different phases of the estrous cycle are presented in table 2. It shows that SD was significantly decreased by ketorolac tris salt (15 and 30 µg) and methylprednisolone acetate (0.3 and 0.6 µg), compared to the control's dose dependently (P<0.05), while there was no significant difference between the 0.15 µg dose of methylprednisolone, 7.5 µg of ketorolac, and the control (P>0.05).

As can be seen in table 3, the MR of seizures during various phases of the estrous cycle were significantly decreased by ketorolac tris salt (7.5, 15, and 30 µg) and methylprednisolone acetate (0.3 and 0.6 µg), compared to the control's dose dependently (P<0.05), and 0.6 µg of methylprednisolone completely inhibited MR in all phases.

In all experiments of ITMS, ITTS, SD, and MR, there was no significant difference neither between follicular phases (proestrus and estrus) nor between luteal phases (metestrus and diestrus) (P>0.05), while antiepileptic parameters were significantly more prominent during the luteal phase than the follicular phase (P<0.05).

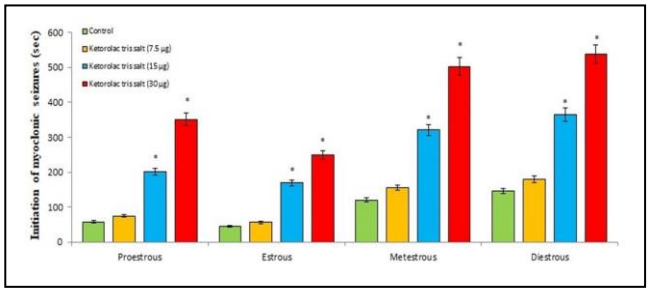

Figure 1. Antiepileptic effects of Ketorolac tris salt (7.5, 15 and 30 µg) on the initiation time of myoclonic seizures (ITMS) (sec) during the estrous cycle. *Asterisks indicate significant difference in each phase of the estrous cycle compared with the control group (P < 0.05). Data are presented as mean ± SEM

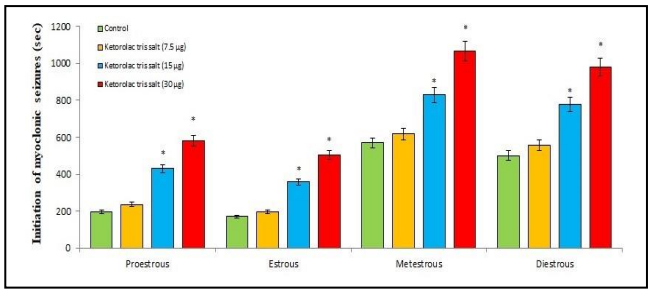

Figure 2. Antiepileptic effects of Ketorolac tris salt (7.5, 15 and 30 µg) on the initiation time of tonic-clonic seizures (ITTS) (sec) during the estrous cycle. *Asterisks indicate significant difference in each phase of the estrous cycle compared with the control group (P < 0.05). Data are presented as mean ± SEM

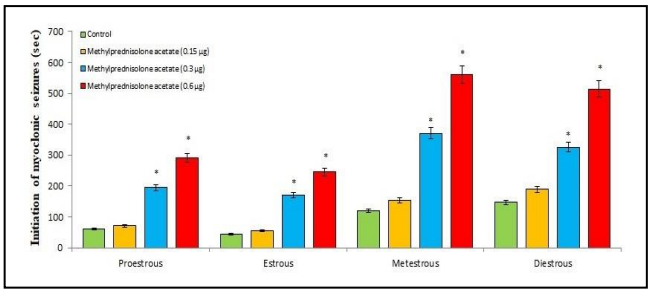

Figure 3. Antiepileptic effects of Methylprednisolone acetate (0.15, 0.3 and 0.6 µg) on the initiation time of myoclonic seizures (ITMS) (sec) during the estrous cycle. *Asterisks indicate significant difference in each phase of the estrous cycle compared with the control group (P < 0.05). Data are presented as mean ± SEM

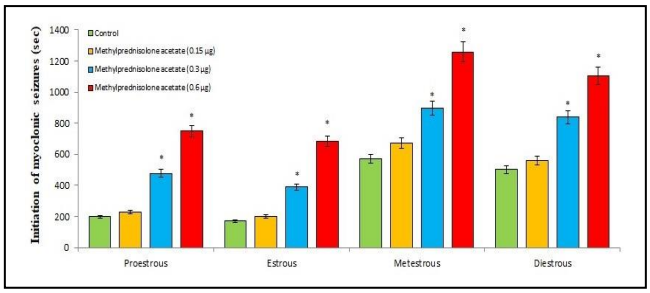

Figure 4. Antiepileptic effects of Methylprednisolone acetate (0.15, 0.3 and 0.6 µg) on the initiation time of tonic-clonic seizures (ITTS) (sec) during the estrous cycle. *Asterisks indicate significant difference in each phase of the estrous cycle compared with the control group (P < 0.05). Data are presented as mean ± SEM

Table 2. Effects of Ketorolac tris salt (7.5, 15 and 30 µg) and Methylprednisolone acetate (0.15, 0.3 and 0.6 µg) on the seizure duration (SD) (sec) during the estrous cycle. Different letters (a, b or c) in each column indicate significant difference (P< 0.05) between various treatments in each phase of the estrous cycle; data are presented as mean ± SEM

Group Estrous Cycle	Proestrous	Estrous	Metestrous	Diestrous
Control	745 ± 68^{a}	811 ± 65^{a}	473 ± 42^{a}	584 ± 52^{a}
Ketorolac tris salt (7.5 µg)	658 ± 52^{a}	783 ± 58^{a}	429 ± 35^{a}	519 ± 60^{a}
Ketorolac tris salt (15 µg)	408 ± 39^{b}	475 ± 46^{b}	259± 36^{b}	326 ± 35^{b}
Ketorolac tris salt (30 µg)	274± 33^{c}	301 ± 43^{c}	187 ± 26^{c}	235 ± 24^{c}
Methylprednisolone acetate (0.15 µg)	635 ± 42^{a}	778 ± 59^{a}	465 ± 47^{a}	513 ± 59^{a}
Methylprednisolone acetate (0.3 µg)	452 ± 47^{b}	438 ± 45^{b}	240 ± 37^{b}	347 ± 38^{b}
Methylprednisolone acetate (0.6 µg)	237 ± 36^{c}	292 ± 37^{c}	170 ± 29^{c}	217 ± 31^{c}

Table 3. Effects of Ketorolac tris salt (7.5, 15 and 30 µg) and Methylprednisolone acetate (0.15, 0.3 and 0.6 µg) on the mortality rate (MR) of seizures (%) during the estrous cycle. Different letters (a, b or c) in each column indicate significant difference ($P< 0.05$) between various treatments in each phase of the estrous cycle; data are presented as mean ± SEM.

Group Estrous Cycle	Proestrous	Estrous	Metestrous	Diestrous
Control	50^a	50^a	20^a	20^a
Ketorolac tris salt (7.5 µg)	40^a	50^a	15^a	15^a
Ketorolac tris salt (15 µg)	20^b	20^b	0^b	0^b
Ketorolac tris salt (30 µg)	0^c	0^c	0^b	0^b
Methylprednisolone acetate (0.15 µg)	50^a	50^a	15^a	20^a
Methylprednisolone acetate (0.3 µg)	20^b	20^b	0^b	0^b
Methylprednisolone acetate (0.6 µg)	0^c	0^c	0^b	0^b

4. Discussion

To the author's knowledge, this is one of the limited studies carried out to investigate the effect of SAIDs and NSAIDs on catamenial epilepsy and showed that both ketorolac, as an NSAID, and methylprednisolone acetate, as a SAID or glucocorticoid, showed dose-dependent antiepileptic efficacy by increasing the ITTS and ITMS, in addition to decreasing SD and MR, during various phases of the estrous cycle. An interaction has been suggested between the cyclooxygenase pathway and SD. Convulsions trigger the arachidonate release and the subsequent prostaglandin formation in brain regions primarily involved in the epileptic process (8). The inhibition of brain prostaglandin formation by NSAIDs decreases the susceptibility of animals to experimental seizures. Furthermore, certain anti-inflammatory drugs produce electrographic changes consistent with the CNS excitation and thus can be epileptogenic in high doses. Novel findings revealed that rapamycin, neurosteroids, epigenetic modifiers, and inhibitors of COX-2, TRK, and JAK-STAT pathways decrease epileptogenesis (14). The ICV injection of the ketorolac tris salt and methylprednisolone acetate increased the ITTS and ITMS during various phases of the estrous cycle.

Steroid hormones and their metabolites are responsible for seizures (15). The seizure threshold is increased by progesterone but decreased by estradiol (16). Seizure frequency is increased in the follicular phase but decreased during the luteal phase. An inverse relationship was observed in the epileptic rats for the estradiol/progesterone ratio, in which estradiol concentrations were enhanced in parallel with a reduction in progesterone concentrations. Therefore, increasing the estradiol levels can promote epileptogenesis, whereas progesterone preserves epileptogenesis. Seizure incidence is increased by a decrease in the progesterone level, which in epileptic women might be related to neurosteroid metabolites, such as allopregnanolone and pregnenolone, which act as positive allosteric modulators of $GABA_A$ receptors (17, 18). Allopregnanolone injection has higher antiepileptic effects during the diestrus phase than the estrus in rats. The neurosteroid modulation of extrasynaptic $GABA_A$ receptors can regulate neural excitability during epileptogenesis. The neuroprotective effect of progesterone is partly due to the reduction of cerebral edema by mechanisms such as the protection and reconstruction of the blood-brain barrier (BBB), the down-regulation of the inflammatory cascade by slowing down the inflammatory reactions of cytokines, such as IL-1, IL-6, and TNFα, as well as the migration and activation of immune cells. It is believed that progesterone limits cell necrosis and apoptosis through several different mechanisms, such as decreasing the concentration of NFkβ and the gene expression of its cellular targets, such as IL-1β, inducible nitric oxide synthetase, and cyclooxygenase enzyme, reducing lipid peroxidation caused by cell damage and oxidative stress by inhibiting the production of TNFα, and increasing the production of antioxidant enzymes (18).

Pre-inflammatory cytokine release can cause the breakdown of BBB and participate in the inflammatory responses of immune cells residing in the brain. Following seizures or the resulting cerebral damage, cytokines, prostaglandins, and other inflammatory molecules are induced along with their receptors in neurons and activate neuroglial and endothelial cells of the BBB in the damaged area of the brain. In rodents, the COX-2 enzyme is expressed in several nerve cells, and under normal conditions, the cortical and hippocampal nerve cells are rich in this enzyme. Following seizure injuries, COX-2 enzyme is induced in the frontal parts of the brain, and following sustained seizures caused by electrical stimulation, COX-2 expression is obviously increased. The increased expression of COX-2 in transgenic mice increases the sensitivity to sustained seizures induced by cyanic acid, while in mice without COX-2 or those in which this enzyme has been decreased or COX-2 inhibitors have been received, convulsions and kindling sensitivity are reduced. Another effect of inflammation on increasing brain excitability comes from inhibiting glutamate reuptake by astrocytes and increasing glutamatergic neurotransmission. The ability of IL-1β and TNFα to reduce GABA-dependent chloride ion flow, the expression of $GABA_A$ receptors, or changes in receptor subunits in nerve membranes decreases GABA-dependent inhibition in brain tissue with edema. Cytokines and prostaglandins can change the sensitivity and excitability of nerve cells by changing the function of voltage-dependent chloride channels. Prostaglandin E2 can initiate excitatory post-synaptic potential in neurons of the CA1 region of the hippocampus, which increases neural sensitivity and excitability. NSAIDs efficacy in epilepsy is influenced by numerous factors, including the timing of administration, the animal model of epilepsy, and the signaling pathways involved in cyclooxygenase induction. Moreover, some clinical studies on the indication of NSAIDs in neurological pathologies accompanied/characterized by seizures indicate that prolonged, low-dose, nonselective NSAIDs treatments may offer protection against seizures and strokes (5, 17, 18).

The results of this study suggest that ketorolac tris salt and methylprednisolone acetate show dose-dependent antiepileptic effects, which are more prominent during the luteal phase than the follicular phase and can be regarded as promising candidates to prevent epilepsy in females. Since there is no similar study, this study can be used as a basic study in this regard.

Authors' Contribution

Study concept and design: J. K., M. Z.
Acquisition of data: Z. F.
Analysis and interpretation of data: M. Z.
Drafting of the manuscript: M. Z. and J. K.
Revision of the manuscript: J. K., M. Z., N. P.

Ethics

All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals to Investigate Experimental Pain in Animals (Zimmermann, 1983) and approved by the Ethics Committee.

Conflict of Interest

The authors declare that they have no conflict of interest.

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