Original Article

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Antiepileptic Role of *β-alanine* on Pentylenetetrazol-Induced Convulsions during Estrus Cycle in Rat

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ABSTRACT

Background & Objective: Epilepsy is one of the main neurological disorders and *β*-alanine has several beneficial in the nervous system.This study aimed to determine the antiepileptic effect of the *β*-alanine during estrous cycle in pentylenetetrazol (PTZ)-induced seizures in rat.

Materials & Methods: Thirty female rats were allocated into five experimental groups as control, sodium valproate (75 mg/kg), and *β*-alanine (15, 30 and 45 mg/kg) during estrus cycle (proestrus, estrus, metestrus and diestrus). Puberty was determined by vaginal smears whereby animals with two normal cycles were chosen. After administration of the sodium valproate or *β*-alanine, PTZ injection (80 mg/kg, i.p) was done. Animals were monitored for 30 minutes with initiation time of myoclonic seizures (ITMS), initiation time of tonic–clonic seizures (ITTS), and seizures' duration (SD) registered.

Results: Based on the findings, *β*-alanine (30 and 45 mg/kg) significantly increased the onset time of ITMS and ITTS compared to the control group ($P<0.05$). *β*-alanine (30 and 45 mg/kg) significantly reduced SD compared to the control group ($P<0.05$). The antiepileptic effect of *β*-alanine was more prominent during metestrus and diestrus than proestrus and estrus ($P<0.05$).

Conclusion: It seems *β*-alanine has antiepileptic effects which are more prominent during proestrus and estrus than metestrus and diestrus.

Keywords: Antiepileptic, *β*-alanine, PTZ, Estrus, Rat

Introduction

The basic pathophysiology of epilepsy is not fully provoked but imbalance between excitatory and inhibitory anticonvulsants play important role **(1)**. Several models of focal epilepsy are based on impairment of cortical GABAergic neurons and most anti-epileptic drugs act by inhibition of Na^+ or Ca^{2+} channels **(2)**. PTZ is commonly used for inducing general seizure in experimental catamenial epilepsy which acts by inhibition of the $GABA_A$ receptor **(3)**. Catamenial epilepsy is a type of the seizure which happens in results of the changes in sexual hormone in women. There is ample evidence that ovarian estrogens contribute to the development of epilepsy, whereas progesterone has a protective effect against seizures **(4)**. Cyclic changes in ovarian hormones during estrous cycle may play in incidence of the catamenial epilepsy. Progesterone or its derivatives allopregnanolone and pregnanolone affect GABA receptors and reduce the occurrence of seizures **(5)**. Enzyme-modulating antiepileptic drugs such as carbamazepine, phenobarbital sodium valproate is prescribed for epilepsy but they can alter the serum level of steroid hormones **(6)**.

β-alanine is the simplest *β*-amino acid within the human central nervous system as a neuromodulator **(7)**. *β*-alanine has high affinity for normal and reactive astrocytes and competes with GABA for glial uptake **(7)**. *β*-alanine interacts with no less than five

acknowledged receptor sites on NMDA and glycine receptor and $GABA_A$, and also inhibits the glial GABA uptake mediated by GAT protein. Additionally, *β*-alanine is able to penetrate the blood brain barrier **(7)**. Oral supplementation of the *β*-alanine improves exercise performance, inhibition of tumor growth and enhanced glucose sensitivity in patients with Parkinson's disease **(9)**. Despite the fact that *β*-alanine play role of the in $GABA_A$, and involvement of this receptors in catamenial epilepsy, this study aimed to determine antiepileptic effect of the *β*-alanine during estrous cycle in PTZ-induced seizures in rat.

Materials and Methods

The sample consisted of thirty female Wistar rats weighing 200±50 g, which were categorized into 5 different experimental groups based on their estrus cycle phase (proestrus, estrus, metestrus, and diestrus). Animals were kept in accordance with European community regulations for laboratory animals, with standard conditions of 22 ± 1°C and a 12-hour dark/light cycle. They had free access to food and water. Puberty was studied by analyzing vaginal smears. Rats with two normal estrous cycles were selected for the study and estrus synchronization was performed on them **(10).** Daily vaginal smears were used to determine the stage of estrous cycles by analyzing the most common cell type **(11,12)**.

Study procedure

In control group rat received saline while in experiment 2, animals received VPA (75 mg/kg, Sigma CAS Number, 1069-66-5). In experiments 3-5, animals received *β*-alanine (15, 30 and 45 mg/kg, Sigma CAS Number, 107-95-9), respectively. Then, animals received i.p administration of PTZ (80 mg /kg). Each experiment done during estrus cycle (proestrus, estrus, metestrus, and diestrus). Following the seizure being triggered, the animals' conduct was observed for a period of 30 minutes in order to evaluate the time it took for myoclonic seizures (ITMS) and tonic-clonic seizures (ITTS) to begin, as well as their duration (SD). Experiments were carried out between 9 and 12 in the morning to minimize the influence of circadian rhythm on seizure vulnerability.

Statistical Analysis

Data was analyzed by SPSS version 21 through a one-way analysis of variances (ANOVA) and Tukey multiple comparison tests, with results presented as mean ± SD ($P<0.05$).

Results

The effects of VPA, *β*-alanine (15, 30, and 45 mg/kg) on the ITMS is presented in figure 1. As observed, valproic acid injection significantly increased ITMS in the stages of proestrus, estrus, metestrus and diestrus compared to the control group ($P<0.05$). *β*-alanine (15 mg/kg) had no significant effect on ITMS in proestrus, estrus, metestrus and diestrus compared to control group ($P>0.05$). *β*-alanine (30, and 45 mg/kg) significantly inhibited the ITMS in the stages of proestrus, estrus, metestrus and diestrus in comparison to control group ($P<0.05$). The effects of the *β*-alanine were more prominent during metestrus and diestrus than proestrus, estrus ($P<0.05$).

As seen in figure 2, VPA injection significantly increased ITTS in proestrus, estrus, metestrus and diestrus stages compared to the control group ($P<0.05$). *β*-alanine (15 mg/kg) had no significant effect on ITTS in proestrus, estrus, metestrus and diestrus ($P>0.05$). *β*-alanine (30, and 45 mg/kg) significantly decreased ITTS in the stages of proestrus, estrus, metestrus and diestrus ($P<0.05$).

According to figure 3, VPA (75 mg/kg) significantly decreased SD in proestrus, estrus, metestrus and diestrus phases compared to the control group ($P<0.05$). *β*-alanine (15 mg/kg) had no significant effect on SD in proestrus, estrus, metestrus and diestrus stages ($P>0.05$). *β*-alanine (30, and 45 mg/kg) significantly decreased SD compared to the control group ($P<0.05$). The effects of the *β*-alanine were more prominent during metestrus and diestrus than proestrus, estrus ($P<0.05$).

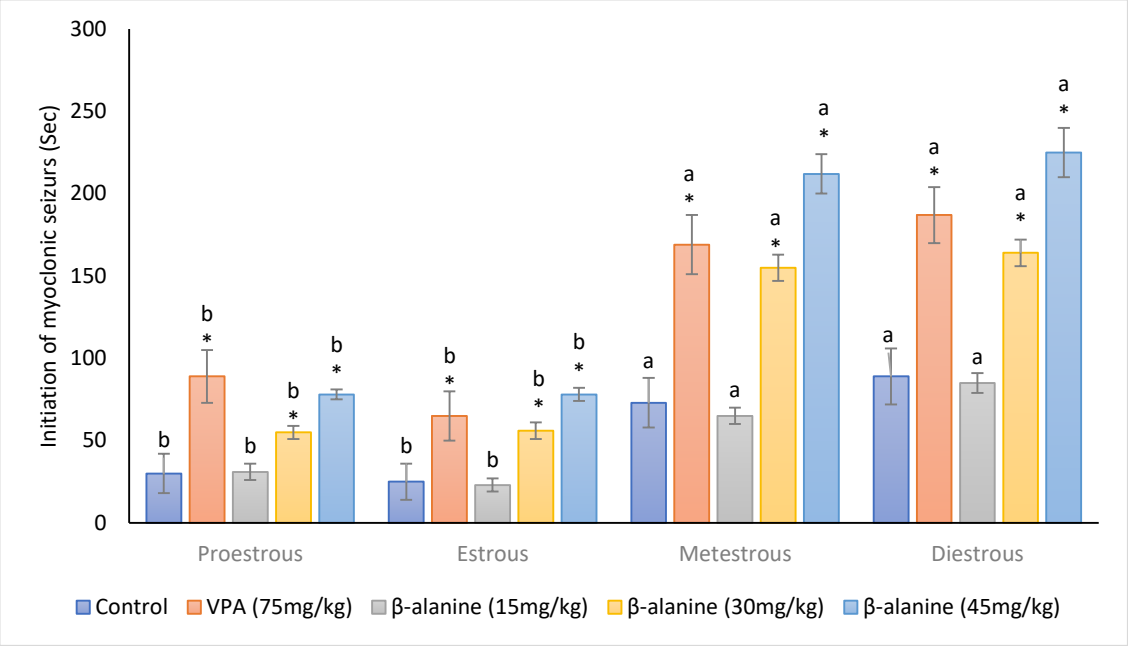

Figure. 1. Antiepileptic effects of *β*-alanine (15, 30, and 45 mg/kg) on the initiation time of myoclonic seizures (ITMS) (sec) during various phases of the estrous cycle. *Asterisks indicate a significant difference in each estrous cycle phase compared with the control group ($P < 0.05$). Different letters (a and b) indicate significant differences for each group in each estrous cycle phase ($P < 0.05$). Data are presented as mean ± SE.

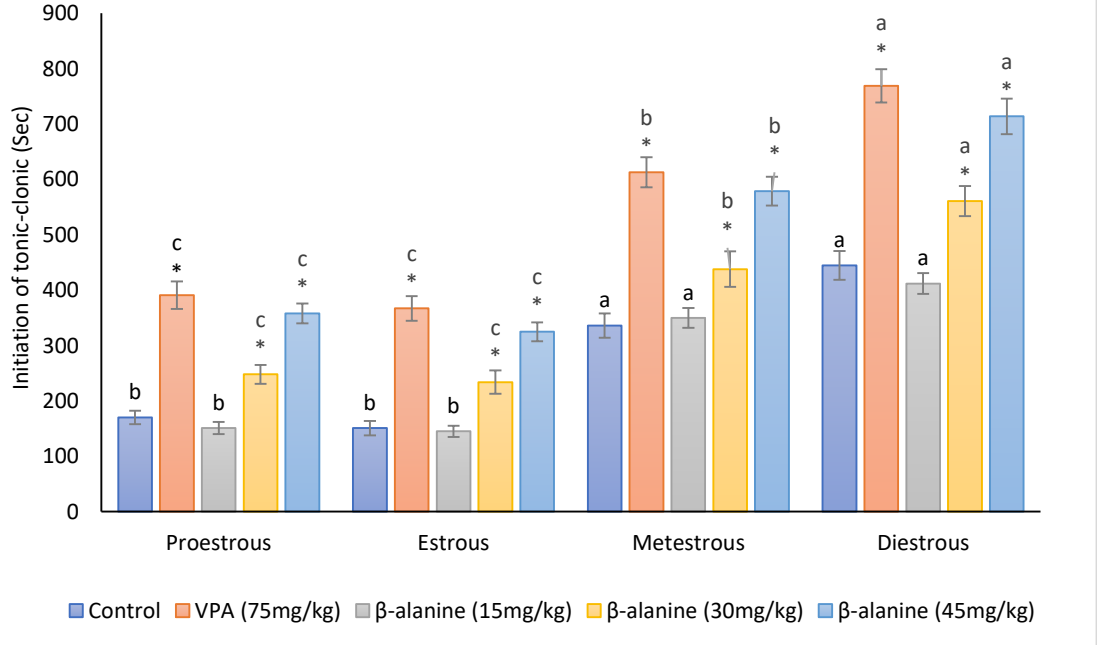

Figure 2. Antiepileptic effects of *β*-alanine (15, 30, and 45 mg/kg) on the initiation time of tonic-clonic seizures (ITTS) (sec) during various phases of the estrous cycle. *Asterisks show a significant difference in each estrous cycle phase

compared with the control group ($P < 0.05$). Different letters (a, b, and c) display significant differences for each group in each estrous cycle phase ($P < 0.05$). Data are presented as mean ± SE.

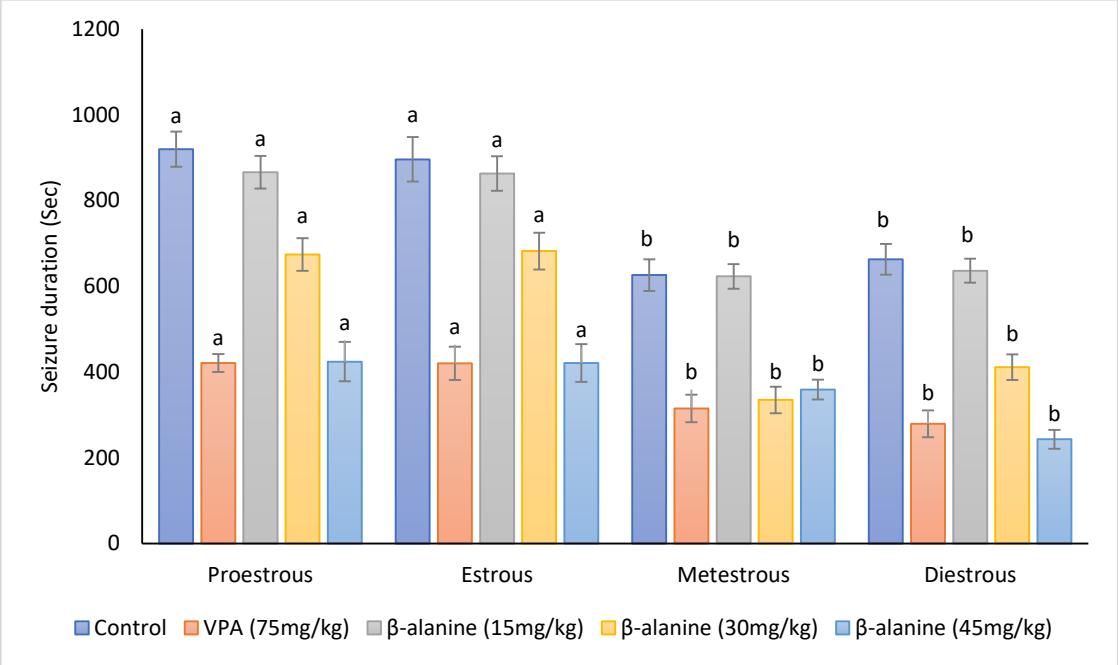

Figure 3. Antiepileptic effects of *β*-alanine (15, 30, and 45 mg/kg) on seizure duration (sec) during various estrous cycle phases. *Asterisks reveal a significant difference in each estrous cycle phase compared with the control group ($P < 0.05$). Different letters (a and b) indicate significant differences for each group in each estrous cycle phase ($P < 0.05$). Data are presented as mean ± SE.

Discussion

In epileptic women, fluctuations in ovarian steroids and their metabolites are responsible for catamenial epilepsy. Seizure incidence increases during the follicular phase and declines during the luteal phase **(13)**. Elevated estrogen levels in the amygdala and hippocampus enhances seizure threshold while progesterone lowers it. Diminished progesterone levels in preovulatory phase leads to increased seizure incidence **(14)**. Allopregnanolone is an allosteric metabolite of the progesterone and acts as modulator of $GABA_A$ receptors **(15).** Based on the main finding of the current study, *β*-alanine (30 and 45 mg/kg) increased the onset time of ITMS and ITTS and decreased SD. The antiepileptic effect of *β*-alanine was more prominent during metestrus and diestrus than proestrus and estrus. *β*-alanine can across the blood brain barrier and serve as a marker for glial GABA uptake **(16)**. Neuronal-glial studies have reveled *β*-alanine has a high affinity for glial $GABA_A$ receptors **(17).**

β-Alanine is easily transported in the CNS, muscles, and other organs. In the brain *β*-alanine transporters by GABA transporter (GAT). The GAT-1 and GAT-4 are found in the brain while GAT-2 and GAT-3 are found in the brain and peripheral tissues. GAT transports *β*-alanine, and *β*-alanine inhibits GABA uptake by GAT-2 **(7)**. Despite direct mechanism for how *β*-alanine regulates GABA uptake is not fully elicited but it seems Na^+ ions are required for *β*-alanine and GABA. *β*-alanine uptake is dependent on the concentration of both Na^+ and Cl^- ions **(18)**.

GABA is the primary inhibitory neurotransmitter in the brain, and its transmission plays a role in controlling mood, memory, and eating habits. Allopregnanolone, a progesterone metabolite, is a powerful modulator of GABA receptors with positive effects. Exposure to steroids that modulate GABA receptors or increased sensitivity to allopregnanolone can lead to hyperactivity or heightened GABAergic tone, and is linked to various symptoms as well as disorders **(19)**. Administration of steroid antagonists that modulate GABAA receptors lowers the intensity of symptoms and conditions **(20)**. The binding of GABA to its receptor leads to an increase in chloride

ion flow, membrane hyperpolarization, and reduction in neuronal firing rates **(21-22)**. Direct GABA antagonists binding to GABAA receptors can lead to increased neuronal excitability and seizures while also interfering with GABA binding sites **(23-25)**. A potential use of *β*-alanine, instead of carnosine, in aquaculture has been suggested to enhance the growth and feeding rate of mussels. *β*-alanine injections disrupted metabolites related to lipid metabolism. Specifically, metabolites involved in linoleic acid metabolism and steroid biosynthesis increased after *β*-alanine injection **(26).**

Conclusion

In conclusion, *β*-alanine seems to have an antiepileptic role which is more prominent during metestrus and diestrus than proestrus and estrus. However, further research is required to determine accuracy of these findings in human trials.

Acknowledgments

None.

Authors' Contribution

Conflict of Interest

The authors declare that they have no conflict of interest.

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None.

Ethics Approval and consent to participate

The research protocol received approval from the Animal Ethics Committee at the Science and Research Branch of Islamic Azad University in Tehran, Iran (IR.IAU.SRB.REC.1401.597; 2023-2-16).

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