ISSN 1819-7124, Neurochemical Journal, 2023, Vol. 17, No. 2, pp. 289–296. © Pleiades Publishing, Ltd., 2023.

EXPERIMENTAL ARTICLES

The Role of Nitric Oxide Pathway in the Anti-Epileptogenic Effect of Valproic Acid in the Pentylenetetrazole-Kindling Model in Rats

Bilal Sahin[a], Ahmet Kemal Filiz[a], and Ziad Joha[b, 1]

[a] *Departments of Physiology, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey*

[b] *Departments of Pharmacology, Sivas Cumhuriyet University, School of Pharmacy, Sivas, Turkey*

Received January 18, 2022; revised July 8, 2022; accepted October 20, 2022

Abstract—Though valproic acid (VPA) is used to treat seizures, more investigations have been needed to understand the effect of VPA on the nitric oxide pathway in epilepsy. Our investigation aimed to study the effect of VPA on cortical and hippocampal NOS isomers in epileptogenesis processes in the PTZ kindling model of epilepsy in the rat to reveal its mechanisms of action. Twenty-four male Wistar albino rats were used in this study. The animals were divided into four groups control, PTZ without VPA, and PTZ with VPA at two doses (100 and 200 mg/kg) Rats were kindled by injections of PTZ (35 mg/kg) with drugs or vehicle 30 minutes before, once every other day for 12 times and their behavior was observed. After completing the epileptic model process, nitric oxide pathway markers (eNOS, nNOS, iNOS, and NO) in the cortex and hippocampus were assessed by using ELISA methods. VPA suppressed seizure stages and decreased nNOS, iNOS, and NO levels while increasing eNOS levels in the hippocampus and cerebral cortex. The effect of VPA on nitric oxide pathway was found to contribute to its anti-epileptic activity.

Keywords: pentylenetetrazole, epilepsy, nitric oxide pathway, valproic acid

DOI: 10.1134/S1819712423020113

INTRODUCTION

Epilepsy is one of the most important chronic diseases that causes recurrent seizures affecting human life mentally and physically [1]. An imbalance between the excitatory and inhibitory control systems in particular areas of the brain leads to the development of epilepsy [2]. Epileptic seizure or convulsion is a period of temporary neurological dysfunction caused by abnormal electrical discharges which occur from time to time in the brain [3]. Valproate was first used in clinical practice around 50 years ago, and its efficacy and tolerability have been well documented in preclinical and clinical studies. It has been the mainstay of anti-epileptic therapy because of its broad spectrum of action for various seizures and epileptic syndromes. Several clinical trials suggest that valproate provides the broadest spectrum of anticonvulsant effects among all currently known anti-epileptic medicines in adults and children with epilepsy [4]. One of the mechanisms of valproate is related to pre-and post-synaptic modulation of GABAergic transmission. Specifically, valproate supports the availability of synaptic GABA and facilitates GABA-induced responses by both pre-synaptic and post-synaptic mechanisms [5]. Valproate, in particular, slows the loss of post-synaptic inhibition activity due to the activation of GABA-A receptors by interacting directly with the benzodiazepine regulatory regions of GABA receptors and enhances the binding of baclofen to GABA-B receptors [6].

Nitric oxide (NO) is a freely diffuse gas synthesized from the oxidation of L-arginine by one of the isoforms of the NO synthase enzyme ((NOS) (neuronal (nNOS), endothelial (eNOS) and inducible (iNOS)). NO acts as a second messenger and a neurotransmitter/neuromodulator in the brain and influences various physiological functions such as interneuron communication, synaptic plasticity, memory, intracellular signal transduction and mediator release [7, 8]. NO has also been linked to the development of pathologies that can lead to neurological problems like ischemia, stroke, and epileptogenic seizures [8].

In experimental animals, pentylenetetrazole (PTZ) is one of the most commonly used agents in the production of primary generalized seizures. [9]. PTZ exerts its stimulating effect in the central nervous system by inhibiting the opening of Cl^- channels via binding to the picrotoxin binding site of the GABA-A/benzodiazepine receptor complex. PTZ inhibits the depolarization of neurons by reducing the activity of GABA synapses via the GABA receptor-benzodiazepine-chloridianophore complex. Repeated PTZ injections have been linked to an increase in the num-

[1] Corresponding author; address: Department of Pharmacology, Sivas Cumhuriyet University School of Pharmacy, Sivas, 58140 Turkey; e-mail: zead-geha@hotmail.com.

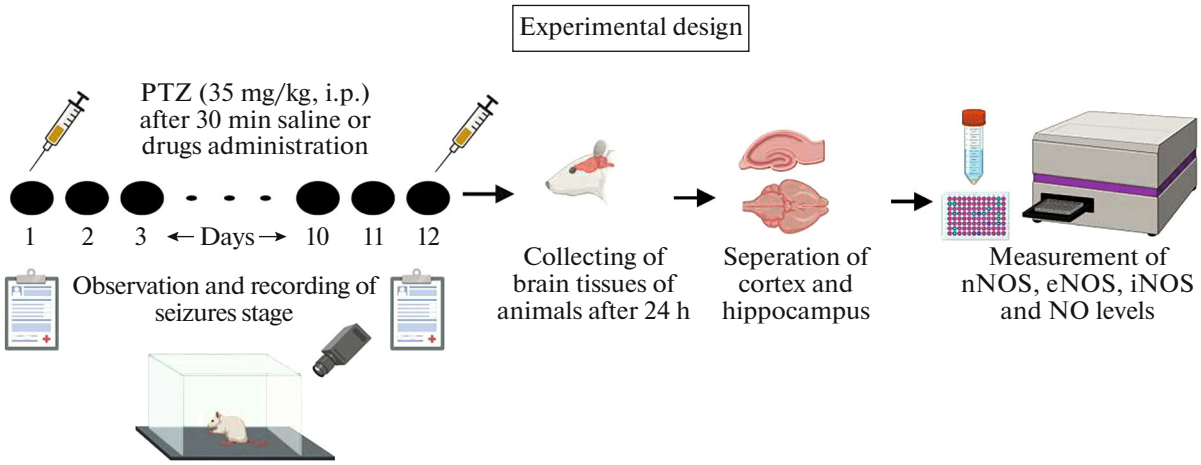

Fig. 1. Experimental design of the study (created with BioRender.com).

ber of benzodiazepine receptors [10]. Seizures induced by PTZ, changes in extracellular and intracellular ion levels, and enhanced excitatory or decreased inhibitory activity have all been linked to impairments in certain membrane functions [11].

Since several clinical trials suggest that VPA has beneficial effects in adults and children with epilepsy, more studies are needed to understand the underlying mechanisms. In this study, we aim to investigate the effect of VPA on cortical and hippocampal NOS isomers in epileptogenesis processes in the PTZ kindling model of epilepsy in the rat to reveal its mechanisms of action.

MATERIALS AND METHODS

Animals. Twenty four adult male Wistar albino rats, 4–5 months old and weighing 225–265 g, (provided by Cumhuriyet University Animal Laboratory, Sivas, Turkey) were used in the study. The rats were kept under standard conditions, including a constant temperature of 23 ± 2°C 35–60% humidity, and a 12:12-hour light-dark cycle. Rats were given unlimited access to food and water. All the experiments took place between 8 a.m. and 5 p.m. The procedures were carried out following the Local Ethics Committee's recommendations for the welfare of experimental animals (Approval no. 65202830-050.04.04-511).

Chemicals. PTZ and valproic acid (Sigma-Aldrich Co., Missouri, USA) were dissolved in physiological saline. All of the treatments were given intraperitoneally (i.p.). All other general agents employed in the research were of analytical grade.

Experimental design. Rats were randomly divided into 4 groups ($n = 6$ per group): control group (vehicle + vehicle), vehicle-treated group (vehicle + 35 mg/kg PTZ), VPA-treated group (100 mg/kg VPA + 35 mg/kg PTZ), VPA-treated group (200 mg/kg VPA + 35 mg/kg PTZ). Rats were kindled by injections of PTZ (35 mg/kg) with drugs or vehicle 30 min before, once every other day for 12 times, and their behavior was observed. All drugs were given through a peritoneal route (Fig. 1).

PTZ kindling. PTZ was given at a subconvulsive dose (35 mg/kg) every other day for 12 times to trigger kindling. Animals in the VPA groups were given VPA (100 and 200 mg/kg) for 30 min before receiving the PTZ injection. After each administration, the rats were separated into plexiglass cages for a 30-min observation period. The following is how a modified Racine's scale was used to score behavioral seizures: In stage zero, there is no convulsion. In stage one, vibrissae and pinnae twitching is observed. In stage two, motor arrest with more prominent twitching is observed. In stage three, generalized myoclonic seizures occur. In stage four, seizures of tonic-clonic type with no loss of postural control are observed. In stage five, seizures of tonic-clonic type with a lack of the righting reflex are observed. In stage six, a lethal seizure is observed. The rats with stage 4 seizures for three consecutive days were defined as "kindled" [12].

Preparation of brain tissue homogenates. Once the experiment was finished, the rats were decapitated, and their brains were removed for additional biochemical parameter analysis. The cortical and hippocampal tissues were separated from the brain. A mechanical homogenizer (a light-duty Ultra-Turrax homogenizer, ISOLAB, Germany) was used to homogenize the cortical and hippocampal tissues in a cold phosphate buffer solution (pH 7.4). The homogenates were centrifuged at 4000 rpm for 10 min at a temperature of 4°C [13]. The supernatants were then collected for biochemical examination. The total protein levels in each sample were calculated using a Bradford protein assay kit (Merck, Germany) [14].

Measurement of nNOS, eNOS, iNOS and NO levels. The nNOS, eNOS, iNOS and NO levels in the

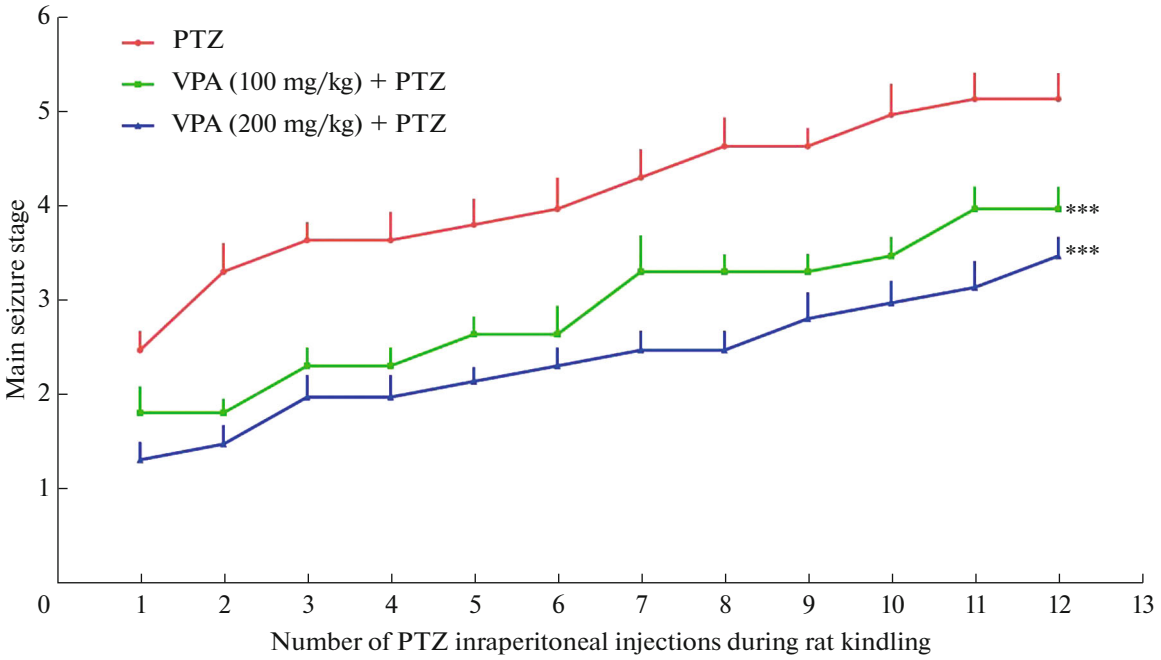

Fig. 2. Effect of the pretreatment of valproic acid on seizure stage during epileptogenesis process. Values are presented as mean ± SEM ($n = 6$ rat in each group). ***$P < 0.001$, compared with the PTZ group.

cortical and hippocampal supernatants were measured using rat ELISA commercial kits (YL Biont, Shanghai, China). The procedures were carried out in accordance with the manufacturer's guidelines. In a nutshell, tissue samples and standards were placed in a plate and incubated at 37°C for 60 min. After the washing step, staining solutions were added and incubated at 37°C for 15 min. An ELISA reader (Thermo Fisher Scientific, Altrincham, UK) was used to read the results at 450 nm after the stop solution was added. Standard curves were plotted to determine the value of samples. The coefficients of variance between and within plates were less than 10%.

Statistical analysis. Data were expressed as the mean ± standard error of the mean (SEM). The drug × time interaction on the seizure stages was investigated using a two-way ANOVA followed by a Bonferroni posttest. One-way ANOVA followed by the Tukey, multiple comparison post hoc test, was used to analyze nNOS, eNOS, iNOS and NO levels in the cortex and hippocampus. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of valproic acid on epileptogenesis. Chemical kindling was induced by repeated injections of the PTZ at a sub-convulsive dose (35 mg/kg, i.p.) every other day for 12 times, with a gradual rise in seizure score reaching a mean value of 5 after the 10th injection. Administration of the VPA at doses of 100 and 200 mg/kg i.p., 30 min before the injection of PTZ, remarkably reduced the seizure score from the first day until the last day compared to the PTZ-induced seizures ($P < 0.001$) (Fig. 2).

Effect of valproic acid on nNOS levels in the cortex and hippocampus. The means of nNOS levels in cortical tissues were 49.24 ± 1.44 ng/g protein in the control group, 68.55 ± 3.56 ng/g protein in the PTZ group, 41.54 ± 2.59 ng/g protein in the VPA (100 mg/kg) group, and 33.60 ± 1.51 ng/g protein in the VPA (200 mg/kg) group. The PTZ increased the cortical nNOS levels compared to the control ($P < 0.001$; Fig. 3a). In addition, the VPA at the two doses reduced the cortical nNOS levels compared to the PTZ group ($P < 0.001$; Fig. 3a).

The means of nNOS levels in hippocampal tissues were 64.95 ± 2.34 ng/g protein in the control group, 80.51 ± 3.27 ng/g protein in the PTZ group, 68.25 ± 0.97 ng/g protein in the VPA (100 mg/kg) group, and 58.31 ± 3.86 ng/g protein in the VPA (200 mg/kg) group. The PTZ increased the hippocampal nNOS levels compared to the control ($P < 0.01$; Fig. 3b). In addition, the VPA at the two doses reduced the hippocampal nNOS levels compared to the PTZ group ($P < 0.05$ to $P < 0.001$; Fig. 3b).

Effect of valproic acid on eNOS levels in the cortex and hippocampus. The means of eNOS levels in cortical tissues were 15.46 ± 1.01 ng/g protein in the control group, 7.77 ± 0.71 ng/g protein in the PTZ group, 17.83 ± 0.39 ng/g protein in the VPA (100 mg/kg) group, and 29.14 ± 0.92 ng/g protein in the VPA (200 mg/kg) group. The PTZ increased the cortical

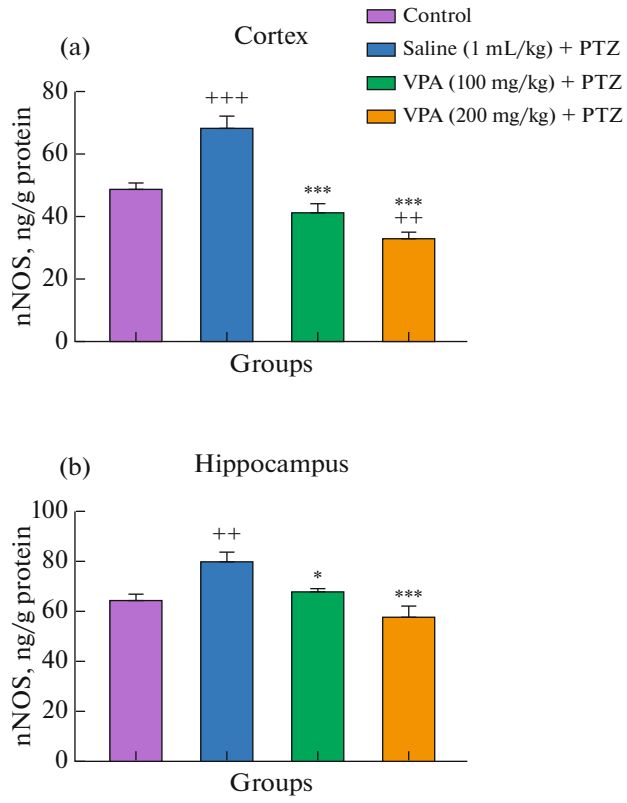

Fig. 3. Effect of the pretreatment of valproic acid on nNOS levels in the rat cortex and hippocampus. Values are presented as mean ± SEM. (n = 6 rat in each group). $^{++}P <$ 0.01 and $^{+++}P < 0.001$, compared with the control group; $^{*}P < 0.05$, and $^{***}P < 0.001$, compared with the PTZ group.

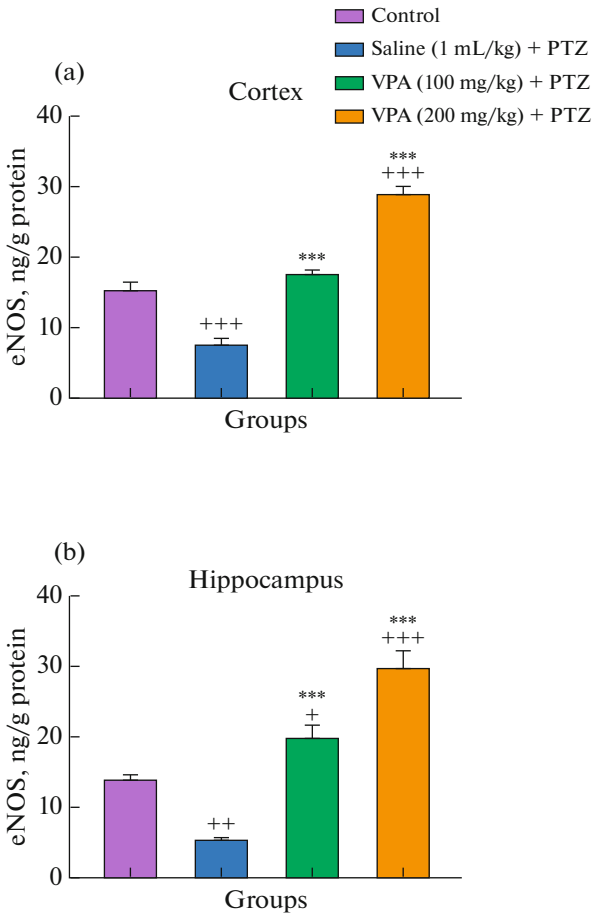

Fig. 4. Effect of the pretreatment of valproic acid on eNOS levels in the rat cortex and hippocampus. Values are presented as mean ± SEM. (n = 6 rat in each group). $^{+}P <$ 0.05, $^{++}P < 0.01$ and $^{+++}P < 0.001$, compared with the control group; $^{***}P < 0.001$, compared with the PTZ group.

eNOS levels compared to the control ($P < 0.001$; Fig. 4a). In addition, the VPA at the two doses reduced the cortical eNOS levels compared to the PTZ group ($P < 0.001$; Fig. 4a).

The means of eNOS levels in hippocampal tissues were 14.06 ± 0.57 ng/g protein in the control group, 5.49 ± 0.18 ng/g protein in the PTZ group, 20.01 ± 1,68 ng/g protein in the VPA (100 mg/kg) group, and 29.93 ± 2.34 ng/g protein in the VPA (200 mg/kg) group. The PTZ increased the hippocampal eNOS levels compared to the control ($P < 0.01$; Fig. 4b). In addition, the VPA at the two doses reduced the hippocampal eNOS levels compared to the PTZ group ($P <$ 0.001; Fig. 4b).

Effect of valproic acid on iNOS levels in the cortex and hippocampus. The means of iNOS levels in cortical tissues were 74.21 ± 3.98 ng/g protein in the control group, 102.01 ± 4.96 ng/g protein in the PTZ group, 79.74 ± 2.62 ng/g protein in the VPA (100 mg/kg) group, and 71.42 ± 2.48 ng/g protein in the VPA (200 mg/kg) group. The PTZ increased the cortical iNOS levels compared to the control ($P < 0.001$; Fig. 5a). In addition, the VPA at the two doses reduced the cortical iNOS levels compared to the PTZ group ($P < 0.01$ to $P < 0.001$; Fig. 5a).

The means of iNOS levels in hippocampal tissues were 86.63 ± 1.47 ng/g protein in the control group, 113.91 ± 2.18 ng/g protein in the PTZ group, 96.83 ± 3.77 ng/g protein in the VPA (100 mg/kg) group, and 84.57 ± 1.58 ng/g protein in the VPA (200 mg/kg) group. The PTZ increased the hippocampal iNOS levels compared to the control ($P < 0.001$; Fig. 5b). In addition, the VPA at the two doses reduced the hippocampal nNOS levels compared to the PTZ group ($P <$ 0.001; Fig. 5b).

Effect of valproic acid on NO levels in the cortex and hippocampus. The means of NO levels in cortical tissues were 8.14 ± 0.19 μM/g protein in the control group, 15.17 ± 1.09 μM/g protein in the PTZ group, 9.86 ± 0.71 μM/g protein in the VPA (100 mg/kg) group, and 7.85 ± 0.29 μM/g protein in the VPA (200 mg/kg) group. The PTZ increased the cortical NO levels compared to the control ($P < 0.001$; Fig. 6a).

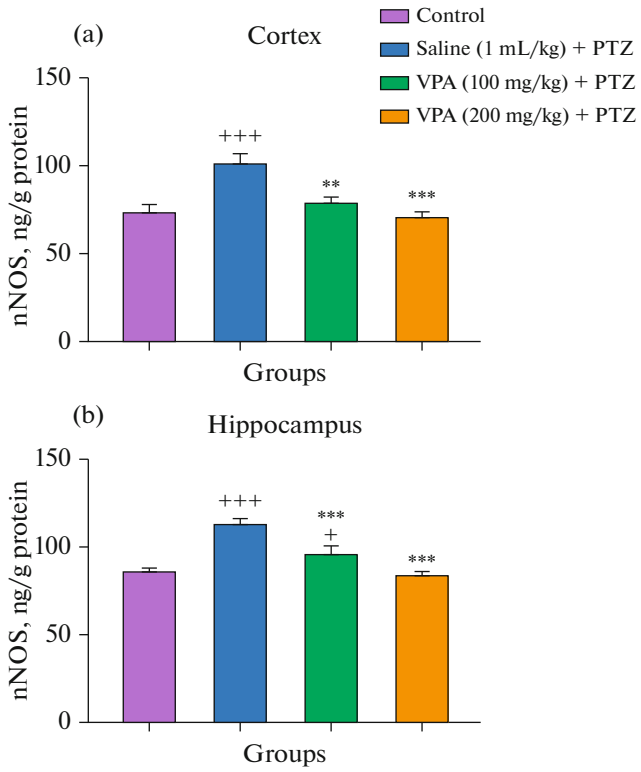

Fig. 5. Effect of the pretreatment of valproic acid on iNOS levels in the rat cortex and hippocampus. Values are presented as mean ± SEM. (n = 6 rat in each group). $^{+}P < 0.05$ and $^{+++}P < 0.001$, compared with the control group; $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with the PTZ group.

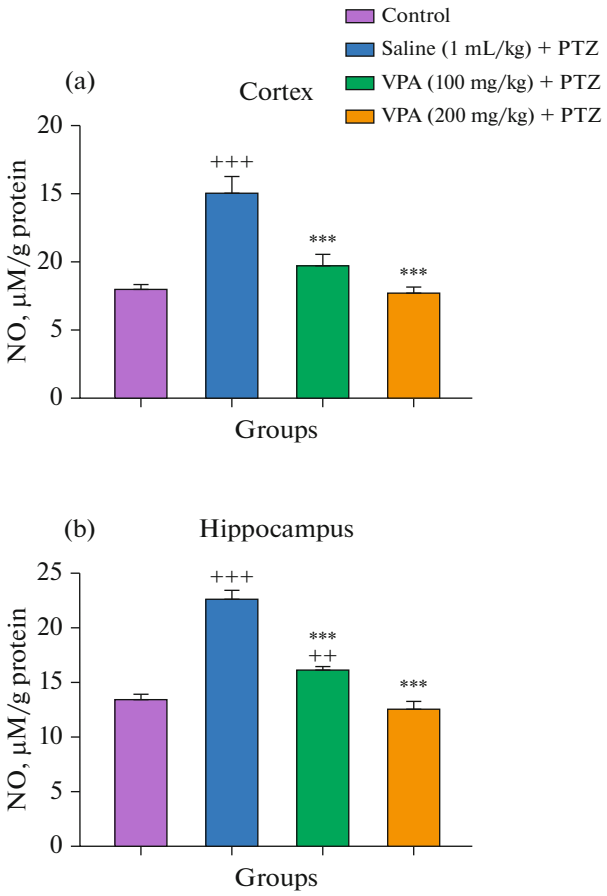

Fig. 6. Effect of the pretreatment of valproic acid on NO levels in the rat cortex and hippocampus. Values are presented as mean ± SEM. (n = 6 rat in each group). $^{++}P < 0.01$ and $^{+++}P < 0.001$, compared with the control group; $^{***}P < 0.001$, compared with the PTZ group.

In addition, the VPA at the two doses reduced the cortical NO levels compared to the PTZ group ($P < 0.001$; Fig. 6a).

The means of NO levels in hippocampal tissues were 13.65 ± 0.27 μM/g protein in the control group, 22.74 ± 0.73 μM/g protein in the PTZ group, 16.28 ± 0.19 μM/g protein in the VPA (100 mg/kg) group, and 12.73 ± 0.57 μM/g protein in the VPA (200 mg/kg) group. The PTZ increased the hippocampal NO levels compared to the control ($P < 0.001$; Fig. 6b). In addition, the VPA at the two doses reduced the hippocampal NO levels compared to the PTZ group ($P < 0.001$; Fig. 6b).

DISCUSSION

In this study, the role of nitric oxide pathway in the anti-epileptogenic effects of VPA on PTZ-induced epileptic seizures in rats was evaluated for the first time. It was found that PTZ administration every other day for 12 times resulted in typical epileptic seizures that were associated with decreased levels of eNOS and elevated levels of nNOS, iNOS, and NO in the hippocampal and cortical regions of rats. In addition, treatment with VPA caused a significant reduction in seizure stages, increased decreased levels of eNOS, and decreased elevated levels of nNOS, iNOS, and NO in the cortex and hippocampus after PTZ-induced kindling. VPA's antiepileptic activity has been thoroughly validated in experimental research in diverse models of generalized and localized seizures [15].

VPA enhanced the latency to seizure initiation [16] despite being ineffective in avoiding status epilepticus in a pilocarpine model [17]. VPA acute intraperitoneal treatment dramatically improved the seizure threshold in the PTZ model of epilepsy [18], with anticonvulsant action potentiated by long-term treatment [19]. The efficacy of VPA was also demonstrated in the electroshock-induced seizures model, where VPA reduced seizure frequency in a dose-dependent manner [20]. VPA increased the number of after-discharges needed to elicit epileptic seizures in a dose-dependent manner in an experimental design based on a kindling model [21]. VPA inhibited seizures by modulating membrane permeability, inhibiting T-type voltage-activated Ca^{2+} currents and voltage-dependent sodium channels, and

amplifying GABA-mediated inhibition [22]. VPA also reversed the neuronal damage caused by persistent convulsions in the hippocampus formation in the kindling model, enhancing neuroprotection and preventing behavioral abnormalities [23]. In the kainate model of status epilepticus, similar effects were observed [24]. Furthermore, VPA effectively reduced seizure frequency and was capable of achieving seizure-free status in a rat or human temporal lobe epilepsy model [25]. VPA not only reduces seizure frequency but also lowers mortality [26]. Despite controlling seizures, the great majority of antiepileptic drugs are still unable to slow the progression of epilepsy's underlying natural history. As a result, considering their importance in seizure management, most antiepileptic medicines lack a demonstrated anti-epileptogenic potential [27]. Even though various antiepileptic medications have neuroprotective activities, only a few achieve anti-epileptogenic properties [28]. Findings from the kindling pharmacoresistant epilepsy model imply that VPA reduces the formation of kindling and inhibits kindled seizures completely, acting as both a symptomatic and disease-modifying agent [22, 29]. In line with previous studies, it was found in our study that VPA produced remarkable antiepileptic effects, as proved by a significant decrease in the seizure stages in the VPA group.

eNOS, iNOS and nNOS are the NOS isoenzymes. eNOS and nNOS are produced in endothelial cells and neurons. iNOS is produced in reactive astrocytes and mediates immune processes. Whereas the augmented activity of nNOS and iNOS is associated with excitotoxicity, the eNOS causes a protective influence against neurotoxicity [30]. As a result, medicines that decrease iNOS and enhance eNOS activities have been suggested as a potential alternative antiepileptic drug [31]. It was shown by Bahcekapili et al. that PTZ-induced seizures caused down expression of eNOS [30]. In this study, it was shown that repeated injections of the PTZ at a sub-convulsive dose caused a decrease in eNOS levels in the cortex and hippocampus of the rats. On the other hand, VPA increased eNOS levels in the hippocampus and cortex after seizures induced by repeated injections of the PTZ.

nNOS is widely generated in neurons [32]. The stimulation of nNOS causes a rise in NO levels in postsynaptic neurons, which causes the soluble guanylate cyclase (sGC)/guanozine monophosphate (cGMP) pathway to be activated. cGMP is a secondary messenger that induces glutamate release in presynaptic neurons and excitement in postsynaptic neurons. As a result, the nNOS/sGC/NO/cGMP pathway is critical for presynaptic and postsynaptic neuron excitation [33]. It's also been claimed that nNOS is involved in epileptic seizures, generating neuronal stimulation after the seizures [34]. Furthermore, it has been observed that nNOS is linked to epileptogenesis and PTZ-induced epileptic seizures [35]. In this study, it was shown that repeated injections of the PTZ at a sub-convulsive dose caused an increase in nNOS levels in the cortex and hippocampus of rats. On the other hand, VPA decreased nNOS levels in the hippocampus and cortex after seizures induced by repeated injections of the PTZ.

iNOS is abundantly expressed in glial cells [36]. It was shown that iNOS plays a role in epilepsy and is responsible for the induction of neuroinflammation, which results in neuronal stimulation after seizures [37]. Furthermore, it was found that iNOS synthesis increased after PTZ-induced epileptic convulsions [30]. In this study, it was shown that repeated injections of the PTZ at a sub-convulsive dose caused an increase in iNOS levels in the cortex and hippocampus of rats. In addition, VPA decreased iNOS levels in the hippocampus and cortex after seizures induced by repeated injections of the PTZ. In agreement with our results, VPA was found to inhibit iNOS in the spinal cords of encephalomyelitis rats [38] and reduce nNOS in the basolateral amygdala of animal models [39]. However, the involvement of NOS isoform in antiepileptic effect of VPA has not been investigated. We demonstrated in this study that pretreatment of VPA remarkably suppressed iNOS and nNOS levels and elevated eNOS levels in the cortex and hippocampus in PTZ administration rats. We hypothesized that one of the antiepileptic mechanisms of VPA in PTZ-induced epilepsy may be through decreased iNOS and nNOS and increased eNOS expression in the cortex and hippocampus, the regions most susceptible to seizure-induced damage. VPA may create a neuroprotective effect by increasing eNOS levels, an anti-inflammatory effect by decreasing iNOS levels, and an anti-excitatory effect by lowering nNOS levels, all of which support the antiepileptic effect of VPA.

The term "oxidative stress" refers to an imbalance between the oxidant and antioxidant systems. The two primary sources of oxidant systems are reactive nitrogen species (RNS) and reactive oxygen species (ROS) [40]. In physiological circumstances, ROS and RNS play critical roles in redox control. Increases in ROS and RNS, on the other hand, generate oxidative and nitrosative stress, both of which are linked to epileptogenesis and epileptic seizures. Nitrosative stress is another source of NO, in addition to the iNOS, nNOS, and eNOS systems [41]. Furthermore, nitrosative stress has been found to play a role in the activation of T-type voltage-gated calcium channels and *N*-methyl-*d*-aspartate (NMDA) receptors [42]. Hyperexcitability in neurons results from the opening of calcium channels in response to nitrosative stress [43]. In our study, it was shown that repeated injections of the PTZ at a sub-convulsive dose caused an increase in NO levels in the cortex and hippocampus of rats. On the other hand, VPA diminished NO levels in the hippocampus and cortex after seizures induced by repeated injections of the PTZ. One of the proposed mechanisms underlying VPA's antiepileptic

characteristics is its influence on NO as part of nitrosative stress.

CONCLUSION

This research determined, in line with previous studies, that VPA administration to rats provided anti-epileptogenic properties in PTZ-induced epileptic seizures. The anti-epileptogenic effect could occur by alteration of nitric oxide pathway (eNOS, nNOS, iNOS and NO pathway). These findings support the neuroprotective and anti-epileptogenic properties of VPA. More research is needed to answer the questions concerning the possible mechanisms involved.

ACKNOWLEDGMENTS

The authors would like to thank the Sivas Cumhuriyet University, School of Medicine, CUTFAM Research Center, Sivas, Turkey, for providing the necessary facilities to conduct this study.

FUNDING

No external funding was received.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors have no conflict of interest to disclose.

Ethical approval. The procedures were carried out following the Local Ethics Committee's recommendations for the welfare of experimental animals. (Approval no. 65202830-050.04.04-511).

Authorship contribution statement. Bilal Sahin and Ahmet Kemal Filiz**:** Investigation, Conceptualization, Writing – review & editing. Bilal Sahin and Ziad Joha: Methodology, Data curation, Formal analysis, Writing – original draft.

REFERENCES

1. Copple, P.J., *Arch. Pediatr. Adolesc. Med.,* 1994, vol. 148, no. 7, pp. 769–70.
2. Brailowsky, S., and García, O., *Arch. Med. Res.*, 1999, vol. 30, no. 1, pp. 3–9.
3. McGonigal, A., *Neurosurgery Clinics of North America,* 2020, vol. 31, no. 3, pp. 373–385.
4. Löscher, W., *CNS Drugs*, 2002, vol. 16, no. 10, pp. 669–694.
5. Cunningham, MO., Woodhall, GL., and Jones, R.S.G., *Neuropharmacology,* 2003, vol. 45 no. 7, pp. 907–917.
6. Löscher, W., and Schmidt, D., *Epilepsia,* 1980, vol. 21, no. 6, pp. 611–615.
7. Hoffman, M., *Science,* 1991, vol. 252, no. 5014, p. 1788.
8. Moncada, S., Palmer, RM., and Higgs, EA., *Pharmacol. Rev.,* 1991, vol. 43, no. 2, pp. 109–142.
9. Li, B., Wang, L., Sun, Z., Zhou, Y., Shao, D., Zhao, J., Song, Y., Lv, J., Dong, X., Liu, C., Wang, P., Zhang, X., and Cui, R., *PLoS One,* 2014, vol. 9, no. 4, e93158.
10. Coenen, A.M.L., and Van, Luijtelaar. E.L.J.M., *Behav. Genet.,* 2003, vol. 33, no. 6, pp. 635–655.
11. Miraucourt, LS., da Silva, J.S., Burgos, K., Li, J., Abe, H., Ruthazer, ES., and Cline, H.T., *PLoS One,* 2012, vol. 7, no. 1, e29086.
12. Filiz, A.K., Gumus, E., Karabulut, S., Tastemur, Y., and Taskiran, A.S., *Epilepsy & Behavior,* 2021, vol. 118, 107915.
13. Taskiran, A.S., Ergul, M., Gunes, H., Ozturk, A., Sahin, B., and Ozdemir, E., *Cellular and Molecular Neurobiology,* 2021, vol. 41, pp. 173–183.
14. Kruger, N.J., *The Protein Protocols Handbook*, 2009, p. 17–24.
15. Löscher, W., *Seizure,* 2011, vol. 20, no. 5, pp. 359–368.
16. Mehrabi, S., Sanadgol, N., Barati, M., Shahbazi, A., Vahabzadeh, G., Barzroudi, M., and Golab, F, *Metabolic Brain Disease*, 2018, vol. 33, pp. 107–114.
17. Kandratavicius, L., Alves Balista, P., Lopes-Aguiar, C., Ruggiero, R.N., Umeoka, E.H., Garcia-Cairasco, N., and Leite, J., *Neuropsychiatr. Dis. Treat.,* 2014, vol. 10, pp. 1693–1705.
18. White, H.S., *Epilepsia*, 2003, vol. 44, no. s7, pp. 2–8.
19. El-Azab, M.F. and Moustafa, Y.M., *Pharmacological Reports,* 2012, vol. 64, no. 2, pp. 305–314.
20. Luszczki, J.J., Trojnar, M.K., Ratnaraj. N., Patsalos. P.N., and Czuczwar. S.*J., Epilepsy Research,* 2010, vol. 90, no. 3, pp. 188–198.
21. Blanco, M.M., Dos Santos, J.G., Perez-Mendes, P., Kohek, S.R.B., Cavarsan, C.F., Hummel, M., and Mello, L.E., *Epilepsia,* 2009, vol. 50, no. 4, pp. 824–831.
22. Srivastava, A.K. and White, H.S., *Epilepsy Research,* 2013, vol. 104, pp. 26–34.
23. Löscher, W., and Brandt, C., *Pharmacological Reviews*, 2010, vol. 62, no. 4, pp. 668–700.
24. Pitkänen, A., *Neurology,* 2002, vol. 59, no. 9 suppl 5, pp. S27–S33.
25. Nissinen, J., and Pitkänen, A. *Epilepsy Research,* 2007, vol. 73, no. 2, pp. 181–191.
26. Ghiglieri, V., Sgobio, C., Costa, C., Picconi, B., and Calabresi, P., *Progress in Neurobiology,* 2011, vol. 94, no. 2, pp. 102–114.
27. Shinnar, S., and Berg, A.T., *Epilepsia,* 1996, vol. 37, no. 8, pp.701–708.
28. Radzik, I., Miziak, B., Dudka, J., Chrościńska-Krawczyk, M., and Czuczwar, S.J., *Pharmacological Reports*, 2015, vol. 67, pp. 663–668.
29. Romoli, M., Mazzocchetti, P., D'Alonzo, R., Siliquini, S., Rinaldi, V.E., Verrotti, A., and Costa, C., *Curr. Neuropharmacol.*, 2019, vol. 17, no. 10, pp. 926–946.
30. Bahçekapili, N., Akgün-Dar, K., Albeniz, I., Kapucu, A., Kandil, A., Yağız, O., and Üzüm, G., *International Journal of Neuroscience*, 2014, vol. 124, no. 10, pp. 762–770.
31. Murashima, Y.L., Yoshii, M., and Suzuki, J., *Epilepsia,* 2002, vol. 43, Suppl 5, pp. 130–135.

32. Zhou, L., and Zhu, D.Y., *Nitric Oxide,* 2009, vol. 20, no. 4, pp. 223–230.

33. Tricoire, L., and Vitalis, T., *Frontiers in neural circuits*, 2012, vol. 6, p. 82.

34. Kovács, R., Rabanus, A., Otáhal, J., Patzak, A., Kardos, J., Albus, K., Heinemann, U., and Kann, O., *The Journal of Neuroscience,* 2009, vol. 29, no. 26, pp. 8565–8577.

35. Zhu, X., Dong, J., Han, B., Huang, R., Zhang, A., Xia, Z., Chang, H., Chao, J., and Yao, H., *Frontiers in Cellular Neuroscience,* 2017, vol. 11, p. 377.

36. Pawate, S., Shen, Q., Fan, F., and Bhat, NR., *Journal of Neuroscience Research*, 2004, vol. 77, no. 4, pp. 540–551.

37. Mao, K., You, C., Lei, D., and Zhang, H., *International Journal of Clinical and Experimental Medicine*, 2015, vol. 8, no. 6, pp. 8820–8827.

38. Zhang, Z., Zhang, Z.Y., Wu, Y., and Schluesener, H.J., *Neuroscience*, 2012, vol. 221, pp. 140–150.

39. Wang, X., Guo, J., Song, Y., Wang, Q., Hu, S., Gou, L., and Gao, Y., *Frontiers in Cellular Neuroscience*, 2018, vol. 12, p. 251.

40. Moylan, S., Berk, M., Dean, O. M., Samuni, Y., Williams, L.J., O'Neil, A., Hayley, A.C., Pasco, J.A., Anderson, G., Jacka, F.N., and Maes, M., *Neuroscience and Biobehavioral Reviews*, 2014, vol. 45, pp. 46–62.

41. Aguiar, C.C., Almeida, A.B., Araújo, P.V., de Abreu, R.N., Chaves, E.M., do Vale, O.C., Macêdo, D.S., Woods, D.J., Fonteles, M.M., and Vasconcelos, S.M., *Oxidative Medicine and Cellular Longevity,* 2012, vol. 2012, p. 795259.

42. Todorovic, SM. and Jevtovic-Todorovic, V., *Antioxid Redox Signal*, 2014, vol. 21, no. 6, pp. 880–891.

43. Taskiran, A.S. and Tastemur, Y., *Experimental Brain Research,* 2021, vol. 239, no. 2, pp. 591–599.