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The Effects of Valproic Acid on NO/cGMP in Pentylenetetrazole-Induced Acut Epilepsy Model in Rats

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Absract: Epilepsy is a disease which causes neuronal damage and loss of consciousness in consequence of recurrent seizures. Nitric oxide as a neuromodulator in brain is a gas which can penetrate into cells. It has a significant role on physiological cases,pathology of many diseases such as inflammation and degenerative diseases. The purpose of this research is to search the activity of NO/cGMP pathway of valproic acid in an experimental acute epileptic model which is induced with pentylenetetrazol in rats.18 adult male Wistar Albino rats were used in the study. The rats were randomly divided into 3 groups (n=6) as control group, pentylenetetrazol (PTZ+salin) 45 mg kg-1, valproic acid (PTZ+VPA) 150 mg kg-1. After 24 hours of PTZ application, all rats brain tissues were removed and then cortex and hippocampus were separated. While PTZ increased the hippocampus and cortex NO/cGMP levels compared to control ($p < 0.01$), VPA decreased the hippocampus and cortex NO levels by comparison with PTZ ($p < 0.001$). On the other hand, while VPA decreased cortex cGMP levels ($p<0,05$) it did not change cGMP levels in hippocampus ($p> 0.05$). This study has suggested that VPA can show antiepileptic activity via NO/cGMP pathway.

Sıçanlarda Pentilentetrazol ile İndüklenen Akut Epilepsi Modelinde Valproik Asidin NO/cGMP Üzerindeki Etkileri

Anahtar Kelimeler
Epilepsi, Pentilentetrazol, Valproik Asit, NO/cGMP

Öz: Epilepsi, tekrarlayan nöbetlerle nöronal hasara, bilinç kaybına neden olan bir hastalıktır. Nitrik oksit (NO); beyinde nöromodülatör olarak ve hücreler arasında yayılabilen bir gazdır. Fizyolojik olaylarda, iltihaplanma ve dejeneratif hastalıklar gibi birçok hastalığın patolojisinde önemli rolü vardır. Bu araştırmanın amacı, sıçanlarda pentilentetrazol ile indüklenen deneysel akut epileptik modelde valproik asidin NO/cGMP yolağının aktivitesini araştırmaktır. Çalışmada 18 adet yetişkin erkek Wistar Albino sıçan kullanıldı. Sıçanlar rastgele kontrol grubu, pentilentetrazol (PTZ) 45 mg kg^{-1}, valproik asit (VPA) 150 mg kg^{-1} olarak 3 gruba (n=6) ayrıldı. PTZ uygulamasından 24 saat sonra tüm sıçanların beyin dokuları çıkarıldı, korteks ve hipokampus ayrıldı. PTZ hipokamp ve korteks NO/cGMP düzeylerini kontrole göre artırırken ($p <0,01$), VPA hipokamp ve korteks NO düzeylerini PTZ'ye göre düşürdü ($p<0,001$). Ancak VPA korteks cGMP düzeylerini düşürürken ($p <0,05$), hipokampus cGMP düzeylerini değiştirmedi ($p> 0,05$). Bu çalışma, VPA'nın antiepileptik aktivitesini NO/cGMP yolu ile gösterebileceğini düşündürmektedir.

1. INTRODUCTION

In the central nervous system, NO functions as an intercellular spreadable signalling molecule [1]. NO is produced from L-arginine in an NADPH-dependent reaction by NO synthase (NOS) in brain. [2]. NO is involved in many physiological and pathological events as a neurotransmitter/neuromodulator in the brain. These

events are involved in the pathology of many diseases such as depression, learning, memory, synaptic plasticity, inflammation, epilepsy, and long-term degenerative diseases [3]. Epilepsy, one of the neurodegenerative disorders, is reported to be caused by NO. Epilepsy is a neurodegenerative disease that affects 3% of the world population [4] and occurs with recurrent seizures [5].

Nitric oxide performs this function by activating the ryanodine receptors. Ryanodine receptors increase the release of intracellular calcium stores and thus neuronal damage occurs [6]. Nitric oxide is a potent stimulator of guanilyl cyclase and causes an increase in intracellular second messenger cyclic guanosine monophosphate (cGMP) levels [7]. Cyclic guanosine monophosphate levels are regulated by cyclic adenosine monophosphate (cAMP) and cyclic nucleotide phosphodiesterases (PDEs) that catalyze the hydrolysis of cGMP [8]. Nitric oxide (NO) is a seizure sensitivity modulator known for its dose-dependent anti-convulsant and pro-convulsant effects in epileptogenesis [9].

Although the mechanism of action of antiepileptic drugs is uncertain, GABA-A receptors are thought to be responsible. One of these drugs, valproic acid (VPA), increases GABA production, reduces GABA transaminase, and inhibits excitatory neurotransmission [10]. They reported that VPA has an anticonvulsive effect on the PTZ-kindled model [11].

It has been suggested that NO may also play a role in the antiepileptic effect mechanism of VPA [12].

In the central nervous system, NO can act as a second messenger, neuromodulator and neurotransmitter, suggesting that NO plays an important role in epilepsy and epileptogenesis. The aim of this study is to investigate the activity of the NO/ cGMP pathway on the antiepileptic efficiency of valproic acid in an experimental acute epileptic model induced by pentylenetetrazole in rats.

2. MATERYAL VE METOT

The research was conducted in Sivas Cumhuriyet University Faculty of Medicine Experimental Animals Laboratory. Not having been exposed to stress, 18 Wistar Albino rats, 4-5 months old (230±20 g), were kept in cages to able to use in the study. All rats in the study were kept at 22-24°C with a 12-hour light/dark cycle, isolated from sound, with %55±6 humidity, and they were fed at an appropriate rate. The experimental application was carried out between 09:00 and 16:00. The light and sound level of the experimental environment were kept under constant control. For experimental procedures, permission was obtained from Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (license number 2020/327).

2.1. PTZ-Acute Epilepsy Protocol

A single dose of 45 mg kg^{-1} PTZ was applied to rats due to observe acute model epilepsy. After PTZ application, rats were placed in plexiglass cages (40 cmX40 cmX30 cm) and the seizures of each rat were observed within 30 mins. The seizure grade record was used to evaluate the seizures Modifies Racine's Convulsion Scale (RCS) as in the following, at initial phase there was no convulsion; at 1st phase there was twitching of vibrissae and pinnae; at 2nd phase there was a distinct twitching; at 3rd phase there were myoclonic jerks; at 4th phase there was tonic–clonic seizure while the animal remained on its feet; at 5th phase there was tonic–clonic seizure with loss of the righting reflex; at 6th phase there was tonic–clonic seizure with wild climbing and jumping; and 7th phase there was a lethal seizure [13].

2.2. Drugs

Pentylenetetrazole (PTZ) and valproic acid (VPA) were purchased from Sigma-Aldrich (St Louis, MO, USA). NO and cGMP Elisa kits were provided from Santa Cruz Biotechnology. PTZ and VPA were dissolved in saline and prepared in accordance with the doses stated below. PTZ and VPA were freshly dissolved before administering injections.

2.3. Experimental groups and Procedure

18 rats were randomly divided into 3 group (n=6). All injections were performed intraperitoneally.

Control group: Rats were administered 1 mg kg^{-1}ml single dose of saline and were applied another 1 mg kg^{-1}ml single dose of saline dose 30 min after the first injection. 24 hours after, white matter was removed, cortex and hippocampus were separated.

PTZ group: Rats in the PTZ group were applied 1ml/kg saline and administered a single dose of 45 mg kg^{-1} pentylenetetrazole 30 min after the saline injection. 24 hours after PTZ application, white matter was removed, cortex and hippocampus were separated.

VPA group: Rats were applied 150 mg kg^{-1} VPA and administered 45 mg kg^{-1} pentylenetetrazole 30 min after the VPA injection. 24 hours after PTZ application,white matter was removed, cortex and hippocampus were separated.

2.4. Preparation of Brain Tissue Homogenates

24 hours after the experimental protocol was completed, all experimental rats were euthanized. White matter of rats was removed immediately and the NO and cGMP levels in each sample were centrifuged after homogenization and frozen at -80° C to measure the NO and cGMP levels with Elisa kit.

2.5. Measurement of NO and cGMP

Rat ELISA commercial kits were used to determine the NO and cGMP levels in the supernatants of tissues taken from each group (YL Biont, Shanghai, China). Process protocols were done according to the manufacturer's instructions. Briefly, standard and tissue samples were added to the plate and incubated for 60 minutes at 37°C.

After the washing step, staining solutions were added and incubated for 15 minutes at 37° C. Stop solution was added and read at 450 nm. Standard curves were drawn to determine the value of the samples. The coefficients of variation within and between plates were found to be less than 10%.

2.6. Statistical Evaluation

All experimental results and biochemical analysis were converted into numerical values. Statistical analysis and graphing was used SPSS 25.0 for windows and GraphPad Prism 7. Results were presented as Mean ± SEM (standard error of mean). The results were evaluated by One-way ANOVA and followed by Tukey HSD (post-hoc test). $p<0.05$ values were defined.

3. BULGULAR

Hippocampus and Cortex NO Levels

As shown in Figure 1 of our research, a statistically important increase in hippocampus and cortex NO levels were observed in the PTZ administered group compared to the group control ($p<0.001$). However, a statistical decrease in hippocampus and cortex NO levels was found in the VPA administered group compared to the PTZ administered group ($p<0.001$).

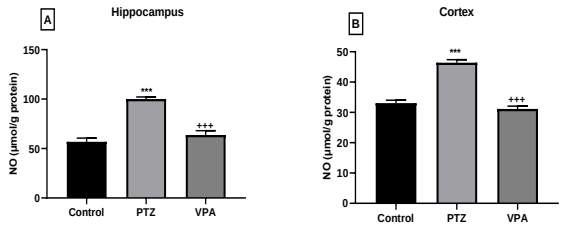

Figure 1. Effect of drugs on NO levels in the Hippocampus and Cortex after PTZ-induced seizure. * * * $p<0.001$ different from control; +++ $p<0.001$ different from PTZ.

3.2. Hippocampus and Cortex cGMP Levels

While a statistically important rise in hippocampus cGMP level was observed in the PTZ group compared to the control group ($p<0.001$), no significant difference was found between the VPA and PTZ group ($p>0.05$). However, a statistically significant increase in cortex cGMP level was observed in the VPA group compared to the PTZ group ($p<0.05$) (Figure 2).

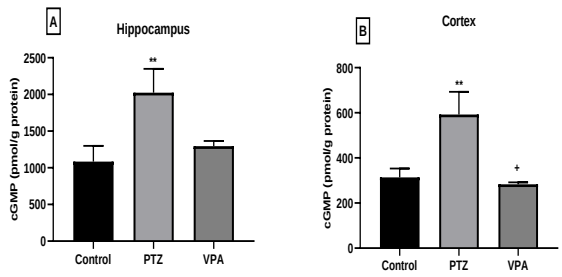

Figure 2. Effect of drugs on cGMP levels in the Hippocampus and Cortex after PTZ-induced seizure. * * $p<0.001$ different from control; + $p<0.001$ different from PTZ.

4. DISCUSSION

Epilepsy is a most common neurodegenerative disease that trigger progressive neuronal degeneration [14]. Over the past two decades, many studies have been conducted to discern the mechanisms underlying the epileptogenesis process and to treat epilepsy. Many events, such as apoptosis, inflammation, and oxidative stress play a role in the pathogenesis of epilepsy [15]. Nitric oxide, a known neurotransmitter/neuromodulator in the brain, plays a role in many physiological and pathological processes [16]. Nitric oxide is a potent stimulant of guanylyl cyclase, causing increased levels of cyclic GMP (cGMP) [7]. In the pathophysiology of epilepsy, NO shows anticonvulsant or a proconvulsant effects [17,18].

Han et al. [19] found that the 7-nitroindazole (7-NI) which is a NO inhibitor reduced hippocampus NO levels in the PTZ-induced rats, while PTZ caused seizures in rats as a result of an increase in hippocampus NO levels. This has been attributed to the increased density of PTZ's glutamate receptors as the activation of N-methyl-D-Aspartic acid (NMDA) receptors causes an increase in NO levels [20]. The hippocampus is activated after PTZ-induced seizure, as assessed by fos expression. But the mechanism of PTZ to increase NO production in the hippocampus is not clear [21]. PTZ can increase glutamate released by decrease GABA release, and thus stimulate NO production [22]. In our study, there was an important increase in both hippocampus and cortex NO levels in the PTZ group.

Nitric oxide triggers epileptic activity through cGMP formation. Glutamate activation stimulates NMDA receptors, activates nNOS by providing calcium flow to the cytosol, resulting in NO formation. Nitric oxide activates guanylate cyclase to synthesize cGMP, which is thought to initiate seizures [23]. Several studies on the role of NO in epileptic activity have mostly used proconvulsive drugs and NOS inhibitors. Some researchers have suggested that many factors, such as specific proconvulsive drugs, the type and concentrations of NOS inhibitor, method of administration, and employed specific strains or species, influence outcomes [24].

Another study researched the effect of Taurine on cortex NOS level in PTZ-induced rats and found that PTZ increased the cortex NOS level. The reason for this increase was explained by the stimulation of NMDA receptors by glutamate, catalyzing NO production and activating NOS, or by the fact that PTZ increases the NOS level by blocking GABA receptors. Taurine, on the other hand, has been found to significantly reduce the level of NOS [25]. We found that PTZ increased the level of cortex NO of rats, while VPA decreased it. The mechanism underlying this effect was demonstrated by PTZ, a GABA-A receptor antagonist, stimulating the NMDA receptor and activating neuronal nitric oxide synthase (nNOS) [26]. Therefore, in epileptic effect, it is suggested that nNOS increases NO as a result of stimulation with NMDA receptor [27]. Mülsch et al. [28] found that NO levels increased in the amygdala and cortex

during kainate-induced seizures in rats, and NO levels decreased in the group treated with 7-nitroindazole and diazepam. In our study, VPA, which was used as an antiepileptic, reduced NO levels in both the hippocampus and cortex, while decreased the cGMP levels in the cortex, but did not affect the hippocampus cGMP level. In another study, when looking at serum nitrite and nitrate levels in epileptic children using valproic acid or carbamazepine, nitrite and nitrate levels were found to be significantly higher in both valproic acid and carbamazepine groups compared to the control group. Based on these results, it has been suggested that valproic acid and carbamazepine may have an antiepileptic effect through nitric oxide [29]. It has been shown that 7-nitroindazole(7-NI) inhibitor improves the anticonvulsant effect of classic and second-generation antiepileptics, with the exception of tiagabin, felbamate and topiramate. However, the effect of NG-nitro-L-arginine methyl ester has not been clearly found. In the seizure models of pentylenetetrazole, pictotoxin and N-methyl-Daspartat, the inhibitor exhibited both convulsive and anticonvulsive effects depending on the dose. NG-nitro-L-arginine methyl ester enhanced the effectiveness of diazepam and clonazepam, decreased valproate and phenobarbital, but did not affect the anticonvulsant effect of phenytoin and ethosuximide [30].

The data of this research were consistent with previous works view that VPA caused a decrease in NO-cGMP levels in white matter, but an increase in NO-cGMP levels after PTZ-induced seizures.

5. SONUÇ

Our results show that NO plays an important role in acute epileptic model. However, VPA can demonstrate its antiepileptic effect by reducing the level of NO/cGMP, thus creating a protective effect against neuronal damage. Further research is needed to answer questions raised about possible involved mechanisms.

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Conflict of İnterest

The authors report no any conflict of interest

REFERENCES

[1] Dawson TM, Snyder SH. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J Neurosci*. 1994;14(9):5147-5159.
[2] Moncada S, Palmer RM, Higg, EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. 1991;43(2):109-142.
[3] Bruckdorfer R. The basics about nitric oxide. Mol Aspects Med. 2005;26(1-2):3-31.
[4] Wyllie E. Wyllie's treatment of epilepsy: principles and practice. 6th ed. Philadelphia, PA: Wolters Kluwer Health; 2015.
[5] Tastemur Y, Gumus E, Ergul M, Ulu M, Akkaya R, Ozturk A, et al. Positive effects of angiotensin-converting enzyme (ACE) inhibitor, captopril, on pentylenetetrazole-induced epileptic seizures in mice. Trop J Pharm Res. 2020; 19(3):637–643.
[6] Mikami Y, Kanemaru K, Okubo Y, Nakaune T, Suzuki J, Shibata K, et al. Nitric Oxide-induced Activation of the Type 1 Ryanodine Receptor Is Critical for Epileptic Seizure-induced Neuronal Cell Death. EBio Medicine. 2016; 11:253-261.
[7] Snyder SH, Bredt DS. Nitric oxide as a neuronal messenger. *Trends Pharmacol Sci*. 1991;12(4):125-128.
[8] Kaster MP, Rosa AO, Santos AR, Rodrigues AL. Involvement of nitric oxide-cGMP pathway in the antidepressant-like effects of adenosine in the forced swimming test. *Int J Neuropsychopharmacol*. 2005;8(4):601-606.
[9] Buisson A, Lakhmeche N, Verrecchia C, Plotkine M, Boulu RG. Nitric oxide: an endogenous anticonvulsant substance. *Neuroreport*. 1993;4(4):444-446.
[10] Johannessen CU, Johannessen SI. Valproate: past, present, and future. *CNS Drug Rev*. 2003;9(2):199-216.
[11] Dziki M, Hönack D, Löscher W. Kindled rats are more sensitive than non-kindled rats to the behavioural effects of combined treatment with MK-801 and valproate. *Eur J Pharmacol*. 1992;222(2-3):273-278.
[12] Peker E, Oktar S, Ari M, Kozan R, Doğan M, Cağan E, et al. Nitric oxide, lipid peroxidation, and antioxidant enzyme levels in epileptic children using valproic acid. *Brain Res*. 2009; 1297:194-197.
[13] Erdogan MA, Yusuf D, Erdogan A, Erbas. Levodropropizine suppresses seizure activity in rats with pentylenetetrazol-induced epilepsy. Epilepsy Res.2019; 150:32–37.
[14] Zhu X, Dong J, Han B, Huang R, Zhang A, Xia Z, et al. Neuronal Nitric Oxide Synthase Contributes to PTZ Kindling Epilepsy-Induced Hippocampal Endoplasmic Reticulum Stress and Oxidative Damage. Front Cell Neurosci. 2017; 11:377.
[15] Kwon YS, Pineda E, Auvin S, Shin D, Mazarati A, Sankar R. Neuroprotective and antiepileptogenic effects of combination of anti-inflammatory drugs in the immature brain. J Neuroinflammation. 2013; 10:30.
[16] Akula KK, Dhir A, Kulkarni SK. Nitric oxide signaling pathway in the anti-convulsant effect of adenosine against pentylenetetrazol-induced seizure threshold in mice. Eur J Pharmacol. 2008;587(1-3):129-134.
[17] Nakamura TA, Yamada K, Hasegawa T, Nabeshima T. Possible involvement of nitric oxide in quinolinic acid-induced convulsion in mice. Pharmacol Biochem Behav. 1995;51(2-3):309-312.
[18] Lallement G, Shih TM Pernot-Marino, I, Baubichon D, Foquin A, McDonough JH. The role of nitric oxide in soman-induced seizures, neuropathology,

and lethality. Pharmacol Biochem Behav. 1996;54(4):731-737.
[19] Han D, Yamada K, Senzaki K, Xiong H, Nawa H, Nabeshima T. Involvement of nitric oxide in pentylenetetrazole-induced kindling in rats. J Neurochem. 2000;74(2):792-798.
[20] Royes LF, Fighera MR, Furian AF, Oliveria MS, Fiorenza NG, Myskiw JC, et al. Involvement of NO in the convulsive behavior and oxidative damage induced by the intrastriatal injection of methylmalonate. Neurosci Lett. 2005;376(2):116-120.
[21] Shehab S, Coffey P, Dean P, Redgrave P. Regional expression of fos-like immunoreactivity following seizures induced by pentylenetetrazole and maximal electroshock. Exp Neurol. 1992;118(3):261-274.
[22] Giorgi O, Orlandi M, Lecca D, Corda MG. MK-801 prevents chemical kindling induced by pentylenetetrazol in rats. Eur J Pharmacol. 1991;193(3):363-365.
[23] Garthwaite J. Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci*. 1991;14(2):60-67.
[24] Balter-Seri J, Yuhas Y, Weizman A, Nofech-Mozes Y, Kaminsky, E, Ashkenazi, S. Role of nitric oxide in the enhancement of pentylenetetrazole-induced seizures caused by Shigella dysenteriae. Infect Immun. 1999;67(12):6364-6368.
[25] El-Abhar HS, El Gawad HM. Modulation of cortical nitric oxide synthase, glutamate, and redox state by nifedipine and taurine in PTZ-kindled mice. Epilepsia. 2003;44(3):276-281.
[26] X. Zhu, J. Dong, K. Shen, Y. Bai, Y. Zhang, X. Lv, et al. NMDA receptor NR2B subunits contribute to PTZ-kindling-induced hippocampal astrocytosis and oxidative stress. Brain Res Bull. 2015;114: 70-78
[27] X. Zhu, J. Dong, K. Shen, Y. Bai, J. Chao, H. Yao Neuronal nitric oxide synthase contributes to pentylenetetrazole-kindling-induced hippocampal neurogenesis. Brain Res Bull. 2016;121:138-147
[28] Mülsch A, Busse R, Mordvintcev PI, et al. Nitric oxide promotes seizure activity in kainate-treated rats. *Neuroreport*. 1994;5(17):2325-2328.
[29] Karabiber H, Yakinci C, Durmaz Y, Temel I, Mehmet N. Serum nitrite and nitrate levels in epileptic children using valproic acid or carbamazepine. Brain Dev. 2004;26(1):15-8.
[30] Banach M, Piskorska B, Czuczwar SJ, Borowicz KK. Nitric oxide, epileptic seizures, and action of antiepileptic drugs. CNS Neurol Disord Drug Targets. 2011;10(7):808-19.