Pharmacological Reports
2011, 63, 1569–1573
ISSN 1734-1140

Short communication

Kynurenic acid: a new effector of valproate action?

Piotr Maciejak[1,2], Janusz Szyndler[2], Danuta Turzyńska[1], Alicja Sobolewska[1], Adam Płaźnik[1,2]

[1]Department of Neurochemistry, Institute of Psychiatry and Neurology, Sobieskiego 9, PL 02-957 Warszawa, Poland

[2]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Krakowskie Przedmieście 26/28, PL 00-927 Warszawa, Poland

Correspondence: Piotr Maciejak, e-mail: piomac@yahoo.com

Abstract:
We investigated the changes in hippocampal kynurenic acid (KYNA) concentrations and the amino acids involved in neuronal activity regulation following valproate (VPA) administration (400 mg/kg *ip*) in pentylenetetrazole-kindled rats (*in vivo*). We found a remarkably long-lasting increase in KYNA levels following VPA administration, and this effect correlated with a rise in GABA levels. No changes in the concentration of other analyzed amino acids were present. It is likely that the antiepileptic and neuroprotective properties of VPA may also be a consequence of an increase in the hippocampal KYNA concentration.

Key words:
PTZ-kindling, microdialysis, valproate, kynurenic acid, GABA, steady state, epilepsy, neuroprotection

Introduction

Valproate (VPA) is a commonly used antiepileptic drug with a well-established efficacy in the treatment of generalized and partial seizures. The action mechanism of VPA has not been fully elucidated [1]. VPA inhibits NMDA-evoked transient depolarization and neuronal voltage-gated Na^+ channels, increases GABA turnover and potentiates GABAergic currents [15, 20].

Kynurenic acid (KYNA) is a metabolite of tryptophan degradation and is synthesized from L-kynurenine in a reaction that is mediated by kynurenine aminotransferases. KYNA is a non-competitive antagonist of α7 nAChRs, and it is also a low-potency, broad-spectrum antagonist of ionotropic glutamate receptors. Similar to GABA, KYNA is one of the most important endogenous inhibitory neuroactive agents. Moreover, KYNA exerts neuroprotective activity [6, 18, 19].

The present study was designed to determine whether KYNA plays a role in the central action mechanism of VPA. Therefore, we investigated changes in the concentrations of KYNA in the hippocampus, which is one of the key structures in the process of epileptogenesis, and other amino acids that are involved in the regulation of neuronal activity following acute VPA administration to pentylenetetrazole (PTZ)-kindled, free-moving rats. All of the examined groups consisted of kindled animals.

Materials and Methods

Male Wistar rats, weighing 200 ± 20 g at the beginning of the experiment, were used in the study. The animals were housed in standard laboratory conditions in a temperature- and humidity-controlled environment. The study was approved by the Committee for Animal Care and Use at the Medical University in Warsaw. The animals received repeated intraperitoneal (*ip*) injections of PTZ at a subconvulsive dose of 30 mg/kg, three times a week. After each injection, the rats were observed for 30 min for the intensity of convulsions according to a five-point behavioral Racine scale [14]. Animals were considered kindled when they exhibited stage 5 seizures in two consecutive trials. Finally, a cohort of 16 kindled animals was used in the microdialysis study: 8 saline-injected (control) and 8 VPA-injected animals. Subsequently (at least seven days after the last seizure episode), the rats were anesthetized with a mixture of ketamine (Ketanest, Parke Davis, USA) and sodium pentobarbital (Morbital, Biovet, Poland) and fixed in a stereotaxic apparatus (Stoelting & Co., USA). The dialysis probe, with an outer diameter of 0.3 mm (hand-made, U-shaped membrane loop of 4 mm long), was implanted unilaterally (randomly on the left/right) into the dorsal hippocampus (AP: 3.5 mm, L: ± 1.5 mm, V: –4 mm) [17]. After 30 h, the microdialysis probes were perfused with artificial cerebral spinal fluid. After an initial 2 h equilibrium period, two 20 min dialysate samples (40 µl each) were collected. The mean of these collections was used as a reference point (baseline = 100%) for the percent changes in the subsequent 8 collections (the absolute values of the KYNA baseline concentration (the means ± SEM, nM) were 2.9 ± 0.6 and 1.7 ± 0.3 for the saline and VPA treated group, respectively; the absolute values of the amino acids baselines are presented in Tab. 1). Immediately afterwards, the animals received a single *ip* injection of VPA at 400 mg/kg (sodium salt; dissolved in 0.9% NaCl and titrated with 1 M HCl to a final pH around the physiological level) or saline, and 8 consecutive samples were collected (40 µl each). VPA was obtained from Sanofi-Aventis (Poland). The dose of VPA was selected based on the pilot study and previous studies.

When the experiment was terminated, the brain of each animal was sliced and examined to verify the probe placement. The extracellular concentrations of amino acids (alanine (ALA), taurine (TAU), GABA, glutamate (GLU), glycine (GLY) and aspartate (ASP)) were determined using an HPLC system with electrochemical detection according to the method described previously [17]. The detection of KYNA and tryptophan (TRP) was performed using HPLC with fluorescence detection [11]. The fluorescence detector was set at an excitation of 344 nm and an emission wavelength of 398 nm for the detection of KYNA, and 254 nm and 404 nm were used to detect TRP. The retention time of KYNA and TRP was 9 and 17 min, respectively. Calibration curves were created by the injection of KYNA and TRP standards in concentrations that ranged from 0.5–120 nM and 0.5–20 µM, respectively.

The differences in amino acids and KYNA concentrations between the experimental groups in relative values (i.e., percentage changes) were analyzed using ANOVA for repeated measures followed by the LSD *post-hoc* test. The correlation analysis was performed using Pearson's r test. (Statistica, Release 8, StatSoft Inc., USA).

Results

VPA produced a significant increase in hippocampal KYNA concentrations compared to the baseline levels and the saline control group (drug effect ($F = 23.58$, $p < 0.005$), time effect ($F = 14.57$, $p < 0.005$), and drug × time interaction ($F = 13.56$, $p < 0.005$)). *Post-hoc* analyses revealed that the KYNA level was significantly increased compared to the baseline level after 60 min ($p < 0.05$), 80 min ($p < 0.01$), 100 min ($p < 0.005$), 120 min ($p < 0.005$), 140 min ($p < 0.005$) and 160 min ($p < 0.005$) and compared to the control group at 60 min ($p < 0.05$), 80 min ($p < 0.05$), 100 min ($p < 0.005$), 120 min ($p < 0.005$), 140 min ($p < 0.005$) and 160 min ($p < 0.005$) post-VPA administration (Fig. 1).

There also appeared to be a significant effect of VPA on the concentration of GABA in the hippocampus (drug effect ($F = 6.00$, $p < 0.05$), time effect ($F = 2.53$; $p < 0.05$), and drug × time interaction, ($F = 2.82$, $p < 0.01$)). The *post-hoc* test showed that GABA concentrations were increased compared to the baseline level after 60 min ($p < 0.05$), 100 min ($p < 0.05$), 120 min ($p < 0.005$), 140 min ($p < 0.005$) and 160 min ($p < 0.005$) following VPA administration. Compared

Tab. 1. Changes in hippocampal amino acid concentrations after saline or valproate injection. The data show the means ± SEM and represent the percentage change (%) compared to the baseline values, which are presented as absolute values (μM). Saline treated group (SAL) n = 8. Valproate treated group (VPA) n = 8. * Differs from baseline. # Differs from control (saline treated). *, # $p < 0.05$, ## $p < 0.01$, ***, ### $p < 0.005$

GROUP	BASELINE (μM)	20 min (%)	40 min (%)	60 min (%)	80 min (%)	100 min (%)	120 min (%)	140 min (%)	160 min (%)
GABA/SAL	0.05 ± 0.01	100.5 ± 12.1	197.1 ± 4.3	101.5 ± 4.1	127.5 ± 24.2	133.1 ± 24.8	118.5 ± 28.9	115.0 ± 17.5	92.1 ± 16.5
GABA/VPA	0.04 ± 0.01	89.4 ± 21.9	142.9 ± 21.1	213.0 ± 30.6*,#	172.7 ± 11.9	191.4 ± 67.1*	235.4 ± 60.4***, #	251.8 ± 83.9***,###	285.4 ± 73.9***,###
GLU/SAL	1.50 ± 0.30	83.1 ± 4.3	94.1 ± 4.2	71.4 ± 4.8	91.6 ± 10.4	74.2 ± 6.6	107.8 ± 39.4	73.9 ± 3.8	90.4 ± 8.9
GLU/VPA	1.51 ± 0.40	84.7 ± 5.7	93.9 ± 12.1	184.7 ± 50.5	81.0 ± 14.2	70.7 ± 9.5	104.7 ± 26.2	110.1 ± 26.0	83.14 ± 9.4
TRP/SAL	0.33 ± 0.04	91.2 ± 4.1	109.8 ± 6.3	88.7 ± 3.8	102.2 ± 16.7	87.0 ± 4.0	87.2 ± 6.9	91.0 ± 8.6	92.9 ± 10.3
TRP/VAP	0.35 ± 0.06	131.4 ± 39.8	92.6 ± 9.2	119.3 ± 5.9	113.0 ± 6.6	117.1 ± 8.5	115.6 ± 8.9	119.3 ± 9.8	110.9 ± 10.5
TAU/SAL	2.77 ± 0.30	85.2 ± 6.6	90.7 ± 5.4	81.4 ± 5.4	87.9 ± 10.2	78.6 ± 5.3	71.0 ± 6.3	82.1 ± 6.5	118.0 ± 29.4
TAU/VPA	3.45 ± 0.50	94.7 ± 3.1	104.8 ± 6.3	100.8 ± 5.2	93.7 ± 5.5	78.9 ± 2.5	77.5 ± 3.4	83.4 ± 6.4	77.1 ± 2.3
GLY/SAL	2.25 ± 0.30	89.4 ± 4.4	115.2 ± 5.5	78.9 ± 5.3	113.8 ± 13.3	88.8 ± 8.0	218.2 ± 132.3	90.8 ± 8.5	132.8 ± 16.0
GLY/VPA	2.94 ± 0.50	95.0 ± 5.2	81.3 ± 8.0	106.0 ± 6.8	87.9 ± 6.5	87.2 ± 12.9	102.4 ± 20.1	72.9 ± 15.7	172.4 ± 62.8
ASP/SAL	0.59 ± 0.10	66.9 ± 10.8	122.3 ± 11.0	50.3 ± 10.6	91.5 ± 16.9	59.8 ± 12.4	190.2 ± 125.8	60.4 ± 9.8	108.7 ± 18.6
ASP/VPA	0.50 ± 0.10	95.0 ± 11.4	89.4 ± 14.4	157.3 ± 32.1	76.6 ± 4.7	72.5 ± 12.9	134.6 ± 27.5	86.9 ± 11.8	126.3 ± 29.3
ALA/SAL	4.43 ± 0.30	87.9 ± 5.4	107.1 ± 5.4	84.1 ± 5.8	103.8 ± 12.6	87.2 ± 5.4	97.4 ± 12.7	89.8 ± 5.0	102.8 ± 9.7
ALA/VPA	5.76 ± 0.70	91.6 ± 4.1	92.8 ± 5.0	96.1 ± 2.5	86.5 ± 4.6	75.6 ± 5.7	83.4 ± 7.7	62.2 ± 4.2	76.9 ± 4.3

to the saline control group, GABA concentrations were increased after 60 min ($p < 0.05$), 120 min ($p < 0.05$), 140 min ($p < 0.01$) and 160 min ($p < 0.005$) post-VPA administration (Tab. 1).

The changes in the concentrations of GABA and KYNA after VPA administration were significantly positively correlated ($r = 0.55$, $p < 0.001$). No significant changes in the concentrations of the other studied amino acids were found.

Discussion

We found a very potent increase (more than 1,600%) in KYNA concentration in the rat hippocampus after valproate (VPA) administration to PTZ-kindled rats. Furthermore, the increase in KYNA was positively correlated with a local rise in the GABA levels. No changes in the concentrations of the other analyzed amino acids were found.

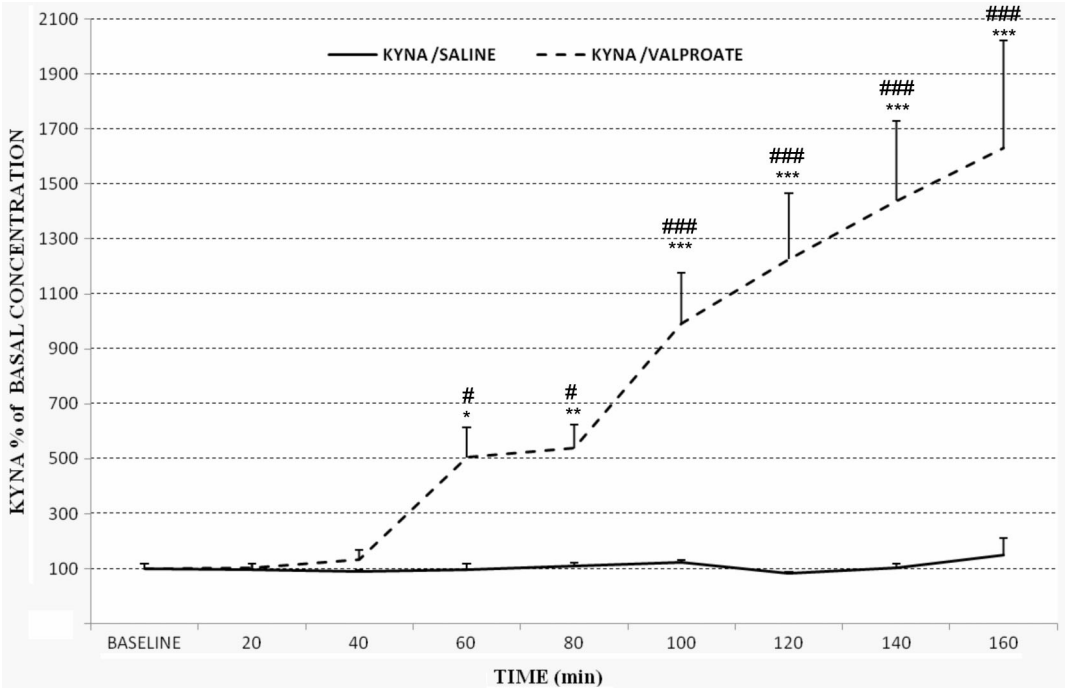

Fig. 1. The effect of valproate or saline on the basal concentration of KYNA in the hippocampus. The data show the means ± SEM and represent the percentage change compared to the baseline values (100%). Eight animals in each group. * Differs from baseline. # Differs from control (saline treated). $^{*,\#}$ $p < 0.05$, ** $p < 0.01$, $^{***,\#\#\#}$ $p < 0.005$

This study did not allow us to determine the mechanism of the VPA-induced increase in KYNA levels. Several hypotheses need to be considered. First, VPA could evoke a peripheral release of kynurenine that is easily transported to the brain and stimulate KYNA production [9, 18]. Second, valproate could stimulate the activity of kynurenine amino transferase (KAT); however, this effect has not been observed *in vitro* [8]. Third, VPA could inhibit kynurenine hydroxylase, which is a major kynurenine metabolizing enzyme, to increase the KYNA concentration [13]. The mechanism of action of VPA requires further study. However, it seems clear that the increased concentration of KYNA may be an important element in its antiepileptic and neuroprotective activities. In *in vitro* studies, KYNA reduces the spontaneous epileptiform firing of neurons, which indicates that this effect could be a part of the acute antiseizure action of VPA [16]. Moreover, VPA exerts its potent neuroprotective activity in different models of neurodegeneration [2, 7, 10], but this mechanism's effect is still not known. In light of our data, KYNA may play an important role in this phenomenon. Furthermore, the fact that the KYNA brain concentration is increased after the peripheral VPA administration creates the possibility of more clinical research on the neuroprotective effects of this antiepileptic drug [4, 5].

Because our study was performed in kindled rats, it was necessary to determine the effects of kindling on hippocampal KYNA concentrations. In our previous work, we found that PTZ-induced kindling was accompanied by a progressive decrease of KYNA in brain structures, including the hippocampus [11]. The current study shows that even in this situation, VPA led to a very potent increase in hippocampal KYNA concentrations.

Another important observation concerns the positive correlation between the KYNA and GABA concentrations based on the inhibitory role of GABAergic innervation of the limbic structures in the regulation of ictal activity [12]. Accordingly, VPA strongly stimulates GABAergic neurotransmission [15]. Further research is needed to understand the central effects of VPA [3]. Our unpublished results show that the effects of VPA on KYNA are also present after intragastric drug administration in a dose-dependent manner. Moreover, an applied dose of VPA (400 mg/kg) protected against PTZ-induced seizures in all rats. It is also important to determine whether the KYNA increase is VPA-specific or a more general effect of

other antiepileptic drugs. This important problem is now under study in our laboratory.

This the first report indicating a very potent stimulatory and concomitantly occurring effect of VPA on the limbic concentrations of KYNA and GABA. Because of the inhibitory role of both endogenously occurring substances on the neuronal activity, the current study strongly suggests their contribution to the process of epileptogenesis and the mechanism of action of VPA. In light of our findings, it is likely that the antiepileptic and neuroprotective features of VPA, at least in part, may be a consequence of the increases in the KYNA levels.

Acknowledgment:
The valuable assistance of Karolina Kołosowska is gratefully acknowledged.

References:

1. Baltes S, Fedrowitz M, Tortós CL, Potschka H, Löscher W: Valproic acid is not a substrate for P-glycoprotein or multidrug resistance proteins 1 and 2 in a number of in vitro and in vivo transport assays. J Pharmacol Exp Ther, 2007, 320, 331–343.
2. Chen PS, Peng GS, Li G, Yang S, Wu X, Wang CC, Wilson B et al.: Valproate protects dopaminergic neurons in midbrain neuron/glia cultures by stimulating the release of neurotrophic factors from astrocytes. Mol Psychiatry, 2006, 11, 1116–1125.
3. Czuczwar M, Cieszczyk J, Czuczwar K, Kiś J, Saran T, Turski WA: Influence of orphenadrine upon the protective activity of various antiepileptics in the maximal electroshock-induced convulsions in mice. Pharmacol Rep, 2009, 61, 732–736.
4. Foster AC, Vezzani A, French ED, Schwarcz R: Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett, 1984, 48, 273–278.
5. Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR: Blood–brain barrier transport of kynurenines: Implications for brain synthesis and metabolism. J Neurochem, 1991, 56, 2007–2017.
6. Hilmas C, Pereira EFR, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX: The brain metabolite kynurenic acid inhibits α7 nicotinic receptor activity and increases non-α7 nicotinic receptor expression: physiopathological implications. J Neurosci, 2001, 21, 7463–7473.
7. Kim HJ, Rowe M, Ren M, Hong JS, Chen PS, Chuang DM: Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: multiple mechanisms of action. J Pharmacol Exp Ther, 2007, 321, 892–901.
8. Kocki T, Wielosz M, Turski WA, Urbanska EM: Enhancement of brain kynurenic acid production by anticonvulsants – Novel mechanism of antiepileptic activity? Eur J Pharmacol, 2006, 541, 147–151.
9. Konradsson-Geuken Ĺ, Wu HQ, Gash CR, Alexander KS, Campbell A, Sozeri Y, Pellicciari R et al.: Cortical kynurenic acid bi-directionally modulates prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. Neuroscience, 2010, 169, 1848–1859.
10. Leng Y, Chuang DM: Endogenous α-synuclein is induced by valproic acid through histone deacetylase inhibition and participates in neuroprotection against glutamate-induced excitotoxicity. J Neurosci, 2006, 26, 7502–7512.
11. Maciejak P, Szyndler J, Turzyńska D, Sobolewska A, Taracha E, Skórzewska A, Lehner M et al.: Time course of changes in the concentration of kynurenic acid in the brain of pentylenetetrazol-kindled rats. Brain Res Bull, 2009, 78, 299–305.
12. Mann EO, Paulsen O: Role of GABAergic inhibition in hippocampal network oscillations. Trends Neurosci, 2007, 30, 343–349.
13. Miranda AF, Boegman RJ, Beninger RJ, Jhamandas K: Protection against quinolinic-acid mediated excitotoxicity in nigrostriatal dopaminergic neurons by endogenous kynurenic acid. Neuroscience, 1997, 78, 967–975.
14. Racine RJ, Gartner JG, Burnham WM: Epileptiform activity and neural plasticity in limbic structures. Brain Res, 1972, 47, 262–268.
15. Rosenberg G: The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? Cell Mol Life Sci, 2007, 64, 2090–2103.
16. Scharfman HE, Goodman JH, Schwarcz R: Electrophysiological effects of exogenous and endogenous kynurenic acid in the rat brain: studies in vivo and in vitro. Amino Acids, 2000, 19, 283–297.
17. Szyndler J, Maciejak P, Turzyńska D, Sobolewska A, Lehner M, Taracha E, Walkowiak J et al.: Changes in the concentration of amino acids in the hippocampus of pentylenetetrazole-kindled rats. Neurosci Lett, 2008, 439, 245–249.
18. Vamos E, Pardutz A, Klivenyi P, Toldi J, Vecsei L: The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection. J Neurol Sci, 2009, 283, 21–27.
19. Wejksza K, Rzeski W, Turski WA: Kynurenic acid protects against the homocysteine-induced impairment of endothelial cells. Pharmacol Rep, 2009, 61, 751–756.
20. Wojtal K, Borowicz KK, Błaszczyk B, Czuczwar SJ: Interactions of excitatory amino acid receptor antagonists with antiepileptic drugs in three basic models of experimental epilepsy. Pharmacol Rep, 2006, 58, 587–598.

Received: March 28, 2011; **in the revised form:** June 14, 2011; **accepted:** June 21, 2011.