



---

Short communication

## Kynurenic acid: a new effector of valproate action?

Piotr Maciejak<sup>1,2</sup>, Janusz Szyndler<sup>2</sup>, Danuta Turzyńska<sup>1</sup>,  
Alicja Sobolewska<sup>1</sup>, Adam Płażnik<sup>1,2</sup>

<sup>1</sup>Department of Neurochemistry, Institute of Psychiatry and Neurology, Sobieskiego 9, PL 02-957 Warszawa, Poland

<sup>2</sup>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<sup>+</sup> 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  $\alpha 7$  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  $200 \pm 20$  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:  $\pm 1.5$  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  $\mu$ l 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  $\pm$  SEM, nM) were  $2.9 \pm 0.6$  and  $1.7 \pm 0.3$  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  $\mu$ l 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  $\mu$ M, 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  $\times$  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  $\times$  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

Download English Version:

<https://daneshyari.com/en/article/2011866>

Download Persian Version:

<https://daneshyari.com/article/2011866>

[Daneshyari.com](https://daneshyari.com)