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DOES VALPROATE ACT AS AN ANTICONVULSANT OR ANTIEPILEPTIC ON METAPHIT-INDUCED SEIZURE IN RATS?

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The effects of valproate sodium (VPA) on metaphit (1-[1-(3-isothiocyanatophenyl)-cyclohexyl]-piperidine)-induced epilepsy were studied. For this purpose, adult Wistar rat males were used and their behavioral, electrocortical activity and power spectra were examined.

Severity of metaphit (10 mg/kg; i.p.) seizures was increasing during the time needit to reach the peak 7-12 h after injection. VPA (100 mg/kg) was i.p. injected to animals with fully developed metaphit seizures after the 8th audiogenic testing. The rats divided in four groups received (i.p.): 1. saline; 2. metaphit alone; 3. metaphit + VPA and 4. VPA alone. They were exposed to sound stimulation 60 min after metaphit administration and further on at hourly intervals. Metaphit produced generalized convulsions and electrocortical abnormalites (high voltage spikes and spike-wave complexes) and power spectrum analysis revealed an increase in total voltage output during the seizure. Valproate, a classical antiepileptic drug prolonged the duration of latency period, decreased the incidence and mean seizure grade but expressed no effect on metaphit-induced electrocortical abnormalites.

The results suggest that VPA (100 mg/kg) acted as an anticonvulsant rather than antiepileptic in the metaphit model of epilepsy.

Key words: audiogenic seizures, EEG, power spectra, metaphit, valproate, rats

INTRODUCTION

Anticonvulsant properties of valproic acid (VPA) were first recognized by Meunier *et al.* (1963). Later on anticonvulsive characteristics of its sodium salt were observed and at present, valproate sodium represents the drug which is frequently applied in the treatment of epilepsy. There is no single mechanism of its action that could completely account for numerous effects of the drug on neuronal tissue and its broad clinical activity in epilepsy (Loscher, 1993), as well

as some other brain diseases, *e.g.* mania, bipolar depression, cyclothymia, *etc*. (Bowden, 2003).

Metaphit (1-[1-(3-isothiocyanatophenyl)-cyclohexyl]-piperidine) is known for its different *in vitro* and *in vivo* effects on living cells, *i.e.* organisms including induction of audiogenic seizures in mice (Debler *et al.*, 1989), Guinea pigs (Šušić *et al.*, 1991) and rats (Stanojlović *et al.*, 2000, 2005). Also, Rafferty *et al.* (1985) observed metaphit-induced long term blockade of PCP receptors in the NMDA receptor complex channel.

In the present work, we were interested to learn whether valproate sodium, a standard antiepileptic drug acts as an anticonvulsant or antiepileptic. Hence we have examined the effects of valproate on metaphit model of epilepsy combined with audiogenic stimulation (AGS) of adult Wistar rat males.

MATERIAL AND METHODS

General

Adult Wistar rat males weighing 180-220 g. were used. The animals were obtained from The Military Medical Academy Breeding Laboratories, Belgrade and kept under controlled environmental conditions (ambient temperature 22-24°C, 50-60% humidity, 12-12 light/dark cycle, light on 8 a.m.) for at least one week before the experiment. They had free access to standard laboratory chow and tap water and were habituated to handling. All animals were allowed to acclimate in a plastic cage (55x35x15 cm; one rat per cage). Only the animals responding clearly to metaphit treatment and AGS were used, while the others that did not respond to AGS and behaved normally during the time period of 8-12 h after metaphit administration were excluded from the experiment.

The animals divided into four groups were i.p. treated with: 1. physiological saline (n=6); 2. metaphit alone (10 mg/kg b.w.; n=12); 3. metaphit (10 mg/kg b.w.) + VPA (100 mg/kg b.w.; n=8) or 4. VPA alone (100 mg/kg b.w.; n=6). They were exposed to sound stimulation 60 min later and further on at hourly intervals.

Seizure model

None of the untreated animals screened for audiogenic susceptibility expressed seizure activity. AGS was applied for 60 s using an electric bell (on the top of the cage) generating 100±3 dB and frequency of 5-8 kHz. Audiogenic convulsive behaviour was assessed by the incidence of motor seizures and seizure severity grade determined using the scale from 0-3 (0 - no response; 1 - wild running only; 2 - wild running followed by clonic seizures; 3 - wild running progressing to generalized clonic convulsions followed by tonic extension of fore- and hind limbs and tail in audiogenic resistant rat (Šušić and Marković, 1993).

For electrophysiological experiments, *i.e.* EEG recordings, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.), positioned in a stereotaxic apparatus and three gold-plated recording electrodes were implanted

over frontal, parietal and occipital cortices. The experiments were performed in accordance with the Helsinki Declaration and animals were left to recover for 7 days after the surgery. An EEG apparatus (RIZ, Zagreb, Croatia) with a modified output degree enabling to transfer output signals to the input circuit of 8-channel, 12-byte AD card PCL-711B (Advantech Co. Ltd.) installed into a computer and the corresponding software were used. The quantification of total voltage power and of single frequency bands was done employing a Berg-Fourier analyzer. In the present study epochs of 13 sec were used for 60 min. The spectrum power was plotted and the integrated energy signals expressed as μV^2.

Data analysis and drugs

Significance of the differences in the incidence of seizures was evaluated by Fisher's exact probability test. All score data were statistically compared by the Kruskal-Wallis one-way ANOVA and Mann Whitney U-test for the differences in the mean seizure grade and Kruskal Wallis ANOVA test for the differences in the mean duration of the latency period (*$p<0.05$, **$p<0.01$).

Metaphit methanesulfonate was a product of Sigma-Aldrich Chemical Co., U.S.A. Valproate sodium was a kind gift of Hemofarm Pharmaceutical Works (Vršac, Serbia and Montenegro).

RESULTS

Effect of metaphit and valproate on electrocortical activity and spectrum power

No EEG changes were recorded in control animals either exposed or unexposed to AGS (Fig. 1.). Baseline state was characterized by desynchronization and a high frequency low amplitude (10-12 Hz) activity in EEG. Total power spectra showed the intensity of 500 - 600 μV^2.

EEG signs of epilepsy are shown in Fig. 2. EEG record of complete motor seizure response (grade 3) with a high amplitude low-frequency synchronized spikes (3-5 Hz) and sleep-like patterns induced by audiogenic stimulation in a metaphit-treated rat can be seen. Electrocortical changes were objectively quantified. Spectrum power analysis of the electrocortical changes induced by metaphit revealed an increased total voltage power (5 000 – 6 000 μV^2).

Valproate in the dose applied (100 mg/kg b.w.) led to a rapid behavioural but not electricortical changes in metaphit-pretreated animals (Fig. 3). Namely, VPA did neither reduce nor eliminate electrocortical metaphit-induced spikes and high amplitude waves within 1-5 Hz range (delta and theta), a very intense power spectrum and a sharp metaphit-induced spiking can be seen.

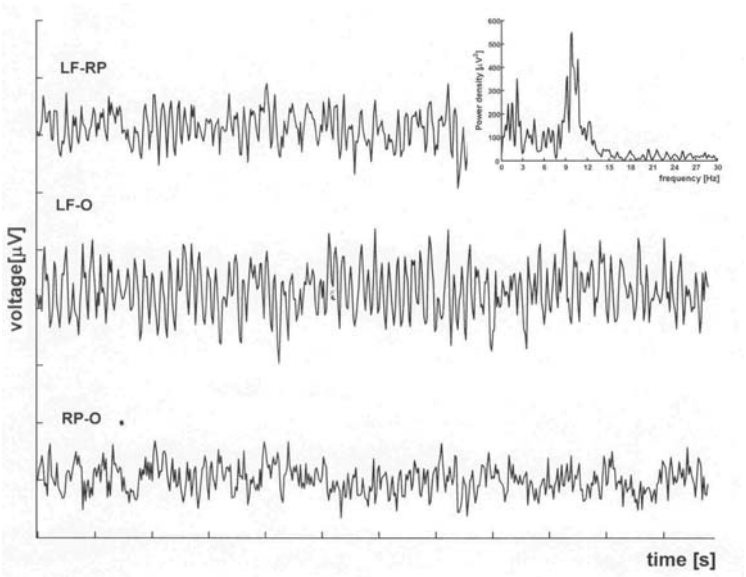

Figure 1. EEG record of baseline state and the corresponding power spectra in physiological saline-injected adult Wistar rat male
Right corner - power spectra (V^2) of the corresponding EEG activity shown on the left.
LF-RP: left fronto-right parietal cortex, LFO: left - fronto-occipital cortex; RP-O: right parietal-occipital cortex. Time calibration 1 sec, amplitude calibration 50 V.

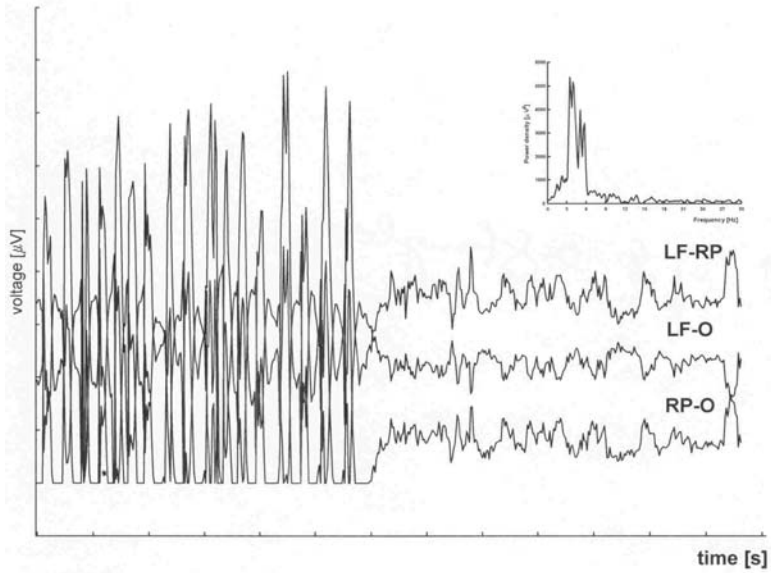

Figure 2. EEG tracings in a metaphit-treated rat (10 mg/kg, i.p.) during sound stimulation. Note a very intense power spectrum that was temporary decreased after the sound offset. For the details on EEG recordings and calibration see caption to Fig 1.

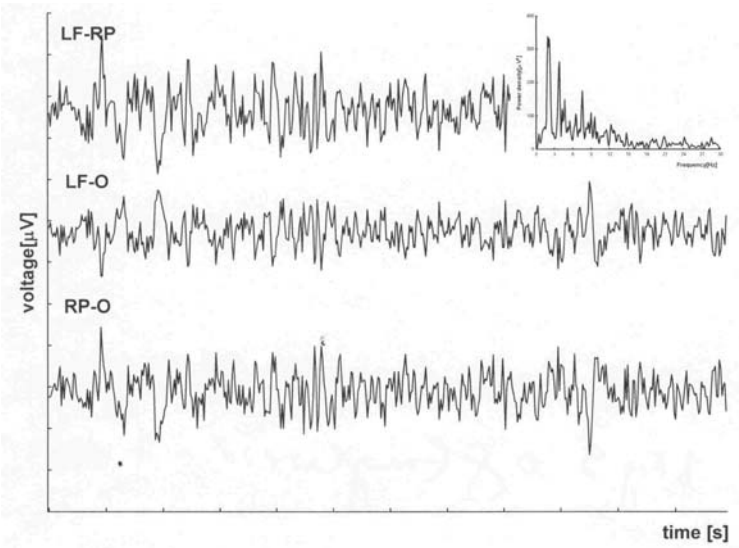

Figure 3. The effect of valproate (100 mg/kg, i.p) on EEG recordings and power spectrum of a rat pretreated with metaphit.
Left - EEG recordings from the cortical leads 60 min after valproate injection. Some metaphit spiking and sleep-like patterns without clinical seizure activity (grade - 0) were observed. A power spectrum of EEG was more intense in a low frequency (2-4 Hz) region. For the details on EEG recordings and calibration see caption to Fig 1.

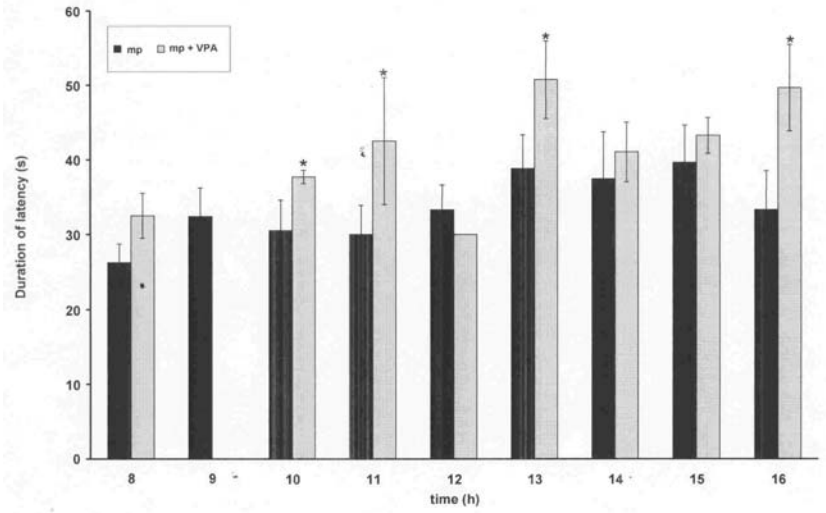

Figure 4. Duration of the latency period (time from the onset of sound stimulation to the onset of running) in metaphit-pretreated and VPA injected rats.
Values are means±S.D. expressed in seconds. Significance of the differences was evaluated by Kruskal Wallis ANOVA test; mp *vs.* mp + VPA, (*$p<0.05$).
For more details see Table I.

Effects of metaphit and valproate on behavior and motor changes

VPA given i.p. in the dose of 100mg/kg rapidly reversed behavioral and motor changes induced by metaphit. As shown in Table 1., mean seizure grade was rapidly reduced after VPA injection, especially during the first four hours after administration, *i.e.* 9-10 h ($p<0.01$) and 11-12 h ($p<0.05$) upon metaphit application in comparison with the group that received metaphit alone. The same holds true for the incidence of audiogenic convulsions at the same experimental points, and at 9 h ($p<0.01$) none of the animals responded (0/8), while between 10 h and 12 h after metaphit ($p<0.05$) the number of convulsing animals was significantly reduced in comparison with the group that received metaphit alone.

Table 1. Influence of valproate on mean seizure grade and incidence of audiogenic convulsions in metaphit-pretreated rats

T (h)	Mean seizure grade		Incidence	
	mp	mp+VPA	mp	mp+VPA
1	0.33		2/12	
2	0.58		3/12	
3	0.92		4/12	
4	1.00		4/12	
5	1.83		9/12	
6	1.92		8/12	
7	2.25		9/12	
8	2.00	3,00	9/12	8/8
9	1.92	0,00**	9/12	0/8**
10	2.25	0,87**	10/12	3/8*
11	1.92	0,38*	9/12	2/8*
12	2.00	0,25*	9/12	1/8*
13	1.58	0,63	8/12	3/8
14	1.75	1,00	7/12	4/8
15	1.50	1,50	8/12	5/8
16	1.42	1,50	6/12	5/8

T – Time after metaphit administration; mp – metaphit, VPA – valproate. The rats treated with metaphit (10 mg/kg; n=12) followed 8 h later by valproate (100 mg/kg; n=8) were exposed to an intense audiostimulation (100±3 dB, 60 s) at hourly intervals after metraphit injection. Significance of the differences in the mean seizure grade between the groups: mp *vs.* mp + VPA was evaluated by Kruskal–Wallis one–way ANOVA and Mann–Whitney U–test (*$p<0.05$, **$p<0.01$) and in the incidence by Fisheržs exact probability test.

In addition, VPA treatment resulted in a significantly increased duration of the latency period 10 h, 11 h, 13 h and 16 h ($p<0.05$) after metaphit administration in comparison with that observed in animals treated with metaphit alone (Fig. 4).

In the rats injected with VPA (100 mg/kg, i.p) alone and subjected to AGS, behaviour and EEGs were similar to those observed in the controls treated with physiological saline.

DISCUSSION

The results of the present work showed that metaphit in the dose of 10 mg/kg b.w. together with audiogenic stimulation triggered increasing locomotor activity (after a latency period) which progressed in running crisis and generalized in clonic convulsions, thus confirming our earlier results (Šušić *et al.*, 1993; Stanojlović *et al.*, 2000, 2004). However, in some cases the locomotor activity progressed into extension of whole body and tail. The picture of convulsive animals was typical for generalized, reflex audiogenic epilepsy. Electrocortical abnormalites seen as spikes and spike-wave complexes progressing into the burst of spike-wave complexes after AGS, either preceded or accompanied motor disorders.

Metaphit binds to the part of the NMDA receptor complex that contains five modulatory sites for binding both glutamate and synthetic agonists and antagonists. AGS as a precipitation factor leads to Na^{+} and Ca^{++} influx through the ion channel connected to the NMDA receptor and depolarization of a critical number of neurons that propagate further to other brain regions (Lipovac *et al.*, 1993). Metaphit acts producing glutamate accumulation by inhibiting the influx mechanism at the luminal side of brain capillaries, as well as the glial uptake of glutamate. In this manner extracellular glutamate level in the brain increases (Pardige *et al.*, 1988, Lipovac *et al*., 2002.).

VPA represents the drug of choice for the treatment of a broad array of seizure disorders. For example in the model of complex partial seizure with secondary generalization or temporal lobe epilepsy (Loscher, 1993, 1998), absence seizure in humans (van Rijn *et al.*, 2004), as well as in acute, reactive or provoked seizures (Sun *et al.*, 2002).

Nistico *et al.* (1980) reported that the antagonistic effect of sodium valproate after *i.v.* injection had a rapid onset, suggesting that this drug may exert a direct effect on the postsynaptic inhibitory mechanism. There are several proposed mechanisms by which exposure to excess VPA could reduce seizures since the details on the mechanism of its action are still unclear. VanErpi *et al.* (1990) demonstrated that VPA acted reducing sodium and potassium conductances, while Loscher (1993) reported that it enhanced GABAergic synaptic transmission by increasing GABA levels in the brain. Also, Gean *et al.* (1994) showed that VPA reduced the excitatory synaptic transmission that leads to synchronized cell firing and epileptic bursting, especially with excitatory synaptic processes, suppressing depolarization induced by NMDA in a dose-dependent manner. It could be hypothesized that under experimental conditions, *i.e.* in metaphit-induced seizure, valproate influenced the inhibition/excitation equilibrium, *i.e.* potentiated GABAergic and inhibited glutamate/NMDA as suggested by Loscher (2002) for anticonvulsant activity of VPA on generalized convulsive seizures.

Our results demonstrate a significant anticonvulsant VPA activity in metaphit model of seizures, *i.e.* this drug rapidly reduced mean seizure grade, diminished the incidence of audiogenic convulsions in some checkpoints and prolonged the latency period. It reversed metaphit-induced behavioral and motor changes but expressed no effect on electrocortical activity. Thus, it could be taken as an anticonvulsant rather than antiepileptic.

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REFERENCES

1. *Bowden CL,* 2003, Valproate, *Bipolar Disorders* 5, 189-202
2. *Burton BS,* 1881, On the propyl derivatives and decomposition products of ethyl acetoacetate, *Am Chem J,* 3, 385-95.
3. *Debler EA, Lipovac MN, Lajtha A, Zloković BV, Jacobson AE, Rice KC, et al*. 1989, Metaphit, an isothiocyanate analog of PCP, induces audiogenic seizures in mice, *Eur J Pharmacol,* 165, 155-9.
4. *Gean PW, Huang CR, Tsai JJ,* 1994, Valproic acid suppresses the synaptic response mediated the NMDA receptors in rat amygdale slices, *Brain Res Bull* 33, 333-6.
5. *Lipovac MN, Debler EA, Zloković BV, Jacobson AE, Rice KC, de Costa B, et al*. 1993, Metaphit-induced audiogenic seizures in mice: II. Studies on N-methyl-D-aspartic acid, GABA and sodium channel receptors and on the disposition of metaphit in the brain, *Epilepsia,* 34, 211-19.
6. *Lipovac MN, Holland T, Poleksić A, Killian C, Lajtha A,* 2003, The possible role of glutamate uptake in metaphit-induced seizures, *Neurochem Res*, 28, 723-31.
7. *Loscher W,* 1993, Effect of the antiepileptic drug valproate on metabolism and function of inhibitory amino acids in the brain, *Neurochem Res* 18, 485-502.
8. *Loscher W,* 1998, Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy, *Prog Neurobiol* 54, 721-41.
9. *Loscher W*, 2002, Basic pharmacology of valproate: A review after 35 years of clinical use for the treatment of epilepsy, *CNS Drugs* 16, 669-94.
10. *Meunier H, Carraz G, Meunier V,* 1963, Proprietes pharmacodynamiques de l´ acide n-propylacetique, *Therapie*, 18, 435-8.
11. *Nistico´ G, De Sarro GB, Rotiroti D, Silvestri R, Marmo E,* 1980, Antagonism by classical antiepileptic and sodium valproate of cefazolin induced experimental epilepsy in rats. *Res. Comm Chem Pathol Pharmacol,* 29, 429- 44.
12. *Pardige WM,* 1988, Recent advances in blood-brain barrier transport, *Annu Rev Pharmacol Toxicol,* 28, 25-39.
13. *Rafferty MF, Mattson M, Jacobson AE, Rice KC,* 1985, A specific acylating agent for the [^{3}H]phencyclidine receptors in rat brain. *FEBS Lett* 181, 318-22.
14. *Stanojlović O, Živanović D, Šušić V,* 2000, N-Methyl-D-aspartic acid and metaphit-induced audiogenic seizures in rat model of seizure, *Pharmacol Res* 42, 247-53.

15. *Stanojlović O, Živanović D, Mirković S, Mikhaleva II,* 2004, Delta sleep-inducing peptide and its tetra peptide analogue alleviate severity of metaphit seizures, *Pharmacol Biochem Behav*, 77, 227-34.
16. *Stanojlović OP, Živanović DP, Mirković SD, Mikhaleva II*, 2005, Antiepileptic activity of delta sleep inducing peptide and its analogue in metaphit-provoked seizures. *Seizure* 14, 240-47.
17. *Sun M, van Rijin CM, Xi Liu Y, Zheng Wang M*, 2002, Combination of carbamazepine and valproate in different dose proportions in maximal electroshock seizure model in mice, *Epilepsy Res,* 51, 5-12.
18. *Šušić V, Reith MEA, Zloković B, Lajtha A, Jacobson AE, Rice KC, et al.* 1991, Electroencephalographic characteristics of audiogenic seizures induced in metaphit-treated small rodents, *Epilepsia*, 32, 783-89
19. *Šušić V, Marković O,* 1993, Potentiation of metaphit-induced audiogenic seizures by REM sleep deprivation in rats, *Physiol Behav,* 53, 1013-20.
20. *Van Rijn CM, Sun MS, Deckers CLP, Edelbroek PM, Keyser A, Renier W*, et al. 2004, Effects of combination of valproate and ethosuximide on spike wave discharges in WAG/Rij rats. *Epilepsy Res* 59, 181–9.
21. *VanErpi MG, VanDongen AMJ, Van den Berg RJ,* 1990, Voltage-dependent action of valproate on potassium channels in frog node of Ranvier. *Eur J Pharmacol* 184, 151-61.

DA LI JE VALPROAT ANTIKONVULZANT ILI ANTIEPILEPTIK U METAFITOM INDUKOVANOJ EPILEPSIJI PACOVA?

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SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja uticaja valproata (VPA) na metafitom (1-[1-(3-isothiocyanatophenyl)-cyclohexyl]-piperidine) izazvanu epilepsiju kod pacova. Za ovu svrhu su korišćeni adultni mužjaci Wistar pacova i proučavano je njihovo ponašanje, elektrokortikalna aktivnost kao i spektralna snaga.

Intenzitet metafitom (10 mg/kg) izazvane epilepsije se povećava tokom vremena i dostiže maksimum 7-12 h posle injekcije. VPA (100 mg/kg) je i.p. aplikovan životinjama, koje su ispoljavale maksimalan odgovor posle 8 audiogenih testiranja. Pacovi su bili podeljeni u četiri grupe koje su primile (i.p.): 1. fiziološki rastvor; 2. metafit; 3. metafit + VPA i 4. VPA. Sve životinje su izlagane audiogenoj stimulaciji 60 min posle administracije metafita i dalje u intervalima od po jednog sata. Metafit kod životinja izaziva generalizovanu konvulziju i elektrokortikalne abnormalnosti (šiljkovi visoke voltaže i šiljak - talas kompleksi), tako da je analiza spektralne snage ukazala na povećanu voltažu tokom napada. Klasični antiepileptički lek, valproat u ovoj dozi prolongira trajanje latentnog perioda, smanjuje incidencu i srednju jačinu napada, ali nema uticaja na električne abnormalnosti EEG-a indukovane metafitom.

Ovi rezultati sugerišu da je VPA (100 mg/kg) u metafitskom modelu epilepsije efikasniji antikonvulzant nego antiepileptik.