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ANTIEPILEPTIC EFFECT OF VALPROIC
ACID - A FRACTAL ANALYSIS

ANTIEPILEPTIČNI EFEKAT VALPROIČNE
KISELINE – FRAKTALNA ANALIZA

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Ključne reči

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dimenzija

Abstract

The aim of this study was to evaluate effect of valproate in animal model of epilepsy. We used fractal dimension to compare state of seizures with control state. Epileptic activity shows increased fractal dimension and antiepileptic effect of valproate could be described as decrease of fractal dimension. However results show that valproate stabilize seizures but not lead to control state and has no effect on primary excitation. There is experimental evidence that valproate increases turnover of gamma-aminobutyric acid (GABA) and thereby potentiates GABA-ergic function in some specific brain regions thought to be involved in control of seizure generation and propagation. Furthermore the effect of valproate on neuronal excitation mediated by the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors might be important for its anticonvulsant effects.

INTRODUCTION

Advances in the understanding of antiepileptic drug mechanisms of action have revealed two main patterns: increasing inhibition either through GABA or glycine, or decreasing excitation due to glutamate. Our ability to improve the treatment of epilepsy requires greater knowledge of the molecular mechanisms of epilepsy. Most of discussion has been focused on the use of drugs as symptomatic treatment. It also may be possible to improve treatment by focusing on regional effects of drug or drug delivery. The currently available drugs have broad side effects such as cognitive impairment, tremors, teratogenicity. To develop more region-specific and more efficacious drugs we need to better understand mechanisms of local central nervous system function. Molecular biological techniques have increased our knowledge of receptors and transporters immensely. Antiepileptic drugs developed before 1980 appear to act on sodium channels, gamma-aminobutyric acid type A or calcium channels (1).

A fractal is a never-ending pattern. Fractals are infinitely complex patterns that are self-similar across different scales. They are created by repeating a simple process over and

over in an ongoing feedback loop. Fractal dimensions have been proposed as a useful measure for the characterization of electrophysiological time series. Higher fractal values point to more complex phenomenon.

Valproate is one of the most widely used in the treatment of both generalized and partial seizures in adults and children. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system. Valproic acid was first synthesized in 1882 by B.S. Burton as an analogue of valeric acid, found naturally in valerian (2). Valproic acid is a carboxylic acid, a clear liquid at room temperature. For many decades, its only use was in laboratories as a „metabolically inert” solvent for organic compounds. In 1962, the French researcher Pierre Eymard serendipitously discovered the anticonvulsant properties of valproic acid while using it as a vehicle for a number of other compounds that were being screened for antiseizure activity. He found it prevented pentylenetetrazol-induced convulsions in laboratory rats (3). It was approved as an antiepileptic drug in 1967 in France (4). Valproic acid is efficient in antiepileptic treatments of epilepsy, in addition to the type of absans, „grand mal”, and

complex-partial seizures are applied in the treatment of migraine and bipolar disorders (5). There is now ample experimental evidence that valproate increases turnover of GABA and thereby potentiates function in some specific brain regions involved in the control of seizure generation and propagation (6). Also effect of valproate on neural excitation mediated by NMDA subtype of glutamate receptors is anticonvulsant. Valproate increases GABA synthesis and release in specific brain regions, such as substantia nigra, attenuate neuronal excitation induced by NMDA type glutamate receptors and alters dopaminergic and serotonergic function (7). Experimental observation in this article describes number of different levels about the mechanisms of action of valproate.

MATERIALS AND METHODS

Experiments were performed in accordance with standards established by the Ethics Committee IBISS times. We used male Wistar rats under laboratory conditions (aged 2-2.5 months, reared and unlimited access to food and water, 22°C). The rats were intraperitoneally treated with a solution of camphor oil. ECoG registration is done under Nembutal anesthesia (Napentobarbital, SERVA, Heidelberg, Nemačka), 45 mg/kg or Zoletil (Virbac S., A. Carros, Francuska), 60 mg/kg. We registered the activity of parietal cortex and the cerebellar cortex. The signals were monitored on an oscilloscope (Textronix, USA). Signal amplification was performed using amplifier Multi-Channel Processor Plus (Alpha Omega Engineering, Israel). Signal filtering was performed with the parameters: DC for highpass filter and 150 Hz for the low pass filter. Analog to digital conversion is performed with a sampling frequency of 256 Hz. Spectral analysis of ECoG activity was performed Fourier transformation for 120s (divided into 15 epochs of life 8s). Fractal dimension is calculated by Higutchi algorithm (5).

The paper observed activity of the group of rats which has epileptic activity. C7 activity of the rats treated with an 450 µl/kg and 525 µl/kg the essential oil is taken as a reference. At these doses it exhibits epileptic activity. Considered the control group of the same strain of rats treated with lower doses of camphor, and in which there has not been epilepsy, but the changes have occurred in the spontaneous activity. The control value of rat and C7 activity was comparable with the changes which are obtained by treatment with valproate (50-100 mg/kg) and can be represented as a reduction of fractal dimension.

RESULTS

Our results show that seizures have two different states. First state is described as initialization and disruption of ion homeostasis. Second state is correlated with epileptic activity. These two states are presented in figure 1. Toxic effects of camphor oil can lead to epileptic activity. Increase of fractal dimension could be used as a measure of change in ion

homeostasis. We can say that we have two patho-physiological states in epilepsy (Fig. 1).

Fractal dimension in animal with epileptic seizures is increased compare to control (Fig. 1). Valproate effect is decrease fractal dimension and stabilization of seizures (Fig. 2).

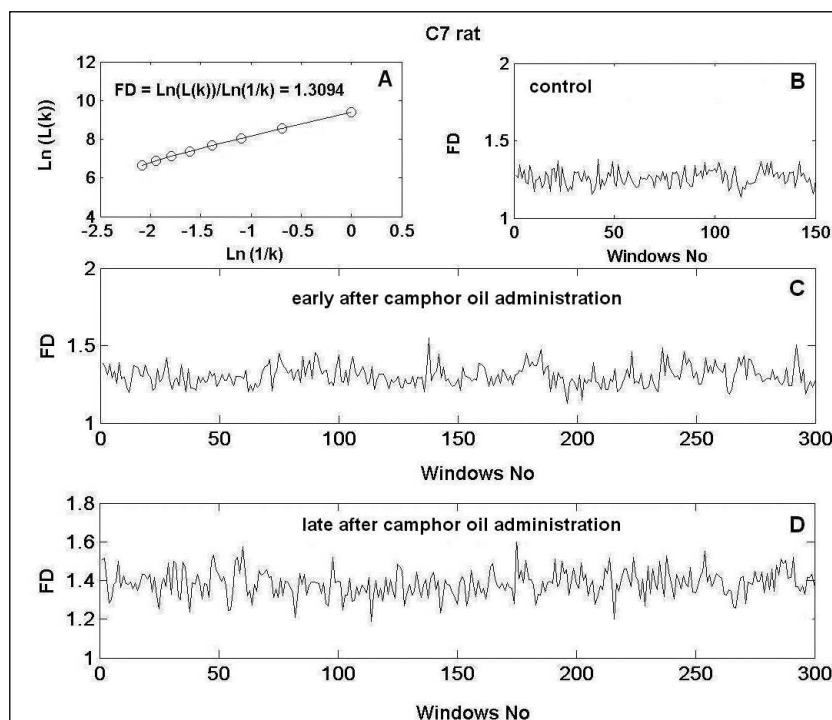


Figure 1. Fractal dimension in control animal and in animal treated with camphor with seizures.

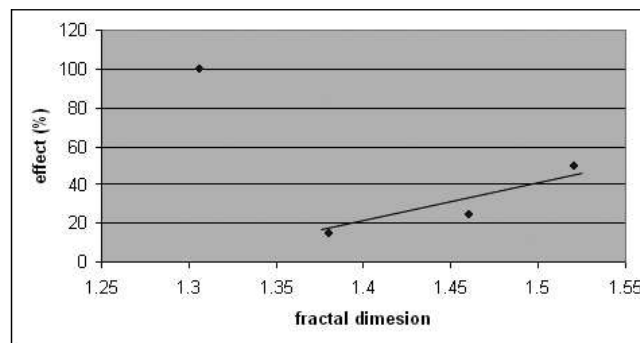


Figure 2. Effect of valproate on fractal dimension.

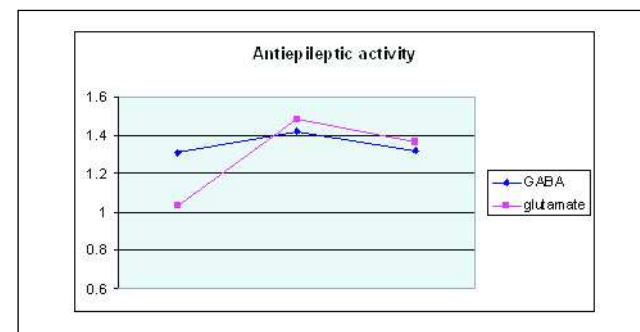


Figure 3. Fractal dimension of GABA and glutamate activity (effect of valproate).

However this effect is partial and do not lead to control value. Maximal effect is 50% and it is dose dependent. Figure 2 shows that effect of valproate is decrease of fractal

dimension. However, this decrease does not lead to homeostasis but stabilized the neuronal activity on different state compare to control. Figure 3 shows correlation between valproate effects and glutamatergic transmission as a change in fractal dimension. We can see stabilization on fractal dimension achieved by GABA inhibition and decrease in glutamatergic basic activity.

DISCUSSION

Mechanisms of action of valproate

Epileptiform discharges and behavioral seizures may be the consequences of the presence of either excessive excitation associated with the neurotransmitter glutamate or from inadequate inhibitory effects associated with gamma-aminobutyric acid (GABA). Valproate may have unique mechanisms of action; specifically, it may affect the removal of glutamate by up regulating GLAST and decreasing GABA transport, which could result in increased tissue concentrations of GABA (8). Valproic acid exerts its effects on ion channels. The effect of VPA on a voltage -dependent channels is reflected in reduced neuronal excitability, acting on Na⁺ and K⁺ channels. It has been shown that VPA acts on a voltage -dependent channels by inhibiting their activity and inhibition of their expression. The function of GABA -aminobutyric acid is realized by binding to its ionotropic receptor ligand binding Cl⁻ channel. Of chloride channel of GABA-A receptors in the brain, activates the valproic acid, and the addition of GABA activated K⁺ conductance in the CNS. Activation of the GABA-A receptor is the stimulation of Na⁺ - K⁺ - 2Cl⁻ cotransporter in the nerve cells, which leads to loss of intracellular Cl⁻ or leads to "up - regulation" KCCl cotransporter, which is important for maintaining low intracellular levels of chlorine (9).

The mechanism of action of valproic acid in the CNS is achieved by:

1. Blocking high-frequency repetitive neuronal currents, blockade of voltage-dependent Na⁺ channel;
2. Increases the activity of glutamic acid decarboxylase and GABA synthetic enzyme;
3. Blocking at high concentrations of GABA transaminase;
4. Blocking Ca²⁺ channel type T;
5. Increasing the concentration of neuropeptide Y in the nucleus reticularis thalami and hippocampus, which plays the role of an endogenous anticonvulsant;
6. Protect against a seizure-induced reduction in phosphatidylinositol (3,4,5)-trisphosphate (PIP3).

Besides effect on GABA valproate also inhibits the TCA cycle and the alpha-ketoglutarate dehydrogenase step which might be responsible for toxic effects of valproate (10). Effect VPA on excitatory neurotransmission have secondary effect on mood-stabilizing and as well as in the treatment of migraine. GABA mediated responses also may be involved in neuropathic pain. (11).

Effects of valproate

Valproate treatment is effective on stabilization of seizures activity (12, 13). The relationship between total dose of valproate concentration is nonlinear, not increase with

increasing dose due saturability of protein. The kinetics of the drug is linear unbound. Valproate bioavailability is over 90%.

However his efficiency is max 50% so it is suitable for all types of seizures. Stabilization of neural activity on state of ion homeostasis has increased fractal dimension compare to control conditions. We might conclude that full recovery might be achieved with time on different drugs. State of epileptic activity has two manifestations: fluctuation and seizures, and valproate dose should be different for this two states and its fluctuate and have linearly effect compare to fractal dimension. However effect of valproate is stable in different neuropsychiatric disorders (14, 15).

Valproate has antiepileptic effect is based on the reduction glutamatergic transmission and stimulation GABA inhibitory effects. It can be said (see Figure 3) that the effect of antiepileptic valproate based on glutamatergic transmission while reducing the inhibition of GABA aims stabilization of excitation and ion homeostasis in individual brain regions. Most of the anticonvulsant activities of the Na⁺ -channel causing the active-dependent blocking effect, that is, the blocking effect is enhanced when the neurons depolarized repetitively at high frequencies. Van Dongen and coworkers on the basis of the results of the experiment with the valproic acid came to the conclusion that the lower concentrations of valproate acts directly on the membrane of the nerve cell and the conductivity of the Na⁺ and K⁺(16). As it is considered that it is basically in the pathogenesis of migraine hiperexcitability condition, despite the cortical spreading depression, application of valproic acid, to normalize inhibit neuronal discharge high frequency channel.

CONCLUSION

We might conclude that valproate efficiency in state of seizures is convenient in all types of excitation. Also valproate stabilized ion homeostasis and neurotransmission in brain regions with excitatory activity. However, he work as stabilization system on excitatory level but with change neurotransmission compared to control state. Valproate is highly efficient antiepileptic but not can be used as a preventive drug.

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Sažetak

Cilj ove studije je bio da se proceni efekat valproata u animalnom modelu epilepsije. Koristili smo fraktalnu dimenziju za poredjenje stanja ekscitacije sa kontrolnim stanjem. Epileptična aktivnost pokazuje povećanje fraktalne dimenzije a antiepileptični efekat valporata može se opisati smanjenjem fraktalne dimenzije. Rezultati ukazuju da valproat stabilizuje pražnjenja ali ne dovodi sistem u stanje kontrole i ne utiče na primarnu ekscitaciju. Eksperimentalni podaci ukazuju da valproat povećava promet GABA i time stimuliše gaba-ergičnu aktivnost u pojedinim moždanim regionima koji su uključeni u regulaciju pražnjenja i ekscitatorne propagacije. Osim toga valproat deluje na neurotransmisiju glutamata preko NMDA receptora što ima antikonvulzivno dejstvo.

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