Kynurenic Acid Inhibition of an Experimental Epileptogenic Focus in the Rat Hippocampus

I. B. Mikhailov, V. I. Guzeva, and N. V. Mel'nikova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 119, № 5, pp. 521-522, May, 1995
Original article submitted June 16, 1994

Kynurenic acid introduced in an epileptogenic focus through an electrochemotrode before the creation of the focus was found to reduce the intensity of epileptiform discharges between seizures and of electrographic correlates of seizures on the electroencephalogram. Administration of kynurenic acid led to the appearance of a slow Θ-rhythm dominating in all the leads.

Key Words: *epilepsy; hippocampus; kynurenines*

Parenteral administration of L-kynurenine to rats is known to appreciably increase the concentration of kynurenic acid (KA) [7] in the striate body and brain cortex [8,9]. KA has a highly affinity to the glycine site of the N-methyl-D-asparagine (NMDA) receptor [6,7] and is its blocker. Some authors [9] have hypothesized that the anticonvulsive effect of kynurenine may be due to its transformation into KA and to blocking of the NMDA receptor. Hence, the aim of this study was to investigate the effects of KA on the activity of an epileptogenic focus in the rat hippocampus, that is, on the function of neurons with pathologically increased excitability.

MATERIALS AND METHODS

Nineteen outbred male rats weighing 120 to 170 g were used in the experiments. The animals were narcotized with Nembutal (50 mg/kg intraperitoneally) before the operation.

The electrode and electrochemotrode were embedded in the hippocampus to a depth of 3.2 mm according to the coordinates in the rat brain atlas [5]. Hippocampal biopotentials were recorded using a 16-channel EEGU-16-0.2 electroencephalograph with visual monitoring by means of an oscillograph attached to the electroencephalograph outlet. The first electroencephalogram (EEG) was recorded on days 6-7 after surgery, and the subsequent experiments (II-VI) with this rat were carried out at 3-day intervals.

Mono- and bipolar leads of bioelectrical activity from the left hippocampus were drawn through an electrochemotrode. This consisted of a needle cannula 0.4 mm in diameter and Nichrome wire electrode 0.18 mm in diameter glued together and insulated with polymethyl methacrylate. The cannula was used to deliver penicillin and KA to the brain. A bipolar electrode embedded in the right hippocampus was supplied with 0.18 mm Nichrome wire instead of a needle cannula. Cortical electrodes consisted of 0.1 mm Nichrome wire. The indifferent electrode was 0.25 mm Nichrome wire fixed to bone crests rimming the upper margin of the orbit.

An epileptogenic focus in the rat left hippocampus was created by introducing 1 μl penicillin sodium solution (100 U) through an electrochemotrode using a micromanipulator. This dose of penicillin was the minimal for induction of a focus in all experiments.

The EEG was recorded for 120 min starting from the moment of penicillin infusion in order to count the solitary epileptiform discharges between seizures per min during each 10-min interval of the experiment and the mean number of electrographic correlates of seizures (continuous long "convulsive" discharge on the EEG) during

Research Center, St. Petersburg Pediatric Medical Institute. (Presented by S. N. Golikov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0005-0504$12.50

Table 1. Changes in Activity of Epileptogenic Focus in Rat Hippocampus under the Effect of KA

Substance injected	Number of experiments	Intensity[1] of	
		epileptiform discharges between seizures	electrographic correlates of seizures
Distilled water (control)	16	90.5*	221.9*
KA, μg:			
1	11	26.8*	66.2*
5	10	10.4*	65.8*
10	12	2.3*	5.6*
20	6	0	0

Note. [1]The intensity of pathological activity was assessed during the second–sixth creation of the focus in the same site of the hippocampus. Here and in Table 2: asterisk shows reliable differences ($p<0.01$) in comparison with the control.

a 10-min interval of the experiment. In addition, the latency was assessed, that is, the time elapsing from the moment of penicillin delivery to the hippocampus and the appearance of the first interseizure epileptiform discharge.

The required amount of dry KA was dissolved in one drop of 1 N NaOH followed by titration of 1 N HCl to attain pH 7.6 and addition of normal saline to achieve the required volume.

KA was delivered to the site of creation of the epileptogenic focus 5 min before penicillin injection in the hippocampus. KA was injected in doses of 1, 5, 10, and 20 μg per μl. Control rats were injected with the same amounts of distilled water in which penicillin sodium was dissolved.

The results were statistically processed using Mann-Whitney's *U* test [2].

RESULTS

The data presented in Table 1 indicate that preinjection of KA in the penicillin epileptogenic focus reduced the intensity of epileptiform discharges between seizures and of the electrographic correlates of seizures on the EEG. It is noteworthy that injection of KA in all the tested doses before penicillin injection caused the appearance of a slow Θ-rhythm on the EEG, which predominated in all the leads, as sometimes occurs in therapy with antiepileptic drugs [1].

The latency of the first epileptiform discharge between seizures was in direct proportion to KA dosage build-up and always higher than in the control (Table 2). Evidently, by blocking the NMDA receptors, KA reduced the rate of penicillin-induced epileptization of the neurons.

Hence, we found proof of a direct suppressing effect of KA, a tryptophan metabolite of the kynurenine pathway, on the excitability of central nervous system neurons.

Previously [4] we demonstrated on the same model that kynurenines alone (for example, anthranilic acid) do not influence the excitability of neurons. Serotonin inhibits the pathological activity of an epileptogenic focus. We proposed [3] allopurinol (a tryptophan pyrrolase inhibitor) as a means of shifting the equilibrium of the intermediate metabolism of tryptophan toward the serotonin pathway at the expense of inhibiting the kynurenine pathway. The findings indicate, however, that the mechanism of this effect is more intricate. It seems that not only the absolute number of kynurenines, but their ratio is of importance. That is why in the search for new approaches to the treatment of epilepsy one should by no means disregard this aspect, that is, the balance between different kynurenines, endogenous convulsants, and anticonvulsants.

Table 2. Latency of First Epileptiform Discharge between Seizures after KA Injection

Substance injected	Latency, min
Distilled water (control)	1
KA, μg:	
1	8*
5	15*
10	52*

REFERENCES

1. R. G. Biniaurishvili, A. M. Vein, B. G. Gafurov, and A. R. Rakhimdzhanov, in: *Epilepsy and Functional States of the Brain* [in Russian], Tashkent (1985), p. 211.
2. E. V. Gubler, in: *Computation Methods of Analysis and Recognition of Pathological Processes* [in Russian], Leningrad (1978), pp. 72-75.
3. V. I. Guzeva, V. A. Gusel', and I. B. Mikhailov, *Zh. Nevropatol. Psikhiatr.*, № 6, 69 (1988).
4. I. B. Mikhailov, *Farmakol. Toksikol.*, № 2, 143 (1981).
5. W. Fifkov and J. Marshall, in: *Electrophysiological Methods in Biological Research* (eds. J. Bures *et al.*), Acad. Press (1961).
6. I. P. Lapin, in: *Quinolinic Acid and the Kynurenines* (ed. T. W. Stone), Boca Raton (1989), pp. 192-211.
7. M. G. Palfreyman and B. M. Baron, in: *Excitatory Amino Acid Antagonists* (ed. B. S. Meldrum), Oxford (1991), pp. 101-129.
8. J. Swartz, J. M. During, A. Freese, and M. F. Beal, *J. Neurosci.*, **10**, № 9, 2965-2973 (1990).
9. L. Vecsei, J. Miller, U. M. Garvey, and M. F. Beal, *Res. Bull.*, **28**, 233 (1992).