Possible Involvement of Nitric Oxide in the Pathogenesis of Experimental Convulsions of Various Genesis

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The content of nitric oxide in convulsions of different genesis is assessed by measuring the formation of paramagnetic mononitrosyl iron complexes with diethylthiocarbamate by electron paramagnetic resonance. A 3- to 4-fold increase in the content of these complexes is found in the brain of rats with thiosemicarbazide- or N-methyl-DL-aspartate-induced seizures in comparison with control animals. A similar increase in the brain NO content was observed in maximum electrical stimulation of metrazol-induced convulsions. These changes were accompanied by elevation of secondary lipid peroxidation products.

Key Words: *thiosemicarbazide;* N-methyl-DL-aspartate*; metrazol; nitric oxide; lipid peroxidation*

The role of nitric oxide (NO) in various processes in the central nervous system such as synaptic transmission, plasticity and memory, and in some pathological states, for instance, glutamate neurotoxicity [15] and convulsions [11] has been generally recognized. There are contradictory data on the role of NO in pathophysiological mechanisms of convulsions: some authors reported an anticonvulsive effect of NO [8,14], while others consider it as a proconvulsant [7,10]. We previously showed an increased NO content in rat brain during convulsions induced by maximum electrical stimulation [2,5] and administration of metrazol [1]. It was interesting to study this phenomena in convulsions of different genesis.

The aim of the present study was to evaluate the role of NO in pathophysiological mechanisms of seizures induced by deficiency of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) or hyperactivation of the N-methyl-D-aspartate (NMDA) subset of glutamate receptors. In parallel, the intensity of lipid peroxidation was assessed.

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MATERIALS AND METHODS

Experiments were carried out on 55 male Wistar rats weighing 180-240 g The animals were maintained under standard vivarium conditions. The experiments were performed in the morning. Seizures were modeled by subcutaneous injection of 30 mg/kg thiosemicarbazide (glutamate decarboxylase inhibitor, Sigma) or by bilateral intracerebroventricular injection of the glutamate receptor antagonist N-methyl-DL-aspartate (NMDLA, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, 28.8 μg in 5 μl physiological saline). The effects were compared with those induced by maximum electrical stimulation (150 mA current, 0.2 sec pulse duration) and metrazol (120 mg/kg, subcutaneously).

The content of NO in the brain was determined using electron paramagnetic resonance, the method

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based on the interaction between NO and Fe^{2+}-diethyldithiocarbamate (Fe^{2+}-DETC) yielding paramagnetic mononitrosyl iron complexes (MNIC) with DETC. These complexes are characterized by an EPR signal with the g-factor being $g_{\perp}$=2.035 and $g_{||}$=2.012 and triplet hyperfine structure at g. Quantitative NO assay has been described in detail [2,3]. Typical spectra of MNIC-DETC complexes are presented on Fig. 1.

The animals were simultaneously intraperitoneally injected with 500 mg/kg Na-DETC and subcutaneously with 37.5 mg/kg $FeSO_4$ and 165 mg/kg sodium citrate, and decapitated 30 min postinjection. The brain cortex was isolated, and the samples were frozen in liquid nitrogen. The content of secondary lipid peroxidation products (thiobarbituric acid-reactive substances, TBARS) in the frontal cortex was measured as described previously [13].

The data were processed statistically using the Student *t* test.

RESULTS

Thiosemicarbazide induced typical repeated clonich seizures, which can be attributed to the deficiency of the inhibitory neutrontansmitter GABA due to blockade of its synthesis. The seizures appeared 80-90 min postinjection and were identical to those described previously [4]. Clonic seizures were also noted after intracerebroventricular injection of NMDLA. The dynamics of NO in the brain cortex in convulsions induced by thiosemicarbazide and NMDLA, as well as of those induced by maximum electrical stimulation and metrazol is shown in Fig. 2. As seen from the figure, control samples exhibited a weak MNIC-DETC signal corresponding to 1.5 nmol/g wet tissue/30 min. A considerably increased content of NO in the brain was observed at the peak of GABA-deficient clonic convulsions. An elevated content of MNIC-DETC was also noted in clonic NMDLA-induced seizures as well as at the peak of tonic extension induced by electrical stimulation and in tonic-clonic metrazol-induced seizure. Thus, the content of NO is practically the same in clonic and tonic seizures, i.e., there is no direct correlation between the mechanism of convulsion (tonic and clonic) and the elevation of NO in the brain cortex.

As seen from Fig. 3, convulsions induced by thiosemicarbazide, NMDLA, maximum electrical stimulation, and metrazol caused a rise of TBARS in the brain, which is consistent with published data [1]. It should be noted that there was no correlation between the contents of NO and TBARS, and the level of TBARS did not depend on the type of convulsions.

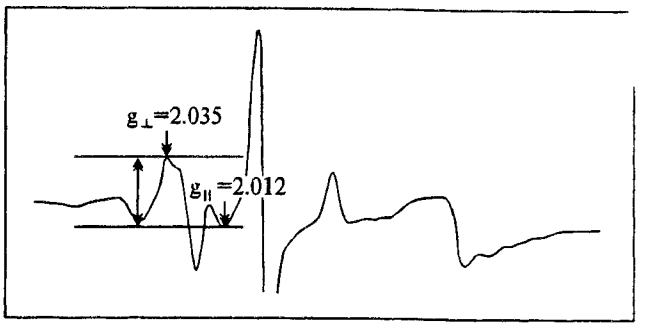

Fig. 1. Typical electron paramagnetic resonance spectrum of mononitrosyl iron complexes with diethyldithiocarbamate.

Our findings suggest that convulsions of various genesis are accompanied by a considerable increase in the content of MNIC-DETC reflecting an increased concentration of NO in the brain cortex. These findings are consistent with the previous observation that NO content increases in the brain in seizures induced by kainate, a non-NMDA glutamate receptor agonist. These is no consensus on the role of NO in the pathogenesis of convulsive disorders. Some researches regard it as an endogenous neuroprotector [6] or anticonvulsant [8,14], which was confirmed by experiments, where inhibition of NO-synthase attained by a 4-day treatment with Nω-nitro-L-arginine potentiates seizures induced by kainate, pilocarpine, or NMDA, i.e., convulsants with different mechanisms of action [8,12]. Previously we reported that Nω-nitro-L-arginine, an inhibitor of NO-synthase, reduces the latency of the early component of metrazol-induced seizure, so-called first start, which probably is a behavioral equivalent of the

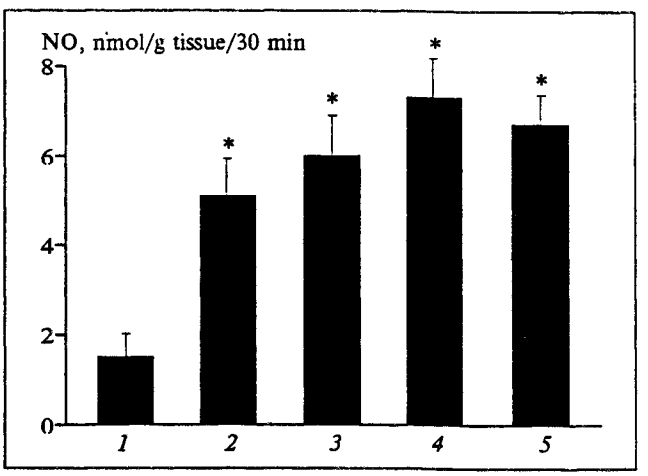

Fig. 2. NO production in rat brain cortex in experimental seizures of different genesis.

*p<0.001 compared with the control. Here and in Fig. 3: *1*) control; *2*) maximum electricl stimulation; *3*) thiosemicarbazide; *4*) metrazol; *5*) NMDLA. Control group comprised 15 rats, each experimental group consisted of 10 animals.

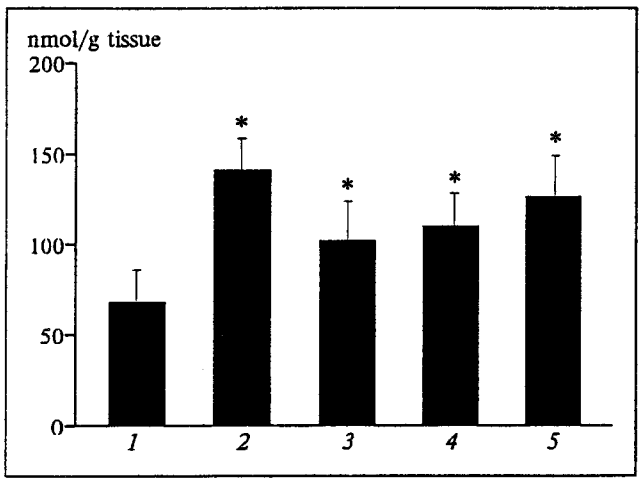

Fig. 3. Content of TBARS in rat brain cortex in seizures of different genesis. *$p<0.05$ compared with the control.

first convulsive discharge in the brain cortex [1]. The inhibitor injected in a low dose (10 mg/kg) considerably reduced the latency of this response, while the development of other seizure components was delayed. These data agree with the concept on retrograde modulation of NMDA receptors involved in seizure generation by NO [9]. It was concluded that NO regulates initiation of convulsive activity. By contrast, other investigators suggest that NO is a proconvulsant exhibiting a neurotoxic effect [7,10]. It is known that generation of NO leads to hyperproduction of free radicals which is accompanied by potentiation of the neurotoxic effect [11]. In our experiments, NO-synthase inhibitor had no effect on activation of lipid peroxidation during seizures suggesting that this effect is not directly related to NO generation.

Our experiments and the data of other scientests who used electron paramagnetic resonance for direct measurements of the NO content in the brain [12] show that generation of NO increases 4- to 6-fold during seizures regardless their genesis. It can be hypothesized that activation of glutamate receptors is an initiating step for consequent events including the neurotoxic effect. Being a retrograde transmitter, NO can exert, at least at the first stage, inhibiting effect apparently via modulation of NMDA receptor activity.

Our findings and published data indicate the involvement of NO in pathophysiological mechanisms of epileptiform reaction are consistent with the concept on the trigger role of NO in convulsive disorders.

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