Role of Nitric Oxide and Lipid Peroxidation in Mechanisms of Febrile Convulsions in Wistar Rat Pups

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Generation of nitric oxide and the content of lipid peroxidation products in the brain are increased in rat pups during febrile convulsions. NO-synthase inhibitor N-nitro-L-arginine in a dose of 250 mg/kg prevented hyperthermia-induced accumulation of nitric oxide, increased the latency febrile convulsions, and had no effect on the content of lipid peroxidation products.

Key Words: *febrile convulsions; nitric oxide; N-nitro-L-arginine; lipid peroxidation; EPR spectroscopy*

Febrile convulsions (FC) during sharp rise of body temperature are observed in 2-13% infants and considerably increase the risk of epilepsy in adults. In children, FC can be provoked by any disease associated with fever of 39-41°C [4]. It is generally accepted that not only long-term hyperthermia, but also increased convulsive activity of the brain (including genetically determined) promote the development of FC. The most adequate experimental model of neonatal febrile convulsions are convulsions induced by hyperthermia in newborn animals, e.g. rats. In rats, sensitivity to hyperthermia is maximum on day 5 of life and then gradually decreases and disappears completely by day 20 of postnatal ontogeny [6]. Neurochemical mechanisms of FC are still poorly understood. Deficiency of GABA-ergic transmission due to inhibition of glutamate decarboxylase [4,12] is investigated as a possible mechanisms of FC. However the role of glutamate, a stimulatory neurotransmitter, in febrile convulsions is little studied.

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The role of nitric oxide (NO) in the development of neurotoxic effect of glutamate has recently attracted special attention [2,8]. NO is a gaseous chemical messenger [8,14] playing a role of a pathogenetic factor in neurodegenerative diseases of the central nervous system, ischemia, brain stroke, and seizures [2, 9]. We demonstrated that the content of NO and secondary lipid peroxidation (LPO) products in the cerebral cortex increases in adult rats with experimental seizures [2,9]. Here we studied possible involvement of NO and LPO products in the initiation and development of experimental FC in rat pups.

MATERIALS AND METHODS

Experiments were performed on 23 Wistar rat pups aged 9-10 days. The day of birth was defined as day 0. Controls (n=5) were injected with 0.9% NaCl without subsequent hyperthermia. Experimental rat pups (n=18) were exposed to hyperthermia, and half of them were intraperitoneally injected with aqueous suspension of N-nitro-L-arginine (L-NNA, 250 mg/kg) with Twin-80 and others received 0.9% NaCl 60 min before induction of convulsions. For FC modeling, the rats were put into an open box, the temperature at the bottom of the box were maintained at 38-39°C with a 200 W

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lamp. The duration of hyperthermia did not exceed 15 min. The intensity and latency of seizures were scored [5] every 10 sec: 0) normal behavior and posture; 1) paddle forepaw movements and tail rigidity; 2) solitary opisthotonic spasm; 3) sporadic myoclonic convulsions; 4) forepaw clonus, extension of hind paws (kangaroo-like posture); 5) loss of equilibrium and falling on one's side. Warming was stopped after appearance of clonic-tonic convulsions with loss of posture. The animals were decapitated, the brain was isolated and frozen in liquid nitrogen.

The content of NO in the brain was measured by electron paramagnetic resonance (EPR) using diethyldithiocarbamate (DETC) as the free radical trap. DETC interacts with endogenous NO and bivalent iron ions forming paramagnetic mononitrosil iron complexes (MNIC—DETC) recorded by EPR. These complexes are characterized by an EPR signal with $g_{\perp}$=2.035 and $g_{||}$=2.012 and a triplet superfine structure at $g_{\perp}$. The method of NO measurement was described in detail [13]. The rats were simultaneously injected with Na-DETC (500 mg/kg intraperitoneally) and a mixture of $FeSO_4$ (37.5 mg/kg) with sodium citrate (165 mg/kg subcutaneously). LPO intensity was evaluated spectrophotometrically by the content of thiobarbituric acid-reactive products [10].

The data were statistically processed using Fisher and Mann—Whitney nonparametric tests and presented as $M \pm m$.

RESULTS

The first signs of FC (paddle movements of the forepaws and tail rigidity) in rat pups treated and untreated with L-NNA were noted on the third minute of warming (158.0±24.0 and 160.0±36.4 sec, respectively). By the end of the 5th min, sporadic myoclonic convulsions (3 points) were observed in both subgroups. The latency of convulsions was virtually the same in two groups. In rat pups treated with L-NNA the latency of generalized seizures was longer than in animals receiving no NO-synthase inhibitor (p<0.05, Fig. 1). Maximum tonic-clonic seizures (5 points) were seen in 67 and 78% animals treated and not treated with L-NNA, respectively.

A weak MNIC—DETC signal, corresponding to 1.3 nmol/g wet tissue/30 min was recorded in brain samples from control animals (Fig. 2, *a*). The content of NO appreciably increased at the peak of hyperthermia-caused seizures and attained 2.1 nmol/g wet tissue, which considerably surpassed the control. Generation of NO notably decreased after injection of 250 mg/kg L-NNA 1 h before hyperthermia.

An essential increase in brain content of secondary LPO products was observed at the peak of FC

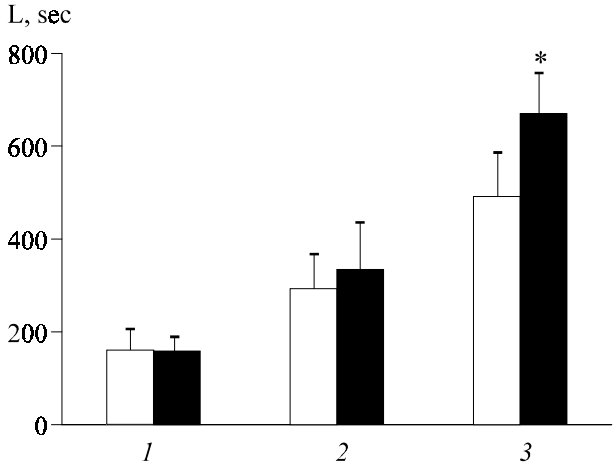

Fig. 1. Effect of NO-synthase inhibitor L-NNA on the latency (L) of hyperthermia-induced convulsions in rat pups. *1, 2, 3*) stages 1, 3, and 5 of convulsions, respectively. **p*<0.05 *vs.* untreated rats. Dark bars: hyperthermia-induced convulsions after injection of L-NNA, light bars: without L-NNA.

(Fig. 2, *b*). Preinjection of L-NNA did not prevent accumulation of TBA-reactive products observed at the peak of hyperthermia-induced seizures.

Published data on the role of NO in the pathogenesis of seizures are contradictory: both the anti- [11] and proconvulsant effect of NO were reported [7]. We detected an increase in the threshold convulsive excitability after injection of L-NNA. The development of FC is paralleled by enhanced production of NO, which is in line with the data of other authors on the involvement of NO in the mechanisms of convulsions caused by various chemical convulsants [1,2,9]. Preinjection with nonselective NO synthase inhibitor L-NNA decreased NO content in the brain of rat pups during convulsive attack below the control and prolonged the latency of convulsions. We assumed that the content of NO in the brain is normally maintained at a constant level, while its deviation modulates the convulsive threshold.

NO is known to play a key role in the formation of highly reactive oxygen forms possessing neurotoxic properties [14], e.g. peroxinitrite, which causes neuronal damage and death. Presumably, the neurotoxic effect of glutamate leads to accumulation of NO in the brain of rat pups during convulsions, while NO synthase inhibitor facilitates FC.

Our findings are in line with published data on the involvement of LPO in the pathogenesis of some brain diseases. In our study L-NNA, decreasing the level of NO during convulsions, did not affect the intensity of LPO. This suggests that both the neurotoxic effect of NO and activation of LPO (NO-independent processes) underlie the development of FC.

Hence, FC in 10-day-old rat pups are associated with increased generation of NO and intensification of

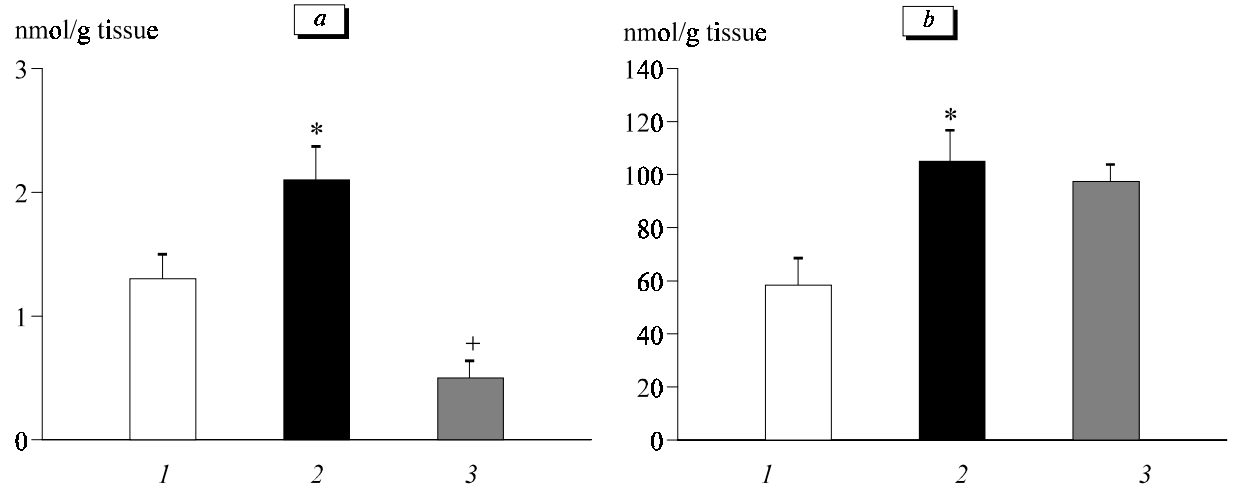

Fig. 2. Effect of hyperthermia-induced convulsions on the content of NO (*a*) and TBA-reactive products (*b*) in the brain of rat pups. *1*) control (no convulsions); *2*) convulsions without L-NNA; *3*) injection of L-NNA (250 mg/kg) 1 h before convulsions. $p<0.05$: **vs.* the control, $^{+}$*vs.* the group without L-NNA.

LPO. In our experiments L-NNA (NO synthase inhibitor) produced a neuroprotective effect by prolonging the latency of clonic tonic convulsions and preventing accumulation of NO, but not affecting LPO processes. Our findings agree with recent concept on the triggering role of NO generation in convulsive states [2].

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