GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Role of Nitric Oxide and Lipid Peroxidation in Pathophysiological Mechanisms of Audiogenic Seizures in GEP Rats and DBA/2 Mice

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We evaluated the role of nitric oxide and lipid peroxidation in the pathophysiological mechanisms of seizures in genetically epilepsy prone (GEP) rats and DBA/2 mice with genetically determined audiogenic epilepsy. In rats and mice acoustic stimulation led to locomotor activation followed by clonic-tonic seizures. The contents of nitric oxide and lipid peroxidation products at the peak of seizures markedly surpassed the control level.

Key Words: *nitric oxide; audiogenic seizures; lipid peroxidation; glutamate neurotoxicity*

According to current concepts, nitric oxide (NO), a gaseous chemical messenger and free radical, acts as a universal modulator of various physiological processes in the organism, including the central nervous system. The involvement of NO into neurotransmitter function of glutamate prompted detailed studies of the role of NO in the pathophysiological mechanisms of epilepsy and seizures. However, there are contradictory data on this problem [7,15]. We previously observed increased NO content in the brain of rats with seizures induced by maximal electroshock, corazole, thiosemicarbazide, and N-methyl-D,L-aspartate [1]. Animals with genetically determined audiogenic epilepsy are of considerable interest with respect to studying the pathophysiological mechanisms of epileptiform activity [11]. These animals are an adequate experimental model for epileptiform syndromes in humans [10]. In the present work we studied seizures induced by acoustic stimulation in genetically epilepsy prone (GEP) rats and DBA/2 mice. These animals demonstrate a stable and reproducible reaction to acoustic stimulation. The latency, type, and intensity of seizures can be estimated quantitatively [8]. However, neurochemical characteristics of the brain in animals with genetically determined audiogenic epilepsy during seizures and in seizure-free periods are little studied. Here we evaluated the role of NO and lipid peroxidation (LPO) in the pathophysiological mechanisms of audiogenic seizures induced by acoustic stimulation.

MATERIALS AND METHODS

Experiments were performed on 19 male GEP rats (200-220 g), 16 male Wistar rats (220-240 g), and 24 DBA/2 mice (7-13 g) obtained from the Mosthly Hospital colony (London). The animals were kept at constant temperature and humidity at 12-h light/dark cycle and had free access to water and food. Experiments were performed in a soundproof chamber. Acoustic signals (120 and 109 dB for rats and mice, respectively) were presented for 60 sec or until the onset

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of clonic-tonic seizures. Acoustic stimulation induced locomotor activation and clonic-tonic seizures. The severity of seizures was expressed in points: 0, no reaction; 1, locomotor activation (wild running); 2, fall on the abdomen and convulsions; 3, fall on the side and clonic seizures; 4, tonic seizures, extension of limbs, and respiratory arrest [8]. In GEP rats locomotor activation was sometimes interrupted with a 5-30-sec rest period followed by the second wave of locomotor excitation culminating in clonic seizures.

The animals were decapitated immediately after acoustic stimulation or during tonic extension, the frontal cortex (from rats) or the whole brain (from mice) was isolated and frozen in liquid nitrogen.

NO content was evaluated by the method of electron paramagnetic resonance (EPR) [4], based on detection of paramagnetic mononitrosyl iron-diethyldithiocarbamate complexes in the brain. These complexes are characterized by an EPR signal with g factors of 2.035 and 2.012 and triplet hyperfine structure at g. EPR signals were recorded on a Radiopan radiospectrometer.

The intensity of LPO was determined spectrophotometrically by the content of thiobarbituric acid-reactive substances (TBARS) [14]. The results were analyzed by Student's t test and Wilcoxon test.

RESULTS

GEP rats (n=19) displayed a pronounced seizure response to acoustic stimulation. Experiments performed at the Mosthly Hospital showed that acoustic signals induce clonic-tonic seizures in 70-75% GEP rats. Seizures did not develop in Wistar rats under similar conditions. Signs of locomotor activation were observed in only 7% Wistar rats. Acoustic stimulation was followed by locomotor activation (1 point) and tonic seizures (4 points) in 56 and 44% DBA/2 mice, respectively. These animals were divided into 2 subgroups. Subgroup 1 included DBA/2 mice characterized by phase 1 attack (locomotor activation, 1 point). DBA/2 mice with clonic-tonic seizures (4 points) and tonic extension of the hindlimbs comprised subgroup 2 (Fig. 1, *a*).

The initial NO content in the cortex was similar in Wistar and GEP rats (Fig. 2). NO generation in GEP rats markedly increased during clonic-tonic audiogenic seizures (3.5±0.4 *vs*. 1.9±0.3 nmol/g in the control). In Wistar rats subjected to acoustic stimulation and not displaying seizures NO content did not differ from the control (Fig. 2). NO concentration in DBA/2 mice with severe clonic-tonic seizures surpassed that in animals characterized by locomotor activation and in mice not subjected to acoustic stimulation (Fig. 1, *b*).

Acoustic stimulation had no effect on TBARS content in the brain cortex of Wistar rats (Fig. 3). In GEP rats with clonic-tonic seizures induced by acous-

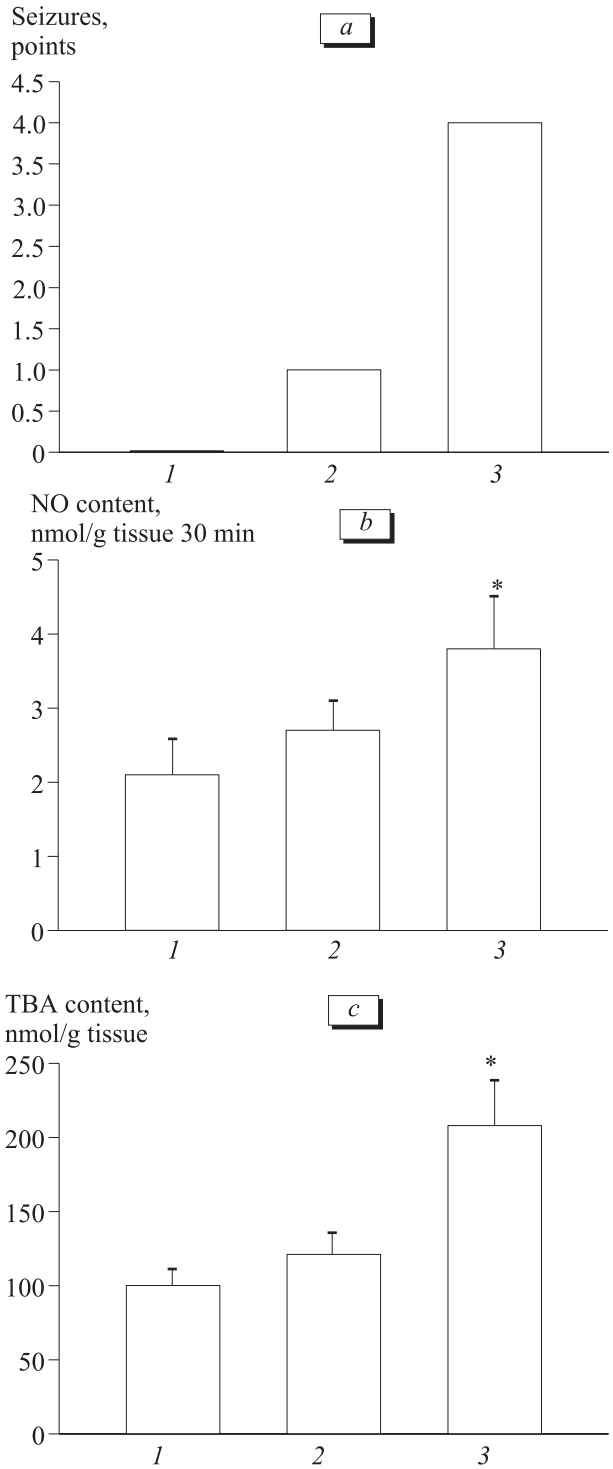

Fig. 1. Effect of acoustic stimulation on the severity of seizures (*a*) and content of NO (*b*) and LPO products (*c*) in DBA/2 mice (*M*±*SEM*). Control, no acoustic stimulation (n=10, *1*); acoustic stimulation, locomotor activation (n=8, *2*); and acoustic stimulation, clonic-tonic seizures (n=6, *3*). Here and in Figs. 2 and 3: *p<0.05 compared to the control.

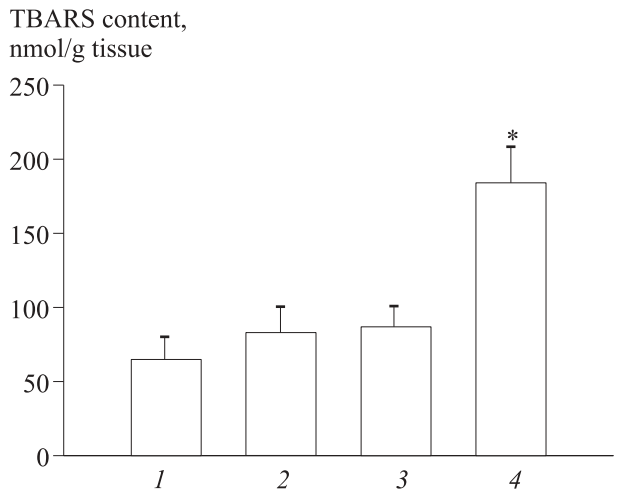

Fig. 2. Effect of acoustic stimulation on NO generation in brain cortex of Wistar and GEP rats (*M*±*SEM*). Here and in Fig. 3: Wistar, control (*n*=8, *1*); Wistar, acoustic stimulation (*n*=8, *2*); GEP, control (*n*=9, *3*); GEP, acoustic stimulation (*n*=10, *4*).

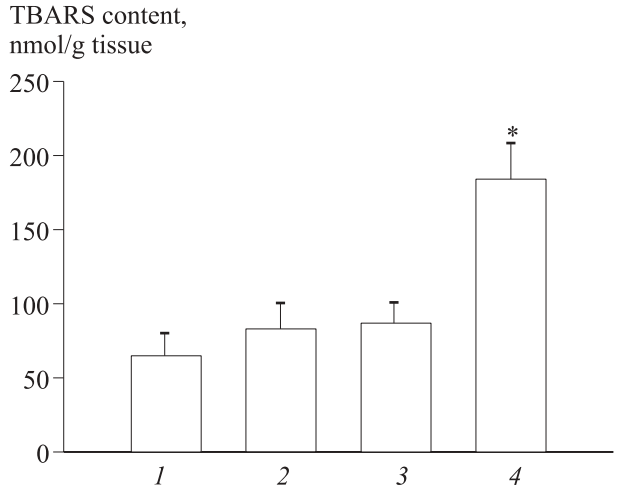

Fig. 3. Effect of acoustic stimulation on the content of LPO products in brain cortex of Wistar and GEP rats (*M*±*SEM*).

tic stimulation the concentration of LPO products surpassed the control by more than 2 times. TBARS content in the brain of mice responding to acoustic stimulation by locomotor activation did not differ from that in control animals (Fig. 1, *c*, *2*). However, the content of secondary LPO products in mice with audiogenic clonic-tonic seizures 2-fold surpassed the control (Fig. 1, *c*, *3*).

Our experiments showed that the intensity of NO generation and the content of LPO products in GEP rats and DBA/2 mice with audiogenic seizures 2-fold surpassed these parameters in intact animals not subjected to acoustic stimulation and in Wistar rats. These results are consistent with published data on convulsions induced by kainic acid [13], corazole, and maximal electroshock [1] and audiogenic seizures in DBA/2 mice [2]. It should be emphasized that the increase in NO content in DBA/2 mice with clonic-tonic seizures was much more pronounced than in animals with locomotor activation. These data illustrate the relationship between the phase of audiogenic seizures and accumulation of NO in the brain of DBA/2 mice. Enhanced NO generation in the brain probably results from activation of NO synthase. This process is mediated by constitutive NO synthase, since activation of inducible enzyme requires more time [12]. Taking into account high anticonvulsant activity of selective inhibitor 7-nitroindazole, it can be hypothesized that neuronal NO synthase plays a major role in the intensification of NO generation [9].

Published data on LPO intensity during experimental epilepsy showed that oxidative stress plays a key role in the onset and development of seizures of different etiology. The reaction between NO and reactive oxygen species yields highly toxic products (*e.g.*, peroxynitrite), which causes neuronal damage and death [3,9]. In our experiments TBARS content markedly increased in the brain of GEP rats and DBA/2 mice with seizures induced by acoustic stimulation. These changes reflect intensification of LPO. These results agree with published data on the intensity of LPO in Krushinskii—Molodkina rats with audiogenic seizures [6] and other models of experimental epilepsy [5].

Audiogenic epileptiform seizures in GEP rats and DBA/2 mice are accompanied by intensive NO generation and activation of LPO. Our results and published data [9,13] indicate that NO is involved in the pathophysiological mechanism of audiogenic seizures. This is consistent with the concept that free radical processes play an important role in seizures of different etiology.

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