Protective Effect of Carnosine on Excitable Structures of the Auditory Apparatus in Albino Rats with Acute Acoustic Trauma

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 1, pp. 112-117, January, 2004
Original article submitted April 18, 2003

We studied the effect of natural antioxidant carnosine on Wistar rats with experimental acoustic trauma of the auditory apparatus. Repeated intraperitoneal injection of carnosine in a dose of 200 mg/kg 12 and 0.5 h before modeling of acute acoustic trauma decreased the severity of degenerative and atrophic changes in the nuclei of hair cells in the cochleae. Carnosine compensated the deficiency of tissue antioxidant systems and suppressed generation of lipid peroxidation products in tissues of the membranous cochlea and auditory cortex of the temporal lobes. Carnosine holds much promise as a nonspecific otoprotector.

Key Words: *hair cells; auditory cortex; carnosine; noise trauma; otoprotection*

Acute and chronic exposure to high-level and low-level noise, respectively, produces irreversible metabolic and micromechanical injury of the auditory apparatus. Metabolic disturbances in cells are determined by generation of reactive oxygen species [8]. Excessive efferent impulses in central structures of the auditory apparatus cause the development of intracellular hypoxia and glutamate excitotoxicity [11]. These changes produce damage to neurons under conditions of oxidative stress [3].

We studied the possibility of protecting the auditory apparatus from oxidative stress. Particular attention was given to biochemical properties of the histidine-containing dipeptide carnosine. This nonspecific physiological substance protects various excitable tissues characterized by anaerobic metabolism and high risk of oxidative stress [3].

Here we studied the effect of physiological antioxidant carnosine on morphofunctional indexes of sensory cells in the cochlea and intensity of lipid peroxidation (LPO) in tissues of the membranous cochlea and auditory cortex during acute acoustic trauma.

MATERIALS AND METHODS

Acute experiments were performed on 28 male Wistar rats weighing 180-250 g. Otoscopy and measurement of Preyer reflex did not reveal somatic and otological diseases [5]. To produce noise trauma the animals were exposed to sound stimulation (5 kHz, 103-107 dB) for 4 h. The sound was generated by a GZ-33 generator and delivered through a low-frequency dynamic in a soundproof chamber (free acoustic field).

We conducted 2 series of experiments. In some animals acoustic trauma was modeled without previous treatment. Other rats intraperitoneally received 200 mg/kg carnosine 12 and 0.5 h before acoustic trauma. Intact animals receiving carnosine and physiological saline served as the control. The rats were decapitated immediately after trauma.

Samples from 16 rats were used for biochemical assay of LPO in series I. Homogenates of the cochlea were studied after removal of the bony capsule. We

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0007-4888/04/1371-0098

also examined the auditory cortex and control tissue of the cerebellum not associated with auditory sensitivity. Supernatants of tissue homogenates were prepared in glass homogenizers, mixed with 50 μM sodium phosphate buffer (pH 7.4), and centrifuged at 1000*g* for 20 min.

Antioxidant properties of carnosine were determined by its influence on LPO intensity and state of the antioxidant system (AOS) in samples. The intensity of LPO was estimated by the amount of malonic dialdehyde (MDA, end product). The state of AOS was evaluated by total superoxide dismutase (SOD) activity and total antioxidant activity (TAA) in tissues. MDA concentration (μmol/g lipids) and total antiradical SOD activity were measured as described elsewhere [2]. TAA was determined by the intensity of $FeSO_4$-induced LPO in homogenates of brain tissue and inner ear [8].

Samples from 14 rats were subjected to morphological examination in series II. The isolation and processing of temporal lobes and cochleae were performed by the method of Vinnikov and Titova [5]. Total plane preparations of the cochlea were examined under an immersion microscope (×90). The structure of hair cell (HC) nuclei was estimated visually by staining nucleic acids (method of L. Einarson *et al.*) [6]. The auditory cortex of the brain was isolated at the level of temporal lobes between 2 frontal sections according to cytoarchitectonics of rodent neocortex [7].

RESULTS

The rats receiving physiological saline or carnosine exhibited a normal (active) Preyer reflex. After sound stimulation the animals failed to elicit or exhibited poor Preyer reflex. The rats injected with carnosine before sound stimulation had reduced Preyer reflex.

MDA content in tissues of the inner ear and brain increased in rats exposed to acoustic stress ($p<0.05$). Carnosine prevented MDA accumulation in the cerebellum ($p<0.05$), but not in tissues of the cochlea and temporal lobe (Fig. 1, *a*). The addition of Fe^{2+} to tissue homogenates activated LPO to a different extent (Fig. 1, *b*) depending on the initial state of AOS in tissues. The intensity of LPO significantly increased in tissues of the inner ear and temporal lobe of rat brain, which

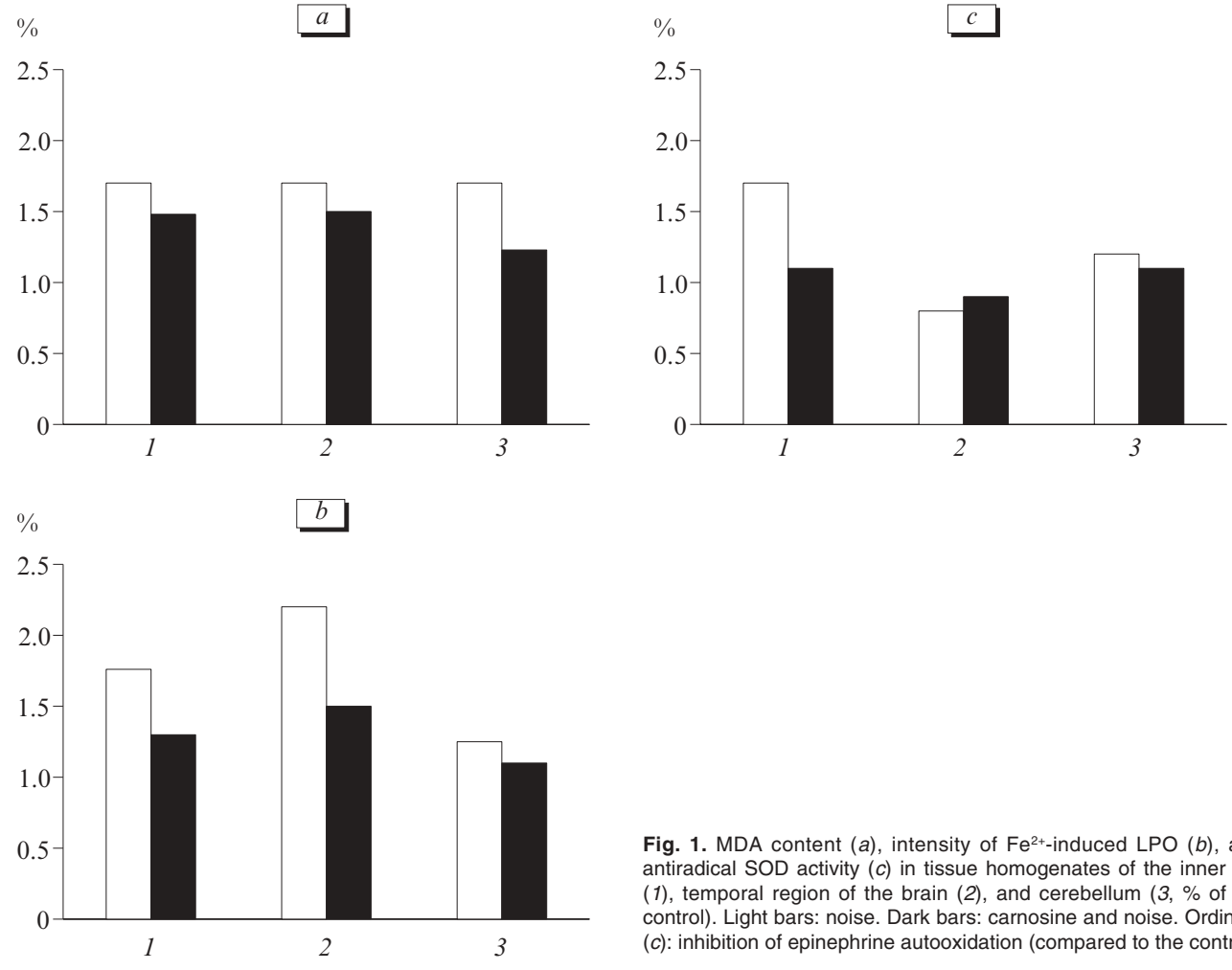

Fig. 1. MDA content (*a*), intensity of Fe^{2+}-induced LPO (*b*), and antiradical SOD activity (*c*) in tissue homogenates of the inner ear (*1*), temporal region of the brain (*2*), and cerebellum (*3*, % of the control). Light bars: noise. Dark bars: carnosine and noise. Ordinate (*c*): inhibition of epinephrine autooxidation (compared to the control).

reflected insufficiency of AOS under these conditions. Carnosine markedly inhibited LPO ($p<0.05$). Acoustic stress increased SOD activity in tissues of the inner ear and cerebellum ($p<0.05$, Fig. 1, *c*). It was probably related to activation of SOD that acts as the major antioxidant enzyme. SOD activity slightly decreased in the temporal lobe of rat brain after noise stress ($p<0.05$). The observed changes reflected insufficiency of AOS. No considerable changes in SOD activity were revealed in animals receiving carnosine.

Examination of plane preparations of cochlear helixes from control rats revealed typical morphological structure of this organ (Fig. 2). Nuclei of the outer HC (OHC) lay in three rows, were monomorphic, and had a regular spherical shape. The nucleoplasm was colored gray-to-blue, which reflected the presence of diffuse RNA in high concentration. Chromatin granules were intensively colored and localized in central regions of the nucleus. Most nucleoli occupied a central position. Nuclei of the inner HC (IHC) were arranged in a single row and had oval shape. They were larger in size and had less colored nucleoplasm compared to OHC nuclei.

Structural characteristics of the cochlea changed after noise stress. It primarily concerned the volume and structure of OHC nuclei. Some OHC were characterized by increased volume of nuclei ($p<0.05$ compared to the control), lightening of the nucleoplasm, and presence of decondensed chromatin (Fig. 3, *a*). Karyopyknosis in other cells was manifested in the appearance of small stellate cells with hyperchromic and hypercondensed chromatin (Fig. 3, *b*). Karyopyknosis is a general morphological sign of coagulation necrosis. Nucleoli were displaced to the margin of the nuclear membrane in normal nuclei of remaining OHC, which reflected the development of adaptive processes. Changes in IHC were similar, but less pronounced than in OHC.

In rats pretreated with carnosine noise stress decreased the volume of OHC nuclei in the cochlea (Fig. 3, *c*). We did not reveal a considerable increase in the volume of OHC nuclei or karyopyknosis. It reflects functional shrinkage of HC nuclei in the cochlea [2] and constitutes a reversible stage of the histophysiological reaction to extraphysiological stimulation. In these rats the count of OHC nuclei with normal structure and volume was higher than in animals not receiving carnosine. Nucleoli were adjacent to the margin of the nuclear membrane (ectopic localization), which serves as a sign of adaptive reconstruction. In rats of this group the state of IHC nuclei did not differ from the control.

Morphological examination of the cochlea was performed after noise exposure. We did not reveal local changes in HR of the cochlear helix that tonotopically corresponded to a frequency of 5 kHz. These data indicate the observed changes were nonspecific. Therefore, sound stimulation was beyond physiological normal and produced traumatic injury. Intensive generation of LPO products in excitable tissues of the cochlea is a biochemical equivalent to sound-induced pathological changes in the cochlea associated with reconstruction of HC nuclei. It should be emphasized that these biochemical signs of oxidative stress in the cochlea are not specific for noise trauma. Oxidative damage to HC is a common stage in the pathogenesis of cochlear dysfunction during aging and aminoglycoside ototoxicity [8-10,12]. It can be hypothesized that

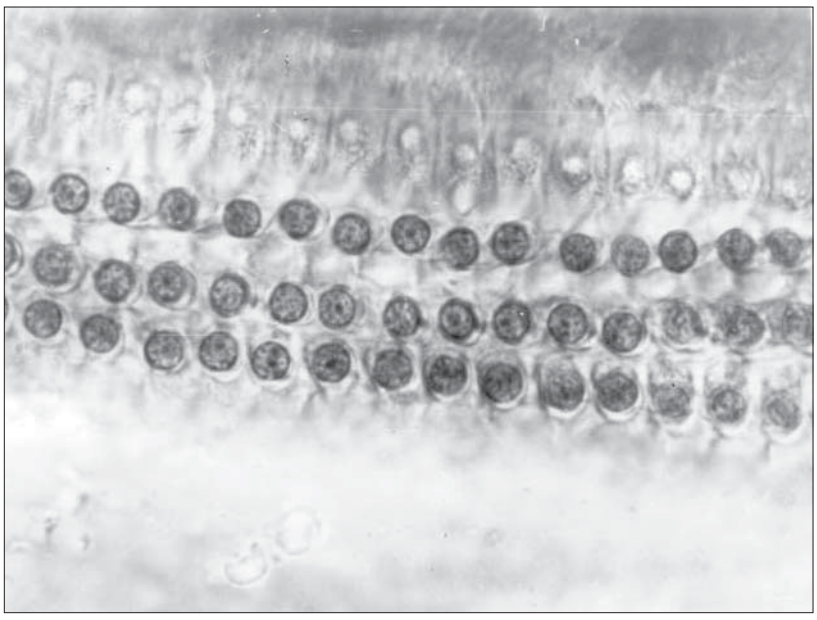

Fig. 2. Cochlea at the level of the major cochlear helix in intact albino rats. Nuclei of outer hair cells (OHC) are arranged in 3 rows. Here and in Fig. 3: Einarson staining, plane preparation (×630).

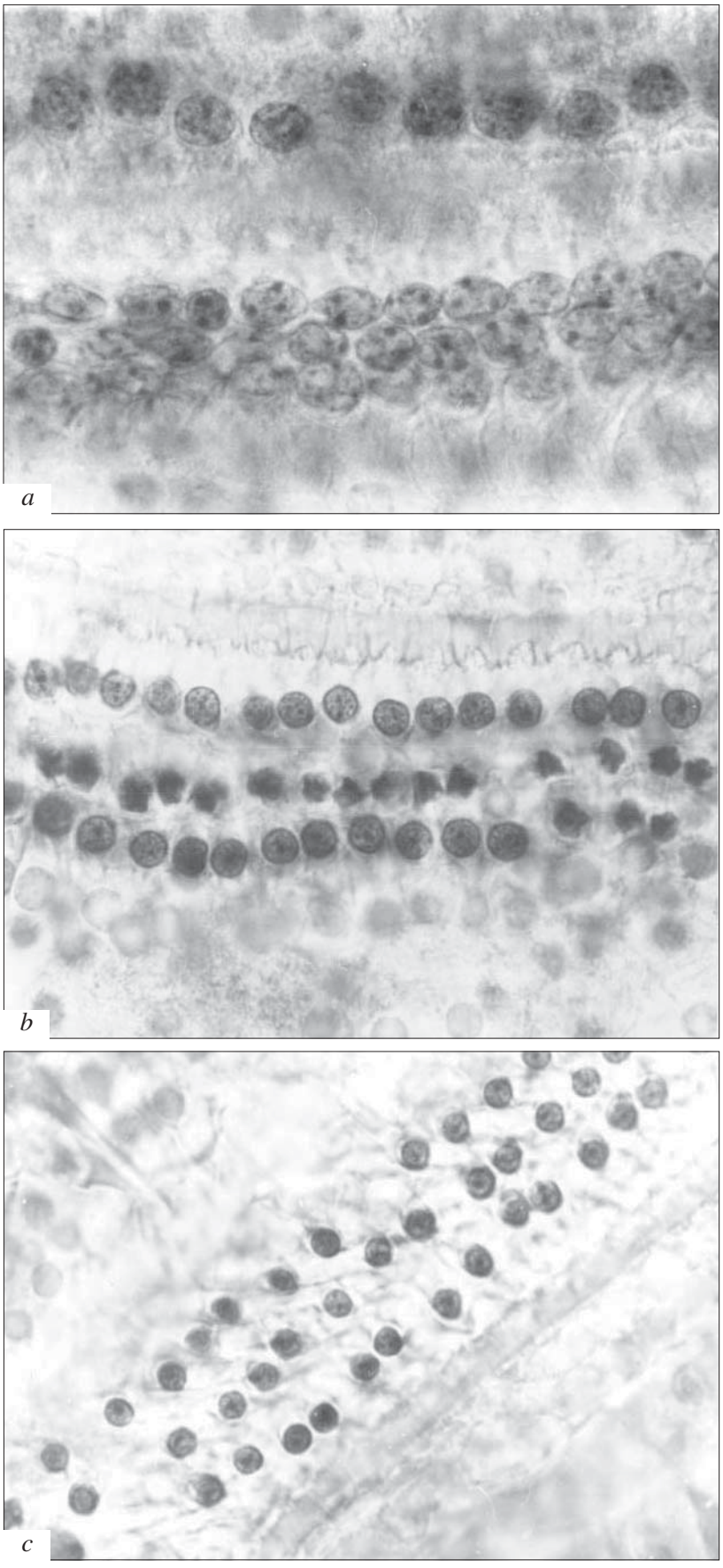

Fig. 3. Cochlea of albino rats after 4-h noise exposure (5 kHz, 103-107 dB, *a*, *b*) and pretreatment with 200 mg/kg carnosine 12 and 0.5 h before simulation (*c*). Single cell with normal nucleus in the first row between sharply swollen nuclei of OHC; no changes in cell nuclei (*a*). First and seconds rows of intact OHC; karyopyknosis in OHC of the second row (*b*). Hyperchromic and shrunk nuclei of OHR; absence of nuclei with sharply swollen nucleoplasm; no signs of karyopyknosis (*c*).

the protective effect of carnosine under conditions of noise trauma is determined by its ability to regulate cell death [4] and will be observed during other damages to the auditory apparatus.

Morphological changes in neurons and microcirculatory disturbances in the auditory cortex produced by high-level noise were studied previously. We first revealed that exposure to noise stress is followed by intensive generation of LPO products in central structures of the auditory apparatus (temporal lobe). Studied indexes in the temporal lobe differed from those in the cerebellum not involved in sound perception.

Our results indicate that carnosine protects the auditory apparatus from oxidative stress during accoustic trauma. Carnosine possesses membrane-protecting activity and produces a positive effect on excitable tissues of peripheral and central structures in the auditory apparatus [3].

The influence of carnosine on central structures of the auditory apparatus is an example of its nonspecific effects in excitable tissues. It is important that carnosine inhibits LPO in peripheral structures of the auditory apparatus (membranous cochlea), contributes to morphological preservation of nuclei in OHC, and reduces the degree of degenerative changes in these cells. The effects of carnosine on cochlear tissues require further investigations. Generation of LPO products during acoustic trauma is probably related to stress-induced metabolic changes not only in HR, but also in other structures of the membranous cochlea (*e.g.*, epithelium of the vascular strip).

We are grateful to Prof. A. A. Boldyrev for his help in this study.

This work was supported by the St. Petersburg foundation for young candidates of sciences (grants No. PD 02-1.0-40, PD 03-4.0-141) and St. Petersburg foundation for students, aspirants, and young specialists (grant No. M 03-4-10).

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