Ultrastructural and Morphometric Characteristics of Nerve Cells and Myelinated Fibers in the Cerebral Cortex after Chronic Exposure to Natural Gas Containing Hydrogen Sulfide in Low Concentrations

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We studied ultrastructural and morphometric characteristics of nerve cells and myelinated fibers in the cerebral cortex after chronic exposure to natural gas containing hydrogen sulfide in low concentrations. Radioisotope assay revealed activation of protein synthesis in nerve cells after chronic exposure to natural hydrogen sulfide-containing gas in low concentrations (10 mg/m^3 by H_2S) for 2 weeks. After 1 month the ultrastructure of myelinated fibers was characterized by sectorial loosening and demyelination.

Key Words: *natural hydrogen sulfide-containing gas; myelin sheaths of nerve fibers; protein synthesis*

The major pollutants of gas fields containing sulfur compounds and localized in France, Canada, Austria, USA, and Russia (Astrakhan and Orenburg) are H_2S and sulfur dioxide. H_2S and other gases in concentrations hazardous to human health are released in the working and service area even if the devices are kept in good repair, and the degree of decompression is technically acceptable. H_2S possesses high corroding activity and, therefore, causes rapid sulfide destruction of technological, locking, regulating, and other devices [1,3].

Acute exposure to H_2S produces hallucinations, amnesia, and discoordination. Chronic exposure to this compound causes mental disorders [1,8]. H_2S affects biological membranes due to high solubility in lipids [8]. H_2S primarily damages the central nervous system (CNS). CNS includes highly organized membrane structures (myelin sheaths) consisting of stretched and modified plasma membranes of oligodendrocytes. A thick layer of myelin envelops the axon and prevents electrical contacts between nerve fibers. Dielectric properties of myelin are associated with an extremely high content of lipids, which is much greater than in other membranes [2,5,7]. H_2S is soluble in lipids present in myelin sheaths of nerve fibers in high concentrations. Here we studied the effect of chronic exposure to the gas mixture with hydrogen sulfide in low concentrations on brain membranes containing myelin.

MATERIALS AND METHODS

Experiments were performed on 90 male outbred albino rats weighing 200-250 g. Control and experimental animals were kept in a vivarium under similar conditions and fed a standard feed. The work was performed according to the Requirements for Studies on Experimental Animals. The rats of groups 1 (n=30) and 2 (n=30) were daily exposed to a gas mixture containing 10 mg/m^3 H_2S for 1 h (2 weeks and 1 month, respectively). The control group included 30 animals not exposed to H_2S.

We used dried natural gas from the Astrakhan gas field. Experiments with the gas mixture containing na-

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tural gas and air were performed using a Geldinskolde seed chamber. H_2S concentration was measured with J-813 gas analyzers (Passport).

The samples were taken under nembutal anesthesia (40 mg/kg) immediately after the last exposure. For light microscopy, AFA fixative containing absolute alcohol (70%), 37% formalin (20%), and glacial acetic acid (10%) was administered transcardially. The brain was removed not less than 6 h after fixation. The samples from the sensorimotor cortex of cerebral hemispheres were treated as described elsewhere [9]. For electron microscopy, the animals were narcotized with 40 mg/kg nembutal and subjected to transcardial perfusion with 2.5% glutaraldehyde on cacodylate buffer (pH 7.3). The samples were treated by the method used at the Laboratory of Experimental Cell Pathology. ^{3}H-Leucine (marker of protein synthesis) was injected intraperitoneally in a dose of 25.9×10^4 Bq/g for histoautoradiographic assay.

The results were analyzed using Scion Image software. We calculated the arithmetic mean and confidence interval. The differences were significant at $p\leq0.05$.

RESULTS

Light microscopy did not reveal pathological changes in nerve cells and fibers of the cerebral cortex from rats exposed to the hydrogen sulfide in low concentrations (10 mg/m^3 H_2S). The maximum permissible concentration of H_2S in the mixture with carbohydrates is 3 mg/m^3 [4]. However, ultrastructural characteristics reflected changes in metabolic processes. After 2-week exposure neuronal nuclei had snaky contours (Fig. 1), the granular endoplasmic reticulum contained a considerable number of fixed ribosomes. Radioisotope assay confirmed activation of plastic processes in nerve cells. After 2-week exposure to natural gas containing hydrogen sulfide, the intensity of leucine incorporation increased from 3.380±0.062 to 5.790±0.135 arb. units. In this period we revealed individual myelinated nerve fibers characterized by loosening of myelin sheaths. Electron microscopy showed that the number of these fibers sharply increased after daily exposure for 1 month. Myelin sheaths of nerve fibers in the cerebral cortex underwent sectorial defiberization. These regions lost normal morphological characteristics and had no myelin. Fibers lost dielectric properties and could not rapidly conduct nerve impulses.

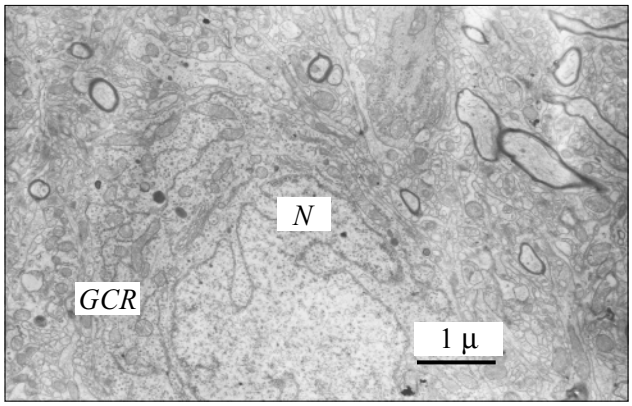

Fig. 1. Layer V neuron in the sensorimotor cortex from rat after 1-h daily exposure to the gas mixture containing 10 mg/m^3 H_2S for 2 weeks. *N*: nucleus. *GCR*: granular cytoplasmic reticulum.

During formation of the myelin sheaths, the axon should induces depression on the surface of Schwann cells. Experiments with tissue cultures showed that 1 turn is completely formed over 44 h [5]. In our experiments inhalation of the gas mixture was performed daily for 1 h in the same time. Therefore, damages to myelinated fibers were produced only in certain regions. However, this exposure did not cause structural changes in axons observed after treatment with the gas mixture containing H_2S in higher concentrations.

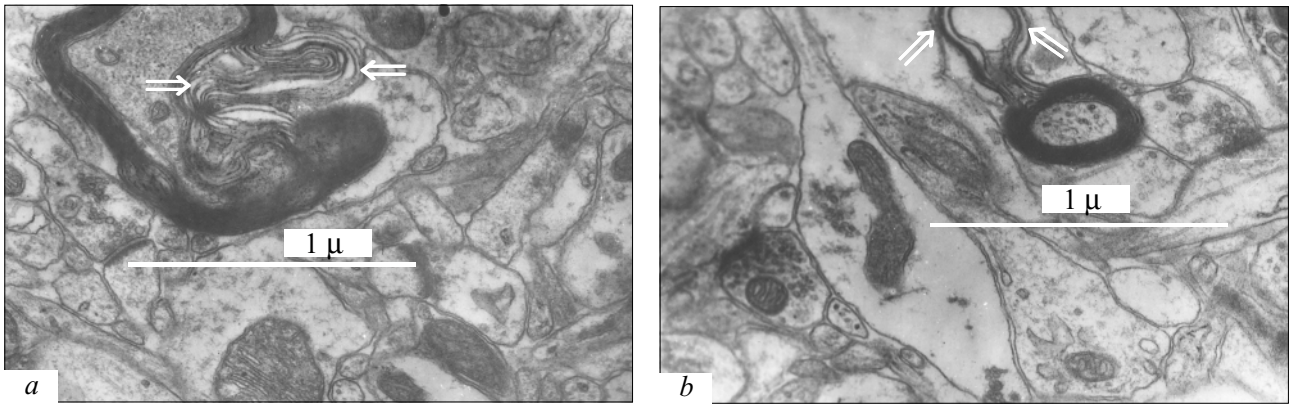

Fig. 2. Myelinated nerve fiber of the sensorimotor cortex from rat after 1-h daily exposure to the gas mixture containing 10 mg/m^3 H_2S for 1 month: sectorial loosening of the myelin sheath to 0.7 μ (arrows, *a*); changes in the shape of electron dense lines in the myelin sheath (arrows, *b*).

The thickness of myelinated fibers in control animals was 0.118±0.004 μ. After chronic exposure to natural hydrogen sulfide-containing gas the thickness of morphologically unchanged myelinated fibers did not differ from the control (0.0919±0.0190 μ). However, the thickness of morphologically transformed myelinated fibers increased to 0.334±0.145 μ. It should be emphasized that the thickness of loosened regions reached 0.7 μ (Fig. 2, *a*). We observed not only quantitative, but also qualitative morphological changes. The intermediate zone presented by myelin disappeared; in these regions myelin was absent. The major zone had an irregular and unusual shape (Fig. 2, *b*).

Our results indicate that H_2S in concentrations slightly surpassing the maximum permissible dose produces considerable destructive changes in brain structures. Pronounced morphological changes in myelinated fibers led to activation of protein synthesis after chronic exposure to the hydrogen sulfide-containing gas.

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