Effect of Hypoxia on Morphological and Functional Parameters of Astrocytes in a Confluent Culture

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The effects of hypoxia on the morphological and functional parameters are studied in confluent cultures of astrocytes derived from the brain of newborn rats. Morphofunctional alterations emerge 16-20 h after hypoxia and manifest themselves as nuclear and cellular hypertrophy, cell vacuolization, and decreased mitotic activity. The intensity of alterations depends on the duration of hypoxia. It is demonstrated that protective activity of the preparation T-3 is similar to that of α-tocopherol and ionol.

Key Words: *cultured astrocytes; hypoxia; free-radical oxidation; antioxidants*

Hypoxia of the nervous tissue leads to cell damage manifesting itself as degeneration of neurons and reactivation and hypertrophy of astrocytes [12,13]. Generally, destructive alterations in neurons occur 2-3 h after hypoxia, while astrocytes display no signs of damage [5,14]. Previously, it was demonstrated that astrocytes are closely associated with neurons: they are involved in the energy metabolism, ionic homeostasis, transport of some inorganic ions during exchange of the neurotransmitter amino acids, and regulation of the immune response of the brain [3,9-11]. Therefore, astrocytes attract more attention in the investigation of the mechanisms of hypoxic damage to the brain [3,14].

The mechanism of hypoxic damage to astrocytes still remains obscure. Several factors contributing to hypoxic damage have been identified [1,3,6,12]. Intensification of free-radical oxidation (FRO) of lipids is one of the major factors determining the damaging effect of hypoxia [2,5,8]. The effectiveness of antioxidant (AO) protection against hypoxia of different etiologies has been demonstrated [2,5].

Therefore, the development of new AO [2] seems relevant. It was reported that steritically hindered phenols have a high antioxidant activity [4].

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We think that astrocyte culture is a convenient tool for the investigation of hypoxic damage to the nervous tissue and screening of antioxidant drugs.

In this study we examined the effect of hypoxic hypoxia on morphological and functional parameters of cultured astrocytes and the protective effects of AO.

MATERIALS AND METHODS

Astrocyte cultures were initiated from the brain cortex of rat pups aged 7-10 days by the method [7] with our modifications. Cells were grown on coverslips in plastic and glass flasks. Culture medium was supplemented with hemohydrolysate (5%), 10% fetal calf serum (DIALEK, Belarus), gentamicin (50 μg/ml), and glutamine (300 μg/ml). Primary confluent cultures (2-week-old) and cultures on days 4-5 after the first passage (seeding dose 10^5 cells/ml) were used. The content of glial fibrillary acidic protein (GFAP), a specific marker of astrocytes, was measured by indirect immunofluorescence using anti-GFAP antibodies and rabbit antiserum (Sigma). Hypoxia was attained by placing the cultures in a gas chamber with 95% NO_2/5% CO_2 for 2-24 h. Gas composition in the chamber was regulated with an SDG-3 dispenser (Institute of Biophysics, Pushchino). The gas chamber was put in a

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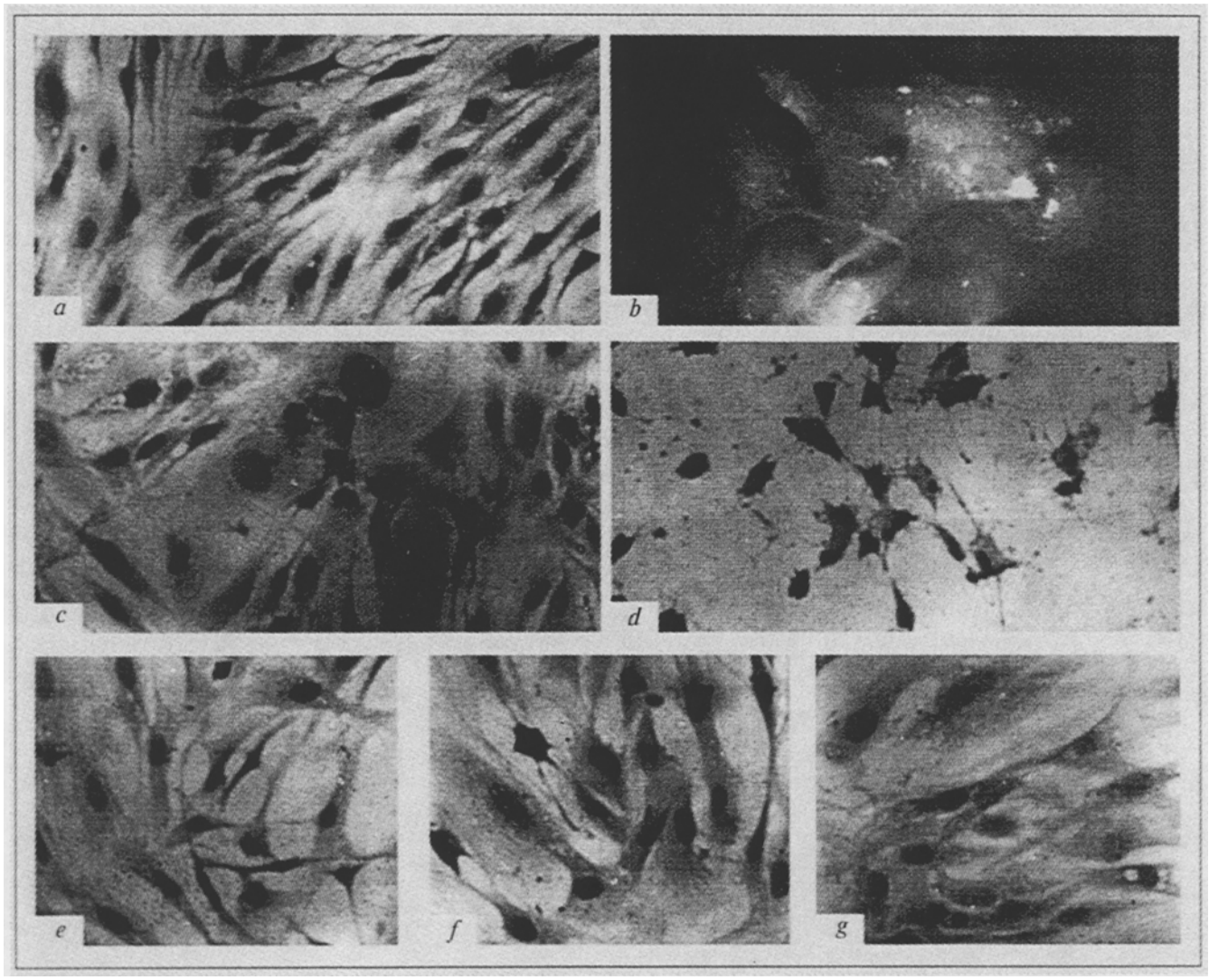

Fig. 1. Protective effects of antioxidants on astrocytes cultured under conditions of hypoxia. *a*) astrocytes cultured under normal conditions (control), staining with hematoxylin and eosin, ×200; *b*) localization of glial fibrillary acidic protein in cells, ×630; *c*) hypertrophy of astrocytes after a 24-h incubation under conditions of hypoxia; *d*) destruction of astrocytes after 24 h of hypoxia; *e-g*) absence of pronounced morphological and functional changes in cultures after a 20-h hypoxia with α-tocopherol (*e*), ionol (*f*), or T-3 (*g*), *c-g*) staining with hematoxylin and eosin, ×200.

thermostat at 37°C. The antioxidant T-3, a pyrocatechin derivative, was added to the growth medium to a final concentration of 0.05 μg/ml. This compound was synthesized at the Byelorussian State University. The toxicity of T-3 for cultured astrocytes was determined in preliminary experiments. The state of cultures was assessed after 2, 4, 8, 12, 18, 20 and 24 h of hypoxia. The cultures were examined under a phase-contrast microscope and light microscope after fixation with Duboscq—Brasil—Bouin fluid and staining with hematoxylin and eosin. The monolayer integrity, morphology and size of the nucleus and cytoplasm, and the state of nuclear chromatin and nucleolus were assessed under a light microscope. Proliferative potential was evaluated by mitotic activity. For this purpose mitoses were counted per 1000 cells in the field of view and expressed in promille; 3000-5000 cells were analyzed per experimental point. The significance of differences was evaluated by the Student—Fisher *t* test.

RESULTS

The cultures derived from the brain of newborn rats contained 95% GFAP-positive cells, which confirms the high degree of their functional differentiation and maturity (Fig. 1, *b*). Other cells were macrophages, fibroblasts, and cells at earlier stages of differentiation. The cultures contained no neurons.

Mitotic activity in control cultures was 15.6± 3.4‰, and the content of hypertrophic cells was 1±0.7% (Fig. 1, *a*).

In vitro hypoxia causes morphofunctional and destructive alterations in astrocytes. The intensity of

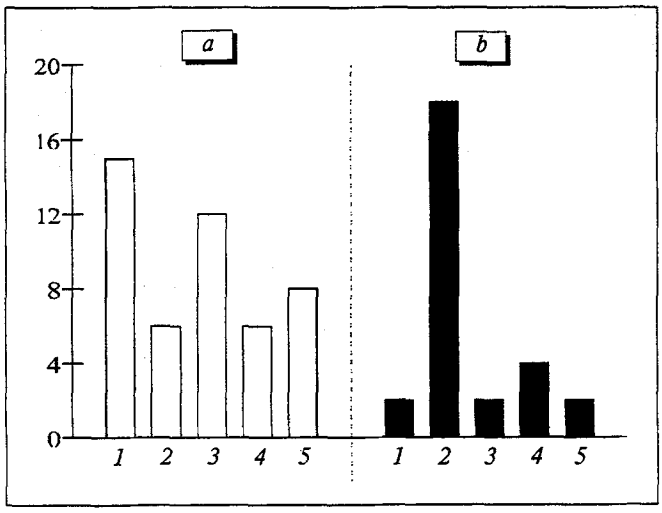

Fig. 2. Effects of various antioxidants on morphofunctional parameters of astrocytes incubated under conditions of hypoxia. *a*) mitotic activity, ‰; *b*) number of hypertrophied cells, %. *1*) control; *2*) hypoxia; *3*) hypoxia+T-3; *4*) hypoxia+ionol; *5*) hypoxia+α-tocopherol.

these alterations depends on duration of hypoxia. After 12-24 h of hypoxia, mitotic activity of astrocytes decreased, and the number of cells with hypertrophied nucleus and cytoplasm with subsequent destruction increased. In passaged and primary cultures, cell damage was observed after 16 and 18 h of hypoxia, respectively. The following manifestations of cell damage were observed: hypertrophy of the cytoplasm and nucleus (Fig. 1, *c*) and mitotic disorders (chromosomal rearrangements in meta- and anaphase). These alterations were more pronounced after a 20-h hypoxia, involving the nuclei. The nuclei became transparent, chromatin was condensed, and the nucleoli disappeared. The cytoplasm became eosinophilic, and mitotic activity dropped. Cell damage was focal, and the monolayer integrity was preserved. After 24 h of hypoxia, the monolayer was disrupted, and some cells detached from the coverslips (Fig. 1, *d*).

Similar results were obtained by others [14]. At the same time, it was demonstrated that the resistance of cultured astrocytes to hypoxia is higher than that of neurons [3,8].

In order to standardize experimental conditions and quantitate the tentative protective effect of various compounds on astrocyte cultures under conditions of hypoxia, temporal (20 h of hypoxia) and morphofunctional (size of cells and nuclei and mitotic activity) criteria were defined. Cultures grown under normal conditions served as the control. Alterations were revealed in cultures incubated under hypoxic conditions with and without AO.

The addition of ionol, T-3, or α-tocopherol blocked cell destruction compared with the control (Fig. 1, *e-g*). In cultures incubated without AO, the mean content of hypertrophied cells was 17.0±1.0%, while cultures incubated in the presence of α-tocopherol and T-3 contained 1.6±0.7 and 1.0±0.4% hypertrophied cells, respectively. For control cultures this parameter was equal to 1.0±0.7%. After hypoxia, mitotic activity of cultured astrocytes dropped to 6.3±1.0‰ (*vs.* 15.6±3.4‰ in the control). It increases in cultures treated with T-3 (12.0±3.0‰) and reached the control level ($p<0.05$). Mitotic activity did not reach the control level in cultures treated with ionol or α-tocopherol, remaining lower than in T-3-treated cultures (Fig. 2).

Thus, the studied AO elicited protective effect on astrocyte cultures under conditions of hypoxia.

Taken together with previous observations [5,8], our data suggest that FRO products contribute to the astroglial damage caused by hypoxia. This study shows that T-3, a disubstituted phenol, is superior to the synthetic AO ionol and naturally occurring AO α-tocopherol in protective activity assessed by mitotic activity of cultured astrocytes. Presumably, this is due to complexing, protonating, and cross-linking abilities of the functional groups of this compound, which implies that T-3 is involved in the regulation of FRO in the membranes. Our results may be useful for further investigation of the protective activity of T-3 and its effect on metabolic reactions in astrocytes, neurons, and other cell types of the central nervous system.

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