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Reorganization of the Ultrastructure of Neocortical Neurons in Rats Treated with Extracellular DNA

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Neuron ultrastructure in layers III–V of the cerebral cortex was studied in rats 24 h after i.p. (n = 3) or i.v. (n = 3) administration of extracellular DNA (ecDNA) at a dose of $7.7 \cdot 10^{-5}$ g/kg. Plastic rearrangements of nuclear chromatin were seen, along with nucleolar hypertrophy, deep invaginations of the nuclear envelope, mitochondrial hyperplasia, and tight contact between mitochondria and other organelles, including nuclei, formation of tubulovesicular bodies in the cytoplasm which could promote the transport of synaptic vesicles to the presynaptic terminals of axons, and activation of the astrocyte glia. These data provide evidence that administration of ecDNA leads to ultrastructural rearrangements of neocortical neurons directed to activating protein synthesis, increasing the efficacy of synaptic transmission, and enhancing energy metabolism, which may promote repair and compensatory processes in ischemic brain pathology.

Keywords: cerebral cortex, neurons, ultrastructure, plasticity, extracellular DNA.

The scope of studies of the mechanisms of ischemic brain damage and the search for pharmacological neuroprotectors required for stimulating regenerative and compensation-recovery processes in cerebral ischemia have significantly widened in recent years. Animal studies using a model of cerebral ischemia have shown that Toll-like receptors, including the TLR9 receptor, which is involved in the binding and endocytosis of DNA fragments, has a preconditioning neuroprotective effect [4, 11]. The highest level of TLR9 expression in the brain is seen in cortical neurons [12]. In the acute stage of ischemic stroke there is an inverse relationship between the volume of the cerebral infarct and the dynamics of neurological deficit on the one hand and the plasma concentration of extracellular DNA fragments (ecDNA) on the other, these latter being TLR9 agonists [2]. ecDNA has been shown to play the role of an endogenous, constantly acting blood factor regulating cerebral hemodynamics both in normal conditions and when the circulation is impaired. ecDNA actively interacts with a variety of body cells [8], including neurons [7]. Addition of low doses of ecDNA to cultures of cerebellar granule cells has neuroprotective actions in conditions of glutamate excitotoxicity, suppressing the production of reactive oxygen species in neurons and activating astrocytes [1].

These data impelled us to study the actions of ecDNA on nerve cell ultrastructure, on the suggestion that plastic changes to cellular ultrastructure may underlie the induction of repair and compensatory-restorative processes on administration of ecDNA in conditions of cerebral ischemia.

Materials and Methods

Male Wistar rats weighing 250–300 g received single i.p. boluses (n = 3) or i.v. infusions (0.06 ml/min; n = 3) of homologous DNA extracted from brain tissue at a dose of $7.7 \cdot 10^{-5}$ g/kg, while control animals (n = 6) received the same volume of isotonic (0.9%) sodium chloride solution. At one day, an-

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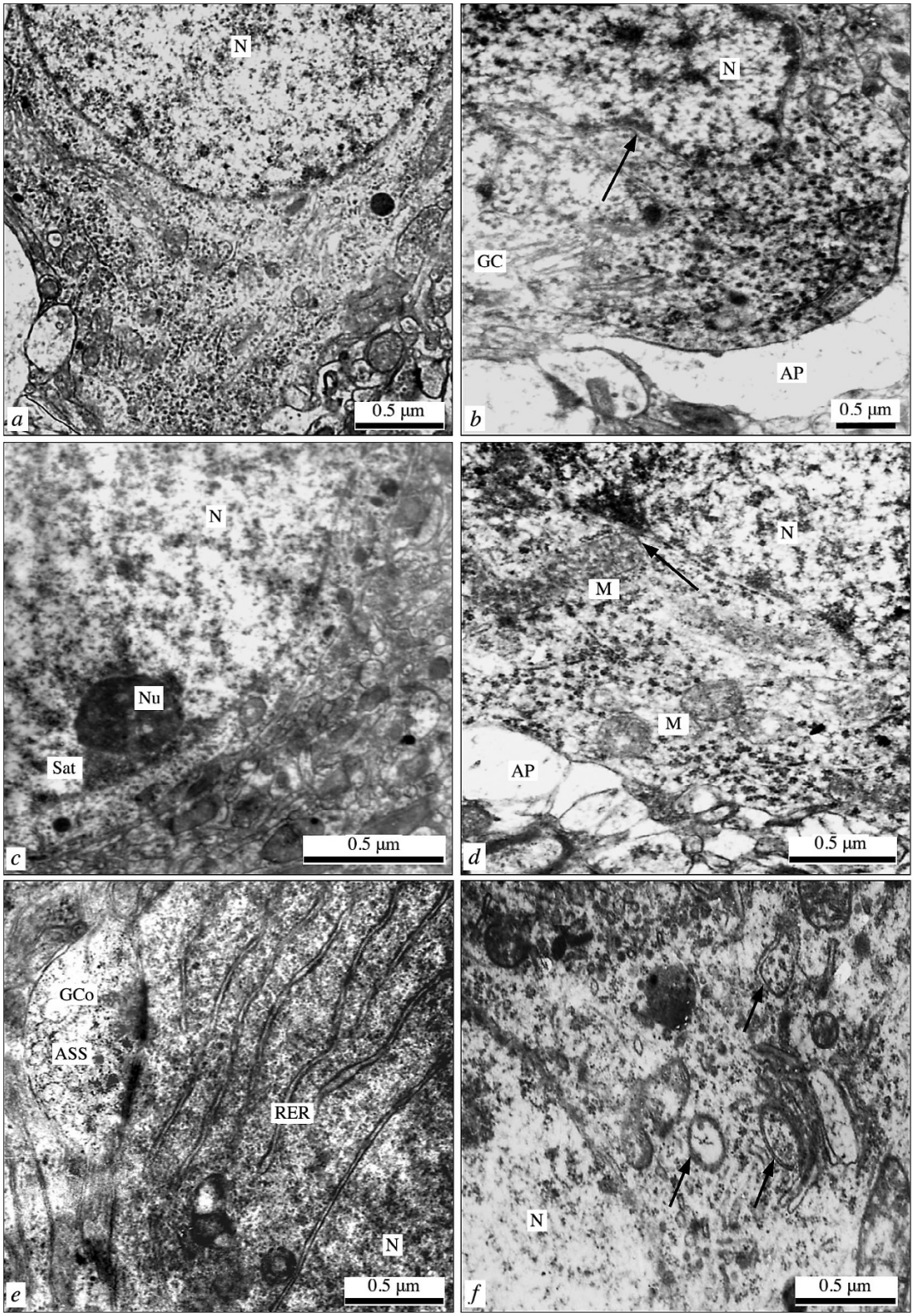

Fig. 1. Neuron ultrastructure in control rats (*a*) and after i.p. administration of extracellular DNA (*b–f*). *b*) Clumped structure of nuclear chromatin and deep invagination into the nuclear envelope (arrow); *c*) contact between hypertrophied nucleolus and the nuclear envelope via satellite; *d*) mitochondria tightly appressed to the outer nuclear membrane at a clumped chromatin accumulation zone (arrow); *e*) ordered rows of extended rough endoplasmic reticulum cisterns; *f*) tubulovesicular structures in the neuron cytoplasm (arrows). AP – astrocyte processes; ASS – axosomatic perforated synapse with growth zone (GCo); RER – rough endoplasmic reticulum; GC – Golgi complex; M – mitochondria; Sat – satellite; N – nucleus; Nu – nucleolus.

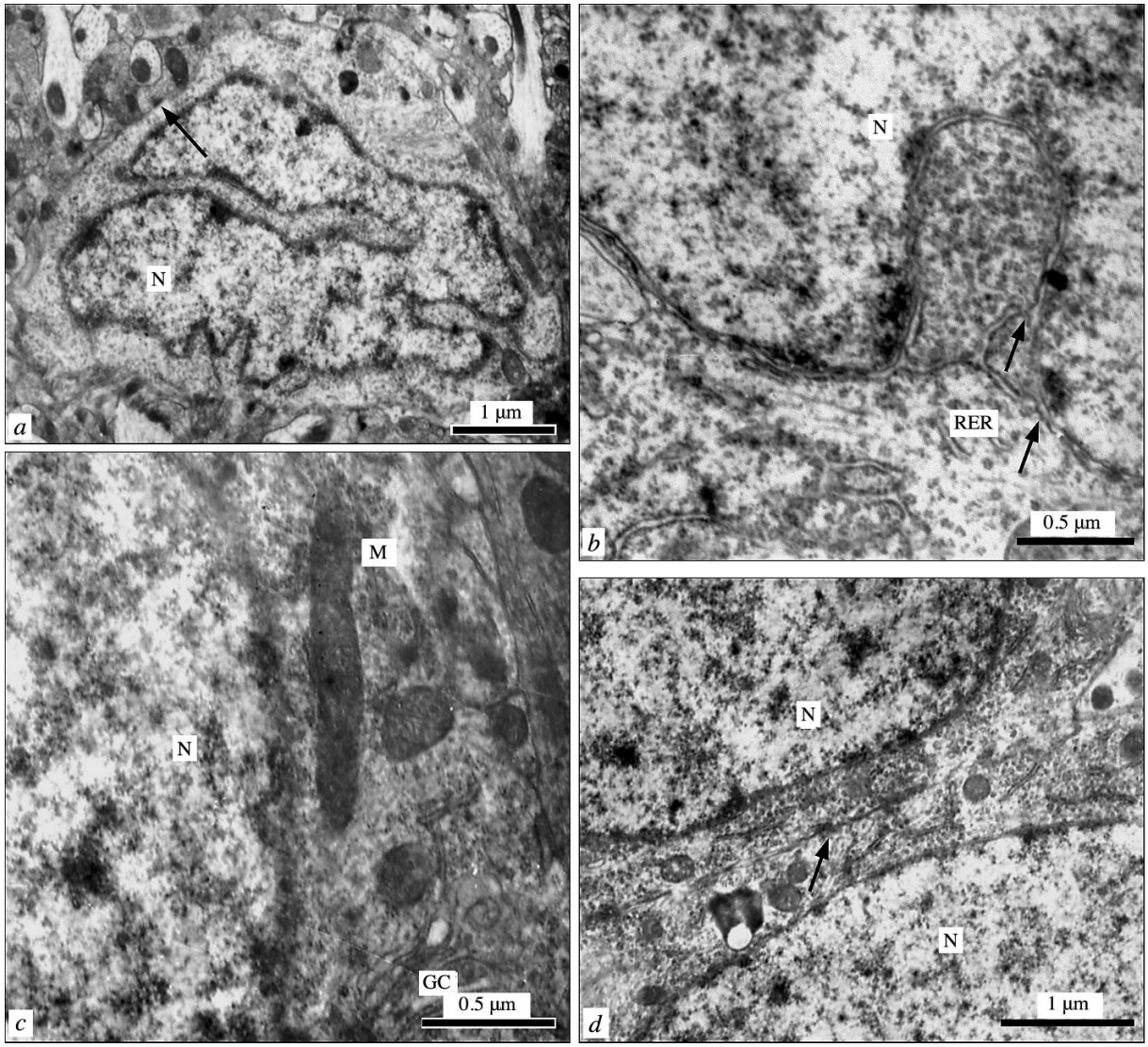

Fig. 2. Ultrastructure of neurons after intravenous administration of extracellular DNA. *a*) Deep invaginations of the nuclear envelope involving elements of the protein-synthesizing apparatus, axosomatic synapse (arrow); *b*) rough endoplasmic reticulum cisterns formed de novo from the outer nuclear envelope and contacts between these at nuclear envelope invagination zones (arrows); *c*) contacts between mitochondria and other mitochondria, the nucleus, and other organelles of the neuron cytoplasm; *d*) paired neurons connected via desmosome or gap junction type contacts. For further details see caption to Fig. 1.

imals were anesthetized with chloral hydrate and underwent vital fixation of the brain by intracardiac administration 2.5% glutaraldehyde solution containing 2% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. After decapitation, samples of the parietal-temporal area of the cortex were collected, which were additionally fixed in 1% osmium tetroxide solution using standard electron microscopy techniques and embedded in Epon-812. Neurons in cortical layers III–V were studied using an H-600 microscope (Hitachi, Japan).

Results

After i.p. administration of ecDNA, chromatin in the nucleoplasm and along the inner nuclear membrane the nuclei of neurons, in contrast to those of controls (Fig. 1, *a*) acquired a clumped structure (see Fig. 1, *b*). Hypertrophic nucleoli (sometimes with 1–2 satellites) directly or indirectly, via accumulations of chromatin, contacted the inner membrane (see Fig. 1, *c*) of the nuclear envelope, which had deep invaginations. The cytoplasm contained large polyribosomal rosettes, well-developed Golgi complexes (see Fig. 1, *b*), electron-dense energized mitochondria which were tightly appressed to the outer nuclear membrane usually close to areas of clumped chromatin (see Fig. 1, *d*), as well as numerous extended rough endoplasmic reticulum cisterns in ordered rows (see Fig. 1, *e*). Neurons were surrounded by lightened astrocyte processes (see Fig. 1, *b*, *d*), while the bodies of these cells showed perforated synapses (see Fig. 1, *e*).

As compared with controls, neuron cytoplasm and processes contained rounded or extended ringlike or tub-

ulovesicular structures, resembling multivesicular bodies (see Fig. 1, *f*). However, in contrast, these latter have double membranes and are filled with vesicles similar in size to synaptic vesicles.

A characteristic feature of normochromic neurons seen after i.v. administration of ecDNA was the presence of extremely deep invaginations of the nuclear envelope (Fig. 2, *a*), giving the nucleus a lobulated structure. Invaginations carried ribosomes and polysomes into the depth of the nucleus, creating an additional nuclear surface and, thus, the conditions for strengthened nuclear-cytoplasmic interactions and increased protein synthesis activity. In addition, rough endoplasmic reticulum cisterns formed from the outer nuclear membrane; these branched and were in close contact with its outblebbings at invaginations (see Fig. 2, *b*). The cytoplasm, rich in a variety of organelles, showed Golgi complex components, as well as giant mitochondria in contact with various organelles or tightly appressed to the nucleus (see Fig. 2, *c*). Some neurons formed pairs with local areas of contact of the desmosome or gap junction type (see Fig. 2, *d*).

Discussion

The data obtained here provide evidence that administration of ecDNA, particularly intravenously, leads to heterogeneity in neuron ultrastructure, this being apparent as changes to both the nucleus and the cytoplasm. Neuron nuclei showed clear clumping of the nuclear chromatin, especially at the inner nuclear membrane, along with nucleolar hypertrophy and eccentricity of nucleolar localization, appearance of nucleolar satellites, large numbers of nucleolar pores, and deep invaginations of the nuclear envelope, pointing to increased nuclear-cytoplasmic interactions and protein hypersynthesis. These changes were accompanied by plastic ultrastructural reorganization in the cytoplasm, as evidenced by the appearance of large polysome particles, energized mitochondria, large numbers of rough endoplasmic reticulum cisterns formed from the outer nuclear membrane, contact between organelles, and the tight contact of organelles with the outer nuclear membrane [3]. The presence of multiple tubulovesicular structures in the neuron cytoplasm was indirect evidence for enhanced neurotransmission. These structures may be morphological variants of endosomal organelles, spherical or tubular in shape and operating as carriers of synaptic vesicles [6, 9, 10, 13]. Tubulovesicular structures located in the neuron cytoplasm may be moved from this area to synaptic contact zones by axonal transport. The presence of contacts of the desmosome or gap junction types between pairs of neurons may support a significant spontaneous synchronization effect via electrical synapses [5].

Thus, administration of ecDNA induces plastic rearrangements in neurons in the brain, directed to activating protein synthesis in neurons and increasing the efficacy of synaptic transmission, which may promote repair and compensatory-recovery processes in cerebral ischemia.

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