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Amitriptyline-Induced Ultrastructural Changes in Various Structures of Rat Brain

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The immediate effect of amitriptyline on various brain structures is characterized by the standard ultrastructural changes in neurons: emergence of numerous coated vesicles in the cytoplasm and formation of cisternae within a 3-h period. These changes are most pronounced in the sensorimotor cortex. Changes in the structure of cytoplasmic organelles are reversible; they disappear 24 h after administration of amitriptyline. Astrocytes respond to the preparation by activation of lysosomes, being the most sensitive cell type of the neuroglia. Numerous synaptic vesicles are seen in the axodendritic synapses. Newly formed fibers in the neuropile may be associated with the antidepressant effect of amitriptyline.

Key Words: *brain; amitriptyline; ultrastructure*

Depression is thought to be associated with disorders in noradrenergic [9] or serotoninergic [6] synaptic transmission. Tricyclic antidepressants suppress the reversible neuronal uptake of norepinephrine or serotonin, thus increasing the efficiency of these neurotransmitters at the postsynaptic membrane. Another hypothesis assumes that amines released from axonal terminals diffuse in the extracellular space, which comprises 12-24% of brain volume [8], and modulate adjacent neurons, glia, and capillaries. Nonsynaptic modulation may change the processing of information in the central nervous system (CNS) [11].

Although clinical effects of antidepressants manifest themselves after a long latent period (2-3 weeks), the immediate response of brain structures at the ultrastructural level is of particular interest. Our aim was to examine the effects of amitriptyline (AT) on some structures of rat brain by electron microscopy.

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MATERIALS AND METHODS

Twelve male rats weighing 180-200 g were injected with AT (5 mg/kg intraperitoneally). In three control experiments sterile isotonic saline was injected. The rats were rapidly decapitated under light Nembutal anesthesia. The sensorimotor cortex (PA area and PAs field), hippocampus, central gray matter, and cerebellum were studied. Material for electron microscopy was collected 30 min, 3, and 24 h after sacrifice, fixed with glutar osmium, processed by conventional methods, and embedded in Epon. Ultrathin sections were cut in an LKB 8800 ultratome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-100S electron microscope.

RESULTS

Coated vesicles formed in all studied brain structures within the first 30 min after injection of AT, the process being most intense in the sensorimotor cortex. Three hours after the injection, endocytosis reached the maximum in hippocampal and central

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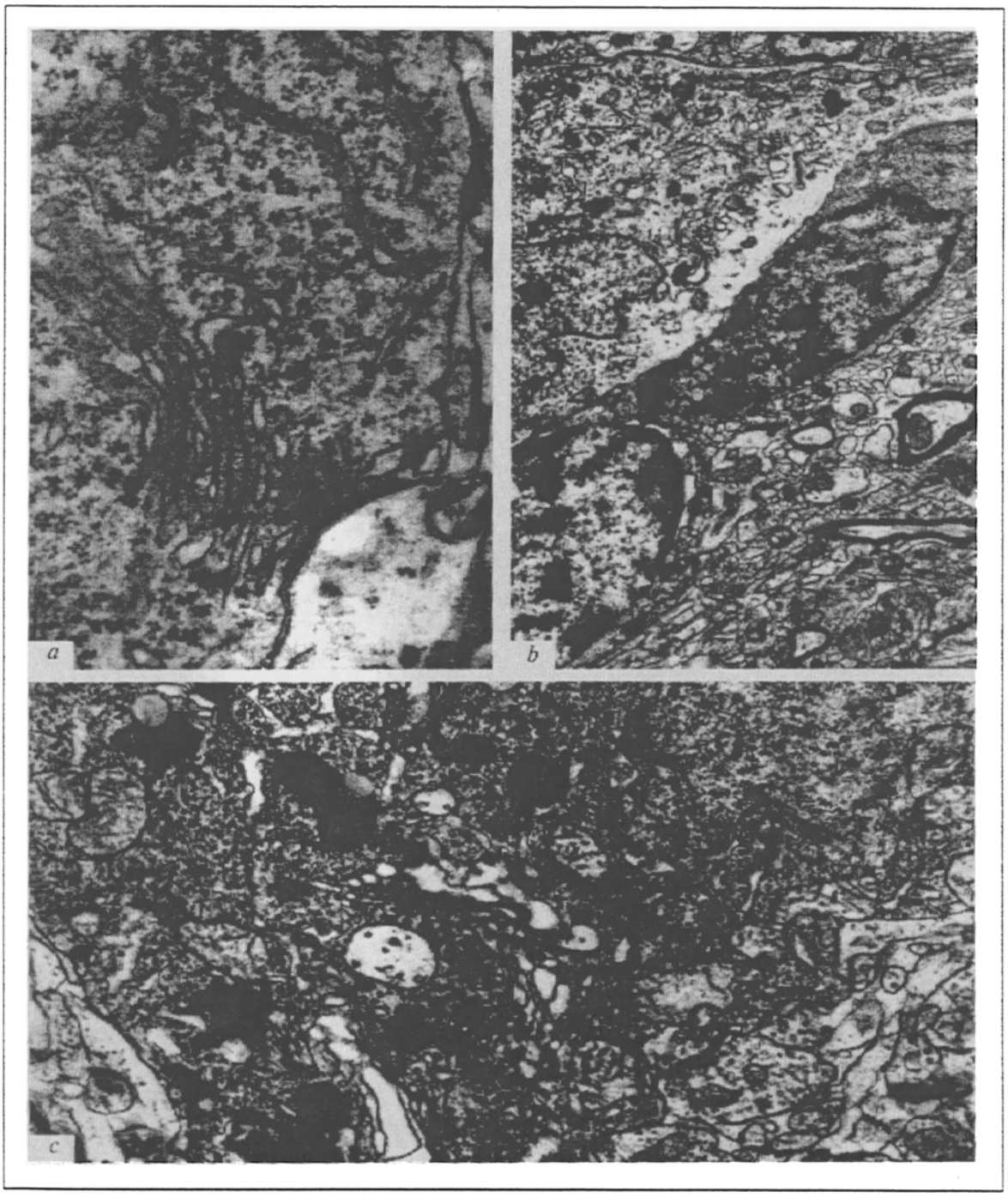
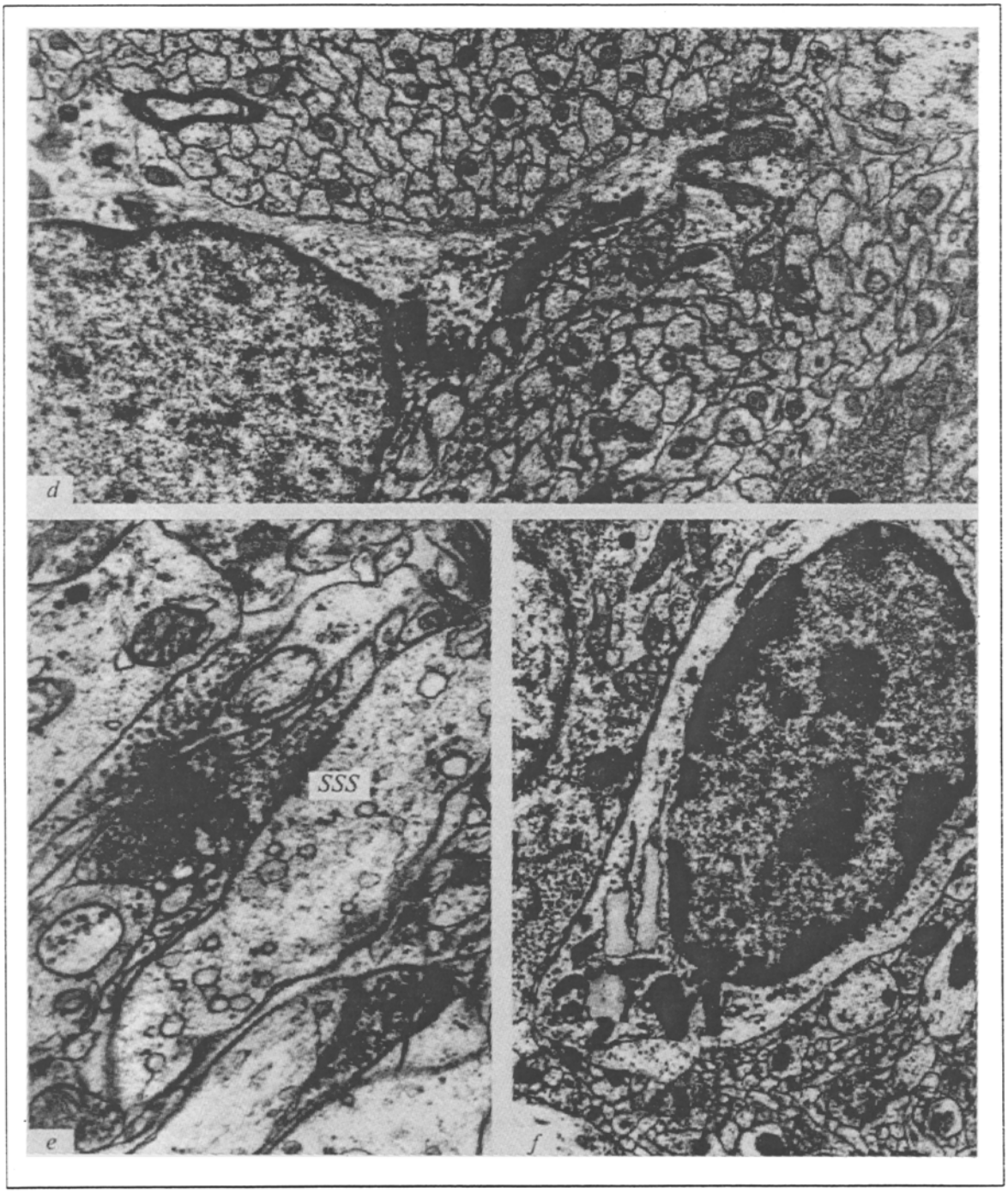


Fig. 1. Ultrastructural changes in the brain after administration of amitriptyline (AT). *a*) large subsurface cisterna in neuronal cytoplasm (hippocampus), ×21,000; *b*) chromatolysis in the neuron contacting with oligodendrocyte (central gray matter), ×5600; *c*) activation of lysosomes, a multivesicular corpuscle is seen (hippocampus), ×10,000;

gray matter neurons, being slightly decreased in cortical neurons.

Twenty-four hours after the injection, the intensity of specific interactions increased in cortical and cerebellar neurons, while in hippocampal and central gray matter neurons it was the same as in the control. The formation of subsurface cisternae coincided with fluctuations in the formation of coated vesicles: activation of specific endocytosis correlated with the rearrangements in neuronal plasmalemma (Fig. 1, *a*).

It should be noted that any ligand (not necessarily a psychotropic agent) induces the standard neuronal response: intensification of vesicle formation and appearance of subsurface cisternae as a compensatory reaction [1-5].



d) AT-induced sprouting in the hippocampus, ×7000; *e*) axodendritic synapse in the central gray matter, ×14,000; *f*) conversion of reticular tubules into lacunae in the sensorimotor cortex astrocyte, ×5600. *SSS*: subsynaptic streak.

Amitriptyline induced slight changes in the mitochondria of cortical neurons and more pronounced changes in dendrites (swelling and division of mitochondria). In the hippocampal and central gray matter neurons, the mitochondria were swollen, while in cerebellar neurons they preserved the original shape. After 24 h, the mitochondria in all studied brain structures were the same as in the control.

In the nuclei, invagination of the karyolemma and its fragmentation were observed; sometimes the nucleolus was lying in the cytoplasm. These changes were less pronounced 3 h after administration of AT, i.e., they were reversible. Our findings suggest that the nuclei of the central gray matter neurons and Purkinje cells of the cerebellum are more sensitive to AT than neuronal nuclei in other

brain structures. The nucleoli remained activated at least for 24 h.

Peripheral or segmented chromatolysis (disappearance of ribosomes and some components of the rough endoplasmic reticulum) was observed in some neurons, predominantly in the central gray matter (Fig. 1, *b*). It is noteworthy that oligodendrocytes, which are capable of transferring RNA into neurons, are located near cells with chromatolysis. Transport of ribonucleoprotein has been documented [7] and may serve as a means of replenishing the neuronal RNA pool after treatment with AT. This reaction was observed within the first 30 min after administration of AT.

Enhanced endocytosis coincided with activation of neuronal lysosomes and an increase of the number of multivesicular corpuscles in which the internalized material was degraded (Fig. 1, *c*). Intensification of intracellular cytolysis may be caused by membrane damage.

New synapses formed between thin nerve fibers (sprouting), which was associated with dendritic damage (Fig. 1, *d*). The contacts between newly formed thin fibers were different from typical synapses.

Thirty minutes after AT injection, numerous synaptic vesicles formed almost in all axon terminals. A subsynaptic streak was sometimes seen in the axodendritic synapses of the central gray matter (Fig. 1, *e*). This structure consists of electron-dense corpuscles located near synaptic membrane. Subsynaptic streaks with a high monoamine oxidase activity were originally described in the frenulum and interpedicle nuclei [10].

In the neuroglia, AT affected predominantly astrocytes, the maximum damage developing within 30 min after administration. This coincided with massive formation of secondary lysosomes in the astrocytes of sensorimotor cortex, central gray matter, and hippocampus. The intensity of intracellular proteolysis decreased after 3 h; however, in hippocampal astrocytes it increased after 24 h. The endoplasmic reticulum cisternae were widened in cortical and hippocampal astrocytes. Using serial sections, we managed to pinpoint the sites where narrow tubules dilate to form lacunae filled with a low-electron-density substance (Fig. 1, *f*).

Only minor disorders were observed in the microcirculatory bed at the peak of AT effect. They consisted in increased transendothelial pinocytosis in the capillaries and arterioles predominantly of the hippocampus. No changes were seen in the astrocyte processes, while in the cerebellar pericytes proteolysis was activated.

Thus, certain amounts of AT penetrating the blood-brain barrier enter brain neurons via coated pits. After lysosomal degradation of coated vesicles, AT affects cell-to-cell contacts. Judging from ultrastructural rearrangements, this preparation is metabolized within a 24-h period.

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