STRUCTURE OF NEURONS AND INTERNEURONAL SYNAPSES AFTER AMPHETAMINE EXCITATION

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Experiments on albino rats showed that 24 h after amphetamine excitation, when the animals' behavior was fully restored to normal, significant changes in the structure of the neurons and interneuronal synapses were present in the caudate nucleus at the microscopic and submicroscopic levels. These changes in the ultrastructure of the synapses were manifested chiefly as a decrease in the number of synaptic vesicles in the overwhelming majority of synapses. The increase in size of the small neurons of the caudate nucleus is based on a combination of destruction and formation of intracellular structures in different quantitative proportions. This accounts for differences in the intensity of compensatory regeneration in the caudate nucleus.

In the modern view the stimulant action of amphetamine on the CNS is due to its effect on adrenergic structures.

The authors previously [7, 8] demonstrated changes in the neurons and interneuronal synapses in the caudate nucleus which is rich in dopamine [11] and in dopamine-containing terminals [12, 13], at the period of maximal intensity of the central action of the drug.

To investigate the finer details of the morphological changes in neurons of the caudate nucleus in the later stages after pharmacological stimulation the microscopic and submicroscopic changes in neurons and interneuronal synapses were studied 24 h after administration of amphetamine.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats. Amphetamine was injected intraperitoneally in a dose of 10 mg/kg, which causes the development of stereotyped movements resembling subcortical automatisms. Brain sections were stained by the Nissl and Golgi methods. The area of cross section of 50 neurons and their nuclei (magnification 420 times) was determined by means of an ocular micrometer in three experimental and three control rats. Statistical analysis of the results was carried out by the Student–Fisher method. The number of spines for each 10-μ length of the secondary branches of the dendrites was counted.

For electron-microscopic investigation the brain was fixed by intravital perfusion with a mixture of 1% glutaraldehyde solution and 2% formaldehyde solution in phosphate buffer (pH 7.2-7.4) with the addition of 0.01% $CaCl_2$ solution and 7% glucose solution, and then postfixed in 2% osmium tetroxide solution. Pieces from the dorsal region of the caudate nucleus, after appropriate processing, were embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate.

EXPERIMENTAL RESULTS

In normal rats the area of cross section of the nucleus of the small neurons (the chief cell population) was 56.2 ± 0.5 μ^2, and the area of cross section of the body was 83.0 ± 0.8 μ^2. The cell nuclei in the

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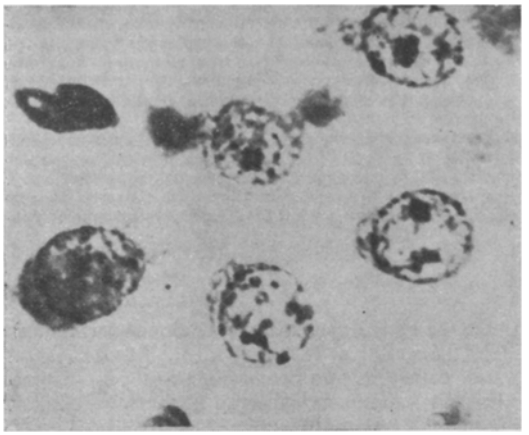

Fig. 1. Swelling of neurons of caudate nucleus, chromatolysis of Nissl's substance, thickening of nuclear membrane, and activation of intranuclear granules 24 h after injection of amphetamine (mg/kg). Nissl's method, 750×.

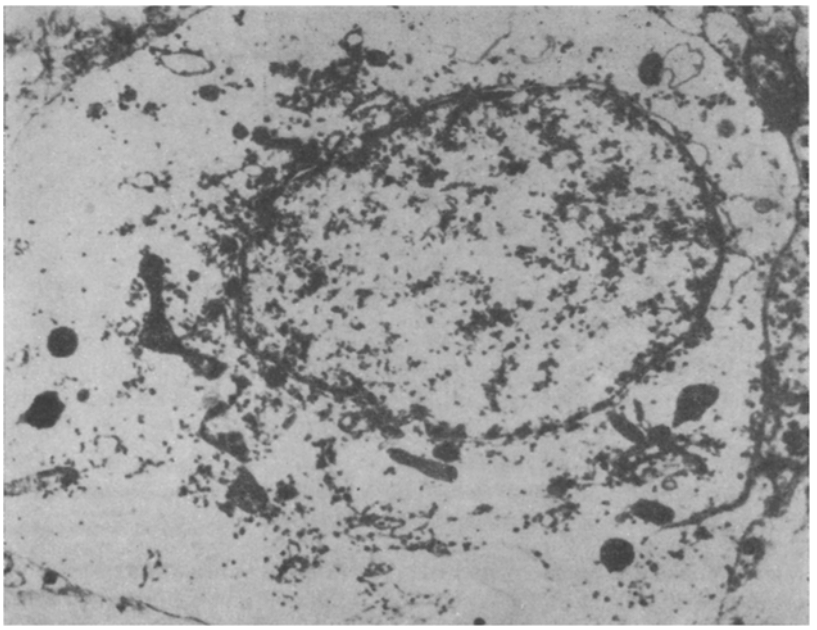

Fig. 2. Ultrastructure of small neuron of caudate nucleus 24 h after amphetamine excitation; 8000×.

caudate nucleus are large, relatively poor in chromatin, and they usually have one dark nucleolus. The nucleus is surrounded by a small border of cytoplasm which stains rather more deeply than the nucleus. Individual neurons show signs of chromatolysis of the Nissl's substance. The dendrites of many neurons are thickly covered with spines, with a mean number of 7.3 ± 0.1 per 10-μ length of dendrite. The distinguishing feature of the submicroscopic structure of the cytoplasm of the small neurons of the normal caudate nucleus is the poor degree of development of the endoplasmic reticulum, which usually consists of a few short and moderately distended cisterns and vacuoles, isolated from one another and lying a considerable distance apart. The Golgi complex incorporates flat cisterns and small and large vacuoles. Most of the vacuoles are situated at its periphery. Ribosomes are most numerous in the cytoplasm; most of them lie freely in the matrix of the cytoplasm as single granules or they are clustered into rosettes. Some ribosomes and polysomes are fixed to the membranes of the endoplasmic reticulum. There are few

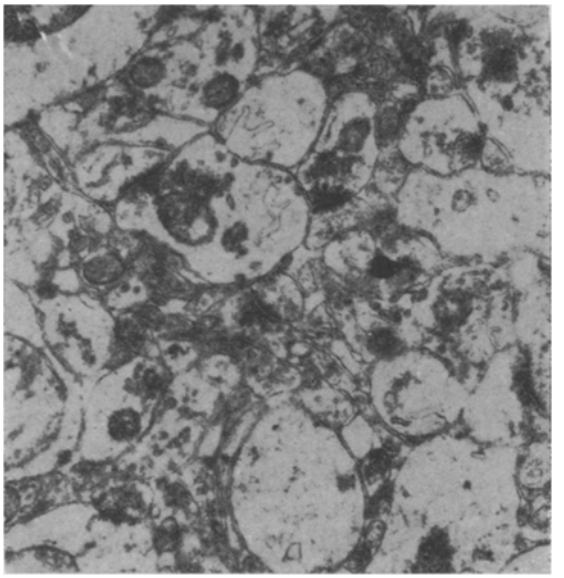

Fig. 3. Ultrastructure of synapses in caudate nucleus 24 h after amphetamine excitation; 20,000×.

mitochondria, most of them are small or medium-sized with densely packed cristae, round or oval in shape, and distributed diffusely throughout the cytoplasm; only individual mitochondria are in close proximity to the cisterns of the endoplasmic reticulum. The nuclei of the nerve cells are even in shape, the nuclear membranes are clearly outlined, and the perinuclear space is narrow.

The caudate nucleus has a thick neuropil in which two types of axon terminals can be distinguished. Most terminals contain small spherical agranular vesicles (400-450 Å), usually in large numbers. The agranular synaptic vesicles in the type II axon cross sections are larger (500-600 Å) and are also spherical in shape. Terminals containing, besides spherical vesicles, elongated or round, granular synaptic vesicles measuring up to 800 Å, are also found among these axons. As a rule the synaptic vesicles in the type II terminals are smaller than in the type I.

The predominant forms of synapses in the caudate nucleus are axo-dendritic synapses and synapses on the dendritic spines, in which the synaptic vesicles are uniformly distributed in the axon terminal or are concentrated near the presynaptic membrane. Most synapses have one active zone of contact measuring 2000-2300 Å, but there are some synapses in which two or even three active zones of contact cover a total distance of up to 4500 Å. A characteristic feature of the caudate nucleus is the presence of synaptic contacts between one axon and two spines or one dendrite and one spine. Dark mitochondria with closely packed cristae are typical of synapses in a state of relative rest. Large, pale mitochondria with fewer cristae, in which the membranous spaces of the inner septa are dilated, are also found in the synapses.

The behavior of the rats 24 h after motor excitation evoked by amphetamine was outwardly indistinguishable from normal. However, significant changes were found in the structure of the neurons and interneuronal synapses in the caudate nucleus. The mean area of cross section of the nuclei ($65.0 \pm 0.7\ \mu^2$) and of the bodies of the neurons ($102 \pm 0.9\ \mu^2$) is higher than in the control animals. Morphological examination showed an increase in size of the intranuclear granules, thickening of the nuclear membrane, and a varied degree of chromatolysis of the Nissl's substance (Fig. 1) in many cells; electron-microscopic examination revealed a decrease in the number of organelles, especially at the periphery of the neuron, which also varied in degree (Fig. 2). The cisterns of the endoplasmic reticulum are dilated and their membranes carry both ribosomes and polysomes. As under normal conditions there are few lysosomes. The mitochondria vary in size, some being small and others large, with a condensed matrix. Local concentrations of mitochondria, some of which are intimately connected with the outer nuclear membrane, are observed. This membrane forms numerous projections of various shapes and sizes into the cytoplasm, and the perinuclear space increases considerably in diameter in the region of these projections. Concentrations of granular material near the inner nuclear membrane are clearly defined.

So far as the structure of the interneuronal connections is concerned, 24 h after administration of amphetamine they numbered 7.4 ± 0.071 per 10-μ length of dendrite compared with 7.3 ± 0.1 in the control animals (the difference is not statistically significant). The spines appear coarser in structure and there are relatively few synapses on them. Electron-microscopic investigation reveals a decrease in the number of synaptic vesicles in the overwhelming majority of axon terminals (Fig. 3), although vesicles are densely packed in some terminals also. Synaptic vesicles in some terminals lie close to the presynaptic membrane while in others most of them are far away from it. In some axon terminals the mitochondria are very swollen and contain few or no cristae. Large membranous inclusions without an internal structure, occupying a large part of the axon terminal, are also seen.

The results of this investigation show that the increase in size of the small cells of the caudate nucleus 24 h after functional stress induced by amphetamine is based on changes in the ultrastructure of the whole series of systems responsible for cell metabolism. Both cytoplasmic and nuclear structures exhibit a high degree of variation in the period of the aftereffects of amphetamine. The functionally determined enlargement of the neurons evidently takes place through a combination of destruction and formation of intracellular structures, including hypertrophy and hyperplasia of individual organelles, the relative intensities of which are varied. This accounts for differences in the intensity of compensatory processes in different cells. The massive formation of lysosomes was not observed in the neurons of the caudate nucleus 24 h after administration of amphetamine, as is seen in the motor cortex of the rat and, in particular, of the monkey [6]; however, mitochondria were found to be concentrated in areas with a high level of organization of the cytoplasmic organelles in these experiments also. The activation of the nuclear structures and contacts between mitochondria and the outer nuclear membrane point to an intensification of exchange processes between the nucleus and cytoplasm. Definite changes also are observed in the ultrastructure of the synapses that can be placed in the category of after-changes distinguished by Aleksandrovskaya et al. [1] when studying changes in RNA in spinal motoneurons after a single functional load.

Changes in the ultrastructure of the synapses and, in particular, the decrease in number of synaptic vesicles could be connected with the increased liberation of mediators. A more likely explanation, however, is that they are due to a decrease in the functional activity of overwhelming majority of synapses, for similar ultrastructural changes in synapses have been observed during dark adaptation in the optic ganglion of insects [3], after picrotoxin convulsions in the tectum opticum of the frog [4], and in the visual cortex of blind rats [2]. There is thus no direct parallel between the restoration of normal behavior and the restoration of the structure of particular nervous centers.

Structural changes in the caudate nucleus 24 h after administration of amphetamine are probably due to the slow elimination of the amphetamine from the body [5]. That is evidently why the noradienalin concentration in the rat brain remains low for a long time after a single injection of amphetamine [10, 14]. The metabolic changes correlate with the functional changes: conditioned-reflex activity of the rat disappears for between 4 h and 2 days after administration of amphetamine, even in a smaller dose [9].

The study of changes in the neurons and interneuronal synapses of the caudate nucleus in the later stages after administration of amphetamine would show how long these morphological changes persist and would provide further details about the mechanisms responsible for restoration of the structures after amphetamine excitation.

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