ELECTRON-AUTORADIOGRAPHIC STUDY OF ADRENALIN-^{3}H DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM

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Interest in mediators of the nervous system has recently increased [3]. Changes in the content of biogenic monoamines and, in particular, of catecholamines have been demonstrated in pathological states and under the influence of various drugs [2, 4, 5]. Original methods of morphological detection of different types of synaptic vesicles have actually been suggested [1]. A special place is occupied by electron-microscopic autoradiography, which is capable of demonstrating the precise incorporation of an exogenous mediator in the fine structures of the neuron that are actively functioning at a given moment.

By making use of the property of neurons of specifically binding exogenous adrenalin [6, 9, 10], in the investigation described below the transport and deposition of adrenalin-7-^{3}H in the cerebral cortex (perikaryon, axon, synapse, dendrite) was studied in rats.

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. Under ether anesthesia, adrenalin-7-^{3}H (specific activity 3.7 Ci/mmole) was injected by stereotaxis into the lateral ventricle. The dose of radioactive mediator (50 μCi) was dissolved in 0.05 ml Ringer's solution. The animals were killed 0.5 h after the injection of adrenalin-^{3}H and pieces of the cerebral cortex were fixed for 2 h in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4. The tissue was then washed with buffer with frequent change of solution and postfixed for 2 h with 1% OsO_4 solution. The specimens were embedded in Epon. To begin with an autoradiographic study was made of semithin sections through the cutaneo-motor area of the cortex, i.e., area PA^m [8]. The motor cortex is regarded as a multisensory system of convergence, which receives information integrated to a certain degree, and completes its processing for subsequent transmission to the pyramidal effector system, i.e., it is the final stage of the afferent pathway. Electron-autoradiographic sections were prepared with type M emulsion as described in [7]. After exposure for 1.5-2 months the sections were developed and studied in the JEM-100B microscope.

EXPERIMENTAL RESULTS

Analysis of the electron-microscopic autoradiographs showed that most grains of silver were located in the neuropil. Glial cells and nuclei of the neurons did not contain the label. Single grains of silver were located above the cytoplasm of the neurons. Axons and dendrites of the nerve cells also were labeled more often with single grains, but the density of the grains above the processes was considerably higher than above the cytoplasm of the neurons, and in some cases two grains could be seen above a cross section through an axon (Fig. 1a). Grains of silver were distributed successively above the presynaptic region, crossing the synaptic space perpendicularly (Fig. 1b), and the label could be clearly differentiated above the postsynaptic part of the axo-dendritic synapse (Fig. 1c). This evidently reflects individual stages of spread of mediator during transmission of the nervous impulse. In most cases the label consisted of a single grain of silver, and this must probably be regarded as natural if the comparatively low specific activity of the adrenalin-^{3}H used in the investigation and the considerable dilution of exogenous mediator by endogenous

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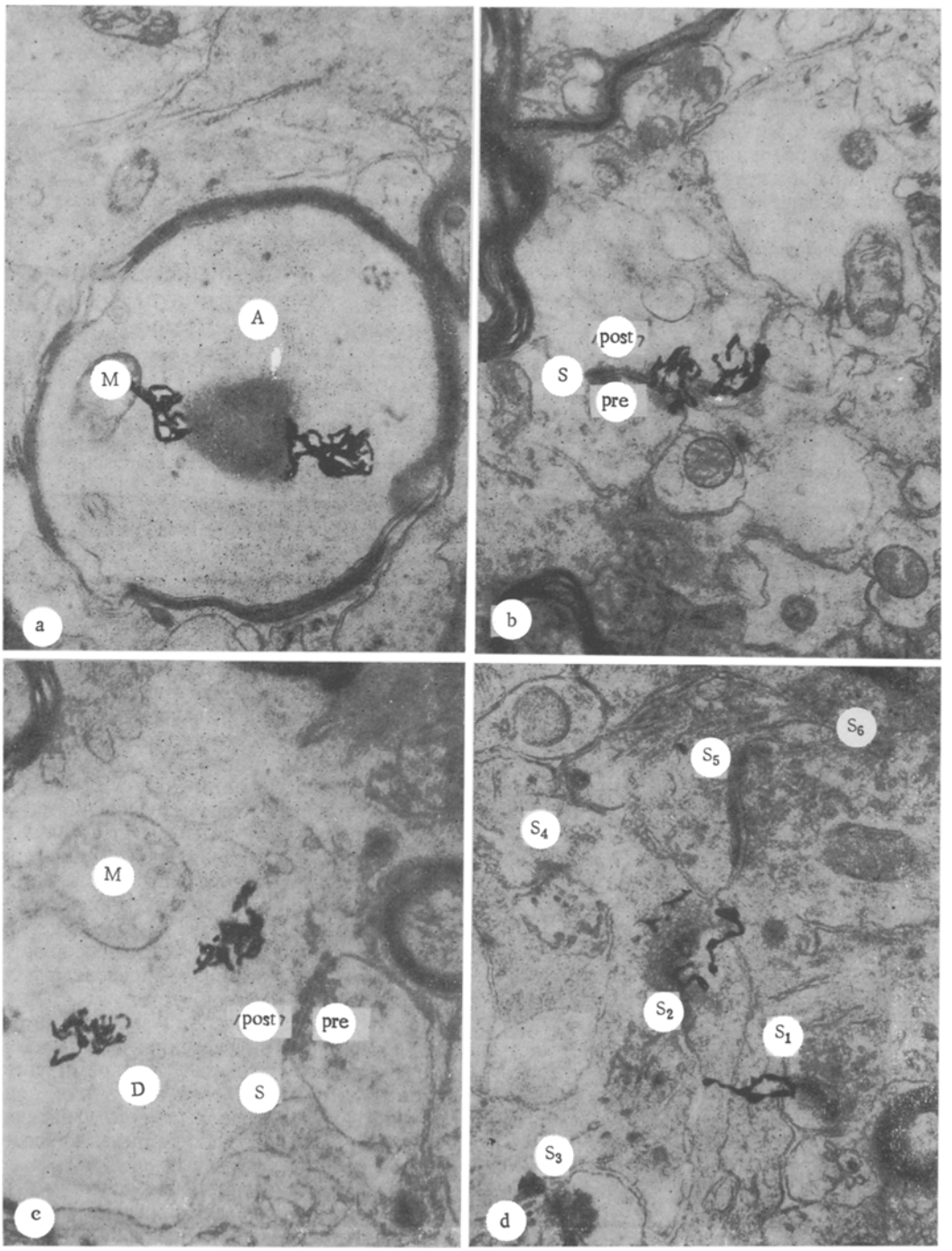

Fig. 1. Distribution of grains of silver above neuropil in rat cerebral cortex, Injection of radioactive mediator adrenalin-7-^{3}H, a) Two grains of silver in myelinated axon (A): b) grains of silver located above synaptic space of axo-dendritic synapse c) dendrite (D) contains several grains of silver; d: S_1 and S_2) axo-dendritic synapses contain exogenous labeled mediator, S_3-S_6) active synapses contain no label, M) Mitochondria, S) axo-dendritic synapse, pre) presynaptic and post) postsynaptic membranes. 60,000×.

are taken into consideration. However, single synapses labeled with several grains of silver also were found under these conditions. The fact that some synapses were labeled with several grains of silver whereas their neighbors contained no label whatever is evidence that the concentration of exogenous mediator in synapses of this group differs sharply (Fig. 1d).

How can this difference in incorporation of exogenous mediator into neighboring synapses be explained? We know that nerve endings in the brain differ in the composition of the mediators they contain. One possible cause of the difference in labeling density may therefore be the presence of a different mediator in the unlabeled synapse, as a result of which the exogenous adrenalin either was not taken up or was not retained.

Yet another explanation can be put forward to explain this unequal labeling of neighboring synapses. A most important principle of the work of the nervous system is unquestionably that excitation and inhibition spread only along strictly defined pathways. In this connection, differences in the intensity of labeling of two neighboring synapses may reflect the fact that only one of them is involved in the process of excitation and, correspondingly, of redistribution of the mediator whereas the neighboring synapse, although not differing in mediator composition, was at rest at the time of the investigation. Finally, yet another explanation of the mechanism of unequal labeling of neighboring synapses may be that an unlabeled but active synapse conducts the nervous impulse on account of residual endogenous adrenalin, whereas the labeled synapse demonstrates incorporation of the exogenous mediator.

The first cause of unequal labeling of neighboring synapses can be abolished by simultaneous injection of several labeled mediators, when it becomes possible to observe in an electron-autoradiograph the functioning of particular parts of the nervous system, such as involvement of a greater or lesser number of synapses in the excitation process and the spread of mediator along the axon–synapse–dendrite sequence. This last mechanism can be excluded by loading the CNS in such a way that maximal utilization of endogenous mediator occurs (shock). Under those conditions the distribution of exogenous mediator in the CNS can be accurately judged.

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