THE INFLUENCE OF ESERINE ON THE CEREBRAL CORTEX OF WHITE RATS

COMMUNICATION II. CHANGES IN THE NEURONES AND IN THE INTERNEURONAL CONNECTIONS OF THE RAT CORTEX INDUCED BY ESERINE

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Our previous communication [6] described the influence of eserine on the conditoned reflex activity of white rats. In the present work we have made an attempt to study the action of eserine on the condition of the neurones and of the interneuronal cortical connections of the rat.

Our work was carried out on the motor and sensor cortices and on the visual cortex, which differs from the other two both morphologically and physiologically. For the topography of these regions we used the maps by Fortuin [14] and Rose [15]; in the motor cortex we studied the outer surface of the hemisphere referred to as f' and Praecentralis granularis, and in the visual cortex, we worked on the central protion.

In these investigations we used Golgi's chrome-silver impregnation, and stained with cresyl violet and with toluidine blue; for the histochemical part of the work we used Brachet's method, and in many cases Feulgen's reaction. Our controls were the neurones from healthy young white rats of the same age.

As a rule, when sodium chloride solution was injected, no noticeable changes in the condition of the neurones or the interneuronal connections in these parts of the cortex was noticed.

When various amounts of eserine were injected, changes in the condition of the neurones and of interneuronal connections were found chiefly in layer V, and in the upper cortical layers; they were more marked in the sensory and motor cortices than in the visual cortex. There was a definite correspondence between the results obtained by staining the sections with toluidine blue and by Brachet's method.

In the motor cortex, after the injection of 0.01 mg per 100 g of eserine, in most cases, besides the cells which were normal in structure and staining properties (Fig. 1,a), a large number stained intensely with basic dyes (they were hyperchromic). Various stages of hyperchromatosis could be seen ranging from cells with a clearly shown intracellular structure to crenated cells. In the initial stage, the Nissl substance in the basal part of the cell body clumped together, and the spaces between the different parts of it were narrowed; in the apical part of the cell the Nissl substance preserved its normal shape and appearance. The intranuclear structure of such cells was maintained, and the nucleolus was either normal or somewhat enlarged. The presence of a large nucleolus and the accumulation of RNA in the cytoplasm indicated that there was apparently an increased synthesis of RNA and, therefore, a greater rate of synthesis of protein than of its breakdown. Besides cells with a clearly shown intracellular structure, there were others which were reduced in size, and hyperchromic, having fine dendrites (Fig. 1,b). The cytoplasm of these cells stained intensely with pironine. When examined with an immersion objective, the nucleus and nucleolus appeared almost completely opaque. In such cells, probably in addition to an accumulation of RNA in the cytoplasm there is also a loss of water and an aggregation of clumps of Nissl substance.

According to D. M. Nasonov and V. Ya. Aleksandrov's theory pf paranecrosis [5], the effect described above is to be interpreted as the initial stage of the denaturation of protein. In the crenated cells, the nucleus cannot be distinguished on the account of dark background of cytoplasm. The body of the cell is either greatly elongated along its long axis, or is irregular. The shape and outline of the nucleus is altered accordingly: it is either extended along the axis, or assumes an irregular outline. In both cases it is smaller than the normal nucleus, and stains

somewhat more strongly in Feulgen's reaction.

After an initial treatment of the sections with ribonculease, which destroys RNA, the cytoplasm of the hyperchromic cells either ceased to stain altogether, or took on a pink shade, which indicates that in additon to RNA, other basophil substances were present in some of the cells.

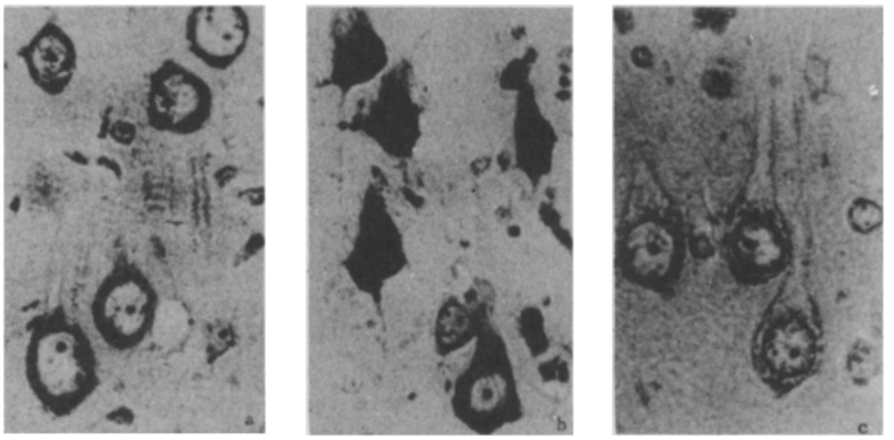

Fig. 1. RNA content of the nerve cells of layer V of the sensory and motor cortex.

We must also note the variability of the reaction of the cortical cells when tested for RNA: besides hyperchromic cells, others are found which are swollen or which are undergoing partial chromatolysis. L. S. Gol'din and V. N. Myasishchev [1] have pointed out a similar variability in the reaction of cells of the motor cortex to intense stimulation [1]. The cause of the variability of the reaction of the cortical cells is probably that at the moment the eserine acts they are in various functional conditions, and therefore react differently to it.

In sections impregnated with silver, the fine structure of the main mass of neurons shows no abnormailty except that occasionally neurones are found with a reduced number of spines on the dendrites, and on some of the neurones the dendrites are varicose.

Thus, 0.01 mg per 100 g of eserine affects cellular metabolism and facilitates the transmission of excitation at synapses without producing any essential change in the structure of the interneuronal connections. Confirmation

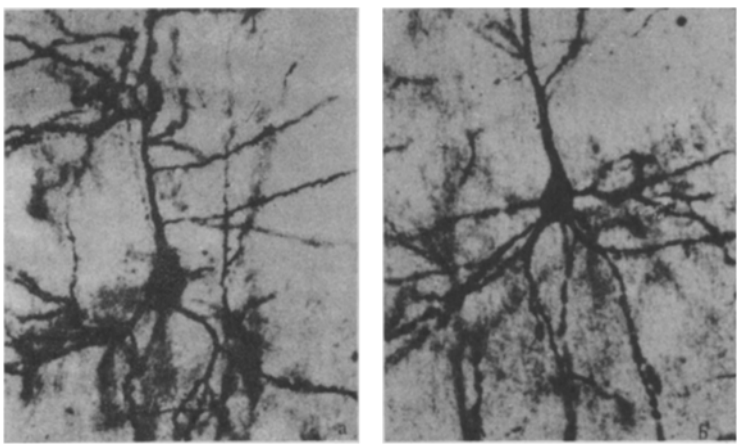

Fig. 2. Pyramidal cell from layer V of the motor cortex. a) In healthy rats; b) after the injection of a convulsive dose of eserine. Golgi's method. Magnification eyepiece 5 x, objective 50 x.

is provided by previous published observations on the influence of the acetylcholine, which accumulates during the action of eserine, on phosphorus metabolism of nervous tissue [12], and the finding of I. I. Ivanov [3], that any weak action on the cortex causes either insignificant changes of the dendrites of the neurones, or none at all.

When the dose of eserine was increased to 0.02 mg per 100 g weight, quite frequently swollen cells were found in the sensory and motor cortex. They had the usual flask shape of cell body, the internal structure was clearly shown, but the dendrites were thickened and appeared at a considerable distance from the cell body. The nucleus and nucleolus showed no particular abnormalities except that cells in which the nucleolus was displaced towards the periphery of the nucleus were found rather more frequently than normally.

With Golgi's silver impregnation, besides unchanged neurones, frequently others could be found whose dendrites were varicosely thickened and had a reduced number of spines. Both quantitatively and qualitatively, neurones were affected by this process to different extents: in some, comparatively few neurones and only occasional dendrites were affected, whereas in others almost all the basal dendrites were deformed. In some of the nerve cells even the apical dendrite was involved.

The structural changes described above occurring in the neurones and dendrites and in the spines of the dendrites affected the neuronal function and the conditioned-reflex activity of the rats.

When a convulsive dose of 0.1 mg per 100 g weight of eserine was given, in some cases, in the sensory and motor cortex of rats killed some time after the convulsion, the main mass of neurons did not differ noticeably from those of normal animals, whereas in other cases (Fig. 1, c), most of the nerve cells were in a condition of chromatolysis (hypochromic cells). All stages could be observed between swollen cells and cells with clear signs of chromatolysis. In the latter case, the cell body had acquired an oval form, the nucleus was elongated transversely, the nucleolus was reduced in size and displaced towards the edge of the nucleus. Dissolution of the Nissl substance had begun and as a rule it started from the outside of the cell body.

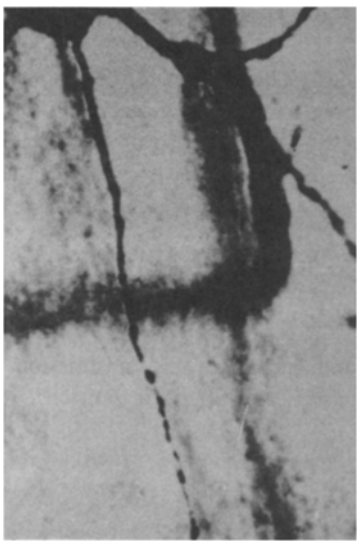

Fig. 3. Axon of a pyramidal cell of layers II + III after the injection of a convulsive dose of eserine. Golgi's method. Magnification: ocular 5x, immersion objective 150x.

Among the hypochromic cells, quite frequently some were encountered which were in a state of transition to shadow-cells.

In all the animals studied, after the injection of a convulsive dose of eserine, there was a more or less marked loss of structure of the interneuronal connections. In the uppermost layers of the cortex (layers II, III, and IV) and in layer V, besides unchanged neurones (Fig. 2, a) there were others in which the basal dendrites were varicose (Fig. 2, b). The number of such neurones and the extent of the change varied in the different animals, and within a single animal. In some neurones, only the terminal protions of the basal dendrites were varicosely thickened, and in others the varicose swellings extended further towards the cell body, the dendrites were more deformed, and in the region of the swellings and thickenings the spines were absent.

When the varicose swelling of the dendrites amounted to a gross deformation, the axon was also involved: near its origin it was normal in appearance, but further from the cell body it became uneven, and thickenings could be seen on it (Fig. 3).

The structural changes in the interneuronal connections caused by convulsive doses of eserine were associated with a marked didturbance of conditioned reflex activity.

Our studies showed that the changes in the interneuronal connections occurred earlier, or at the same time as the changes in the cell body. This result is in line with the findings of S. A. Sarkisov and T. M. Mokhova [9]. However, unlike them we observed most damage not in the upper but in the basal parts of the dendrites.

The reduction in the amount of RNA in the neurones, and the changes in the interneuronal connections due to convulsive doses of eserine appear to depend not so much on the toxic action of the drug as on the development of the convulsion. According to reports, convulsions are associated with chromatolysis and a reduction in the RNA of certain cells [2, 4, 7], and with the deformation of the dendrites [3, 8, 10, 11].

We must note that the sensitivity of different animals to eserine varies. Hyperchromatosis, swelling, and

chromatolysis, are shown more stongly in some animals than in others.

For example, in some animals 0.01 mg per 100 g weight of eserine caused only an insignificant increase in the number of hyperchromic cells of layer V, whereas in other animals such cells form the majority, and not only were those of layer V affected, but so also were cells in higher layers. In some animals, when 0.02 mg per 100 g weight of eserine was injected, besides a swelling of the cells there might also be a well-marked hyperchromatosis.

After the injection of a convulsive dose of eserine, either the structure of most of the neurones differed little from normal, or else most of the cells underwent chromtaolysis. The changes in the interneuronal connections occured various extents in the different animals.

Differences were also found in the degree of spread along the cortex of the structural changes in the neurones and interneuronal connections. The changes could include not only the motor cortex but also other cortical regions; in the visual cortex the changes were less well shown, or absent altogether.

It should be noted that when repeated doses of 0.01 mg of eserine were given, the morphological and cytochemical changes in the neurones were less well shown than after a single injection, probably because of some adaptation to the drug. On the other hand, both we and S. A. Sarkisov and T. M. Mokhova (1958) found that the repeated injection of large doses of eserine causes an enhanced damage to neurones and interneuronal connections. After two injections of a convulsive dose, sclerotic cells could be found in the rat cortex.

The sclerosis showed up as a marked elongation of the cells lengthwise, and in a broadening of the base, The cell body stained deeply with basic dyes, and the fine apical dendrite became wound into a spiral and was seen at some distance from the cell body, and the nucleus was misshapen.

Greater damage was caused to the dendrites and to their spines: they fragmented, and the spines disappeared. The sclerosis of the cortical cells and the structural changes of the interneuronal connections, as manifested by the fragmentation of the dendrites and the disappearance of the spines, appeared to indicate that the morphological changes were irreversible.

Our studies have shown that the morphological changes occurring in the neurones and interneuronal connections in response to the action of various doses of eserine were associated with certain functional cortical changes. What we have called a small dose (0.01 mg per 100 g weight) of eserine caused some changes in the conditioned reflexes and an alteration in the amount of RNA in some of the cells of the sensory and motor cortex, but there was no essential change in the structure of the interneuronal connections. A moderate dose also affected the structure of the interneuronal connections, so that there was interference to the transmission of impulses across synapses and some disturbance of conditioned reflex activity. When given as a convulsive dose, eserine acted primarily on the structure of the interneuronal connections, and in many cases also affected the RNA content of the cells. Then, after the convulsive attack, there is a marked impairment of conditioned reflexes.

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