MORPHOLOGY AND PATHOMORPHOLOGY

CHANGES IN NEURONS OF CERTAIN REGIONS OF THE BRAIN UNDER THE INFLUENCE OF NIVALINE

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The alkaloid nivaline (galanthamine) is rather widely used in the clinic in the treatment of the diseases of the peripheral and central nervous system of various etiology [4, 7, 12, 16, et al].

It has been established [5] that nivaline reduces the activity of cholinesterase and stimulates cholinergic receptors, i.e., it belongs to anticholinesterase substances. The pharmacological characteristic of nivaline has been studied in detail [5, 9].

But there is no single opinion in the problem concerning the mechanism of action of this preparation. Some authors consider that in the central action of nivaline its effect on the reticular formation of the midbrain plays a definite role [2, 6]. The studies of a number of researchers demonstrated a distinct effect of the preparation on the bioelectrical activity of the cerebral cortex [7, 11, 20]. The data are available, according to which nivaline affects both the cerebral cortex and the subcortical formations and reticular formation of the midbrain [3, 8].

In this investigation we attempted to study the changes in the structure of neurons of certain regions of the brain under the effect of nivaline, which will enable us to draw nearer to the solution of the problem concerning the mechanism of its action.

METHOD

The experiments were carried out on 30 white rats and 10 cats. Nivaline was injected subcutaneously and intramuscularly in doses of 1, 6, and 12 mg/kg. The animals were killed 60 min after the injection of the preparation. In the investigation we used the methods of Nissl, and in a number of cases staining by Van Gieson. The control animals were injected with a physiological salt solution. We studied the changes of the neurons in the cortex of the cutaneomotor, auditory, and visual analyzers, in the thalamic nuclei, geniculate bodies, corpora quadrigemina, in the central gray substance of the midbrain, and in certain nuclei of the reticular formation and hypothalamus.

RESULTS

Upon injecting nivaline in a dose of 1 mg/kg the animals became more active, and in certain of them there occurred fibrillations of the head muscles, tremor, spasmodic jerking, which was enhanced under the effect of auditory and tactile stimuli, and minor disorders of vegetative functions. The number of hyperchromatic cells and cells in a state of swelling and chromatolysis in the cortex increased. As a rule, the former was most frequently encountered in the cortex of the cutaneomotor analyzer (Fgi. 1a) and the latter in the cortical regions of the visual (Fig. 1b) and auditory (Fig. 1c) analyzers. The dendrite system of the neurons of the cortex of the cutaneomotor analyzer did not appreciably differ from the control. In the cortex of the visual analyzer we frequently encountered neurons whose dendrites had irregular outlines and a reduced number of branches, whereas, in the cortex of the auditory analyzer we saw neurons with a varicose appearance as the distal segments of the basal dendrites.

In swollen cells in a state of segmental chromatolysis were elicited in the dorsomedial and ventrolateral nuclei of the thalamus more frequently than in the control. The outlines of the dendrites of the neurons were irregular, with few branches; the distal segments of certain dendrites were varicosely thickened and without branches.

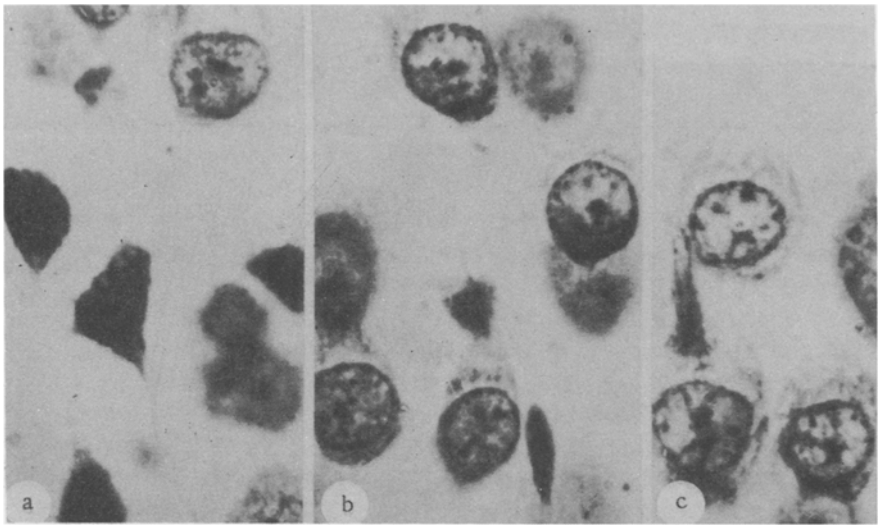

Fig. 1. Neurons of layer III of the cerebral cortex of a rat upon injection of nivaline in a dose of 1 mg/kg. a) Cortex of cutaneomotor analyzer; b) cortex of visual analyzer; c) cortex of auditory analyzer. Staining by Nessl's method. Magnification 750×. (Immersion.)

The number of hypochromatic cells somewhat increased in the medial genticulate body. In the neurons of the corpora quadrigemina we observed, simultaneously with chromatolysis, the phenomena of the disappearance of basophil granules; the distal segments of the dendrites of many neurons were varicosely thickened. In the inferior colliculi we encountered cells resembling shadow cells (Fig. 2a), and the structure of the dendrite apparatus was more appreciably altered (Fig. 2b).

In the neurons of the central gray substance of the midbrain we found: swelling, chromatolysis, vacuolation, sometimes the phenomena of hyperkaryochromia (Fig. 2c). The dendrites of the neurons had irregular outlines, certain of them were varicosely thickened and without branches (Fig. 2d).

The number of hypochromatic and moderately hyperchromatic cells increased in the reticular formation of the brainstem. The small- and medium-sized neurons were mainly subjected to alterations; the hyperchromatic cells were most frequently encountered in the reticular formation of the medulla oblongata (Fig. 3a) and the hypochromatic cells at the level of the midbrain (Fig. 3b). The degree of varicose alteration of the dendrites varied, but the structure of the dendrites of the small neurons, whose varicose swelling extended to all dendrites and over their entire length, was always more severely changed (Fig. 3c).

The changes in the neurons of the hypothalamus were similar to those in the reticular formation of the brainstem, but were always more evident in the anterior and dorsomedial nuclei in comparison with the ventromedial nucleus.

Upon increasing the dose of nivaline to 6 mg/kg, fibrillation of the head muscles and anterior part of the trunk, spastic jerking, and autonomic disorders (increase of respiration, defecation, urination, salivation) occurred in all animals. As a rule hyperchromatic staining and chromatolysis in the cortex were enhanced. In the cortex of the cutaneomotor analyzer the hyperchromatic cells were elicited not only in the upper layers but also in layer V. Along with intense staining of the cell body, swelling of the dendrites was also observed. After silver impregnation many neurons with varicosely modified distal segments of the basal dendrites were found.

The number of modified neurons in the nuclei of the thalamus and in the geniculate bodies increased; there were more hypochromatic cells in the medial geniculate body than in the lateral.

The structure of the neurons of the corpora quadrigemina and the central gray substance of the midbrain was similar to that described above upon injection of nivaline in a dose of 1 mg/kg.

In the reticular formation of the brain stem changes were noted in individual large neurons, the dendrites of which had irregular contours and varicose thickenings in the distal segments.

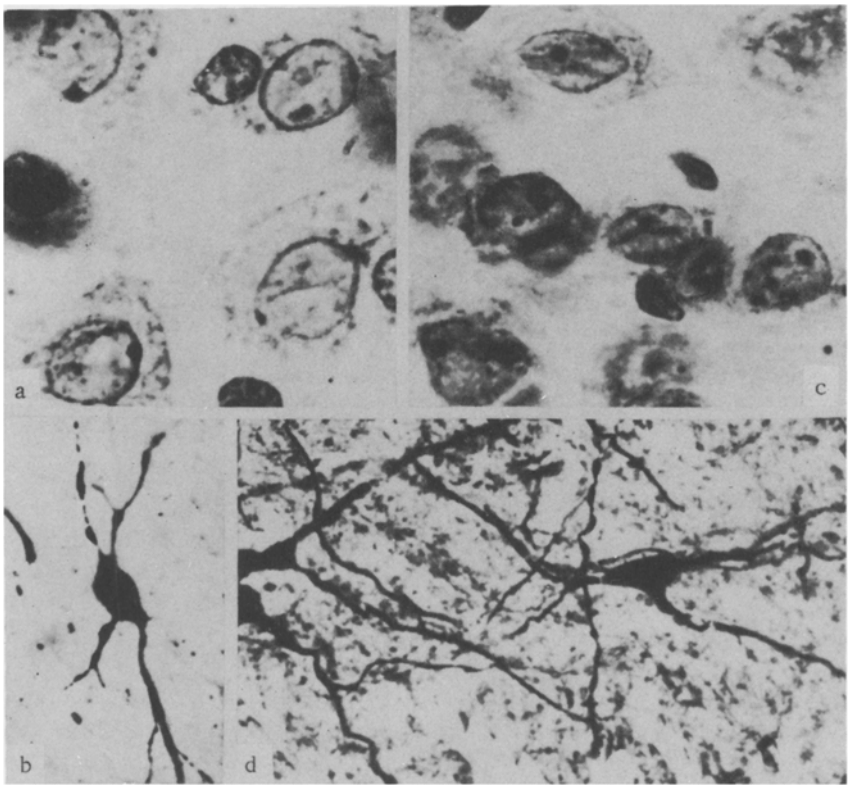

Fig. 2. Structure of neurons of the inferior colliculi and central gray substance of the midbrain upon injection of nivaline in a dose of 1 mg/kg. a, c) Staining by Nissl's method. Magnification 750×; b, d) staining by Golgi's method. Magnification 250 ×.

The number of swollen cells with phenomena of chromatolysis and hyperkaryochromia increased in the hypothalamus and the structure of the dendrites was more severely altered especially in the anterior hypothalamic nucleus where the swelling and thickenings alternated with severely thinned-out sections; the process of varicose swelling reached the perikaryon.

Upon injecting nivaline in a dose of 12 mg/kg strong general excitation of the animals, tonico-clonic spasms, appreciable disorders of the autonomic functions, and subsequently a state of adynamia developed.

The structure of the neurons either resembled that upon injection with 6 mg/kg nivaline, or most cells of the formations studied were in a state of swelling and chromatolysis. In these cases, the structure of not only the basal but also the apical dendrites in the cortex appreciably change.

In cats nivaline caused more evident changes in behavior than in rats. With an intramuscular injection of 6 mg/kg after 15-18 min, and with injection of 12 mg/kg after 7-10 min there developed severe motor excitation, tonico-clonic spasms, impairment in the coordination of movements, and severe disorders of the autonomic nervous system (increase in the respiratory rate, rales, lacrimation, profuse salivation, defecation, urination).

In the morphological investigation we observed in some animals in the upper layers of the cerebral cortex many hyperchromatic cells, whereas in the lower layers we more frequently encountered swollen neurons and individual vacuolated cells. In the cortex of other animals we noted swelling and vacuolation of the cytoplasm of the cells of all layers, which were especially evident in the auditory area of the cortex.

The structural changes of the nuclei of the thalamus were more evident in the cats. A difference was noted in the reaction of the neurons of the geniculate bodies: vacuolation, swelling of the cells, chromatolysis (which was quite considerable) in the medial geniculate body and cells with cytoplasm intensely stained along the periphery or those moderately hyperchromatic made up the ground substance in the lateral geniculate body.

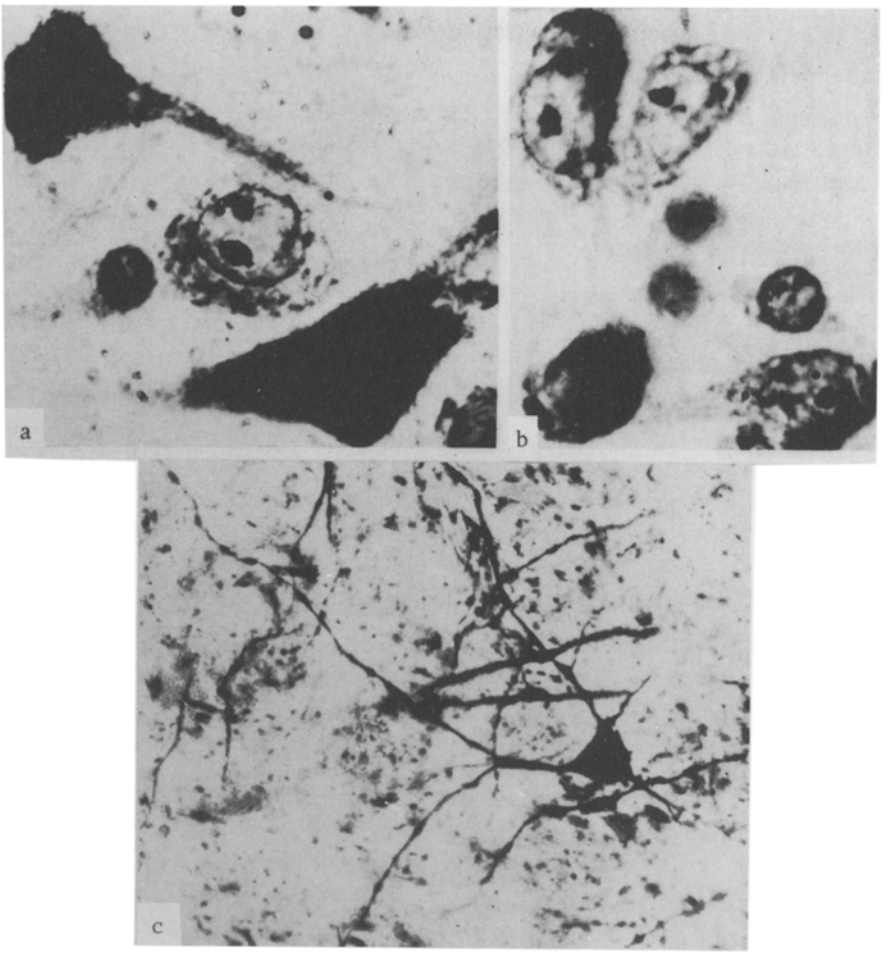

Fig. 3. Structure of the neurons of the reticular formations of the brainstem upon injecting nivaline in a dose of 1 mg/kg. a) Magnocellular nucleus of medulla oblongata; b) cuneate nucleus of midbrain. Staining by Nissl's method. Magnification 750×; c) varicose swelling of dendrites of small neurons of the reticular formation. Staining by Golgi's method. Magnification 250 ×.

In the neurons of the hypothalamus we observed swelling, chromatolysis, and vacuolation of the cytoplasm. In the reticular formation of the brain stem we noted groups of hypo- and hyperchromatic cells. In the latter we were also able to observe swelling of the dendrites. Chromatolysis most frequently had a segmental character. Changes were observed mainly in neurons of small and medium size; large neurons remained almost unchanged. On investigating the brain of cats the phenomena of perivascular and pericellular swelling and pseudoneuronophagia around individual neurons were more significant.

The results of the investigation showed that on injection of nivaline in doses of 1-12 mg/kg, signs characteristic for the action of anticholinesterase substances were noted in the behavior of the animals. Our morphological data indicate that nivaline affects both the cerebral cortex and the subcortical formations and reticular formation of the brain stem.

Disorders of the autonomic functions of the experimental animals were associated in particular with the effect of nivaline on the reticular formation of the brain stem and hypothalamus, where noticeable structural changes of the neurons were noted. We could also observe a definite similarity in the changes of the neurons of the reticular formation and hypothalamus, which, probably, is explained by their morphological similarity [1].

Since disorders of the fine structure of the neurons were elicited at all levels of the reticular formation of the brain stem, it is impossible to agree with the point of view of Rinaldi and Himwich [18], according to which the mesodiencephalic activating system is the specific place of action of cholinergic substances.

It is interesting that in the midbrain itself the morphological changes were elicited not only in the reticular formation but also in the central gray substance and corpora quadrigemina.

The changes of the neurons of the central gray substance can be attributed to the direct effect of nivaline on this formation, but they are apparently connected to a greater degree with the effects from the hypothalamic region which has abundant connections with the central gray substance of the midbrain [15, 19]. As for the corpora quadrigemina, their connections with the cortex of the auditory and visual analyzers and with the reticular formation of the brain stem are well known [13, 17].

Our observed disorders of the cortical neurons under the effect of nivaline can be considered as the result of the direct effect of the preparation on the cortex, for which the presence of cholinoreactive structures is characteristic [11]. This is in agreement with the physiological data indicating the distinctly evident changes of conditioned reflex activity [8, 14] and bioelectrical activity of the cerebral cortex [3, 6, 8, 11, 20] under the effect of nivaline.

When we used neurohistological methods of investigations we were able to demonstrate a difference between the changes arising in the neurons of the cortical regions of a number of analyzers after the injection of nivaline in doses up to 12 mg/kg. If in the cortex of the cutaneomotor analyzer we observed a more or less evident hyperchromatic staining of the cells, then in the visual and especially in the auditory analyzer the processes of swelling, chromatolysis, and varicose changes of the dendrite system of the neurons was appreciably more expressed. The structure of the subcortical regions of the auditory analyzer also changed more profoundly.

The picture of the structural changes of the neurons in various analyzer systems upon the injection of large doses of nivaline revealed similar features, which was apparently caused by severe overexcitation of the nerve cells during spasms and by the development of postconvulsive depression. It should be pointed out that in cats the changes of the neurons were more evident than in the rats, especially in the cerebral cortex and subcortical formations.

It is interesting to note that the structural change of the neurons in the cortex under the effect of nivaline have much in common with those under the effect of eserine, the effect of which on the structure of the neurons, bioelectrical activity of the cortex, and conditioned reflex activity was studied earlier in the investigations of S. A. Sarkisov and associates [9, 10].

SUMMARY

The study was devoted to changes in the neurons of certain parts of the brain in rats and cats under the influence of nivaline. Nivaline was injected subcutaneously and intramuscularly in 1, 6, and 12 mg/kg doses. It was found that nivaline injections in small doses were followed by an increase in the number of hypo- and hyperchromatic cells in the brain cortex and the reticular formation of the brain stem, these cells being represented in various correlations in different parts of the brain cortex.

In the subcortical formations, the central gray matter of the midbrain, and the corpora quadrigemina the changes were mainly manifested by swelling, chromatolysis and vacuolization of the neuron cytoplasm. The changes in the hypothalamus neurons resembled those in the reticular formation of the brain stem.

With an increase in nivaline dosage, changes in the neurons and particularly their dendrite systems increased too. Upon injection of large doses of the preparation the structural changes of neurons in different analyzer systems showed similar features.

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