The morphological observations described above confirm the writers' view [4] that during transmission of the afferent signal the influence of cortical area $S_I$ on the relay neurons of NVP is exhibited directly. Meanwhile, the phasic, facilitatory influence of cortical area $S_{II}$ is manifested indirectly, through other, most probably reticular, structures, for the number of endings of corticofugal fibers arising from cortical area $S_{II}$ is extremely small in that part of NVP where these relay neurons are located in the nucleus.

## LITERATURE CITED


1. Z. V. Eliseeva and R. A. Durinyan, Byull. Éksp. Biol. Med., No. 10, 113 (1975).
2. Z. V. Eliseeva, Tr. Inst. Norm. Patol. Fiziol. Akad. Med. Nauk SSSR, 12, 192 (1969).
3. É. Lang and V. L. Glants, Byull. Éksp. Biol. Med., No. 1, 3 (1975).
4. É. Lang and R. A. Durinyan, Byull. Éksp. Biol. Med., No. 9, 1027 (1976).
5. J. L. Burchfiel and F. H. Duffy, Brain Res., 70, 395 (1974).
6. J. L. De Vito, J. Comp. Neurol., 131, 67 (1967).
7. R. A. Guillery, Z. Zellforsch., 96, 1 (1969).
8. P. Granboulan, in: The Use of Radioautography in Investigating Protein Synthesis, Proceedings (ed. by C. P. Leblond and K. B. Warren), Academic Press, New York (1966), pp. 43-64.
9. E. G. Jones and T. P. Powell, Brain Res., 10, 369 (1968).
10. E. Kawana, Brain Res., 14, 117 (1969).
11. B. M. Kopriwa and C. P. Leblond, J. Histochem. Cytochem., 10, 269 (1962).
12. P. Pasik, T. Pasik, J. Hamori, et al., Exp. Brain Res., 17, 18 (1973).
13. E. S. Reynolds, J. Cell Biol., 17, 208 (1963).


# ULTRASTRUCTURAL MECHANISMS OF SEROTONIN DEMYELINATION


B. A. Saakov, T. A. Khoruzhaya, and É. A. Bardakhch'yan



UDC 616.8-091.934-092:577.175.823



The effect of serotonin on the ultrastructure of the white matter in the CNS of dogs was studied. Intracisternal injection of the amine (6 μg in 0.1 ml physiological saline) led to considerable disturbances in the myelin and glia in regions of the white matter of the spinal cord adjacent to the cerebrospinal fluid channels. Loss of the regular structure and separation of the lamellae of the myelin with rupture and lysis of the myelin sheath and demyelination were observed. Vacuolar degeneration was observed in the oligodendrocytes; the astrocytes were virtually unchanged. After local intracerebral injection of the amine (2 μg in 0.01 ml physiological saline) similar disturbances developed in the white matter of the cerebral hemispheres, but with features of an inflammatory reaction in the late stages of the investigation. In control animals which received injections of physiological saline, changes appeared later and only in the gliocytes. It is concluded that serotonin has the property of injuring myelin and glia.



KEY WORDS: serotonin; ultrastructure of the CNS; myelin; glia; demyelination.


An important role in the pathogenesis of allergic demyelination is played by the intensity and character of the liberation and inactivation of biogenic amines, notably serotonin [2-5]. In the course of experimental allergic encephalomyelitis serotonin has been shown to escape from the brain into the cerebrospinal fluid; on the other hand, intravenous injection of small doses of serotonin into dogs after immunization with encephalito-


Medical Institute, Rostov-on-Don. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.)


Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 606-610, May, 1977. Original article submitted November 12, 1976.

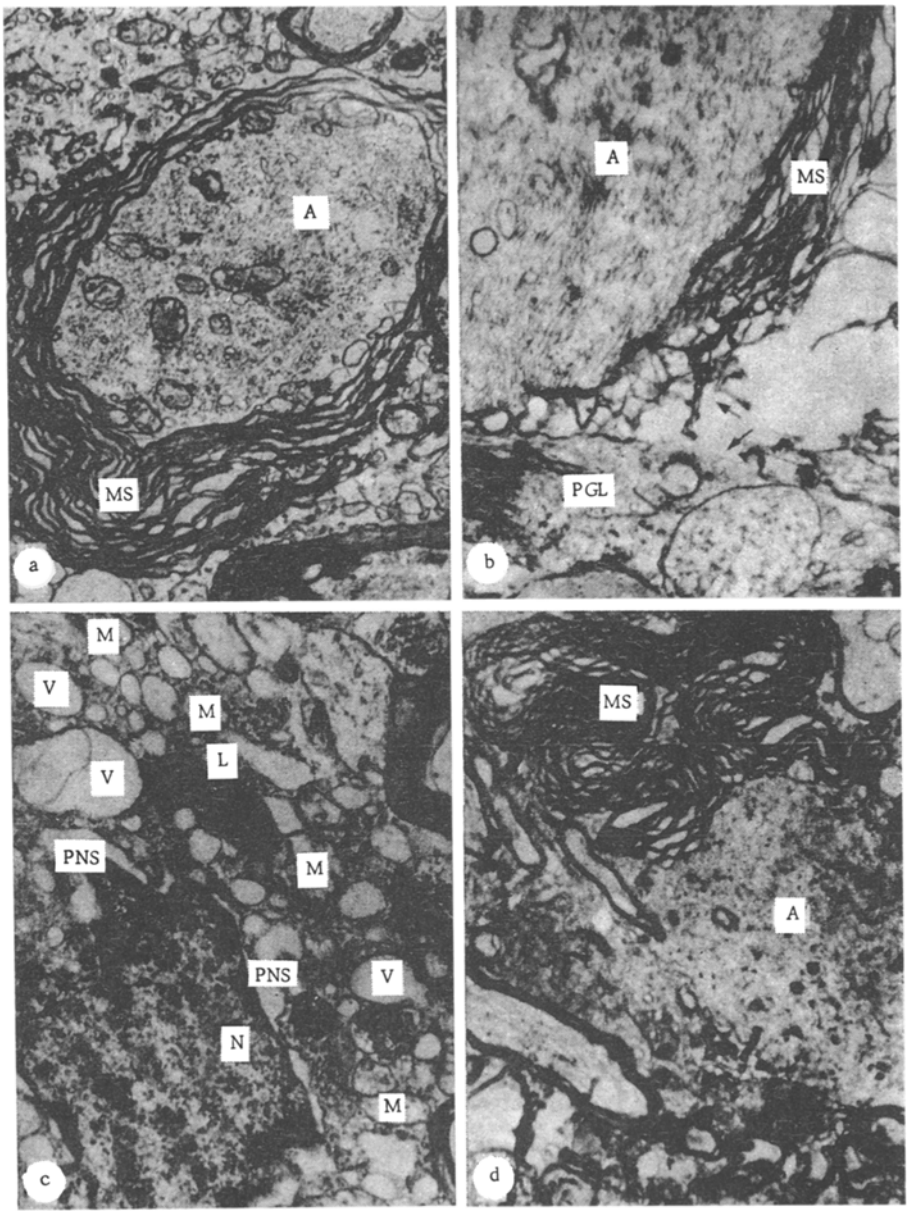


Fig. 1. White matter of spinal cord after intracisternal injection of serotonin: a) separation of lamellae and reduction in thickness of part of myelin sheath, reactive changes in axon (20 min after injection; 12,500×); b) lysis of myelin sheath and plasmalemma of glial cell process (20 min after injection), arrows indicate areas of lysis (14,000×); c) swelling of mitochondria, widening of perinuclear space, and vacuolation of cytoplasm of oligodendrocyte (24 h after injection), secondary lysosomes visible in cytoplasm (14,000×); d) uncovering of axon (24 h after injection; 5000×). Here and in Fig. 2: MS) myelin sheath; MF) myelinated fibers; A) axon; N) nucleus; NOL) nucleus of oligodendrocytes; M) mitochondrion; V) vacuoles; PNS) perinuclear space; PGL) process of gliocyte; MON) monocyte; L) lysis.

genic material potentiates the characteristic disturbances of allergic encephalomyelitis. Even in healthy dogs, destructive changes in the CNS are observed after intravenous injection of serotonin. In small doses serotonin is considered to pass with difficulty through the blood–brain barrier [6]; without appropriate experimental verification, such changes cannot therefore be regarded as the result of the direct action of this amine.

The object of this investigation was to study possible changes in the structures of the CNS following direct contact with serotonin in vivo.

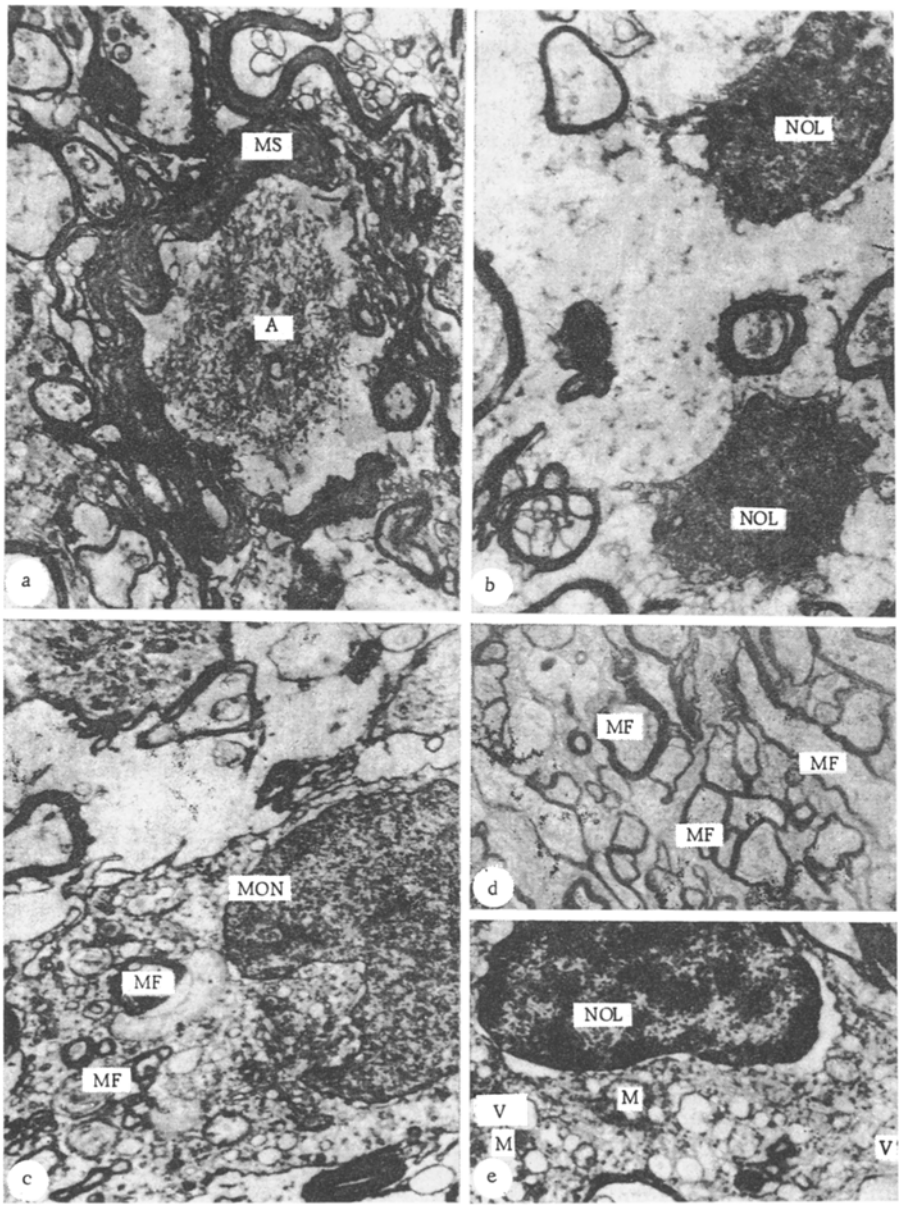


Fig. 2. White matter of cerebral hemispheres after intracerebral injection of serotonin: a) injury to myelin sheath and loss of regularity of lamellae (20 min after injection), destruction of axon (10,000×); b) necrosis of oligodendrocytes and cerebral edema (24 h after injection; 5000×); c) monocyte with phagocytosed myelinated fibers (24 h after injection; 5000×); d) control: group of myelinated fibers 20 min after local injection of physiological saline (3000×); e) control: degenerative changes in oligodendrocyte 24 h after injection of physiological saline (5000×).

## EXPERIMENTAL METHOD

Experiments were carried out on 10 dogs. Serotonin was injected intracisternally (6 μg in 0.1 ml physiological saline) or locally (2 μg in 0.01 ml physiological saline). In the latter case, two burr holes were drilled in the parietal bone under pentobarbital anesthesia. Serotonin was injected into one of them, physiological saline alone into the other (control). The zone of injection was chosen for investigation. After intracisternal injection the material was taken from the upper cervical segments of the spinal cord (from areas adjacent to the cerebrospinal fluid channels). The animals were decapitated 20 min or 24 h after the injection. Pieces of tissue were fixed in 2% osmium tetroxide solution, dehydrated in acetone, and embedded in a mixture of Epon and Araldite. Ultrathin sections, stained with lead citrate and uranyl acetate, were examined in the UÉMV-100K electron microscope.

## EXPERIMENTAL RESULTS

Substantial disturbances in the structure of the myelin and glia were discovered 20 min after intracisternal injection of the amine. Nearly all stages of demyelination, from simple separation of the lamellae of myelin to their complete lysis, could be seen in the myelinated fibers (Fig. 1a, b). Frequently the lamellae were joined together to form separate bands, while neighboring areas showed the characteristic fringed appearance and lysis (Fig. 1b). Of the glial cells, mainly oligodendrocytes were damaged. In their cytoplasm the mitochondria were swollen, increased in volume, and spherical in shape. Their matrix was translucent and the infrequent cristae were fixed to the inner membrane. Some organelles had a dense, homogeneous matrix in which no cristae could be seen. The cisterns of the endoplasmic reticulum were dilated to form large vacuoles. Widening of the perinuclear space was frequently observed. Vacuolar degeneration of the oligodendrocytes was thus an essential feature of the action of serotonin even in the early stages of the investigation. The astrocytes appeared virtually normal under these circumstances.

After 24 h the character of the changes in the oligodendrocytes remained the same (Fig. 1c) but the number of injured cells increased. Often necrotic cells were observed. In this period considerable separation of the myelin lamellae occurred as a prelude to their lysis. In individual myelin sheaths a distinctive vacuolation took place, as the result of a disturbance of the orderliness of arrangement of the lamellae and their separation. Both in the early and later stages of the experiments rupture of the sheath was observed, after which it slid from the axon (Fig. 1d), so that bare axons began to appear in the field of vision.

Ultrastructural changes detectable after intracerebral injection of serotonin were similar to some extent to those described above. A characteristic feature of injury to the myelin sheath 20 min after the injection was a much closer arrangement of the individual lamellae, so that it was impossible to detect their regular pattern even under high power (Fig. 2a). Axons appearing like half-moons because of deformation of their myelin sheath were often seen. In some cases the fibers were so deformed that they ruptured and the contents of the axoplasm spread outside the bounds of the axon itself. Sometimes edema was observed. The glial cells appeared unchanged.

After 24 h the degree of injury to the myelin increased and significant changes also appeared in the glial cells, leading in some cases to lysis of the plasmalemma and necrosis (Fig. 2b). At this stage signs of an inflammatory reaction appeared. The emigrating monocytes exhibited definite myeloaggression (Fig. 2c); neutrophils surrounded by a zone of edema also were often seen.

In the control region of the brain into which physiological saline was injected, no changes were observed in the glia or myelin in the early stages (Fig. 2d). In the late period disturbances were found in the gliocytes: Signs of vacuolar degeneration were observed in some of them (Fig. 2e).

Irrespective of the mode of injection of serotonin, ultrastructural changes were thus observed in the myelin and oligodendrocytes. They appeared within 20 min after injection and gradually increased in severity toward the end of the first day.

The direct effect of serotonin on myelin is evidently to cause labilization of its molecules in the membrane; this is the beginning of its destruction and it is manifested as increased density of the myelin sheaths and the loss of their regular structure. Next follows separation of the lamellae, and in some cases this attains such a degree that the myelin sheath is fragmented. The next stage is lysis of the myelin. Another type of injury also is frequently seen: rupture of the membrane, so that it slides away from the axon.

If Carnegie's hypothesis [7, 8] of the possible receptor role of the basic protein of myelin in relation to serotonin is taken as the starting point, the injurious effect of serotonin can easily be explained by blocking of the corresponding regions of myelin and subsequent labilization of the structure. Injury to the glial cells can be associated with the presence of similar determinants in them. Another explanation could be based on a possible effect of serotonin on ionic interactions [1], which determine the stability of the myelin sheaths. The inflammatory reaction develops after destruction of the myelin and it cannot therefore be regarded as a cause of the destructive changes.

Together with the known properties of serotonin, responsible for this mediator function and regulatory action on the CNS, this amine may also have a harmful action on the brain. Depending on the location of the injuries and on their quantitative characteristics, and also on the reactivity of the organism as a whole, these injuries may become a trigger factor or a factor aggravating the course of the pathological process. The pathogenetic mechanisms of several nervous and mental diseases, especially those for which changes in the endogenous level of the biogenic amines and their synthesis and metabolism are known to exist, must be examined from the standpoint of a possible role of serotonin.

## LITERATURE CITED

1. M. D. Kurskii and N. S. Baksheev, The Biochemical Bases of the Mechanism of Action of Serotonin [in Russian], Kiev (1974).
2. B. A. Saakov, T. A. Khoruzhaya, and É. A. Bardakhch'yan, in: Mechanisms of Some Pathological Processes [in Russian], Vol. 6, Rostov-on-Don (1975), p. 217.
3. T. A. Khoruzhaya, Vopr. Med. Khim., No. 1, 98 (1975).
4. T. A. Khoruzhaya, É. A. Bardakhch'yan and B. A. Saakov, in: Current Problems in Immunology and Immunopathology [in Russian], Rostov-on-Don (1975), p. 110.
5. T. A. Khoruzhaya and B. A. Saakov, Byull. Éksp. Biol. Med., No. 6, 80 (1975).
6. M. Bulat and L. Zupek, Nature, 219, 72 (1968).
7. P. R. Carnegie, Nature, 229, 25 (1971).
8. V. Lennon and P. R. Carnegie, Lancet, 1, 630 (1971).


# RESPONSE OF CARDIOMYOCYTES OF THE RIGHT HEART TO TRAUMA OF THE LEFT


G. B. Bol'shakova



UDC 616.122-001-092.9-07:616.123-018.1-076.5



A myocardial infarct in the left ventricle was produced in adult rats weighing 120-160 g by ligation of the left coronary artery; the left atrium was injured; or a mock operation was performed and the pericardium was removed. On the fifth day after the operation dividing myocytes were found in the right atrium (mitotic index 0.7-8.8‰) and in the subepicardial zone of the right ventricle (mitotic index 0.8-2.9‰). In old rats weighing 300-430 g, on the third day after the various types of injury to the myocardium, mitotic activity was found in the myocytes of the left auricle (1-5.1‰), and in one of eight cases in the right auricle (4.2‰); single mitoses also were found in the subepicardial zone of the left ventricle.



KEY WORDS: myocardial infarct; division of cardiomyocytes; right heart.


This investigation is a continuation of previous work [2] which showed that in response to injury to the left heart (infarct of the ventricle, trauma to the atrium) and, in some cases, in response to a mock operation also, certain cardiomyocytes in the left atrium and the supepicardial zone of the left ventricle start to divide by mitosis. With these observations in mind it seemed important to study the response of the right, intact side of the same heart. There is evidence in the literature of an increase in the number of myocytes of the rat atrium synthesizing DNA in infarction of the left ventricle [4], but no quantitative characteristics of their mitotic activity are given in the paper cited.

The object of this investigation was to study the level of proliferation of the myocytes of the right heart after trauma to the left heart.

## EXPERIMENTAL METHOD

An infarct of the myocardium of the left ventricle was produced in adult noninbred albino rats weighing 120-160 g, the left atrium was injured, or a mock operation was performed with removal of the pericardium only. From the 49 rats 29 were chosen, in which mitoses were comparatively numerous in the left heart in


Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow.


(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 610-612, May, 1977. Original article submitted November 3, 1976.