HISTOCHEMICAL STUDY OF THE SULFHYDRYL GROUPS OF TISSUE PROTEINS IN THE SUPERIOR CERVICAL SYMPATHETIC GANGLION OF THE CAT IN STATES OF REST AND EXCITATION

I. B. Krasnov

Laboratory of Histochemistry (Head – Professor V. V. Portugalov),
Brain Institute (Director – Active Member AMN SSSR Professor
S. A. Sarkisov) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR I. N. Filimonov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 10,
pp. 50-54, October, 1963
Original article submitted June 22, 1962

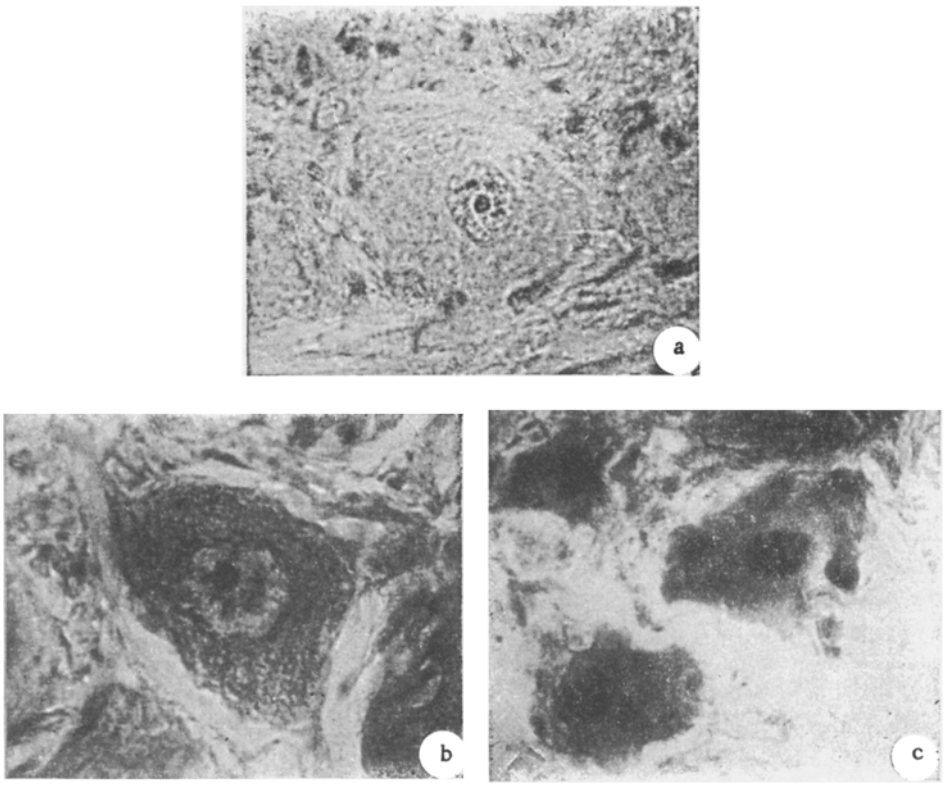
The results of biochemical and cytological investigations [8, 9, 12a, 20] have shown that the change in the functional state of nerve tissue from relative rest to excitation leads to a significant rise of the rate of protein metabolism and to a change in the structure of the protein molecules, similar to the process of reversible denaturation of protein and accompanied by an increase in the number of detectable protein reactive groups (sulfhydryl, etc.). The increase in the number of detectable SH-groups has been used as a test for these denaturation-like changes [20]. The increase in the number of SH-groups in nerve tissue proteins during excitation has been demonstrated by biochemical methods [20], and as yet no confirmation has been adduced by histochemical methods. Yet the use of a histochemical method would enable the intracellular localization of the denaturation-like changes in the proteins of the nerve cell during excitation to be revealed in accordance with the changes in the number of SH-groups.

The object of the present investigation was to study the changes in the concentration of protein SH-groups detectable histochemically in the nerve cells of the superior cervical sympathetic ganglion of the cat in states of rest and excitation. To ensure a more accurate indication of the changes in concentration of SH-groups it was decided to introduce the histochemical sulfhydryl reagent directly into the fluid used for perfusing the ganglion during both rest and excitation. The histochemical reagent used to detect SH-groups was 5-bromoacetyl-3-nitrobenzoic acid (BNB) [5, 6].

EXPERIMENTAL METHOD

Experimental perfusion of the superior cervical sympathetic ganglion was performed on the decerebrate cat. The blood vessels supplying the ganglion were isolated as described by K. M. Bykov and A. M. Pavlova [3]. To cause excitation of the nerve cells of the ganglion, the preganglionic segment of the sympathetic nerve was divided before perfusion and its central portion stimulated electrically (frequency 10 pulses/sec, pulse duration 10 millisec, voltage 3-4 V), by current from a pulse generator type ÉS-4M. The functional state of the neurons of the ganglion was recorded by tracing the contractions of the nictitating membrane on a kymograph.

Twenty minutes after the beginning of stimulation, without interrupting it the natural blood supply of the ganglion was replaced by perfusion. The perfusion fluid was Tyrode's solution, augmented with Na_2HPO_4, forming with the NaH_2PO_4 a buffer system of pH = 7.4 and of capacity adequate to prevent a change in the pH of the solution to the acid side as a result of the liberation of HBr in the course of the reaction between BNB and the SH-groups. The perfusion fluid containing BNB in a final concentration of 0.0005 M was supplied to the perfusion system at a temperature of 38° and a pressure of 120-130 mm. Perfusion continued without interruption of stimulation of the sympathetic nerve for 35 min, after which the perfusion fluid containing BNB was flushed from the ganglion with similar fluid not containing BNB. After being flushed, the ganglion was excised, and sections cut to a thickness of 20 μ in a cryostat.



Distribution of SH-groups of proteins in a neuron of the superior cervical sympathetic ganglion of the cat. a) State of rest; b, c) state of excitation. Objective 90 ×, ocular Gomal' VI.

The sections were fixed in 10% neutral formalin solution, washed free from BNB adsorbed by the tissue with ethanol, and the nitro group of the BNB reduced to an amino group, the amino group diazotized, and an azo-compound formed with 1-amino-8-naphthol-3,6-disulfonic acid, in the conditions described by Maddy [19].

The method of performing the experiment to determine the histochemical picture of the resting ganglion was the same, excluding the stimulation of the preganglionic trunk of the sympathetic nerve by the electric current.

The histochemical study of the protein content of the neurons of the ganglion in states of rest and excitation in decerebrate cats was preceded by an operation to gain access to the superior cervical sympathetic ganglia. The preganglionic trunk of the sympathetic nerve was divided bilaterally, and the central end of one of these nerves was stimulated for 20 min with an electric current (frequency 10 pulses/sec, voltage 3-4 V, pulse duration 10 millisec). The state of excitation of the ganglion cells was recorded by tracing the contractions of the nictitating membrane kymographically. The contralateral ganglion was used to study the state of rest. At the end of stimulation the ganglion was excised, fixed for 2 h in a 3:1 mixture of ethanol and glacial acetic acid, and embedded in paraffin wax. Protein was demonstrated histochemically by Geyer's method [16], using amido black 10 B as stain.

EXPERIMENTAL RESULTS

The intracellular distribution of protein SH-groups, as revealed histochemically in sections of the ganglion after perfusion in a resting state (see figure, a) was characterized by a comparatively high concentration of these groups in the nuclei of the neurons, where proteins rich in SH-groups were uniformly distributed in the form of large masses. The highest concentration of SH-groups, however, was found in the nucleolus. Far fewer SH-groups were detected in the cytoplasm than in the nucleus; the proteins containing SH-groups were distributed uniformly throughout the cell body in the form of small granules. This distribution of SH-groups in the neurons coincides with their localization as described in sections of fresh, unfixed ganglion tissue [5].

In sections of the sympathetic ganglion subjected to electrical stimulation (see figure, b and c), the cytoplasm of the overwhelming majority of neurons was sharply differentiated by the greater intensity of its histochemical

reaction compared with the neurons of the ganglion in a resting state, indicating an increase in the content of detectable SH-groups in the cytoplasm of the neuron during excitation. The nuclei of these nerve cells could be subdivided into two types: one was characterized by a homogeneous distribution of proteins with a high content of SH-groups (see figure, b), while in the nuclei of the other type the proteins rich in SH-groups were localized in the nucleolus, the chromatin granules, and the nuclear membrane (see figure, c).

The study of the protein distribution in sections of the ganglion in a state of rest showed that the proteins were distributed uniformly in the cytoplasm of the neurons in the form of tiny granules of or a homogeneously stained mass. In the nucleus they were situated in the nuclear membrane, nucleolus, and chromatin granules. The protein concentration in the nuclear structures was much higher than in the cytoplasm. It should be noted that the protein concentration in the bodies of the neurons was slightly less than in the intercellular structures. The nuclei of the capsular neuroglia possessed a comparatively high protein concentration.

After electrical stimulation of the ganglion the distribution and concentration of histochemically detectable protein in the neurons and intercellular structures were practically unchanged from those observed in the resting state. The only difference observable was a slightly higher protein concentration in the nuclei of the capsular neuroglia.

The increase in the concentration of histochemically detectable SH-groups in the cytoplasm of the neurons of the excited sympathetic ganglion could hardly be regarded as the result of an increase in the concentration of free cystein and SH-glutathione in the cytoplasm. In the conditions in which the reaction was carried out in the histological section, the possibility of an increase in the intensity of the histochemical reaction on account of mercaptans of low molecular weight was extremely small because these had been flushed out of the tissue sections. The total protein concentration in the neurons of the ganglion during excitation, as these results show, remained at the same level as during rest. The increase in the concentration of protein SH-groups in the neuron during excitation must, therefore, be interpreted as the results of denaturation-like changes in the structure of the cytoplasmic proteins, similar to those described in paranecrosis by D. N. Nasonov and his school.

The increase in the concentration of protein SH-groups discovered in these experiments was observed relatively uniformly throughout the cytoplasm of the neuron, indicating changes in the structure of the proteins in all parts of the neuron and confirming the findings of D. N. Nasonov [8] and of other authors [4, 11, 12] indicating that the whole cytoplasm of the excited nerve cell is involved in the process of modification of its physico-chemical state.

It was stated above that excited neurons with a high concentration of SH-groups in the cytoplasm contain a much higher concentration of protein SH-groups in their nucleus than is found in the nuclei of cells in a state of rest. These observations may also be interpreted as the result of denaturation changes in the nuclear proteins. There are reports in the literature of a sharp increase in the concentration of SH-groups in the nuclear proteins during denaturation brought to light by both biochemical [15] and histochemical [5, 10] investigations. It is apparently explained by the presence of a high proportion of "masked" SH-groups [15] in the nucleoproteins of the nucleus.

Following Bell's claims [13, 14] that the SH-groups are the connecting link between proteins and nucleic acids in nucleoproteins, it may be postulated that a proportion of the total number of SH-groups detected in the nuclei of the neurons of the sympathetic ganglion after electrical stimulation consists of protein SH-groups freed from their bond with RNA as a result of the decomposition and utilization of the latter as one of the sources of energy during excitation of the cell [1].

The change in the physico-chemical state of the neuroplasm of the neuron during excitation – the change from sol to gel – and the increase in the number of detectable protein SH-groups are evidently not simply parallel processes, but are closely interconnected and mutually dependent. As Huggins and co-workers [17, 18] have shown, the SH-groups of the globular proteins play an important role in the process of modification of the aggregate state of protein solutions and in the change from sol to gel, by forming disulfide intermolecular bonds as a result of oxidation and thereby facilitating the aggregation of protein molecules. Similar results were obtained by I. M. Bulankin and co-workers [2] when studying the formation of alkaline protein gels. Huggins and co-workers [17] also described the gelatinization of protein solutions after the addition of mercaptans of low molecular weight, destroying the three-dimensional configuration of the protein molecule by breaking the intramolecular disulfide bonds. Subsequent aggregation of the protein molecules occurs as a result of the formation of hydrogen bonds and is accompanied by an increase in the number of detectable SH-groups. These processes probably also occur in the cytoplasm of the excited neuron, where the changes in the structure of the protein molecules involve the demasking of SH-groups.

It should be noted that these histochemical findings agree with the results of the amperometric titration of SH-groups in a tissue extract of the superior cervical sympathetic ganglion after electrical stimulation [7].

SUMMARY

Histochemical demonstration of the protein SH-groups in the neurons of the superior cervical sympathetic ganglia of cat in vivo was carried out by perfusing the ganglia with fluid, containing 5-bromoacetyl-3-nitrobenzoic acid. Distribution of protein SH-groups in the neurons detected during perfusion of the ganglion at rest did not differ from that in the same neutrons of fresh tissue sections studied in vitro. During perfusion of the excited sympathetic ganglion an increased concentration of protein SH-groups was detected histochemically; this pointed to the presence of denaturation-like changes in the protein structures of the nerve cells during excitation.

LITERATURE CITED

1. V. Ya. Brodskii, in book: Lectures at the First Conference on Problems in Cyto- and Histochemistry [in Russian], p. 7. Moscow (1960).
2. I. M. Bulankin, E. V. Parina, R. I. Agranovich , et al., Ukr. biokhim. zh., 3, 307 (1961).
3. K. M. Bykov and A. M. Pavlova, in book: Collection to Celebrate Academician I. P. Pavlov's 75th Birthday [in Russian], p. 413. Leningrad (1924).
4. A. A. Vishnevskii and B. I. Lavrent'ev, Byull. éksper. biol. 8, 6 502 (1939).
5. I. B. Krasnov, Tsitologiya, 5, 608 (1961).
6. I. B. Krasnov, Zh. obshchei khimii, 1, 293 (1962).
7. I. B. Krasnov, Byull. éksper. biol., 6, 105 (1962).
8. D. N. Nasonov, Tsitologiya, 6, 605 (1959).
9. A. V. Palladin and G. E. Vladimirov, in book: Application of Isotopes to Technology, Biology, and Agriculture [in Russian], p. 227. Moscow (1955).
10. V. V. Portugalov, I. B. Krasnov, and T. V. Ball', in book: Abstracts of Sectional Proceedings of the Fifth International Biochemical Congress [in Russian], Vol. 1, p. 399. Moscow (1961).
11. N. A. Smitten, Izv. Akad. Nauk SSSR. Otdelenie biol. nauk, 3, 257 (1945).
12. S. S. Stepanova and E. M. Krokhina, Arkh. biol. nauk 61, 2, 107 (1941).
12a. R. Vrba, Uspekhi sovr. biol. 41, 3, 321 (1956).
13. L. Bell, Nature, Vol. 182, p. 1088 (1958).
14. L. Bell, Exp. Cell Res., Vol. 16, p. 615 (1959).
15. H. Ernst and U. Hagen, Z. Naturforsch., Bd. 14b, s. 104 (1959).
16. G. Geyer, Acta histochem. (Jena), Bd. 10, s. 286 (1960).
17. Ch. Huggins , D. Tapley, and E. Jensen, Proc. nat. Acad. Sci. (Wash.), Vol. 36, p. 695 (1950).
18. Ch. Huggins, D. Tapley, and E. Jensen, Nature, Vol. 167, p. 592 (1951).
19. A. Maddy, Exp. Cell. Res., Vol. 22, p. 169 (1961).
20. D. Ungar, Tsitologiya, 6, 627 (1959).