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

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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 meta-
obolism 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 de-
tectable 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 5S-4M. The functional state of the neurons of the ganglion was re-
corded 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 gang-
lion was replaced by perfusion. The perfusion fluid was Tyrode's solution, augmented with Na₂HPO₄, forming with
the NaH₂PO₄ 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 0.0065 M was supplied to the perfusion system at a tem-
perature of 38° and a pressure of 120-130 mm. Perfusion continued without interruption of stimulation of the sympa-
thetic 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.
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