MORPHOLOGY AND PATHOMORPHOLOGY

SUCCINIC DEHYDROGENASE ACTIVITY AND SH-GROUP CONTENT IN CAT SUPERIOR CERVICAL SYMPATHETIC GANGLION IN REST AND EXCITATION

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Many investigators are directing their attention to problems of functional histochemistry and biochemistry in the nervous system. Histochemical and biochemical investigations [2, 3] have shown that electrical stimulation of the sciatic nerve in the rat produces changes in succinic dehydrogenase, cytochromoxidase and NAD-diaphorase activities,and SH-group content of the motor cortex. It was, therefore, of some interest to carry out examinations under more truly physiological conditions in combination with physiological examination of the functional state of the nerve structures under investigation.

This paper reports the results of quantitative biochemical determinations of succinic dehydrogenase activity and SH-group content and of histochemical examination of succinic dehydrogenase activity in neurons of the superior cervical sympathetic ganglion in the cat, in the resting state and in excitation.

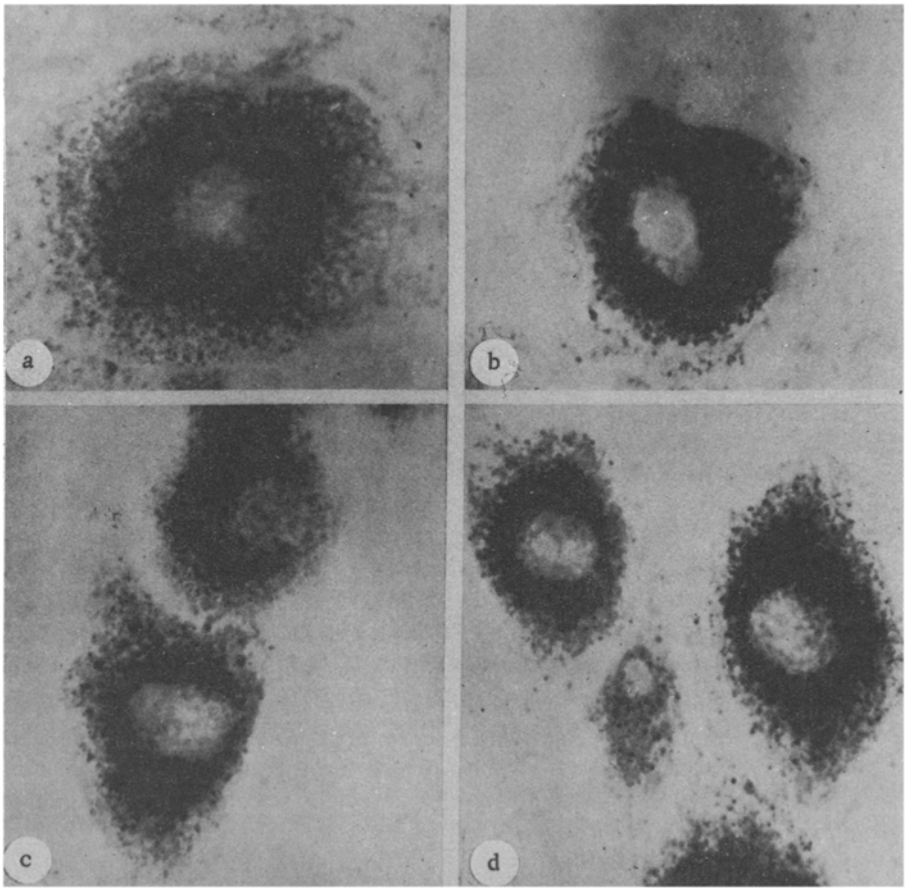
METHOD

The superior cervical ganglia were exposed and the preganglionic sympathetic trunks were divided in decerebrate cats of both sexes, weighing from 1.5 to 3.0 kg. The central segment on one side was stimulated electrically (10 c/s, 3-4 V, pulse length 10 msec). The functional state of the neurons in the ganglion was determined from kymographic records of the contractions of the third eyelid. The sympathetic ganglion on the opposite side was used as a control for study of the resting state.

The ganglion was excised at the end of stimulation, frozen with carbon dioxide snow, and cut into 20 μ sections in a cryostat for histochemical demonstration of succinic dehydrogenase activity. The method employed for this was that of Pearse [6] with 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTH). The sections were incubated in a medium containing saccharose in a final concentration of 0.44 M.

In preparation for quantitative biochemical estimation of succinic dehydrogenase activity and of sulfhydryl groups, the excised ganglion was rapidly freed from its connective tissue membrane, weighed in a torsion balance and homogenized in a small quantity of Ringer's solution at 2-4°C. A quantity of the homogenate was then added to Ringer's solution to give a concentration of 20 mg fresh tissue in 1 ml. 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) was used as hydrogen acceptor in the estimation of succinic dehydrogenase activity. The incubation medium consisted of 0.25 ml 0.5 M solution of sodium succinate in 0.1 M phosphate buffer (pH 7.6), 0.25 ml freshly prepared aqueous solution of INT (3 mg/ml) and 0.25 homogenate containing 5 mg tissue. Incubation was at 37°C for 20 min. The formazan formed during incubation was extracted with 1.25 ml of a 1:1 mixture of ethyl acetate and ethanol. The otpical density of the extract was determined in a photoelectrocolorimeter with green light filter. Parallel tests were made without the addition of succinate to the material incubated to enable endogenous activity to be determined. Results were expressed in micrograms formazan formed from INT by enzymatic reduction per microgram fresh tissue. A reference standard curve was established by nonspecific reduction of INT with ascorbic acid.

For estimation of SH-groups in the extract of ganglion tissue, a fraction of homogenate was placed in a water bath and kept at 38°C for 15 min, centrifuged at 3000 rpm, 20.3 μl of the supernatant fluid then being taken for estimation of SH-groups. Part of this fluid was first dialyzed at 2-4°C against 25 volumes of Ringer's solution containing



Distribution of succinic dehydrogenase in nerve cells of superior cervical sympathetic ganglion of cat, in rest (a, b) and after stimulation of preganglionic nerve (c, d). (5 V, 30 min.) Pearse method with MTT.

$3 \cdot 10^{-5}$ EDTA. The method of estimation employed was amperometric silver titration in tris-buffer [1]. The results were expressed in millimicromoles of SH-groups per milligram fresh tissue. Thirty-two cats were used in the experiments. Results were processed statistically.

RESULTS

Succinic dehydrogenase activity was assessed on the size and number of formazan granules deposited in tissue structures. In the neurons of the resting sympathetic ganglion formazan granules were seen only in the cytoplasm of the soma. The granules were generally all of about the same size (0.5-1.0 μ), with only a few perhaps larger. Electrical (3-4 V) stimulation of the preganglionic nerve for 20 min resulted in considerable increase in the number of large formazan granules. Stronger (5-7 V) and more prolonged (30-40 min) stimulation increased still more the number of large formazan granules in the bodies of neurons situated in the lower third of the ganglion. In the main mass of neurons, however, the granules in these preparations were found to be even finer and, consequently, less intensely stained than in neurons of the resting ganglion. The total number of formazan granules per neuron was not materially changed by the electrical stimulation (figure).

The appearance of large granules of monoformazan or of agglomerates in nerve cells after prolonged excitation may, it is thought, be regarded as a manifestation of intensified and accelerated aging of mitochondria resulting from severe functional strain. At the same time, as the formazan granules in a great majority of the neurons were smaller than the granules in neurons of the resting ganglion, the histochemical picture observed was regarded as indicative of reduced succinic dehydrogenase activity.

Succinic Dehydrogenase Activity and Content of SH-Groups in Superior Cervical Sympathetic Ganglion of Cat, in Rest and in Excitation (M±m)

Functional state	Succinic dehydrogenase activity (μg INT/mg fresh tissue)	SH-groups (mμ/mg fresh tissue)		
		total	nondialyzable	dialyzable*
Rest	34.00±5.03	3.867±0.556	2.833±0.448	1.034±0.192
Excitation	23.00±3.51	4.969±0.448	3.468±0.516	1.501±0.295
Change from rest to excitation	−11.0 ±4.27	+1.102±0.269	+0.635±0.374	+0.467±0.319

* Calculated mathematically as difference between total and nondialyzable SH-groups.

The results of the quantitative estimations of succinic dehydrogenase activity are shown in the table. This activity in the sympathetic ganglion was much greater in the resting state than in excitation. Stimulation of the sympathetic nerve reduced succinic activity by 31%.

Both histochemical and biochemical results thus pointed to reduction of succinic dehydrogenase activity.

Reduction in the activity of oxidative enzymes, and of succinic dehydrogenase in particular, after single stimulations has been observed in investigations on other materials [4], but it is still difficult to discover the exact significance of this. One possible cause may be change in the structure of the mitochondrial membrane, associated with change in the volume of the mitochondria and leading to diminished access of substrate to intramitochondrial enzyme. Supporting evidence for this suggestion is perhaps provided by the observations made in this laboratory by G. P. Gulidova in an examination of succinic dehydrogenase activity in suspensions of mitochondria from different parts of the nervous system in rabbits and cats. In her experiments the swelling of mitochondria, evoked by the action of the hypotonic solution, was always accompanied by increase of enzyme activity, which could be demonstrated spectrophotometrically.

The results of the SH-group titrations of ganglion extracts, given in the table, reveal that 73% of the sulfhydryl groups were connected with the nondialyzable fraction - apparently proteins. When excitation was produced in the ganglion, "total" SH-group increased by 28.5%, groups connected with nondialyzable molecules by 22.4%, and those dialyzable molecules by 45.1%, these changes being statistically significant ($P < 0.001$). The increase in the quantity of SH-groups titrated in the nondialyzable ("protein") fraction should probably be regarded as a sign of denaturation-like change in the structure of protein molecules, developing in the course of nerve cell excitation, whereas the increase in the quantity of "dialyzable" SH-groups, being evidence of increase of the low-molecular mercaptans - SH-glutathione and cysteine in the tissue, is apparently the result of intensified protein metabolism (breakdown and synthesis). The latter is the more probable in that, according to Oeriu [5], the cysteine content of nerve tissue is increased in excitation as well as the contents of free tryptophan, histidine, and cystine.

These results on the behavior of SH-groups in excitation of the superior cervical sympathetic ganglion coincide with results obtained by electrical stimulation of the rat cortex [2, 3, 7] and by excitation of the rat brain.

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