CATECHOLAMINE LUMINESCENCE REACTION IN NEURONS OF THE SPINAL SENSORY GANGLION

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The spinal ganglia of 45 cats were studied by the method of Falck and Hillarp in Krokhina's modification, and parallel investigations were made by Nissl and Unna's methods, staining for lipofuscin, and by the Masson–Fontana method for serotonin. Two types of neurons were discovered: neurons of one type had yellowish-white granules in the region of the axon hillock and speckled yellowish granules and diffuse greenish luminescence in the perikaryon, while those of the second type had orange granules, gathered into small clumps all over the region of the perikaryon. Neurons of both types were surrounded by single adrenergic nerve fibers.

KEY WORDS: neurons of spinal ganglia; catecholamines; adrenergic nerve fibers.

Most of the views of the nerve cell at present held have been based on results obtained by the silver impregnation and Nissl's methods and by staining with methylene blue.

A few investigations in which the properties of the neuron have been studied by the luminescence–histochemical method of Falck and Hillarp have been published [4-7]. However, no attention in them was paid to neurons of the spinal sensory ganglia.

The object of this investigation was to obtain information on the sensory nerve cell and its surroundings by the use of the catecholamine luminescence method of Falck and Hillarp.

EXPERIMENTAL METHOD

Spinal sensory ganglia of 45 cats were studied. The following techniques were used: the luminescence–histochemical method of Falck and Hillarp in Krokhina's modification [1] to detect adrenergic structures, Nissl's method to prove that the intraneuronal granules found were not identical with tigroid, Unna's method to eliminate granules of mast cells from the study, staining for lipofuscin, and the Masson–Fontana method for detecting serotonin. As an additional measure, 15 min before sacrifice the animals received a single injection of noradrenalin in a dose of 1-2 mg/kg, and reserpine was given in a dose of 0.1-0.5 mg/kg daily for 5-7 days. The reserpine treatment, combined with examination of the sections for autoluminescence, acted as a control of the specificity of the reaction.

EXPERIMENTAL RESULTS

The method of Falck and Hillarp showed that the spinal ganglia contain two types of neurons. The overwhelming majority of cells belong to the first type and cells of the second type are extremely rare.

The neuron of the first type has a round or, less frequently, oval body; in the region of what is evidently the axon hillock, bright yellowish-white granules were localized as a "cap" consisting of tightly packed granules of a slightly angular shape (Fig. 1). Besides these "caps," the perikaryon also contained a few speckled yellowish granules.

In control sections stained by Nissl's and Unna's methods, the granules were shown to be neither granules of mask cells nor tigroid of the neuron itself. The localization of the luminescent granules in the

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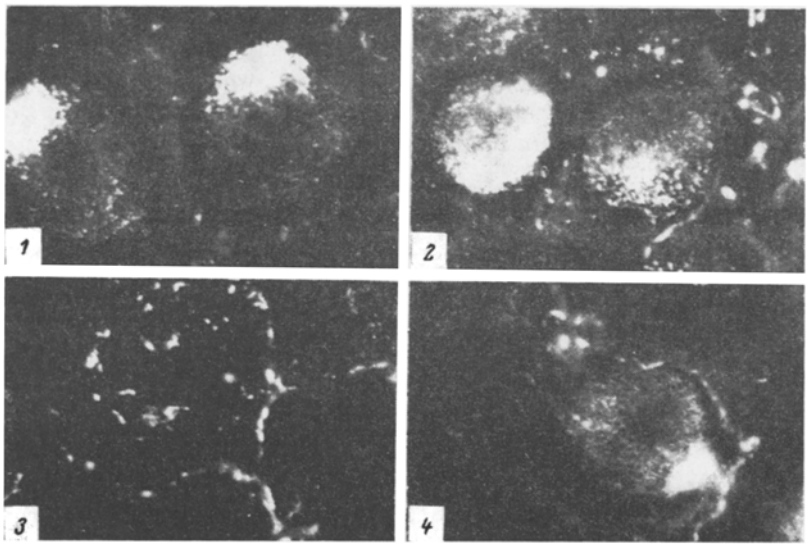

Fig. 1. Granules forming "cap" on body of sensory neuron. Spinal ganglion of cat. Here and in Figs. 2–4: Falck–Hillarp method, ocular 90, Gomal 3.

Fig. 2. Neurons of first type with different intensities of diffuse luminescence. Spinal ganglion of cat.

Fig. 3. Adrenergic nerve endings on type 2 neuron with clumps of granules. Spinal ganglion of cat.

Fig. 4. Adrenergic nerve endings on type 1 neuron. Spinal ganglion of cat.

type 1 neurons coincided with products of positive reactions for both lipofuscin and serotonin. This suggests that luminescence of the granules in the neuron was due not to lipofuscin itself but to serotonin adsorbed on the lipofuscin (the possibility cannot be ruled out that lipofuscin may be a carrier of other biogenic amines which decolorize the luminescence). Nerve cell nuclei do not luminesce.

The whole territory of the cell also was occupied by a very weak, diffuse greenish luminescence, which differed in intensity in different cells. Administration of exogenous noradrenalin intensified this luminescence, but reserpine almost abolished it within 24 h, indicating its specifically catecholamine nature. A dark halo, coinciding in situation with the zone occupied by gliocytes, was present around the body of each neuron. It disappeared after administration of exogenous noradrenalin, when the area was filled, like the body of the neuron, with pale-green luminescence.

Considering the specific character of the green luminescence, indicating the presence of a free fraction of catecholamines, it may be that in the experiments with administration of noradrenalin the perikaryon of the neuron and the perineuronal space absorb both the exogenous catecholamine and substances liberated under these circumstances from the tissue depots (adrenalin, serotonin) [3]. Monoamines are thus present in the cell both bound with granules and in the free state.

The type 2 neurons of the spinal ganglion (Fig. 2), in their luminescence, resemble more closely neurons of the intramural ganglia of the small intestine [2]. They also contained granules, but these were orange in color and were aggregated into clumps over the whole territory of the perikaryon. In view of the great rarity of these findings, no firm conclusions can be drawn but the possibility cannot be ruled out that these neurons are not in fact cerebrospinal in character.

Neurons of types 1 and 2 were surrounded by single adrenergic nerve fibers (Figs. 2–4). Clearly, a cell around which a nerve fiber can distinctly be seen (Fig. 2) contained hardly any of the free catecholamine fraction. Conversely, the fibers surrounding brightly luminescent cells appeared paler or were invisible (Fig. 1).

Observations by other workers in the USSR and elsewhere, and also our own observations on other organs, show that an increase in the level of free catecholamines in the blood plasma of experimental animals is accompanied by increased luminescence of susceptible working tissues. A particularly clear ex-

ample of this is the elastic tissue of blood vessel walls [4-8]. Our own observations and experiments with adrenoblockers on other organs have shown that maximal reception of neuromediator by a working tissue is often accompanied by "emptying" of the afferent nerve fiber, whereas the blocking of reception by an adrenoblocker, on the other hand, reduces the luminescence of the working tissue and retains catecholamines in the nerve fiber, strengthening its luminescence.

These facts suggest that the free fraction of neuromediator, flowing from the adrenergic nerve fiber, is periodically absorbed by the neuron bearing the relationship of working tissue (adrenergic receptor) to the fiber. Absorption of adrenergic mediators may perhaps excite the sensory neuron to activity, including to the production of its intrinsic mediator. This hypothesis naturally requires special verification.

LITERATURE CITED

1. E. M. Krokhina and P. N. Aleksandrov, Kardiologiya, No. 3, 97 (1969).
2. Z. I. Mikheeva, in: Morphophysiology of the Nervous and Cardiovascular Systems [in Russian], Cheboksary (1973).
3. V. E. Solov'ev, Dynamics of the Content of Serotonin and Cholinergic Factors in the Blood and Tissues at Different Levels of Thyroid Function. Candidate's Dissertation, Barnaul (1968).
4. A. Björklund, L. Cegrell, B. Falck, et al., Acta Physiol. Scand., 78, 334 (1970).
5. O. Eränkö and M. Härkönen, Acta Physiol. Scand., 58, 285 (1963).
6. L. G. Elfvin, J. Ultrastruct. Res., 22, 37 (1968).
7. C. Owman and N. O. Sjöstrand, Z. Zellforsch., 66, 300 (1965).
8. D. Jacobowitz and J. K. Woodward, J. Pharmacol. Exp. Ther., 162, 213 (1968).