RETRACTION OF PROCESSES OF RESIDUAL CELLS OF THE LEECH NERVOUS SYSTEM INDUCED BY DEATH OF THE NEURON

I. S. Valitov, A. R. Nizamov,
R. A. Safin, and R. F. Sitdikov

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Processes of regeneration of axons after their injury and subsequent restoration of synaptic contacts in the CNS have been the subjects of much research [4, 5, 7]. Meanwhile the consequences of death of single cells for the structure of the neuron net have received little study. The nervous system of the leech Hirudo medicinalis, which is distinguished by relative simplicity and accessibility for experimental procedures in vivo [2], provides a convenient model for such investigations. In the segmental ganglion of the leech, containing about 400 neurons, 14 mechanosensory neurons of three modalities have been identified (four type N cells, four type P cells, and six type T cells), and form synaptic contacts with motoneurons of the same ganglion [10, 13]. Mechanosensory neurons have contacts through interganglionic connectives with motoneurons in neighboring ganglia also [1, 4]. According to the data of electron microscopy, neurons of one modality make contact with each other [5], whereas functional synapses between them have not been detected by electrophysiological methods. Elimination of some mechanosensory cells leads in certain cases to widening of the receptor zones of the residual cells of the same modality [9]. It is not clear, however, whether the topography of intraganglionic and intersegmental processes in the latter changes under these circumstances. Yet data on this subject would provide the answer to the question whether death of single neurons causes changes in the structure of the neuronal assembly or whether the reorganization process is limited to growth of axons into new regions of the skin.

The aim of this investigation was to discover whether elimination of a mechanosensory neuron activates mechanisms of sprouting in cells with a similar function in the assembly, or whether it activates retraction of processes responsible for interneuronal communication.

EXPERIMENTAL METHODS

Leeches were anesthesized in 8% ethanol solution. An incision was made in the skin of the leech above the 10th ganglion; the vessel in which the nerve trunk lies was opened, and a 0.5% solution of pronase (Sigma, USA), made up in potassium chloride solution (50 mM) with the addition of the dye Fast Green (Serva, West Germany), was injected through a microelectrode into one of the N or P cells [11]. If the injection was done properly, the cell body stained green, and after 30-40 min it could no longer generate action potentials and the potential difference of its membrane disappeared. Total degeneration of the processes of a neuron, according to data in [11], is complete by the end of the 2nd day after injection. After the operation the thin walls of the blood vessel were tightly wrapped around the ganglion, isolating it reliably, and the skin incision, due to reflex contraction of the abdominal wall muscles, was hermetically closed and healed by a scar after 5-6 days. Between 3 and 80 days after injection of pronase the ganglion or chain of ganglia was removed, and a 2% solution of the fluorescent dye Lucifer Yellow (Serva) was injected into the mechanosensory cells [12]. After fixation, dehydration, and mounting of the preparation in medium containing polyvinyl alcohol and glycerol, series of "optical sections" were made with the aid of a fluorescence microscope, and from them the tree of processes of the neuron was subsequenly reconstructed [5, 8].

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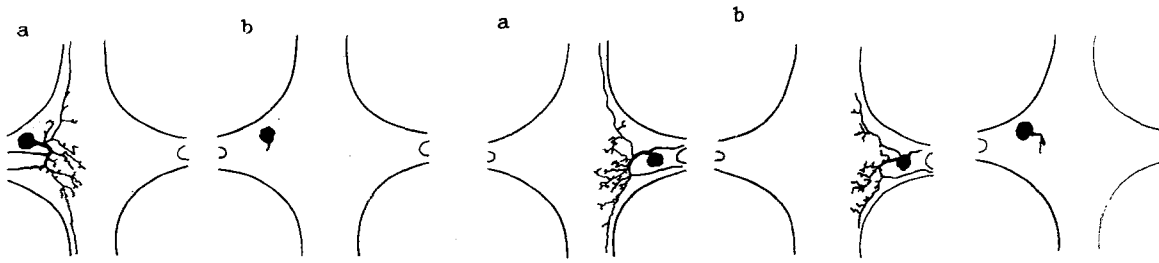

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Topography of processes of left lateral N cell of 10th segmental ganglion of H. medicinalis. a) Control, b) 20 days after elimination of left medial N cell of the same ganglion. Here and in Figs. 2 and 3, stained with Lucifer Yellow, reconstruction from "optical sections."

Fig. 2. Topography of processes of right medial P cell of 10th segmental ganglion of H. medicinalis. a) Control, b) 20 days after elimination of right lateral P cell of the same ganglion.

Fig. 3. Topography of processes of lateral N cell of 10th segmental ganglion of H. medicinalis 73 days after elimination of medial N cell in the same ganglion.

EXPERIMENTAL RESULTS

The first changes in the topography of the mechanosensory neurons were observed as early as on the 3rd day after the operation, and they became well marked by the 7th day. In the experiments with elimination of left medial N cell of the 10th ganglion, Lucifer Yellow was injected after 7-20 days either into the lateral left N cell of the same ganglion or into an N cell of neighboring ganglia. Under normal circumstances N cells form a branched network of processes in the neuropil region, and they send processes into the lateral roots and interganglionic connectives (Fig. 1a). The reaction of the lateral N cell to elimination of the above-mentioned neuron was tested in six experiments. In three cases the processes disappeared completely from the connectives and roots, and in the rest, the principal neurites were preserved, but the number of secondary and tertiary branches was significantly reduced (Fig. 1b).

Retraction of the processes was discovered in some cases of neurons of N type and in neighboring ganglia. In eight of the 12 N cells studied in the 9th ganglion, processes were absent either in the lateral roots or in the connectives. In the 11th ganglion three of 10 N cells studied responded by retraction of their processes to death of the N cell of the 10th ganglion. The response of the neurons described was characteristic not only of cells of N type. Elimination of the medial P cell in the 10th ganglion also led in half of the cases (eight experiments) to retraction of the processes of the P cells of the 10th and the two neighboring ganglia on the 7th-20th day (Fig. 2a, b).

In the control group of leeches a solution of potassium chloride (50 mM) with the addition of Fast Green was injected into the medial N and P cells of the 10th ganglion. In none of the 13 experiments did injection of Lucifer Yellow into the N and P cells of the 9th, 10th, and 11th ganglia induce retraction of the processes of the neurons tested on the 7th-20th days after the operation.

It has been shown that neurons connected with a cell killed by pronase are not injured, according to their electrophysiological and morphological criteria, during the first 2 days after the operation [2, 11]. However, these data do not rule out the possibility that the reaction of the mechanosensory neurons described above may be connected with the direct action of pronase on them. Accordingly, a solution of pronase in a dose four or five times greater than the dose injected intracellularly, was injected extracellularly into living anesthetized leeches, into the neuropil region. Experiments conducted on the 10th-20th days after this procedure revealed no differences in the topography of the processes of the N and P cells from the neurite tree of intact animals.

Death of some mechanosensory neurons may lead to widening of the receptor zone of the remaining cells of the same modality [9]. This must be the result of growth of axons into new areas of skin, i.e., to a process of sprouting. Widening of the receptor zone takes place a few months after the operation [9]. It can be tentatively suggested that retraction precedes

sprouting. Evidence that this is so is given by the presence of thin processes on terminal segments of first-order neurites, which have undergone retraction, in some cases (Fig. 3).

It can be concluded from these experimental data that cells of a mechanosensory neuron may respond to elimination of cells of the same modality by retraction of their processes after a short time. The neuron net is evidently a dynamic structure, reorganizing itself in response to death of some of its cells.

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EFFECT OF SUBSTANCE P AND ITS FRAGMENTS ON PHYSIOLOGICAL AND PATHOLOGICAL PAIN

Academician G. N. Kryzhanovskii,*
S. I. Igon'kina, V. V. Trubetskaya,
P. Oehme, and M. Bienert

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Among the neuropeptides with an antinociceptive effect, a special place is occupied by substance P (SP). As a neurotransmitter of primary sensory neurons, it participates in the transmission of nociceptive impulsation, and at the same time, it possesses regulatory properties, and under certain conditions it depresses pain responses [2-6]. To explain this, at first glance, paradoxical phenomenon, several suggestions have been made. Some workers consider that large doses of SP induce depolarization of the postsynaptic membrane, which has an algesic effect, whereas small doses have an analgesic effect through the release of endogenous opioid peptides from presynaptic endings [2]. Other workers consider that the effect of SP depends on the initial state of the recipient, on binding of SP and (or) its fragments with receptors of various types [3, 4]. Investigations in recent years have revealed spatial demarcation of functions in the SP molecule, and the promising nature of the study of biological effects of SP fragments.

The aim of this investigation was to study the effect of SP and its fragments on physiological and pathological pain.

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*Academy of Medical Sciences of the USSR.

Laboratory of General Pathology of the Nervous System, Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Institute of Physiologically Active Substances, Academy of Sciences of the GDR, Berlin.

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