AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE GIANT NEURONS OF CERTAIN PULMONATE MOLLUSKS

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Because of their large size (100-200 μ) and their superficial position in the ganglia, the giant unipolar neurons of mollusks are very suitable for application of a microelectrode technique for the study of both the electrophysiological properties of the superficial membranes of excitable cells and the simpler neuronal networks [7, 13]. The comparatively few giant neurons in each ganglion of these animals, and their fairly constant localization, provide an excellent opportunity for comparing electrophysiological data with the results of morphological investigations.

In the present paper we describe the results of a study, using a microelectrode technique, of the electrical activity of the giant neurons of the gastropod pulmonate mollusks Planorbis corneus and Limnea stagnalis.

METHOD

The nervous system of Planorbis consists of 6 pairs of symmetrical ganglia and one asymmetrical ganglion (Fig. 1, I and II). The nervous system of Limnea is similar in structure [2]. The diameter of the soma of some of the neurons in the ganglia of these animals exceeds 100 μ. A photomicrograph of the medial part of a visceral ganglion of Planorbis is shown in Fig. 1, III. The cells, similar in form to the giant cells of Helix [1], are round or oval in shape and give off a thick axon. A large nucleus can easily be seen in the cytoplasm of the cells.

Most experiments have been conducted on giant neurons (A and B neurons of Planorbis) of the parietal ganglia. In all experiments two microelectrodes have been inserted into the same chosen neuron simultaneously through the connective-tissue membrane: pulses of current have been passed in either direction through one microelectrode, while with the other microelectrode changes in the transmembrane potential difference and the action potentials have been recorded [4].

Before the present investigation began, the points of the polarizing and recording electrodes were placed in the solution under the control of a binocular microscope (×32) side by side and about 20 μ apart. After the preliminary setting of the microelectrodes, they began to be inserted into the soma of the chosen giant neuron, lying on the surface of the ganglion. In these conditions pulses of hyperpolarizing current with a strength of 1×10^{-8} or 2×10^{-8} A were passed in a continuous stream through the polarizing electrode. The moment of successful penetration of the two microelectrodes into the giant neuron was marked by the appearance of a transmembrane potential difference and an electrotonic potential in the recording channel. Since both microelectrodes were introduced into the cell through the membrane of the ganglion, in order to ensure successful puncture of the membrane carefully selected microelectrodes with a point less than 0.5 μ in diameter had to be used.

For direct stimulation of the neurons by means of the microelectrode and for indirect stimulation (stimulation of neural branches) a rectangular pulse generator with a radiofrequency element at the output was used. As basic solution in which the ganglia of the animals were immersed, a solution of the following composition was used: NaCl) 50 mmole; KCl) 1.6 mmole; $CaCl_2$) 4 mmole. The pH of the solution was maintained with the range 7.1-7.4. When the temperature of the irrigating solution was about 20°, continuous recordings could be made of the electrical reactions of one neuron for several hours.

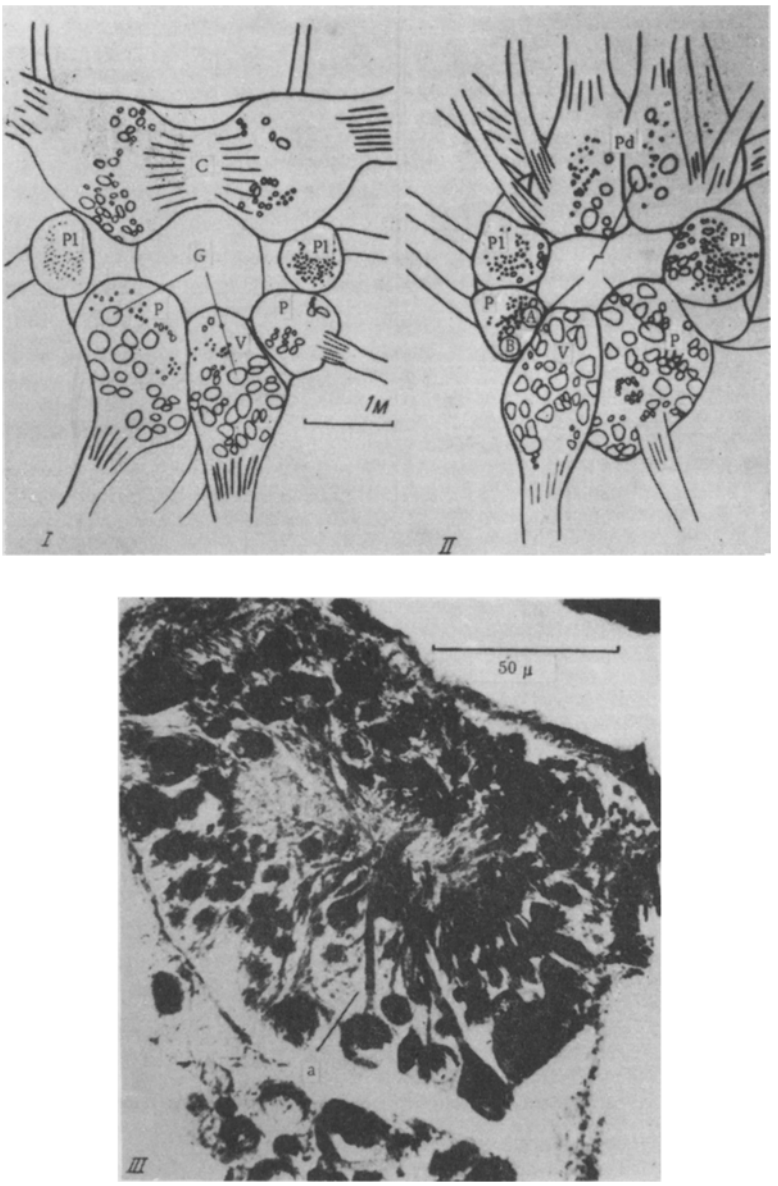

Fig. 1. Nervous system of Planorbis corneus. I) Seen from above; II) seen from below; C) cerebral ganglia; Pd) pedal ganglia; Pl) pleural ganglion; P) parietal ganglion; V) visceral ganglion; G) giant neurons on the surface of the ganglia; in the parietal ganglion two giant nerve cells can be seen (the buccal ganglia are not shown on the scheme); III) photomicrograph of the medial part of the visceral ganglion. Hexatoxylin, silver. The arrow indicates the axon leaving the soma of a giant neuron (a).

RESULTS

The mean value of the resting potential of the giant nerve cells (I and II, Planorbis) was 53.7±4.4 mV, and of the action potential 86.9±5.8 mV. Hence, the action potentials of these cells were almost twice the amplitude of the resting potentials. The giant neurons of Planorbis (I, II) usually generated action potentials even without direct stimulation of their membrane, and also independently of stimulation of afferent pathways. This type of rhythmic activity, which may be described as "spontaneous," was characterized by an extremely stable and low frequency (0.5-2 cps) (Fig. 2, 1). Often this slow rhythmic activity was replaced by long pauses (1-5 min). The factor triggering off the spontaneous rhythmic activity was the slight depolarization of the membrane; in some cases it was brought about by a waveform change in the resting potentials.

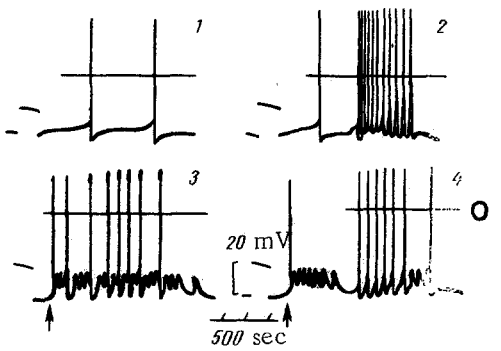

Fig. 2. Electrical activity of giant neurons (Planorbis). 1) Spontaneous rhythmic activity; 2) grouped discharge of a giant neuron; 3-4) grouped discharges of a giant neuron during strong orthodromic excitation (the arrow indicates the moment of application of the stimulus). At the beginning of each sweep of the beam a pulse of standard duration and amplitude is given; O) level of zero potential.

In our experiments, in the course of the spontaneous rhythmic activity the appearance of grouped discharges was regularly (every 30 sec) observed, as had previously been reported during investigation of the giant neurons of Aplysia [6], when they were called "motives." These grouped discharges, spontaneous in character, were always the result of the development of a slow wave of depolarization of the membrane, against the background of which the action potentials were generated. Their number in a "motive" varied from 2 to 20 impulses (Fig. 2, 2). The number, the frequency of repetition, and the changes in the form of the impulses in the grouped discharge gave the "motive" its distinctive appearance.

Similar grouped discharges were observed to appear in response to strong orthodromic excitation, when many neurons were concerned in the discharge. In this case (Fig. 2, 3 and 4) summation of the excitatory postsynaptic potentials was seen, and when a certain critical level of depolarization was reached, this led to the generation of action potentials by the neuron. Both during the spontaneous appearance of "motives" and during orthodromic excitation, the factor predetermining the frequency of discharge of impulses was the level of depolarization of the membrane, as was observed particularly clearly in the case of direct depolarization of the membrane through the microelectrode (Fig. 3, 4 and 5).

Responses of the giant neurons of Planorbis and Limnea to different forms of stimulation are shown in Fig. 3. The action potential of a giant neuron, caused by an antidromic pulse, is shown in oscillogram 1. A two-component type of action potential is seen. The change from the first component to the second takes place at the level of approximately 20 mV. If there is difficulty in the spreading of the pulse to the soma of the giant neuron, or in the presence of slightly artificial hyperpolarization of the membrane, then only the first component may appear (of the order of 20 mV) in response to antidromic stimulation of the cell. The appearance of analogous potentials in the motor neurons of vertebrate animals has been attributed to excitation of the nonmedullated part of the axon and the axon hillock (the "initial segment") alone [5, 8, 10]. We have observed potentials of this type in the giant neurons of Helix [1].

In response to orthodromic stimulation of the giant neurons of Planorbis, the first reaction was an excitatory postsynaptic potential, on which an action potential developed. With a decrease in the strength of stimulation, only the excitatory postsynaptic potential appeared, without the action potential; its latent period was about 50 msec, while the extinction time reached 100 msec (Fig. 3, 2 and 3).

The distinguishing feature of the electrical responses of the giant neurons of Planorbis and Limnea were their longer duration than that of the analogous reactions of the previously investigated neurons of the central nervous system of the higher animals [3, 9]. The specific properties of the membrane of the giant cells of mollusks are indicated by its greater time constant, demonstrated in our experiments during direct polarization of the cell membrane.

The oscillograms 4 and 5 (Fig. 3) show the electrotonic potentials and action potentials of the giant neurons of Planorbis and Limnea during direct polarization of the cells through the microelectrode. In the case of polarization of the membrane with an outgoing current the number of impulses was dependent on the strength of the current passed through the membrane. The maximal frequency of the discharges was about 20 impulses/sec. With weak depolarizing currents only catelectrotonic potentials sometimes were observed, not changing into action potentials. With a considerable increase in depolarization, a decrease in the value of the action potentials or even their total disappearance was observed immediately after the first peak (the inactivating action of depolarization).

In our experiments it was possible to measure accurately the value of the hyperpolarizing current and the value of the anelectrotonic potential. For this reason the entry resistance of the neuron could easily be calculated (the resistance between the internal electrode and the external medium). In the experiments considerable variations in the entry resistance were observed (from 5 to 10.3 MΩ). The mean value of this resistance for 11 neurons was 7.58 ± 0.68 MΩ.

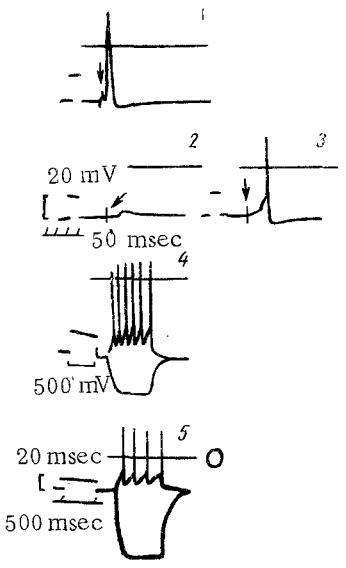

Fig. 3. Electrical reactions of giant neurons to different methods of stimulation. 1) Antidromic stimulation; 2, 3) orthodromic stimulation; 4) electrotonic potentials and action potentials during direct polarization of the cell membrane in Planorbis; two sweeps of the beam are shown together on the oscillogram, during cat- and anelectrotonic polarization (polarization current 1×10^{-8} A); 5) electrotonic potentials and action potentials during direct polarization of the cell membrane in Limnea (hyperpolarization current with a strength of 2×10^{-8} A); O) level of zero potential.

Exact information about the specific resistance of the membrane can be obtained only by taking into account the geometry of the neuron [4, 11]. In this respect the nerve cells of the mollusk have great advantages, for they are directly visible under the microscope and comparatively simple in shape (spherical or ellipsoidal), giving off a cylindrical process with a diameter of about 10 μ (Fig. 1, III). Taking the surface area of the soma of the investigated giant neurons of Planorbis (I, II) as 4.9×10^{-4} cm^2 (diameter of soma 125 μ), we obtained a mean value of 3710 $\pm$ 332 Ω/cm^2 for the specific resistance of the membrane of 11 neurons (ignoring axonal conductivity). The mean value of the specific capacitance of the membrane, calculated from the time constant of the increase of anelectrotonic potential on the membrane, was found in this way to be 24.2 ± 2.9 μF/cm^2. Using the equations of the cable theory [4], the ratio between the axonal conductivity and the conductivity of the soma membrane could easily be calculated. Such calculations showed that for the giant neurons of Planorbis the ratio between the axonal conductivity and the conductivity of the soma membrane was close to 0.5 : 1.

In respect of the motor neurons it was practically impossible to measure the axonal conductivity. The conductivity of the dendrites, on the other hand, was several times greater than the conductivity of the soma membrane [11].

We know that the site of the synaptic endings on the axon lies at a considerable distance from the soma of the giant unipolar neurons of mollusks [12]. The fact that the conductivity of the soma membrane of the neurons in Planorbis does not exceed the axonal conductivity is evidently of decisive importance for facilitating the passage of excitation from the axon to the soma of the giant neuron.

LITERATURE CITED

1. V. D. Gerasimov and V. A. Maiskii, Fiziol. zh. SSSR, No. 9 (1963), p. 1099.
2. V. A. Dogel', A Course of Comparative Anatomy of Invertebrates [in Russian], No. 2, Leningrad (1925).
3. P. G. Kostyuk, Fiziol. zh. SSSR, No. 10 (1961), p. 1241.
4. V. A. Maiskii, Fiziol. zh. SSSR, No. 12 (1963), p. 1468.
5. T. Araki and T. Otani, J. Neurophysiol., 18 (1955), p. 472.
6. A. Arvanitaki and N. Arvanitaki, Ibid., 9 (1955), p. 115.
7. N. Chalazonitis and A. Arvanitaki, Ibid., 10 (1956), p. 291.
8. J. S. Coombs, D. R. Curtis, and J. C. Eccles, J. Physiol., 139, London (1957), p. 232.
9. J. C. Eccles, The Physiology of Nerve Cells, Baltimore (1957).
10. K. Frank and M. G. F. Fuortes, J. Physiol., 130, London (1955), p. 625.
11. W. Rall, Exp. Neurol., 1 (1959), p. 491.
12. L. Tauc, J. Gen. Physiol., 45 (1962), p. 1077.
13. L. Tauc and H. M. Gerschenfeld, J. Neurophysiol., 25 (1962), p. 236.