EXCITABILITY OF THE GIANT NERVE CELLS OF VARIOUS LUNGED MOLLUSCS (*Helix pomatia*, *Limnea stagnalis*, AND *Planorbis corneus*) IN SOLUTIONS FREE FROM SODIUM IONS

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A characteristic feature of the giant nerve cells of the edible snail is its ability to maintain normal excitability in sodium-free solutions [1]. We found that most cells continued to produce action potentials in isotonic solutions of calcium or barium chloride. Furthermore the amplitude of these action potentials was directly proportional to the logarithm of the external concentration of these ions. We therefore concluded that the calcium or barium ions take over the role normally played by sodium ions. It is known that the concentration of calcium ions in the hemolymph of the edible snail reaches quite high values at certain periods [3].

The object of the present work has been to make a comparative study of the excitability of the giant nerve cells of the edible snail (*Helix pomatia*), of the planorbis mollusc (*Planorbis corneus*), and of *Limnea stagnalis* in similar sodium-free solutions. The last two species were chosen because despite their close systematic relationship the concentration of calcium in their hemolymph does not undergo any great seasonal variation, and is several times less than the edible snails.

EXPERIMENTAL METHOD

Stimulae were applied and potentials led off by means of two separate micro-electrodes introduced into the neurone. We studied chiefly the giant cells of the parietal ganglia. The original standard solution for Planorbis and Limnea contained 50 mM NaCl, 1.6 mM KCl, and 4 mM $CaCl_2$; the concentration of these salts corresponded to the hemolymph. The standard solution for edible snails contained 75 mM NaCl, 5 mM KCl, and 10 mM $CaCl_2$. The sodium-free solutions were prepared from a standard by replacing in it the whole of the sodium with isomotic sucrose or with chloride solutions of bivalent ions (manganese, calcium, strontium, or barium). The method has been described in more detail in our previous works [2, 4]. The investigations were carried out in summer on newly hatched snails.

EXPERIMENTAL RESULTS

There was a marked resemblance in the amplitude and the duration of the action potentials and in the frequency of the discharges [2]. However, in sodium-free solutions (in the isotonic solutions of the chlorides of calcium, manganese, strontium, or barium, or in solutions in which sodium chloride had been replaced by isosmotic sucrose) there were definite differences. The nerve cells of the edible snail continued to give a complete action potential for many hours. There was a considerable increase in the membrane resistance. Figure 1 shows oscillograms which represent the responses of the giant cells of the edible snail in isotonic calcium chloride. By the end of the third minute in the solution (Fig. 1, 4) the anelectronic response of the cell increased by 15 mV, and the resting potential by 7 mV, while the amplitude of the action potentials increased by 18-20 mV; these changes were well maintained subsequently, but disappeared completely when the cells were washed with a solution containing the normal amount of calcium (Fig. 1, 5-8).

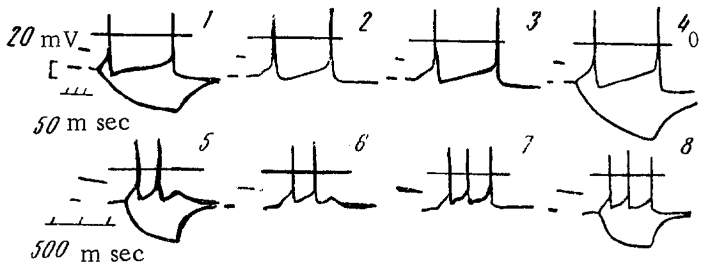

Fig. 1. Reaction of a giant nerve cell of the edible snail (Helix pomatia) in response to catelectronic or anelectronic polarization in isotonic calcium chloride. 1) In sodium-free solution with a normal concentration of calcium ions; 2, 3, 4) in isotonic calcium chloride solution after 1, 2, and 3 min respectively; 5, 6, 7, 8) cells once more placed in a sodium-free (sucrose) solution containing a normal concentration of calcium ions, after 1, 2, 3, and 4 min (sweep speed reduced). Strength of depolarizing current equals $0.3 \cdot 10^{-8}$ A, depolarizing current $1 \cdot 10^{-8}$ A. Calibration pulse included at start of scan. 0) Zero level of resting potential.

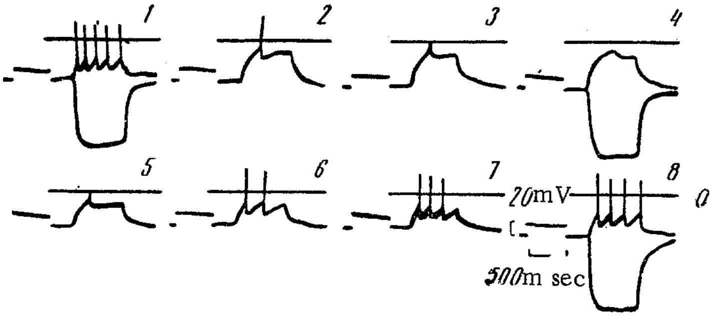

Fig. 2. Reaction of a giant neurone of Limnea stagnalis in an isotonic solution of calcium chloride. 1) Action potential and anelectronic response in physiological saline; 2, 3, 4) in an isotonic solution of calcium chloride, after 1, 2, and 3 min (strength of depolarizing current pulse increased); 5, 6, 7, 8) after washing in physiological saline after 1, 2, 3, and 4 min. 2) strength of hyperpolarizing current pulse $1 \cdot 10^{-8}$ A.

Quite a different state of affairs was found under similar conditions in nerve cells of Planorbis and Limnea. The amplitude of action potentials in these cells was reduced in sodium-free solutions; the number of spikes in the discharge was also reduced, and at the same time their duration increased. By the 3-5th min the action potentials had disappeared, and all that remained was a low-amplitude local response, Figure 2 shows oscillograms of the responses of the edible snail in isotonic calcium chloride, and after the cells had been washed in physiological saline. After removal of sodium ions from the surrounding solution the nerve cells of this animal, just like those of the edible snail, were hyperpolarized and their absolute threshold of excitation was increased. After 3 min (Fig. 2, 4) the cell lost all excitability, despite the high level of the resting potential, and the maintenance of the initial value of the anelectronic response (initial resistance of membrane). When the cells were first washed there was quite a marked depolarization, after which there was a gradual recovery of the resting and action potentials to their original values (Fig. 2, 5-8).

The disappearance of the action potentials in Planorbis took place rather differently. Figure 3, 1-4 shows the responses of a giant cell of this animal to direct stimulation in isotonic calcium chloride. Hyperpolarization of the cells was brief, and was followed by quite a rapid depolarization, which was associated with a reduction of membrane resistance. At this time there was a sudden drop in the action potential; the amplitude fell until it had disappeared completely. Despite the great reduction in the amplitude of the resting potential and of the membrane

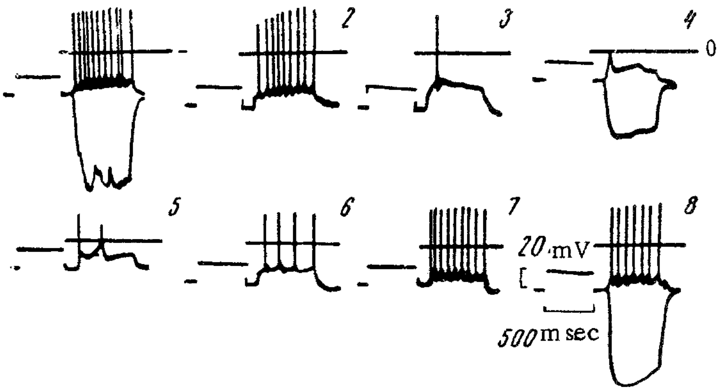

Fig. 3. Reaction of a giant nerve cell of Planorbis corneus in isotonic calcium chloride. 1) In physiological saline (during the anelectronic response spontaneous excitatory postsynaptic potentials were observed); 2, 3, 4) in isotonic calcium chloride after 1, 2, and 3 min (strength of depolarizing current pulse increased); 5, 6, 7, 8) cells were washed in physiological saline after 1, 2, 3, and 4 min. Strength of hyperpolarizing current pulse $1 \cdot 10^{-8}$ A.

resistance this process was reversible, and the cells completely recovered their normal functional properties after they had been washed (Fig. 3, 5-8). The giant nerve cells lost their excitability not only in isotonic calcium chloride but also in isotonic manganese, strontium, or barium chloride, or in solutions in which sodium had been replaced by sucrose.

We must note that in many experiments the nerve cells of Planorbis lost their excitability and ceased to generate action potentials in the sodium-free solutions without any noticeable depolarization. On the other hand, in some cases the cells of Limnea showed a loss of resting potential after they had been in the solutions for a long time.

Removal of the sodium ions from the solution during the first few minutes produced a similar hyperpolarizing action on the nerve cells of each of the animals studied. Apparently under natural conditions the membranes of the giant cells are partially permeable to sodium ions, a circumstance which leads to a reduction of the trans-membrane potential difference in relation to the equilibrium for potassium ions. When sodium ions are removed from the surrounding solution the trans-membrane potential difference shifts towards the latter value. When the sodium-free solution is washed away with normal Ringer in many cases (Fig. 2, 3) there is a considerable influx of sodium ions into the cells, which leads to a marked depolarization of the membrane at this time.

In most cells of the edible snail the generation of action potentials must occur without sodium ions, whereas the cells of the other two species of lunged molluscs lose their excitability in the absence of these ions. This difference is apparently not associated directly with the fact that the edible snails lead a terrestrial life, while Planorbis and Limnea are aqueous species. The giant nerve cells of such aqueous molluscs as Onchidium verruculatum are also able to maintain their excitability in sodium-free solutions [7]. There is no doubt that the relatively high concentration of bivalent ions in the hemolymph of the edible snails, which is also subjected to great seasonal variations [6], is of far greater importance in this respect. The concentration of calcium ions, for example, increases in the hemolymph during the time that these animals are building up quite a strong shell; the calcium stores in the liver and kidneys in the form of calcium phosphates fall considerably at this time [8]. We may suppose that it is for this reason that the nerve cells of the edible snail are able to utilize the alkaline-earth metals as carriers of charge associated with the generation of action potentials.

The cells of Planorbis and Limnea, whose hemolymph contains a low and quite stable concentration of bivalent atoms, possess no such properties. The surface membranes of these cells have other distinctive features. As the results of our investigations have shown, the curves relating the membrane resistance and resting potential to the logarithm of the external calcium ion concentration indicate that they are more sensitive to this ion than are the nerve cells of the edible snail. This circumstance is probably related to the different potassium concentration in the hemolymph (1-2 mM in Planorbis and Limnea, and 4-5 mM in the edible snail).

The frequently observed reduction in the action potentials of the giant cells of Planorbis in sodium-free solutions (a reduction which is considerable but reversible) indicates a marked increase of permeability of the cell membrane under these circumstances. This depolarization does not occur in the nerve cells of the edible snail, although it usually develops in them in isotonic sucrose (calcium-free). The marked depolarization in calcium-free (sucrose) solutions is also found in the giant nerve cells of Onchidium verruculatum, where it is associated with structural disturbance of the membrane [7]. Thus in this respect there is a considerable difference in the membranes of neurones which retain or do not retain their excitability in sodium-free solutions.

The reason for the disturbance of the barrier function of the membrane in sodium-free solutions is not clear. Possibly, as has been shown in many investigations, the absence of sodium ions may result in inactivation of certain enzymatic systems of the cell membranes on which depend the maintenance of the normal permeability and electrical polarization [5 ,9]. We must note that the presence of sodium ions in the solution used to wash the cells is necessary for recovery of the normal functional properties of the nerve cells of the edible snail kept for a certain period in isotonic sucrose; if the cells are washed with isotonic calcium chloride, no such result is obtained. Subsequently the cell may again retain excitability for a long time in isotonic solutions of bivalent ions of the alkaline-earth metals.

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