COMPARISON OF EFFECTS OF ANTIBODIES TO S-100 PROTEIN AND OUABAIN ON *Helix pomatia* NEURONS

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The nature of depolarization of the nerve cell membrane during application of antibodies to S-100 bovine brain protein has been studied previously [2]. It was suggested that the cause of this depolarization may be blocking of electrogenic active transport of monovalent cations by means of Na,K-ATPase.

The aim of this investigation was a comparative analysis of the action of ouabain, a specific blocker of monovalent cationic transport, and of antibodies to S-100 protein on the neuron membrane of the snail *Helix pomatia*.

EXPERIMENTAL METHOD

Experiments were carried out on unidentified isolated CNS neurons of *H. pomatia*. The isolated neuron was placed in a chamber with a continuous flow of normal Ringer's solution (100 mM NaCl, 4 mM KCl, 7 mM $CaCl_2$, 4 mM $MgCl_2$, 10 mM Tris pH 7.7-7.8) and two glass microelectrodes, filled with 2 M potassium acetate solution, with a resistance of 4-8 MΩ, were inserted into it. One electrode was used to measure membrane potential (MP), the other to pass a current in order to clamp MP. The standard apparatus from "Nihon Kohden" (Japan) was combined with the use of a two-electrode method of voltage clamping. Electrical activity of the cell was recorded on an automatic ink-writer from the same firm. Ouabain (Serva, West Germany) was added to the external solution bathing the cell in a concentration of 5×10^{-4} M. Antibodies to S-100 protein, isolated by an immunoaffinity technique, were provided by A. B. Poletaev [1], P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR; they were applied under pressure to the surface of the neuron through a micropipet by the method described previously [2]. The protein concentration in the micropipet was 0.05 mg/ml. The experiments were carried out at room temperature (18-20°C) in May to September.

EXPERIMENTAL RESULTS

Neurons in a good functional state with resting potentials (RP) of −30 to −60 mV, which responded to intracellular stimulation by high-amplitude complete action potentials, were used for the investigation. Addition of ouabain in a concentration of 5×10^{-4} M to the external solution led to a shift of the MP of the neurons toward depolarization by 12 ± 5 mV (15 experiments). The maximal value of depolarization was maintained for 2-3 min, after which the potential slowly (for up to 20 min) returned to its initial value or fell below it (by 2-5 mV; Fig. 1a). When the membrane potential was clamped at the RP level, ouabain caused the development of an inward current, the time course of which coincided with that of depolarization. Steady-state current–voltage characteristic curves (CVC) of the neuron membrane were plotted before and during the action of ouabain (Fig. 1a, b). The current induced by ouabain was estimated from the difference between the strengths of currents corresponding to the same clamped voltage on the cell membrane in normal physiological saline and in a solution containing ouabain. The investigations showed that in 80% of cases the current induced by ouabain had no marked dependence on MP in the region from −40 to −110 mV and was not reversed at these voltages,

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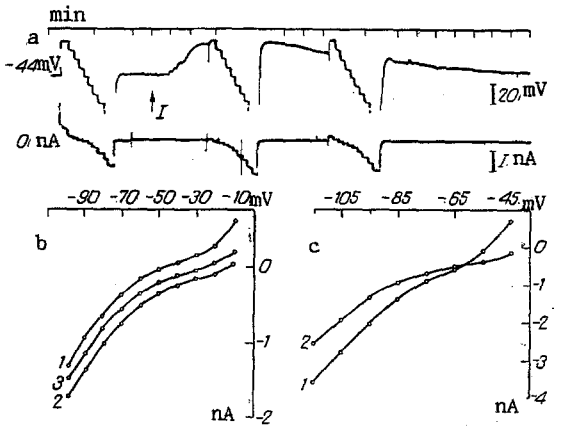

Fig. 1. Action of ouabain in concentration of 5×10^{-4} M on neuron membrane. a) Top curve — membrane potential (MP), bottom curve — MP clamping current (beginning of application of solution with ouabain indicated by an arrow); b) CVC of membrane corresponding to (a), in control physiological saline (1), in solution with ouabain at maximum of depolarization (2), and during restoration of RP (3); c) case of reduction of membrane conductance under the influence of ouabain: 1) CVC of membrane in control physiological saline; 2) CVC of membrane in solution with ouabain at maximum of depolarization.

as is clear from the absence of any point of intersection of the CVC curves, recorded before and during the action of ouabain on the neuron membrane (Fig. 1b). The inward current observed in these cases is probably the result of blockade of the outward current generated by Na,K-ATPase during the active, uncompensated transfer of monovalent sodium and potassium cations through the membrane. In 20% of cases the inward current induced by ouabain was reversed at MP = —60 to —70 mV. Depolarization was accompanied by reduction of the passive conductance of the membrane, and the CVC curves recorded before and during the action of ouabain intersected in the region of values of the potassium equilibrium potential characteristic of the snail (Fig. 1c). Since at rest the neuron membrane is permeable for potassium ions, and at MP values below the electrochemical equilibrium potential for potassium the potassium current is outward, the inward current induced by ouabain, reversed in the region of MP between —60 and —70 mV, is evidently the result of reduction of the potassium conductance of the membrane. Restoration of the RP level in solution with ouabain took place with a decrease in strength of the inward current, while the character of dependence of the current on the clamped voltage remained unchanged (Fig. 1a, b). In cases with the development of an inward current, reversing in the region of —60 to —70 mV, the reversal potential either remained unchanged or was shifted by 2-7 mV toward more positive values of the potential.

In seven experiments successive and simultaneous presentation of ouabain and of antibodies to S-100 protein to the same neuron was tested (Fig. 2). Changes in CVC under the influence of ouabain and the antibodies were similar in character (Fig. 2b, c). On cells on which ouabain reduced membrane conductance, the antibodies gave the same effect. The order of presentation of ouabain and the antibodies was unimportant (Fig. 2c).

Four experiments were carried out to test additivity of the effects of ouabain and antibodies; they showed that additivity is not observed, i.e., in solution with ouabain depolarization either was absent on application of the antibodies or it was significantly less than when antibodies were applied in normal Ringer's solution (Fig. 3a, b).

The results of this and the previous [2] investigation thus indicate that the depolarizing action of ouabain, a specific blocker of monovalent cation transport, and of antibodies to S-100 protein on the surface membrane of the nerve cell is of the same nature, and they confirm that Na,K-ATPase is inhibited on application of the antibodies.

The results of experiments in which reduction of the potassium conductance of the membrane was observed under the influence of ouabain are in agreement with data obtained in [3], in which transport of cations by Na,K-ATPase was investigated in proteoliposomes, and which showed that the native enzyme forms a leakage K^+ channel in liposomes. The passive transport

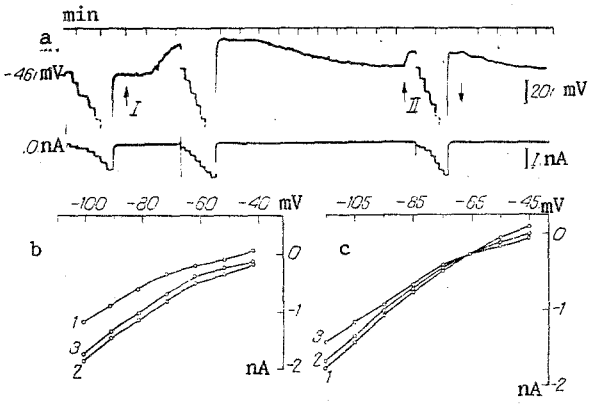

Fig. 2

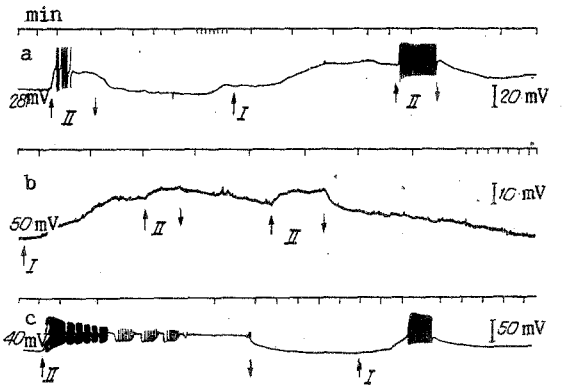

Fig. 3

Fig. 2. Simultaneous and successive presentation of ouabain and antibodies to S-100 protein to neuron membrane. a) Top curve — MP, bottom curve — MP clamping current [arrows pointing upward indicate times of application of ouabain (I) and antibodies (II)]. Arrow pointing downward indicates end of application of antibodies; b) CVC of membrane corresponding to (a) in control physiological saline (1), in solution with ouabain at maximum of depolarization (2), and on application of antibodies in solution with ouabain (3); c) case of reduction of membrane permeability under the influence of antibodies and ouabain: 1) CVC of membrane in control physiological saline; 2) CVC of membrane in response to application of antibodies in control physiological saline (level of depolarization 8 mV); 3) CVC of membrane of same neuron in solution with ouabain at maximum of depolarization (14 mV).

Fig. 3. Examples of absence of additivity of effects of antibodies and ouabain on neuron membrane of *Helix pomatia*. a) Successive and simultaneous presentation of antibodies to S-100 protein (II) and ouabain (I); b) simultaneous presentation of ouabain and antibodies; c) successive presentation of antibodies and ouabain.

observed is inhibited by ouabain and vanadate — Na,K-ATPase inhibitors. The possibility cannot be ruled out that under natural conditions the Na,K-ATPase of the cytoplasmic neuron membrane also forms leakage K^+ channels, and they determine permeability of the resting neuron membrane for potassium ions. Under the influence of ouabain the channels are blocked, so that membrane conductance and depolarization of the neuron are reduced. However, it is still not clear why this happens in only 20% of the neurons used in the experiments.

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