NATURE OF NERVE CELL MEMBRANE DEPOLARIZATION ON APPLICATION OF ANTIBODIES TO PROTEIN S-100

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UDC 612.622.017.1-06:612.822.1.015.348/.014.421.7

KEY WORDS: antibodies to protein S-100; molluscan neurons; Na,K-ATPase

The study of the effect of antibrain antibodies on nerve cell function is interesting for two main reasons. Antibodies to brain-specific proteins, if they pass through the blood-brain barrier [7], can interact with their own antigens on the surface of the nerve cell and play the role of endogenous modulators of neuronal activity. This process may take place in a number of mental diseases, accompanied by a raised blood level of antibodies to brain-specific proteins [2, 3]. Meanwhile the antibodies can be used as a unique tool to determine the functional role of particular brain-specific proteins.

In this investigation the writer studied the electrophysiological effect of antibodies to species-nonspecific protein S-100. It has been shown that protein S-100 is present in the surface membrane of neurons of *Helix pomatia* [10], the giant nerve cells of which can be used as a convenient model with which to study effects of antibodies to this protein. Depolarization of *Helix* neurons by application of antibodies to protein S-100 was described previously [1, 8, 9], but its causes have not been studied. The aim of this investigation was to study the nature of nerve cell membrane depolarization by the action of antibodies to protein S-100.

EXPERIMENTAL METHOD

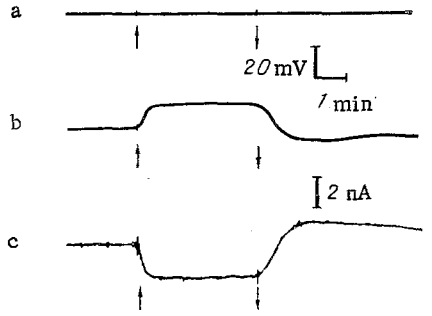
Experiments were carried out on unidentified isolated neurons of the CNS of *H. pomatia*. The snails were kept in a warm room and fed for 1-2 weeks before the experiments. The nerve preparation was incubated in a 1% solution of trypsin in normal Ringer's solution (100 mM NaCl, 4 mM KCl, 7 mM $CaCl_2$, 4 mM $MgCl_2$, and 10 mM, Tris, pH 7.7-7.8) for 20 min at 37.38°C. After the ganglion had been washed with normal Ringer's solution, single neurons were isolated by means of metal needles under the control of a stereoscopic microscope. The isolated soma of the nerve cell with the remnant of the axon was placed in a chamber through which normal Ringer's solution flowed continuously. The solution in the experimental chamber, with a volume of 0.14 ml, flowed at the rate of 0.6 ml/min. Two microelectrodes were inserted into the test neuron at an angle of 150° to one another. One electrode was used to record the membrane potential (MP), the other to pass a current in order to clamp the voltage on the membrane. The microelectrodes were filled with 2M potassium citrate solution and their resistance varied from 4 to 8 mΩ. For voltage clamping and to record the current flowing through the membrane, a standard system from Nihon Kohden (Japan) was used. Antibodies to protein S-100, isolated by an immunoaffinity method (generously provided by A. B. Poletaev, of the P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR) or nonimmune γ-globulins were dissolved in normal Ringer's solution to a concentration of 0.05 mg/ml, pH 7.7-7.8. A glass micropipet, the tip of which was gently broken off under control of the stereoscopic microscope, was filled with the above solutions. The micropipet was placed at a distance of 70-80 μ from the cell surface. Antibodies were applied under a pressure of 1-2 atm. The flow rate of the protein solution from the micropipet was 0.1-0.3 μl/min. Electrical activity of the neurons was recorded on an automatic ink recorder (Nihon Kohden). Experiments were carried out at room temperature (18-20°C) in December-March.

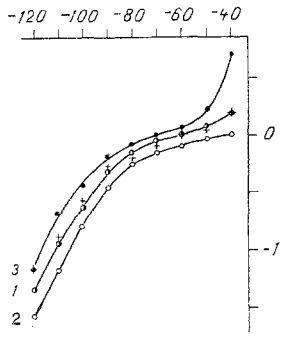
EXPERIMENTAL RESULTS

The neurons used were in a good functional state with resting potential of between —35

Laboratory of Functional Synaptology, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 3-5, January 1187. Original article submitted December 25, 1985.

0007-4888/87/0103-0001$12.50





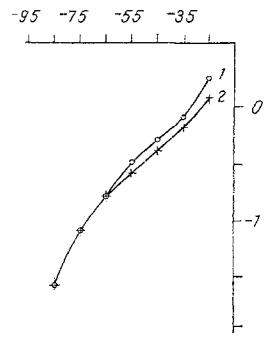


Fig. 1 Fig. 2 Fig. 3

Fig. 1. Effect of antibodies to protein S-100 on MP of a neuron. a) Nonimmune γ-globulins. MP = —40 mV. Arrows pointing upward indicate time of application of protein solution, arrows pointing downward indicate end of application. Concentration of nonimmune γ-globulins in micropipet 0.05 mg/ml; b) antibodies to protein S-100. MP = —40 mV. Antibody concentration in micropipet 0.05 mg/ml; c) current developing during application of antibodies and after rinsing out antibodies to protein S-100. Holding potential —40 mV.

Fig. 2. Effect of antibodies on CVC of neuron membrane. Abscissa, voltage (in mV); ordinate, strength of current (in nA). 1) Control; 2) application of antibodies (0.05 mg/ml); 3) 5 min after end of application of antibodies. Crosses indicate blocking of outward current, developing after rinsing out of antibodies with ouabain in a concentration of $5 \cdot 10^{-4}$ M.

Fig. 3. Effect of ouabain on CVC of neuron membrane. 1) Control; 2) ouabain ($5 \cdot 10^{-4}$ M). Remainder of legend as in Fig. 1.

and —60 mV, and capable of generating high-amplitude action potentials in response to intracellular stimulation by square pulses. Application of antibodies induced membrane depolarization of the neurons with an amplitude of 13 ± 4 mV (Fig. 1b), and under voltage clamp conditions, it induced an inward current (Fig. 1c). Application of the control γ-globulin caused no such changes (Fig. 1a). After rinsing out the antibodies with normal Ringer's solution, the MP level was observed to rise (Fig. 1b), and this was accompanied by an outward current of up to 2.5 nA (Fig. 1c). The outward current steadily diminished to zero in the course of 3-15 min, while the resting potential simultaneously regained its previous level.

To discover the nature of the inward current arising under the influence of the antibodies, its dependence on MP was studied. For this purpose the stationary current-voltage characteristic curves (CVC) of the neuron membrane were obtained before and during application of the antibodies. From the difference between the values of the currents corresponding to the same clamped potential on the cell membrane in normal physiological saline and during application of the antibodies, the current arising through the action of antibodies on the membrane was estimated. The results of tests on 14 neurons showed that the inward current which appeared is independent of voltage in the region from —50 to —110 mV. CVC of the membrane of one neuron before and during application of antibodies are shown in Fig. 2. Depolarization of the neuron in response to application of antibodies took place without any change in passive membrane permeability, as was shown by the parallel shift of the CVC relative to its initial position (see Fig. 2: 1, 2). The outward current appearing after rinsing out of the antibodies depended only weakly on voltage between —60 and —110 mV (Fig. 2: 3).

The absence of changes in passive membrane permeability during depolarization evoked by application of antibodies suggested that the observed effects of the antibodies is connected with active ionic transport in the nerve cell membrane. It was accordingly deciced to carry out experiments with ouabain, a specific blocker of active transport of monovalent cations. Experiments on five neurons shows that, after addition of ouabain in a concentration of $5 \cdot 10^{-4}$ M to the external solution surrounding the cell, depolarization of the cell amounted to 6 ± 1 mV, which was less on average than during application of the antibodies. The "pump" current, blocked by ouabain, gradually decreased in the course of membrane hyperpolarization, and tended toward zero at levels below —80 mV (Fig. 3).

Investigation of the outward current developing after rinsing out the antibodies showed that it was blocked completely by ouabain (Fig. 2). An outward current of this kind was observed neither after replacement of the physiological saline with ouabain by normal Ringer's solution nor after artificial holding of the cell for 5-6 min (the usual time of application of the antibodies) at a depolarization level corresponding to that present during application of the antibodies.

The appearance of currents without any change in membrane conductance, and without any reversal potentials, is usually linked with the activity of metabolic electrogenic pumps [4-6, 12]. For instance, during application of antibodies the inward current may be the result of inhibition of the outward Na^+ current, created by Na,K-ATPase. However, the experiments to study the action of ouabain on MP showed that the depolarization arising under the influence of ouabain was lower in value and the current blocked by ouabain was strongly dependent on MP. Thus, we did not find that the effects of ouabain or of the solution of antibodies, in the concentrations used, on the neuron membrane were completely identical. Membrane depolarization in response to application of antibodies cannot be explained purely by suppression of the outward "pump" current, similar to the inhibitory action of ouabain. Application of antibodies leads to more complex changes in Na,K-ATPase activity or to modulation of the activity of other transport ATPases of the cell surface membrane.

Support for the view that active transport of Na^+ and K^+ is involved in the effect of antibodies under investigation is given by the appearance of an outward current, blocked by ouabain, which develops after washing out of the antibodies. Considering that the current is potential-independent between −60 and −110 mV, its genesis can be attributed to activation of transport Na,K-ATPase which, as was shown in [4], can create an outward current of up to 3 nA in magnitude, independent of the clamped voltage in the region between −50 and −90 mV, in the presence of a raised intracellular sodium level. What can cause this activation of Na,K-ATPase as a result of application of antibodies? Depolarization induced by antibodies is not accompanied by a change of membrane permeability, and for that reason an increase in the intracellular Na^+ concentration during depolarization cannot take place on account of increased membrane permeability for Na^+. At the same time, it can be tentatively suggested that antibodies do nevertheless inhibit the work of the transport system, even though the effects of ouabain and of antibodies are not identical. In that case, an increase in the intracellular Na^+ concentration can take place on account of leaking of Na^+ into the cell, uncompensated by Na,K-ATPase activity. However, after the blocking of active ion transport of the cell by ouabain for a period of time equal to the time of application of the antibodies and their subsequent washing out by ouabain, no outward current was found. Consequently, if an increase in the intracellular Na^+ concentration does take place when Na,K-ATPase is blocked, it is not sufficient to activate the transport mechanism. After artificial depolarization of the cell for 6 min at a level corresponding to that of depolarization during application of antibodies, no outward current arises. It can be tentatively suggested that activation of transport of Na,K-ATPase takes place as a result of conformational changes in protein S-100 when the antibody—antigen complex is destroyed.

On the basis of these results we suggest that Na,K-ATPase participates in the effect of antibodies on the nerve cell membrane studied in these experiments.

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MECHANISM OF ACTION OF AMINO ACIDS ON GASTRIC SECRETION

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UDC 612.323-06:612.332.74:547.466

KEY WORDS: amino acids; gastrin; stomach; secretion; regulation.

It was I. P. Raznikov who first showed that proteins contained in food, when absorbed in the intestine and entering the blood stream, may act as stimulators of gastric secretion. These investigations pointed to the primary role of amino acids in that process. The study of the effect of amino acids on function of the digestive system has been continued in Shlygin's laboratory [5, 6].

However, the question of the mechanism of action of amino acids circulating in the blood stream on gastric secretory function has not yet been finally settled. Gastric secretion in its 3rd phase not only enables the final stage of digestion in the stomach to be completed, but it also helps to maintain intestinal digestion, by inducing the release of intestinal hormones. Elucidation of the mechanism of action of amino acids on the functions of the digestive tract is also directly linked with the solution to the clinical problems of parenteral feeding.

For a long time research workers are unable to distinguish between the action of amino acids entering the blood stream and the gastrin mechanism.

It was only by means of radioimmunoassay that it was shown that protein food from the intestine does not cause the serum immunoreactive gastrin level to rise [10]. However, the role of gastrin in the mechanism of action of parenterally administered amino acids on gastric secretion has not yet been solved.

To study this problem several series of investigations were conducted on dogs: experiments were set up using gastrin inhibitors and with direct determination of the blood gastrin level after intravenous injection of various amino acids (lysine, glutamine).

EXPERIMENTAL METHOD

Milid-proglumide and secretin (Boots, England) were used as gastrin blockers. Milid was synthesized in Italy (Milid 200 ^{50}, Rottalab, Monza, Milan). It contains a benzene ring and two isopropyl groups. As a result of substitution of the terminal amino acid of gastrin by these groups, milid acts as a competitive inhibitor of gastrin at the cell receptor level.

In experiments on three dogs with an isolated Pavlov gastric pouch the action of milid was studied on gastric secretion induced by intravenous injection of glycine and of casein hydrolysate. Milid was injected intravenously in a dose of 400 mg in 5 ml of solution before parenteral injection of nitrogenous substances; secretin was infused at the rate of 50 units/h intravenously after injection of the amino acid.

To study the blood gastrin level, experiments also were carried out on four dogs with an intact innervation of the stomach, but which had undergone operations by the techniques of Basov and Pavlov. The blood gastrin level was determined by radioimmunoassay using standard kits from CIS International (France). Blood samples were taken before and 1 h after the beginning of injection of the amino acid, and again h later, to coincide with the end of in-

Laboratory of Physiology and Biochemistry of Digestion, Institute of Nutrition, Academy of Medical Sciences of the USSR. Laboratory of Molecular Pathology and Biochemistry, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental' Biologii i Meditsiny, Vol. 103, No. 1, pp. 5-7, January, 1987. Original article submitted April 1, 1986.

 0007-4888/87/0103-0004$12.50