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MECHANISMS OF THE STIMULATING EFFECT OF ANTIBRAIN ANTIBODIES ON Ca^{++} CURRENTS IN THE NEURON MEMBRANE

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The study of the mechanisms of interaction of antibrain antibodies with antigens of the neuron membrane is an urgent problem in normal and pathological physiology. It was shown previously that antibodies to the microsomal fraction and to synaptic vesicles of the rat brain can potentiate the Ca^{++}-component of action potentials of snail neurons [6, 7].

The aim of this investigation was to study the mechanisms of this effect of antibodies in voltage clamp experiments with recording of Ca^{++}-currents.

EXPERIMENTAL METHOD

Experiments were carried out on isolated unidentified neurons of *Helix pomatia* by the voltage clamp method, using two microelectrodes. Before isolation of the neurons, the nerve ganglia were incubated in an 0.5% solution of trypsin, made up in standard Ringer's solution for snails, at 30°C for 20-30 min. The isolated neurons were placed in a perfusion chamber with a volume of 0.14 ml. The rate of flow of the solution in the chamber was 0.6 ml/min. To record Ca^{++}-currents the standard Ringer's solution in the chamber was replaced by a sodium-free solution containing K^+-channel blockers: 10 mM $CaCl_2$, 4 mM KCl, 4 mM $MgCl_2$, 95 mM tetraammonium bromide, 5 mM 4-aminopyridine, 5 mM Tris-HCl (pH 7.6). To study the effect of Cd^{++} ions on Ca^{++}-currents, $CdCl_2$ was added to the above solution in a concentration of $5 \cdot 10^{-6}$ to $5 \cdot 10^{-4}$ M.

The microelectrodes were filled with 2 M KCl and their resistance was 5-10 MΩ. A standard apparatus (Nihon Kohden, Japan) was used to clamp the voltage on the membrane and record ionic currents. The preparation of transition processes during a stepwise change of potential in these experiments was 0.3-1 msec.

The microsomal fraction of the rat brain and antibodies to this antigen were obtained by methods described previously [5, 8]. In the control experiments, blood serum γ-globulins of an unimmunized rabbit were used. Antibodies or control γ-globulins were diluted in the solution used to record Ca^{++}-currents, either without Cd^{++} ions or with the addition of $2 \cdot 10^{-5}$ M Cd^{++}, depending on the experimental conditions. A glass micropipet with broken off tip, lo-

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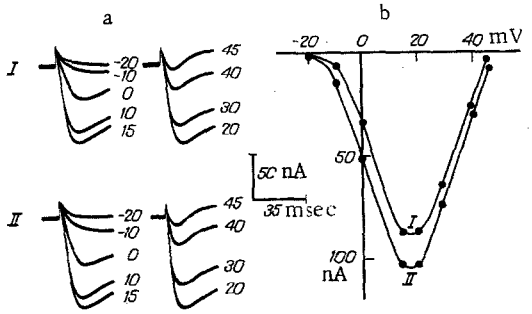

Fig. 1

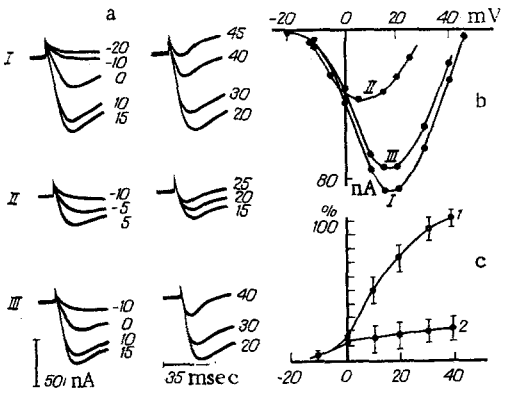

Fig. 2

Fig. 1. Stimulating effect of antimicrosomal antibodies on Ca^{++}-currents of snail neurons. a) Traces of Ca^{++}-currents in control (I) and after application of antibodies (II). Values of testing potential (in mV) shown on right of traces; b) current-voltage characteristic curves of Ca^{++}-currents corresponding to traces.

Fig. 2. Decrease of sensitivity of Ca^{++}-current to blocking action of Cd^{++} ions under the influence of antimicrosomal antibodies. a) Traces of Ca^{++}-currents in control (I), under the influence of $2 \cdot 10^{-5}$ M Cd^{++} (II), and of the combined action of Cd^{++} and antibodies (III). Values of testing potentials (in mV) shown on right of traces of currents; b) current-voltage characteristic curves of Ca^{++}-currents corresponding to traces; c) dependence of inhibitory effect of $2 \cdot 10^{-5}$ M Cd^{++} on Ca^{++}-currents on potential before application of antibodies (1) and in presence of antimicrosomal antibodies (2); abscissa, testing potential (in mV); ordinate, ratio of difference of Ca^{++}-current in control (I_{Ca}) and in presence of Cd^{++} ($I_{Ca/Cd}$) to I_{Ca}. Complete blocking of I_{Ca} by Cd^{++} ions taken as 100%. Mean values of inhibitory effect of Cd^{++} for eight cells indicated by filled circles.

cated 70-80 μ from the neuron, was filled with this protein solution (4 mg/ml). The proteins were applied under a pressure of 1-2 atm. The experiments were conducted at room temperature from March to July.

EXPERIMENTAL RESULTS

The resting potential of the isolated neurons in sodium-free solution with K^+-channel blockers varied from −40 to −70 mV and the resistance of the membrane close to the resting potential was 20-40 MΩ. The holding potential was stablized at −60 mV. By stepwise depolarization for 35-45 msec with an interval of 6-10 sec the membrane potential was shifted up to values of between −30 and +50 mV. During testing depolarization of the membrane inward Ca^{++} currents (I_{Ca}) with an amplitude of 20-200 nA could be recorded. The amplitude of I_{Ca} was estimated as its peak value. The threshold potential for recording I_{Ca} varied from −20 to −10 mV. A further increase in the testing shifts of potential led to a gradual decrease in the amplitude of the inward current, so that on testing with a potential of 40 to 60 mV only outward currents could usually be recorded (Fig. 1).

Application of antibodies to the microsomal fraction of rat brain to the surface of the nerve cell caused an increase in the amplitude of I_{Ca} without any appreciable change in the stationary current. The increase in the maximal I_{Ca} reached 7-38%, so that on average its value was 16 ± 3% (n = 15, Fig. 1a). An increase in I_{Ca} was recorded at all levels of potential (Fig. 1b). The effect of an increase in I_{Ca} developed a few seconds after application of the increased pressure in the micropipet containing antibodies and it ceases 1-2 min after the end of removal of the pressure. Control experiments with the γ-globulin fraction of blood serum from unimmunized rabbits revealed no significant change in I_{Ca} (n = 11).

One possible explanation of the stimulating effect of antimicrosomal antibodies on I_{Ca} is a decrease in Ca^{++} binding by the anionic groups of the channel. To test this hypothesis the effects of antibodies were studied in the presence of Cd^{++} ions, a specific competitive blocker of Ca-channels.

In the present experiments, just as in those of other workers [1, 2], Cd^{++} ions caused dose-dependent inhibition of I_{Ca}. The threshold concentration for blockage of I_{Ca} by Cd^{++} ions was $5 \cdot 10^{-6}$ M, but in the presence of $5 \cdot 10^{-4}$ M Cd^{++} I_{Ca} was completely inhibited. Reduction of the maximal I_{Ca} by 50% was observed when the Cd^{++} concentration was $2 \cdot 10^{-5}$ M (Fig. 2a). However, unlike data published previously [1, 2], in the present experiments the effectiveness of I_{Ca} blockage by Cd^{++} ions increased with an increase in the intracellular potential, as can be seen in Fig. 2b, c, which shows the current-voltage characteristic curve of I_{Ca} after application of $2 \cdot 10^{-5}$ M Cd^{++} and the curve of dependence of the inhibitory effect of Cd^{++} ions on I_{Ca} on voltage.

The effect of antimicrosomal antibodies on I_{Ca} was studied with Cd^{++} ions in a concentration of $2 \cdot 10^{-5}$ M. The experiments showed that application of antibodies significantly reduced the effectiveness of I_{Ca} blockage by Cd^{++} ions, as was shown by an increase in the amplitude of I_{Ca} and also by normalization of the position of the current-voltage characteristic curve of I_{Ca} on the voltage axis (Fig. 2). If the maximal value of I_{Ca} in the presence of $2 \cdot 10^{-5}$ M Cd^{++} was taken as 100%, the increase in I_{Ca} under the influence of antibodies under these conditions averaged 85 ± 10% (n = 8). The effect of the antibodies was quickly reversed on rinsing, so that cessation of application of the antibodies from the micropipet under conditions of continuous flow of the solution through the chamber led after 1-2 min to restoration of the blocking action of Cd^{++} ions on I_{Ca}. Nonimmune γ-globulin did not possess the effect of the antimicrosomal antibodies and did not lead to a decrease in the sensitivity of I_{Ca} to the blocking action of Cd^{++} ions (n = 10).

According to views expressed by Kostyuk et al. [4], permeability of Ca-channels for bivalent cations is controlled by their differential binding with the carboxyl group of the inner selective filter: permeability of the channel for this cation is increased by a decrease in affinity of the latter for the carboxyl group. The blocking action of Cd^{++} ions relative to Ca-channels has been explained by the high affinity of the selective filter of the Ca-channel for these ions [1, 2]. The unusual dependence of the blocking effect of Cd^{++} ions on potential, revealed by the present investigation, does not contradict the above-mentioned model of the Ca-channel, if the suggestion of Byerly et al. [3], that Cd^{++} ions can pass through the channel in the presence of a motive force, is accepted.

Abolition of blockade of Ca-channels by Cd^{++} ions under the influence of antimicrosomal antibodies, observed in the present investigation, can be explained by weakening of binding of these ions by the selective filter of the channel. These changes in the channel, in our view, may be due both to screening of the field of the anionic group of the channel by the positive charges of the protein molecule of the antibody and to structural modification of the channel filter with a decrease of affinity for bivalent cations. These changes in the channel may be responsible, in our view, for the observed strengthening of Ca^{++}-currents under the influence of antibodies in the absence of Cd^{++} ions.

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