ELECTRICAL RESPONSES OF RETZIUS CELLS OF THE LEECH TO INHIBITION OF ACTIVE IONIC TRANSPORT BY OUABAIN

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The effect of the cardiac glycoside ouabain in a concentration of 10^{-4} M on the electrical properties of the Retzius cells in a segmental ganglion of the central nervous system of Hirudo medicinalis was studied by a microelectrode technique. The first phase of the response is depolarization of the cell membrane by 4-11 mV and an increase in the frequency of spontaneous activity with no change in the electrical resistance, both of which develop after about 1 min. The second phase, taking 2-10 min to complete, is characterized by the cessation of spike activity and by a decrease in the resistance of the membrane, and also by the total disappearance of electrical transmission such as is normally observed between the two cells of the ganglion. The response is reversible by prolonged rinsing to remove the ouabain.

KEY WORDS: ouabain; nerve cells of the leech; ionic transport; interneuronal electrical transmission.

Active ionic transport in nerve and other excitable tissues may be closely connected with their specific function [4, 5, 9, 11, 12, 14, 16, 18, 20]. In the investigation described below the effect of inhibition of the active transport through the membrane of the Retzius cells in the segmental ganglion of Hirudo medicinalis on the intracellular potentials, the electrical conductivity of the membrane, and the electrical transmission coupling the two cells of Retzius in each ganglion was investigated [8, 15].

Many physiological and biochemical properties of these cells have been well investigated [1-3, 8, 10, 13, 15], but the relationship between active transport and the bioelectrical properties of their membranes has not been studied. The cardiac glycoside ouabain, in a concentration of 10^{-4} M, was used as the agent to inhibit the enzymic mechanism of active ionic transport [6, 7, 14].

EXPERIMENTAL METHOD

Two glass microelectrodes were inserted into one of two Retzius cells. Pulses of direct hyperpolarizing current (I_d), up to 20 nA in strength and about 1 sec in duration were passed through one electrode and the intracellular potential was recorded through the other. In most experiments a recording electrode also was inserted into the second cell. The resting potentials (RPs) and their increments (U_1 and U_2, in the polarized and nonpolarized cells, respectively) arising during passage of the current were recorded on the tape of an automatic recording potentiometer. Fast processes were photographed from the screen of a cathode-ray oscilloscope. The input impedance of the cell membrane was calculated by the formula $R_{in} = U_1 I_d$ and the coefficient of intercellular electrical transmission by the equation $K_t = U_2/U_1$. The preparation was bathed continuously in a solution from a pipe system permitting the solutions to be changed in a few seconds. The formula of the normal Ringer's solution and the details of the experimental

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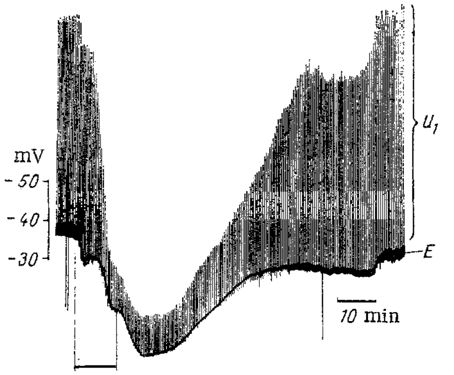

Fig. 1. Effect of ouabain (10^{-4} M) on resting potential E and its increment at times of passage of pulses of polarizing current with a strength of 5 nA and a duration of about 1 sec through the membrane of the Retzius cell. Each pulse of current induces a hyperpolarization increment of intracellular potential U_1, recorded as a vertical straight line about the line E. The horizontal line denotes the time during which the supply system was switched to the solution with ouabain.

procedure were taken from Hagiwara and Morita [8]. Ouabain was dissolved directly in the Ringer's solution to a concentration of 10^{-1} M and the experiments were carried out at 18-20°C.

EXPERIMENTAL RESULTS

A typical example of the response of a Retzius cell to the first application of ouabain is shown in Fig. 1. The response began with rapid depolarization, that developed practically immediately after the ouabain reached the preparation. In 17 cells studied the magnitude of this primary depolarization was 4-11 mV and the mean duration of the intermediate process was about 1 min. At this stage R_{in} was virtually unchanged. The primary depolarization could therefore be due either to a change in the ionic concentration gradients through the cell membrane, which is unlikely for a process at such a rate, or to removal of the component of the RP that can be created by the sodium pump [5-7, 17, 19]. Simultaneously with depolarization a marked increase in the frequency of the spontaneous spike activity characteristic of Retzius cells under electrophysiological experimental conditions was observed [3, 8, 10]. This increase in firing rate can be seen on the records illustrated in Fig. 2A, B, C, obtained in an experiment with the simultaneous recording of intracellular potentials from the two cells. Record A is evidence that a comparatively slow spontaneous spike activity was present before the ouabain started to act. Records B and C were obtained approximately 3 min after the beginning of the response. At this period, besides an increase in frequency, a decrease in amplitude of the action potential (AP) also was observed; this evidently is the usual manifestation of cathodic depression during depolarization. In most experiments after the completion of the rapid primary depolarization R_{in} did not change significantly for some time (up to 2-3 min) longer, and for that reason this state was included in the first phase of the response.

The second phase of the response was characterized by a steady decrease in R_{in} from its initial value of about 9.2 MΩ (within the range from 5.5 to 13 MΩ) to a new stable value averaging about 2.2 MΩ. In the various experiments the new final value of R_{in} was between 20 and 32% of the initial value. The decrease in R_{in} could be judged from the decrease in U_1 when the strength of the polarizing current was unchanged, as will be clear from Fig. 1. Parallel with the fall in R_{in} there was a more marked fall in K_t. This can be seen in the records shown in Fig. 2. Record D was obtained about 45 sec after record C, at the very beginning of the decrease in R_{in}. Clearly K_t had fallen by this time by more than half compared with its initial value (record A), although R_{in} still had its initial value. Record E was obtained at the end of the intermediate process of the decrease in R_{in} and it is evidence of the virtually total absence of electrical transmission. The initial value of K_t could be as high as 0.5-0.6 or more, but at the end of the response it could not be measured accurately, for U_2 was at the artefact level even when the strongest polarizing currents were applied (see Fig. 2E, H). The duration of the second phase was 2-10 min. Later, after the application of ouabain, the value of R_{in} showed little change for several tens of minutes. The excitability of the Retzius cells disappeared completely at this phase of the response. The spontaneous APs were the first to disappear: only two APs, evoked by stopping the hyperpolarization, can be seen on record D, whereas on record E stopping an even stronger hyperpolarizing current no longer evoked an anodal off-response. Depolarization of the cell by a current of any strength likewise could not evoke AP generation after the fall in R_{in}.

At this second stage of the response the behavior of RP is complex. In the experiment illustrated in Fig. 1 the general tendency toward depolarization caused by equilization of the concentration gradients was relatively clear. In many other experiments, however, this secondary depolarization developed much more slowly, so that toward the end of the process of the decrease in R_{in} RP was at about the level reached after primary depolarization. As a rule primary depolarization was followed by one or two depolarization waves superposed, during the decrease in R_{in}, on the more or less well marked secondary depolarization. This complex behavior of the RP was probably attributable to a combination of interconnected changes in

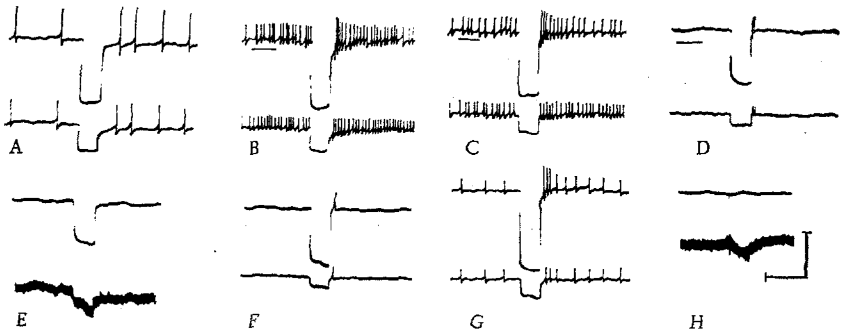

Fig. 2. Records taken at different stages of the response of the two Retzius cells to the action of ouabain (10^{-4} M). Intracellular potential of polarized cell is recorded by the top beam, potential of nonpolarized cell by the bottom beam. Each record shows increment of potentials of the two cells during passage of the pulse of polarizing current: A) initial state; B, C) about 3 min; D) about 4 min; E) about 8 min after beginning of response; F, G) after rinsing for 62 and 75 min, respectively; H) artefact recorded during passage of the current when the microelectrodes were in the surrounding solution. Strengths of polarizing solution 10 nA in A-D, F, and G, 20 nA in E and H. Calibration 2 sec, 50 mV except for bottom beams in E and H, where it is 5 mV.

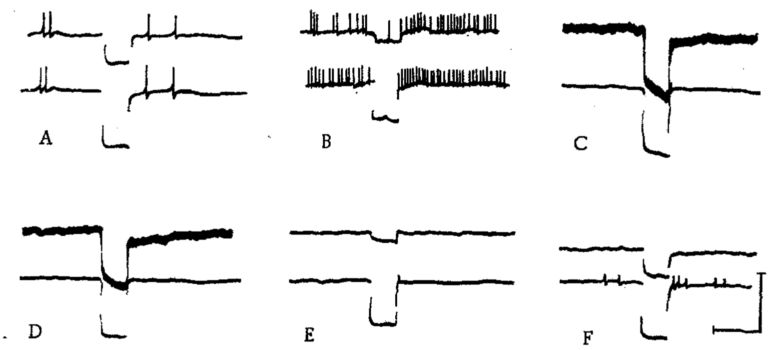

Fig. 3. Records obtained in an experiment with increased concentrations of K^+ ions: A) initial state; B) intermediate process during replacement of normal Ringer's solution by solution containing 30 mM K^+; C) state established by the action of 30 mM K^+; D, E) state established by the consecutive action of 40 and 20 mM K^+; F) a few minutes after returning the preparation to normal Ringer's solution. Strength of polarizing current in A and B 5 nA, in C and D 20 nA, in E and F 10 nA. The cell whose potential is recorded by the bottom beam is polarized. Calibration 2 sec, 40 mV, except for the top beams in C and D, for which it is 4 mV.

the ionic balance of the cell and in the ionic permeabilities of the various parts of the cell membrane, with its complex morphological structure and its functional heterogeneity. The possibility cannot be ruled out that a change in the ionic composition of the intracellular medium leads to changes in the potential of the microelectrode tip, which also are superposed on the RP recorded. The most likely cause of the decrease in R_{in} is considered to be an increase in the concentration of K^+ ions in the narrow intercellular spaces resulting from their liberation from the glial and nerve cells of the ganglion. An increase in the concentration of K^+ ions in the Ringer's solution to 30-40 mM led to the same decrease in R_{in} as was caused by the action of ouabain (Fig. 3).

The cause of the inhibition of electrical transmission during the action of ouabain is a particularly interesting problem (Fig. 2). Clearly when R_{in} falls K_t must also fall on account of an increase in the electrical leaks through the somatic membranes and the membranes of the processes through which electrical transmission takes place in this case. However, on the assumption that the change in electrical transmission is due entirely to changes in the passive electrical characteristics of the cable structure of the cells, a greater or lesser degree of correlation between K_t and R_{in} would have been expected if the initial value of K_t were that recorded between the somata of the Retzius cells, but not the inhibition of transmission as occurred under the influence of ouabain (Fig. 2).

To assess to what extent the passive changes in conductance of the membrane during the action of ouabain themselves affect electrical transmission a series of experiments was carried out in which increased concentrations of K^+ ions were added to the solution bathing the preparations in order to reduce R_{in} to the value observed during the action of ouabain. One such experiment is illustrated in Fig. 3. In this case fairly good correlation is observed between K_t and R_{in}. Comparison of the records in Figs. 2E and 3D shows that equal stimuli in the polarized cell in the first case were virtually not transmitted to the second cell, whereas in the second case the mechanism of electrical transmission itself can be taken to be unchanged, for the fall in K_t here could be completely explained by a corresponding drop in resistance of the somatic membranes. But since this drop was the same in both cases and since it was probably due to the same causes, it can be concluded that ouabain has a specific action on the mechanism of electrical transmission. This in turn means that electrical interaction between the Retzius cells takes place with the direct participation of a membrane enzyme reaction of the sodium pump type.

It will be clear from Figs. 1 and 2 that the action of ouabain on the Retzius cells is almost completely reversible. The RP, the spontaneous spike activity, and R_{in} were restored to their original levels by rinsing the preparation with Ringer's solution for 30-70 min, and R_{in} actually rose a little higher than its initial value (see Figs. 1 and 2). However, under these conditions K_t as a rule did not reach its initial level, and this can be regarded as further confirmation of the hypothesis of the specific action of ouabain on the mechanism of electrical transmission.

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