COMPARISON OF CHANGES IN THE ELECTRICAL ACTIVITY OF A SINGLE NODE OF RANVIER AFTER AN INCREASE IN THE POTASSIUM ION CONCENTRATION IN THE MEDIUM AND THE ACTION OF PROCAINE

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Previous investigations have shown that identical changes take place in the electrical activity of the isolated node of Ranvier when placed in procaine solution and in a medium with a low sodium concentration [1-3]. These changes consisted of depression of the action potential (AP) to a local response, and an increase in the critical level of depolarization, with no change or a slight increase in the resting potential (RP). Whereas, however, an anodic current restored the AP in procaine solution, it increased its amplitude only very slightly in the medium with the low sodium concentration. In both cases the critical level under the anode did not regain its initial value. These facts agree with the hypothesis that procaine inactivates the system of sodium conduction of the membrane.

In the present investigation the changes in the electrical activity of the node were compared during the action of procaine and in the presence of an increased concentration of KCl in Ringer's solution. The action of tetraethylammonium (TEA) and of the anode of a direct current was also studied in these conditions.

METHOD

The test object consisted of isolated fibers of the sciatic nerve of a grass frog (Rana temporaria). A single fiber was isolated from the nerve trunk by means of a slight modification of the method of Kato and Tasaki [24]. The position of the nodes of Ranvier (N_0, N_1, N_2) of the dissected nerve fiber in the experimental chamber is indicated in Fig. 1A. These nodes were connected to the polarizing , stimulating, and detecting circuits of three electrodes ($Cu-CuSO_4$) [11-13]. AP generation of the extreme nodes (N_0 and N_1) was supressed by 0.2% procaine solution. For detection and recording of the potentials at the node (N_1) a cathode repeater was used [4], in conjunction with a dc amplifier and a cathode-ray oscillograph. As test stimuli pulses of direct current with a duration of 6-8 msec were used. The polarizing current was in operation for 1 sec. The experiments were carried out from April to October, 1963. During this period Ringer's solution of the following composition was used: NaCl–111 mM, KCl–1.34 mM, $CaCl_2$–1.81 mM, $NaHCO_3$–2.4 mM. The concentration of NaCl in the solutions with an increased KCl concentration was unchanged. The solution of 20 mM TEA chloride was prepared in Ringer's solution.

RESULTS

An increase in the concentration of KCl in the Ringer's solution to 20 mM depolarized the membrane of the single node of Ranvier, lowered the maximum of the AP and increased the critical level of depolarization of the membrane. With a KCl concentration of 27 mM, besides the AP, a local response developed in the solution, the magnitude of which gradually increased with an increase in the strength of the stimulus. A change in the membrane potential towards the side of depolarization can be seen in Fig. 1B, a, where two responses of a node of Ranvier are superposed: one in normal Ringer's solution and one in a solution with KCl in a concentration of 27 mM. An anodic current, applied to the node, restored both the RP and AP (Fig. 1B, b). The responses of the node in the medium with a high KCl concentration differed from the AP in Ringer's solution by the fact that in the former the phase of repolarization was more drawn out [15].

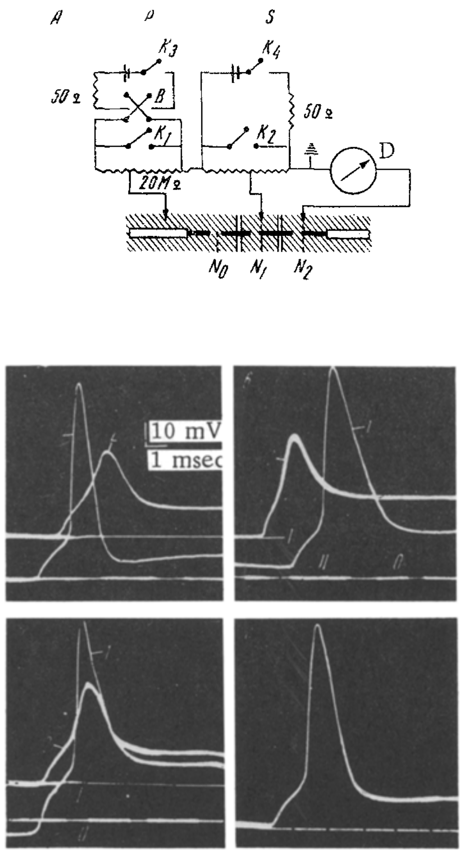

Fig. 1. A) Scheme of experimental apparatus: P) polarizing circuit; S) stimulating circuit; D) detecting circuit, N_0, N_1, N_2) nodes of Ranvier of a single nerve fiber. B) Effect of anodic current and TEA on electrical activity of node in a medium with high potassium concentration; a_1) AP in Ringer's solution; a_2, b_2, c_2) local responses in KCl solution with a concentration of 27 mM; O) original resting potential; I) change in membrane potential indicates the degree of depolarization of membranes in solution of KCl with a concentration of 27 mM; b_1) AP restored by anodic current in solution of KCl with concentration of 27 mM; c_1) AP restored by 20 mM TEA in a solution of KCl with a concentration of 27 mM; II) change in membrane potential in TEA solution shows removal of depolarization; d) AP in Ringer's solution after washing away TEA.

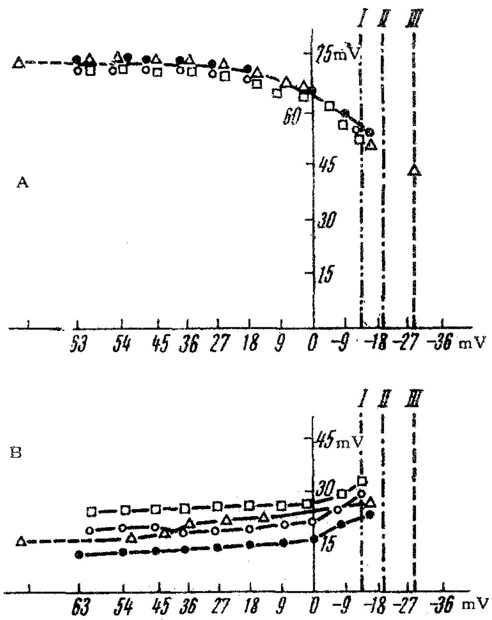

Fig. 2. Relationship between maximum of AP (A) and critical level (B) of depolarization and degree of change of membrane potential during catelectrotonus and anelectrotonus in normal Ringer's solution (black circles) and in KCl solutions with concentrations of 13.5 mM (unshaded circles), 20 mM (squares), and 27 mM (triangles). I, II, III) Changes in membrane potential under influence of KCl in concentrations of 13.5, 20, and 27 mM respectively. Along the axis of abscissas—change in membrane potential in millivolts (a number with a minus sign indicates catelectrotonus). Along the axis of ordinates: A) maximum of AP in millivolts (from zero line to maximum of AP); B) critical level in millivolts (from initial level of membrane potential to beginning of AP).

The relationship between the level of the maximum of the AP of the node in solutions at different concentrations of KCl and the strength of the anodic and cathodic currents applied is shown in Fig. 2A. In the experiment described, restoration of the AP took place after the membrane potential had attained its initial level. However, complete restoration of the AP was not observed until the KCl concentration had risen to 27 mM. With higher concentrations and, in particular, in an isotonic solution of KCl, the anode restored the AP only partially.

A previously published investigation demonstrated that the critical level of depolarization of the membrane in normal Ringer's solution is lowered beneath the anode [11, 12]. In a solution with a high potassium concentration a lowering of the critical level also was observed, but the original values were not restored. The curves given in Fig. 2B illustrate the relationship between the critical level and the degree of change in membrane potential during catelectrotonus in solutions with a high potassium concentration. The critical level under the anode at KCl concentrations of 13.5 and 20 mM was higher than at a concentration of 27 mM. The more abrupt fall of the critical level in KCl solutions of 27 mM or higher concentration was associated with the appearance of hyperpolarization responses [23, 25]. These responses developed in the node after switching on an anode current of threshold strength and they

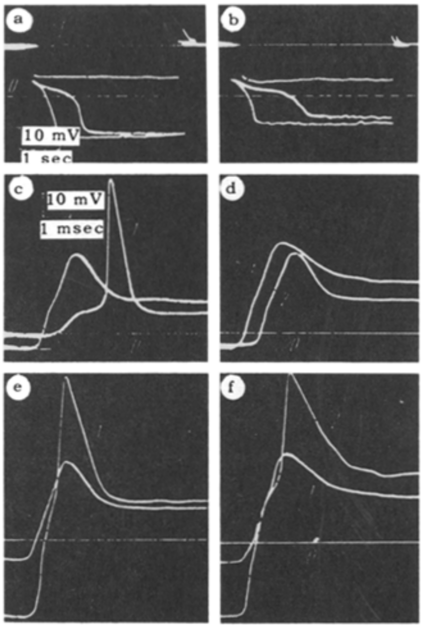

Fig. 3. Change in electrical activity of isolated node of Ranvier. a) Hyperpolarization responses in KCl solution with a concentration of 27 mM; b) the same after addition of procaine to the solution in a concentration of 10^{-4}; O) initial resting potential; I) change in resting potential under the influence of KCl solution with a concentration of 27 mM; c-f) effect of anelectrotonus and TEA on electrical activity of node under the influence of procaine; c_1) AP in Ringer's solution; c_2-d_2-e_2) local responses of node in solution of 10^{-4} procaine; e_1) AP restored by anodic current in solution of 10^{-4} procaine; d_1 and f_1) local responses of node in solution of 10^{-4} procaine and in solution of TEA with a concentration of 20 mM; f_2) AP restored by anodic current in solution of 10^{-4} procaine and in solution of TEA with a concentration of 20 mM; O) initial resting potential; 1) change in membrane potential under the influence of solution of 10^{-4} procaine.

had a latent period which diminished as the strength of the current increased. When the anodic current was switched off, anode-break APs developed (Fig. 3a). The application of the cathode of a direct current to a node in Ringer's solution lowered the amplitude of the AP. This fall was more marked in a solution which an increased KCl concentration (Fig. 2A). Whereas the AP under the cathode fall, the critical level, on the contrary, rose. These changes were observed especially clearly in solutions with an increased KCl concentration (Fig. 2B).

The electrical activity of the node of Ranvier was also depressed by the action of procaine (Fig. 3c). The anode of a direct current, when applied to the node under the influence of procaine, restored the AP and lowered the critical level (Fig. 3e). Summation of the effects of catelectrotonus and procaine took place during their combined action. This was shown by the fact that the increase in the critical level and the depression of the AP under the cathode were more marked after the action of procaine than before.

Several authors [18, 25] have shown experimentally that TEA lowers the depolarization of the membrane in a medium with a high potassium concentration. Our own experiments, carried out together with B. I. Khodorov, confirmed these observations. At the same time, they showed that TEA restores the AP in a medium with a high concentration of KCl. It is clear from Fig. 1B, c that under the influence of a TEA solution with a concentration of 20 mM there was a lowering of the depolarization caused by a KCl solution with a concentration of 27 mM, and the AP was completely restored. In these circumstances the AP was more protracted than the AP during the action of the anodic current (Fig. 1B, b, c). The effect of TEA did not disappear even after many changes of Ringer's solution. The AP remained longer in duration than initially (Fig. 1B, d). In contrast to the effect of TEA in a medium rich in KCl, in procaine solution it did not abolish the depression of AP generation, but simply increased the amplitude and duration of the local response (Fig. 3d). In the node under the influence of procaine, the anodic current in the presence of TEA restored the AP. This AP was still more drawn out than in the other cases we have considered (Fig. 3f). Treatment of the node with procaine did not abolish the hyperpolarization responses and did not change their configuation (Fig. 3b).

The results obtained were similar in principle with those of other workers studying the changes in the electrical activity of the node of Ranvier during an increase in the concentration of KCl solution [17-20, 21, 28]. In our experiments an increase in the concentration of the KCl solution in the medium caused depolarization of the membrane of the node and lowered the AP. Application of an anodic current, adequate in strength to cause repolarization of the membrane in a solution with a high concentration of KCl, restored AP generation [5-9, 15]. According to the theory of Hotchkiss and Huxley, depression of the electrical activity of the node in a medium rich in KCl takes place as a result of inactivation of the sodium conductivity of the membrane. The anode of a direct current, when applied to the membrane depolarized with an excess of KCl, restores the RP to its original level, removes the cause of the inactivation, and restores the AP. The cathode current, on the other hand, by increasing the depolarization of the membrane, at the same time strengthens the degree of inactivation, as a result of which depression of the AP takes place.

Besides lowering the amplitude of the AP, an excess of K^+ ions in the medium caused a considerable rise in the critical level. This rise was dependent on the degree of depolarization of the membrane. Accordingly, during the action of the cathode, the increase in the critical level in the solution rich in KCl became still more marked. Apparently the anode should restore the critical level in an excess of KCl. This, however, did not take place. Although the critical level was lowered under the anode, it still remained much higher than its initial value.

TEA acted like the anode on the node in the medium with a high potassium concentration: it abolished the depolarization of the membrane, in consequence of which the AP was restored. The changes in the AP in the node during an increase in the KCl solution were similar to those observed under the influence of procaine [1-3, 26]. There was one difference, however: in procaine solution the membrane resting potential is unchanged or slightly increased [14], while in a medium with a high potassium concentration it is considerably lowered [17-20]. This evidently may explain the observation that TEA restores the AP in a high-potassium medium but has no such effect on the node when under the influence of procaine.

Various authors have suggested [22, 27] that local anesthetics increase the inactivation of the sodium conductivity of the membrane. The anode of a direct current, when applied to a node under the influence of procaine, abolishes this inactivation partially or completely—depending on the degree of hyperpolarization,—and this leads to restoration of the AP.

Since TEA does not increase the membrane potential of the node under the influence of procaine, this explains why it does not restore the AP. In contrast to the AP, the critical level of depolarization in procaine solution, as also in a medium with a high potassium concentration, is not fully restored by the action of an anodic current. It evidently follows from this that the increase in the critical level under the influence of procaine or of excess of KCl in the solution cannot be attributed entirely to an increase in the degree of inactivation of the sodium conductivity of the membrane. The latter is evidently completely responsible only for the change in the AP.

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