INCREASE IN NEGATIVE AFTER-DEPOLARIZATION OF THE SINGLE RANVIER NODE OF ISOLATED NERVE FIBERS UNDER THE INFLUENCE OF BIVALENT (Ca^{++}, Ba^{++}, Mn^{++}, Zn^{++}, Ni^{++}) AND TRIVALENT (La^{+++}) IONS

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An increase in the Ca^{++} concentration in Ringer's solution and the addition of Ba^{++} and Mn^{++} to it (up to 10 mM) have been shown to increase (by 20-50%) the amplitude and duration of negative after-depolarization (NAD) of the single Ranvier node of the meylinated nerve fiber. Meanwhile the time constant of NAD is virtually unchanged.

Addition of Zn^{++} and Ni^{++} (1, 5, and 10 mM) and La^{+++} (0.1-1 mM) ions to the external solution causes a sudden (3-5-fold) increase in amplitude and an even greater increase in the duration of NAD. In this case the time constant of NAD is increased by 1.5-3 times. It is concluded that the increase in NAD under the influence of polyvalent ions is the result of their screening of the negative charges on the outer surface of the membrane and changes in the time constants of activation and inactivation (τ_n, τ_m, τ_h) of the ion channels.

It was shown previously [1] that NAD of intact nerve fibers consists of two components: short (τ = 1.2 msec) and long (τ = 48 msec). In single nerve fibers isolated by the classical method, however, only the short component of NAD is found, and its duration is only 3-5 msec [12]. The causes of the absence of the long NAD and the exaltation phase associated with it in single Ranvier nodes of isolated nerve fibers have not yet been explained. The present writer has suggested that during the procedure of isolation of the single nerve fiber the structure of the Ranvier node is damaged and, in particular, the integrity of the diffusion barrier formed by outgrowths of the Schwann cells, which in the intact fiber are responsible for the accumulation of potassium ions flowing from the axoplasm in the juxtamembranous space, is disturbed. However, there is no direct proof as yet of this hypothesis. It is also known [8] that the long NAD and the exaltation phase connected with it are absent in the recently dissected frog nerve for 20-40 min. Disappearance of NAD in this case cannot be explained by damage to the structure of the Ranvier node of the nerve fibers, more especially because it is reversible in character.

To continue the systematic analysis of the nature of after-potentials and the causes of their considerable differences in intact and isolated nerve fibers [1], the writer studied how NAD of the single Ranvier node responds to polyvalent ions known to have the property of screening negative charges fixed on the outer surface of the membrane, and of inducing an effect resembling hyperpolarization [2-4, 6, 9, 10].

EXPERIMENTAL METHOD

Experiments (over 50) were carried out by a modification of the classical technique [14] described previously [1]. In the initial state the single Ranvier node was kept in Ringer's solution of the following composition: Ca^{2+}. The action of the polyvalent ions chosen was studied by adding them to the external Ringer's solution. The pH of the solution was kept at 7.3, or 6.6 in the case of addition of La^{+++} ions to the external solution.

EXPERIMENTAL RESULTS

The data on the effect of an increase in the Ca^{++} concentration in the external solution up to 10 mM on the single Ranvier node showed (Fig. 1A, B) a marked increase in the threshold potential. The amplitude and duration of the action potential remained about the same as in a solution with the original Ca^{++} concentration. NAD was increased by 1.3 times by an increase in the Ca^{++} concentration. Addition of 10 mM Ba^{++} (Fig. 1C), and Mn^{++} (Fig. 1D) to the external solution had a similar action. The time constant of NAD was significantly increased (by 3-7%) or unchanged by an increase in the Ca^{++} concentration in the Ringer's solution (Fig. 1E: a, b) and addition of Mn^{++} (Fig. 1E: c, d) and Ba^{++}. The effect of these ions on NAD of the single node of the isolated nerve fiber was thus in principle the same as during hyperpolarization

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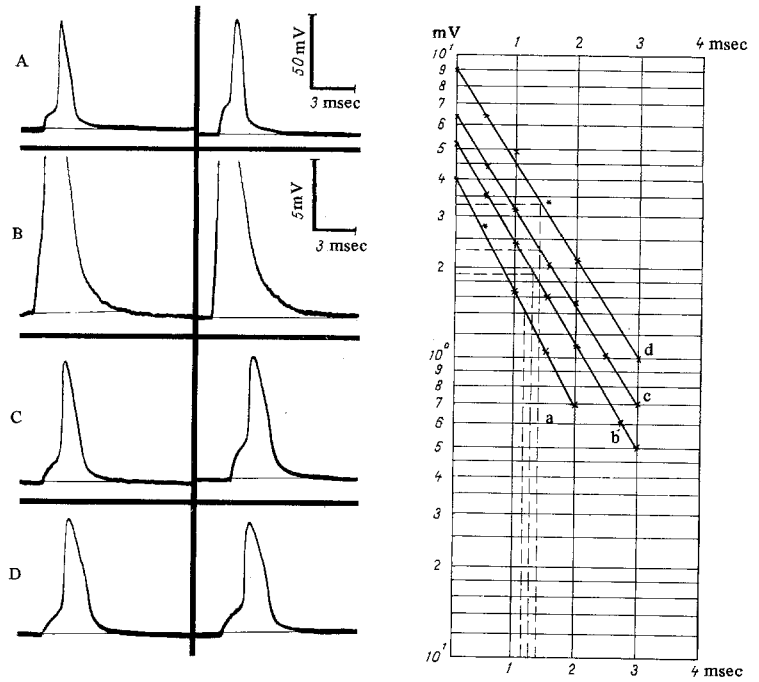

Fig. 1. Effect of increase in Ca^{++} concentration in Ringer's solution and addition of Ba^{++} and Mn^{++} ions to it on action potential and NAD of single Ranvier node. Traces on left recorded from preparations kept in Ringer's solution of normal composition, traces on right recorded after an increase in Ca^{++} concentration in solution to 10 mM (A, B) or addition of Ba^{++} (C) and Mn^{++} (D) ions to it. E) Semilogarithmic graph of NAD of nerve fibers in Ringer's solution (a), after an increase in Ca^{++} concentration in it to 10 mM (b), and also before (c) and after (d) addition of 10 mM Mn^{++} to solution.

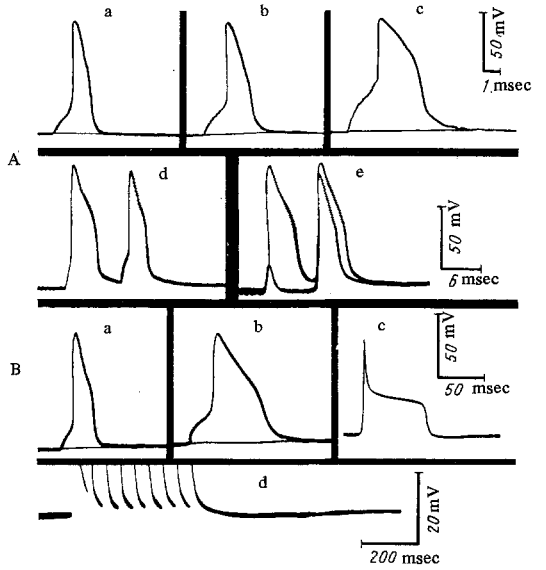

Fig. 2. Change in action potential and NAD of single Ranvier node as a result of addition of Zn^{++} (A) and Ni^{++} (B) ions to the external solution. Explanation in text.

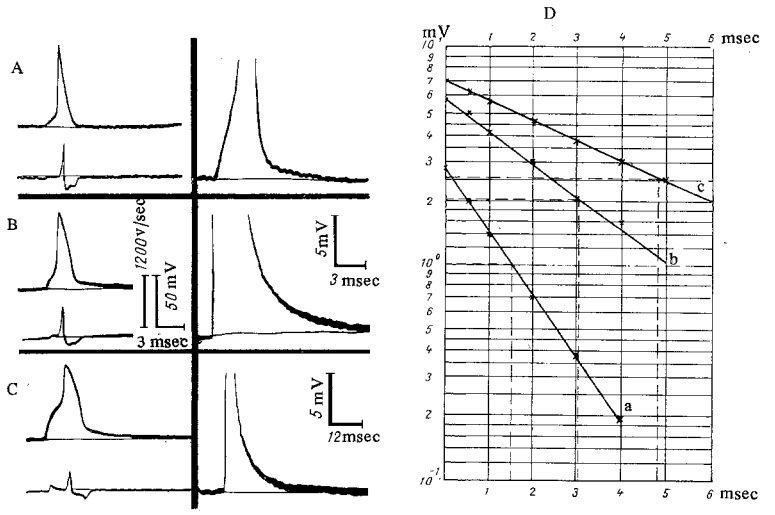

Fig. 3. Changes in action potential and NAD of single Ranvier node following addition of 0.1 mM (B) and 1 mM (C) La^{+++} to Ringer's solution. Traces on left show action potentials of nerve fiber, those on right show NAD recorded after additional tenfold amplification. D) Semilogarithmic graph of NAD of single Ranvier node surrounded by Ringer's solution (a) and after addition of 0.1 mM (b) and 1 mM (c) La^{+++} to solution.

by an anodal current [3].

The other group of so-called plateau-forming [13] bivalent ions had a much stronger and more marked action on the spike and NAD of single Ranvier nodes. Of the members of this group, the effect of Zn^{++} and Ni^{++} ions was studied. It will be clear from Fig. 2A (b) that the addition of 1 mM Zn^{++} to the external solution caused a marked increase in duration of the action potential and NAD of the node. After the addition of 5 mM Zn^{++} to the solution the repolarization phase was slowed so that a plateau appeared at the end of the spike, with the result that the duration of the action potential was increased by 2.5-4 times. NAD also was increased in this case. This became clearly visible when a relatively low level of amplification was used (see calibration). Under the influence of 5-10 mM Zn^{++} the duration of NAD reached 10-25 msec. When paired stimuli were applied (Fig. 2A: d) a characteristic feature was found: The second action potential arising against the background of NAD after the previous potential was reduced in both amplitude and duration. In the next frame (Fig. 2A: e) the two traces are superposed on each other. A response to paired above-threshold stimuli was recorded first, just as in the previous frame, after which the intensity of the first stimulus was reduced to below threshold, and responses to paired stimuli were recorded again. In the absence of an above-threshold response to the first stimulus, the response to the second stimulus was of the same amplitude and duration as that to the first.

Ni^{++} ions had a similar effect on the action potential and NAD to Zn^{++}. The addition of 1 mM Ni^{++} to the external solution increased the duration of the action potential by 1.5-2.5 times and increased the amplitude and, in particular, the duration of NAD considerably (Fig. 2B: b). The time constant of NAD, like the action of Zn^{++} ions, was increased by 1.5-3 times. During repetitive stimulation of a node surrounded by solution containing Ni^{++} ions the amplitude of NAD fell successively, and at the end of tetanization it was several times less than after a single action potential (Fig. 2B: d).

The addition of tetraethylammonium (TEA) to a solution containing Ni^{++} ions very greatly (by 5-15 times) lengthened the plateau of the action potential (Fig. 2B: c). This fact was first described by Khodorov and Belyaev [3], but as yet no satisfactory explanation is forthcoming.

Of the trivalent ions, only the action of La^{+++} was studied. As will be clear from Fig. 3B, the addition of 0.1 mM La^{+++} to the external solution caused an increase in the threshold potential, a small increase in the duration of the spike, a decrease in the maximal steepness of rise of its ascending part (V_{max}), and strengthening of NAD. The time constant of NAD was doubled. These changes in action potential became even more marked with an increase in the La^{+++} concentration to 1 mM (Fig. 3C). However, in this case the action potential did not form a plateau.

After the addition of 1 mM La^{+++} to the external solution NAD increased in both amplitude and duration. In this case, just as in normal Ringer's solution, decay of NAD was approximately exponential. However, the time constant of NAD rose sharply (Fig. 3D).

The results described above thus show that polyvalent ions cause a significant increase in the threshold potential and NAD of the single Ranvier node of the isolated nerve fiber. Membrane voltage clamping

experiments have shown that under the influence of polyvalent ions the curve of dependence of the parameters of ionic permeability of the membrane ($P_{Na}-E$, P_K-E, $h_\infty-E$) is shifted along the voltage axis toward depolarization [2-4, 6, 9, 10, 15]. All these effects can be explained by screening of the surface negative charges on the membrane by polyvalent ions. The action of Ca^{++}, Mn^{++}, and Ba^{++} ions on the Ranvier node can evidently be attributed mainly to this factor.

However, polyvalent ions can also have a specific action on the node membrane. Membrane voltage clamping experiments have shown that Ni^{++} ions affect the kinetics of ionic currents. On the addition of these ions to the external solution the time constants of activation and inactivation (τ_m, τ_n, τ_h) are considerably increased [8-10], with consequent lengthening of the action potential [12, 13, 23]. In this respect the effect of Ni^{++} ions is similar to the effect of cooling [9, 10, 12]. Experiments on the squid giant axon have shown that addition of Zn^{+++} ions (10 mM) to the external solution delays sodium activation. This has recently been confirmed by Armstrong and Gelly [6]. The effect of Zn^{++} ions on the kinetics of ionic currents in the Ranvier node of myelinated nerve fibers has not been studied. However, to judge from the identical character of changes in the action potential and NAD, it can be postulated that the effect of Ni^{++} and Zn^{++} ions on the parameters of ionic permeabilities of the Ranvier node is probably similar.

As Fig. 2 shows the addition of Ni^{++} and Zn^{++} ions to the external solution causes predominantly slowing of the repolarization phase and an increase in amplitude and duration of NAD. In the writers' view, these two phenomena cannot be explained purely by a decrease in the repolarizing potassium current (I_K) [6, 7, 14, 15]; they are evidently also due to weakening of sodium inactivation and by preservation of the inward sodium current (I_{Na}) after the spike.

Blocking the potassium channels of the membrane by TEA is known to lengthen the repolarization phase only a little (by 1.2-1.3 times) [3], substantially less than the lengthening which follows addition of Ni^{++} and Zn^{++} ions to the external solution. It will be clear from Fig. 2A (d, e) and Fig. 2B (d) that the plateau of the action potential and NAD are reduced after repeated stimulation. Probably the only explanation of this fact is that the sodium channels of the membrane, which are left in a conducting and inactivated state, do not take place in generation of the next action potential.

An important feature distinguishing the action of Ni^{++} ions on the Ranvier node membrane is their ability, if added together with TEA, to cause the appearance of an extremely long action potential plateau (Fig. 2B: c), 5-15 times longer than that after the addition of Ni^{++} ions alone to the external solution. NAD in this case also is increased a little. This effect can only be explained by prolonged preservation of sodium permeability after the spike. In all probability the mechanism of sodium inactivation is disturbed by the combined action of Ni^{++} ions and TEA.

According to Vogel [15], when La^{+++} is added to the external solution there is a substantial fall in the leakage current (I_l). Addition of TEA to a solution already containing La^{+++} ions does not cause the appearance of an action potential plateau. This likewise does not take place after the combined action of TEA with Ba^{++}, Mn^{++}, and Zn^{++} ions. All this indicates the presence of specific differences in the action of polyvalent ions on the nerve fiber membrane. The increase in NAD under the influence of bivalent and trivalent ions is the result of screening of negative charges fixed on the outer surface of the membrane, and also of the effect of some of them on the time constants of activation and inactivation (τ_m, τ_n, τ_h) of the ionic channels. In this case restoration of the long component of NAD, characteristic of intact nerve fibers [1], does not take place. This state of affairs suggests that the origin of the long component of NAD is probably not connected with a change in the kinetics of ionic currents arising during the action potential, and in the writer's view it confirms the hypothesis put forward previously [1].

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