CHANGES IN THE ELECTRICAL ACTIVITY OF A SINGLE NODE OF RANVIER OF AN ISOLATED NERVE FIBER DUE TO NOVOCAIN

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The present study is a continuation of investigations of the direct action of novocain on nerve tissue conducted at the A. V. Vishnevskii Institute of Surgery [3-5, 7, 8]. Studies have been made of changes in excitability, conduction, electrotonus, and accommodation of the nerve in vitro and in vivo, in warm- and cold-blooded animals. However, these investigations were carried out on whole nerve trunks, consisting of many thousands of nerve fibers, covered by a perineural sheath not readily permeable to novocain solution, making the accurate analysis of the results difficult.

We have studied the effect of novocain on the electrical activity of the isolated nerve fiber. The special feature distinguishing our experiments was that novocain, in various concentrations, was applied directly to a single node of Ranvier in response to application of cathodic and anodic stimuli of different strength were recorded. The preliminary results were published earlier [1].

EXPERIMENTAL METHOD

Investigations were carried out on isolated nerve fibers of the grass frog, dissected by a slightly modified Kato-Tasaki technique [21]. The perineural sheath of the nerve trunk was incised and removed by means of specially pointed and ground steel needles. The nerve fiber, isolated for a distance of 10-12 mm, with the adjacent parts of the nerve trunk, was transferred to a plexiglass gutter in such a way that one node (N_2) lay in the middle compartment and two other nodes (N_1 and N_3) in side compartments, consisting of movable glass slides with rounded, ground edges. The air-filled gaps between these compartments (0.2-0.5 mm wide) served as insulating bridges (Fig. 1, A). A three-electrode apparatus was used for recording and stimulation (nonpolarizing Cu – CuO_4 electrodes). The Verigo-Khodorov scheme was used to provide simultaneous stimulation and polarization [11].

Action potentials were recorded by means of a cathode repeater [2], a high-stability dc amplifier, and a cathode-ray oscillograph. So that potentials could be taken from only the middle node of Ranvier (N_2), receiving direct stimulation, the Ringer's solution in the side compartments was replaced by a 0.2% novocain solution, depressing the activity of the nodes N_1 and N_3. The frogs were kept for long periods in the refrigerator at 5-6°. The experiments were conducted at 19-21°.

EXPERIMENTAL RESULTS

A rectangular cathodic stimulus of rheobase strength produced passive depolarization of the membrane in a solitary node of Ranvier, changing into an active, slowly increasing local response, developing into the ascending phase of an action potential when it reached the critical level of depolarization [14, 15]. The threshold shift of the membrane potential of the node beneath the cathode will be referred to henceforward as the "threshold of depolarization" ΔV (Fig. 1, B, a).

The action of novocain solution in a concentration of $1 \cdot 10^{-8}$ and $1 \cdot 10^{-7}$ on the test node of Ranvier (N_2) did not alter the amplitude of the action potential and threshold of depolarization. If the novocain concentration was increased to $5 \cdot 10^{-5}$ the amplitude of the action potential gradually increased and the threshold of depolarization rose progressively (Fig. 1, B). In a concentration of $1 \cdot 10^{-4}$, novocain lowered the amplitude of the spike by 75-80%, while the threshold of depolarization rose to 200% (Fig. 1 C).

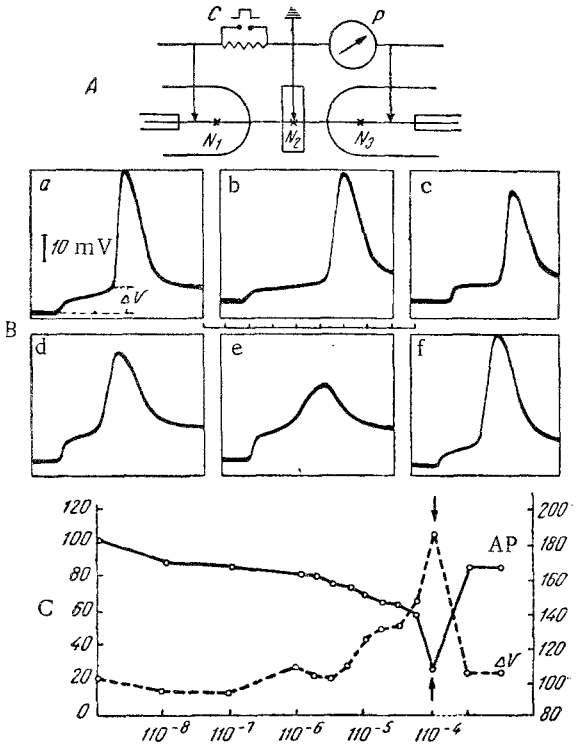

Fig. 1. A) Scheme of arrangement of the nerve fiber in the experimental chamber. N_1, N_2, N_3) nodes of Ranvier; C) stimulating circuit; P) recording circuit. B) Changes in amplitude of action potential (AP) and threshold of depolarization of a solitary node of Ranvier (N_2) under the influence of novocain in different concentrations: a) AP of a node of Ranvier in Ringer's solution; b, c, d, e) responses of nodes of Ranvier in novocain in concentrations of $1 \cdot 10^{-8}$, $1 \cdot 10^{-5}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-4}$; f) AP after rinsing novocain away with Ringer's solution. Time marker 1 millisec. C) Relationship between amplitude of AP and threshold of depolarization, and concentration of novocain. Along the axis of abscissas) concentration of novocain in Ringer's solution. Along the axis of ordinates: on the left) amplitude of AP (in %), on the right) threshold of depolarization (in %). The arrow indicates replacement by Ringer's solution.

Besides these changes in the amplitude of the action potential and the threshold of depolarization, the shape of the spike also changed. It became more drawn out and rounded as a result of the slower development of the ascending phase. At a certain stage of the novocain effect, the action potential ceased to obey the "all or nothing" law. The amplitude and gradient of the increase in the response rose gradually with an increase in the strength of the stimulus. The sharp demarcation between the local response and the action potential became obliterated (Fig. 2, c, d). We distinguish conventionally between the action potential and the local response by the presence of a genu, with its convexity uppermost, on the response curve (Fig. 2, b). In some cases, however, it was practically impossible to distinguish between the local response and the action potential.

These results differ in some respects from the observations made by certain workers who recorded graded responses from solitary nodes of Ranvier of medullated fibers in the frog during weak depolarization caused by a dc cathode and KCl [18] and under the influence of urethane [17, 23], and also of non-medullated giant axons of the squid and the axons of the ventral chain of the earthworm in responses demonstrates that the application of the "all or nothing" law is only relative, and constitutes, in our opinion, an important argument in support of the view that the local response and the action potential have a common origin and are different phases of the process of active depolarization of the membrane.

The changes produced by novocain in the node of Ranvier were not fully reversible. After frequent changing of the Ringer's solution over a period of 10-50 min, the action potential recovered to the extent of 90% of the initial amplitude of the spike and the threshold of depolarization fell from 200 to 110% [20, 22].

There is no general agreement on the question of the mechanism of action of novocain on an excitable membrane. The view most widely accepted is that of Shanes and co-workers [20] and of Taylor [22], who investigated the effect of novocain on the electrical activity of the non-medullated giant axon of the squid by means of microelectrodes, using the fixed potential method. These workers concluded that novocain stabilizes the membrane so that, as a result, during excitation its permeability to potassium and, in particular, sodium ions is reduced.

This point of view may explain the increase in the thresholds of depolarization and the decrease (or even total suppression) of the thresholds of the action potentials observed in our experiments. However, the mechanism of the stabilization of the membrane during the action of local anesthetics is not clear, and in this connection the effects of the dc anode and cathode on the normal and novocainized node of Ranvier are of interest. Previous experiments [12] showed that beneath the cathode the amplitude of the action potential is lowered and the critical level of depolarization rises. The anode opposite to the dc cathode increases the amplitude of the action potential and lowers the critical level of depolarization.

The present experiments showed that these effects become especially prominent during the action of a direct current on the node of Ranvier under the influence of novocain. Beneath the cathode there was a marked lowering

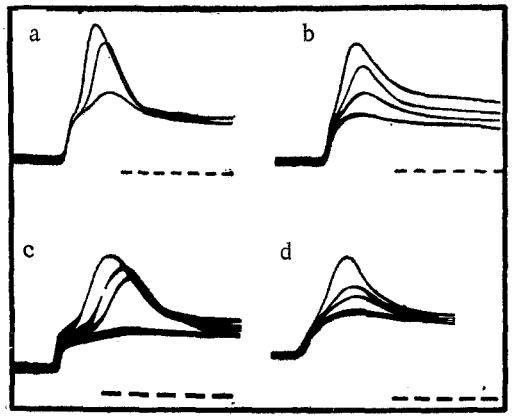

Fig. 2. Effect of an increase in the strength of the stimulating current on the amplitude of the response of the node of Ranvier under the influence of novocain in concentrations of $5 \cdot 10^{-5}$ (a), $1.3 \cdot 10^{-5}$ (b), and $1 \cdot 10^{-4}$ (c, d). Time marker 1 millisec.

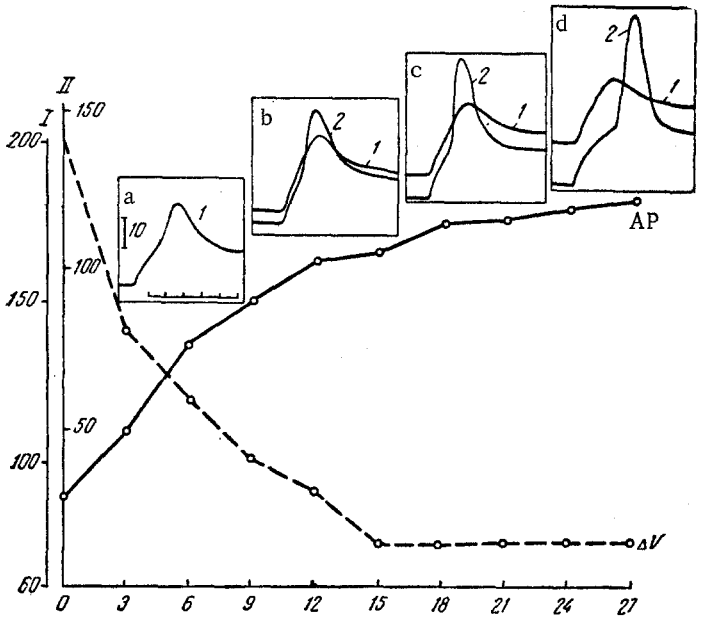

Fig. 3. Restoration of the action potential (AP) and threshold of depolarization of a novocainized node of Ranvier by a dc anode. a_1, b_1, c_1, d_1) responses of a node of Ranvier under the influence of novocain in a concentration of $1 \cdot 10^{-4}$; b_2, c_2, d_2) ditto, in conjunction with application of an anode voltage of 6, 18, and 24 mV. The displacement of the zero line corresponds to the change in the resting potential beneath the anode. Time marker 1 millisec. Relationship between the amplitude of the response and of the threshold of depolarization of the node of Ranvier under the influence of novocain and the strength of the applied anodic current. Along the axis of abscissas) strength of anodic current (in mV). Along the axis of ordinates: I) amplitude of AP (in %); II) threshold of depolarization (in %).

of the action potential and an increase in the threshold of depolarization, or, in other words, the effects of the cathode and of novocain were added together. Beneath the anode, on the other hand, the action potential depressed by the action of novocain increased sharply, while the threshold of depolarization fell. This restorative action of the anode became more marked as the strength of the polarizing current was increased. When the strength of the anodic current reached a certain limit, the action potential rose to its initial value (Fig. 3, b).

The restorative action of the anode on excitable tissues under the influence of novocain was observed by Pasternak and Arnold [19] in bundles of nerve fibers and by Weidman [24] in Purkinje fibers. In order to explain his findings, Weidman used the concept of Hodgkin and Huxley [16] that there are three possible states of sodium carrier systems – resting, active, and inactive –, to suggest two possible mechanisms of the restorative action of the anode: 1) activation of the system of sodium carriers and 2) displacement of the novocain cations from their point of action on the excitable membrane.

We consider that the second possible explanation is improbable, for the anode increases the amplitude of the action potential and lowers the critical level, not only of nerve fibers under the influence of novocain, but also of normal nerve fibers. The differences between the action of the anode in these cases are purely quantitative.

On the other hand, there is no concrete physiological evidence concerning the physico-chemical nature of the processes of inactivation and activation. The system of sodium conductivity is known to be inactivated by depolarization. Novocain, however, as various workers have shown [9, 13] and as our experiments confirmed, does not depolarize membranes. The restorative influence of the anode on the node of Ranvier under the influence of novocain cannot therefore be the direct consequence of a change in the membrane potential, but is apparently connected with metabolic changes taking place in the protoplasm of the nerve fiber beneath the anode.

SUMMARY

A solution of novocain in small concentrations ($1 \cdot 10^{-8}$ and $1 \cdot 10^{-7}$) does not cause changes of action potential (AP) amplitude and threshold of depolarization (ΔV) in the node of Ranvier of isolated frog's nerve fiber. A novocain concentration of $5 \cdot 10^{-5}$ causes a reduction of response amplitude and a rise of the depolarization threshold. Responses are graded, i.e., increase with an increase in the strength of its stimulus. Novocain solution in a concentration of $1 \cdot 10^{-4}$ depresses AP in the node and causes a rise of threshold of depolarization up to 200%. Changes caused by novocain are usually not completely reversible even after long wash-off. Electrical activity, depressed by novocain in the node of Ranvier restored by the action of a direct current anode. If anode voltage is sufficiently large, the AP is completely restored and the threshold of depolarization is at the same level as before. The effect of a direct current cathode is similar to that of novocain. A cathode current causes a sharp fall of AP and a considerable rise of the threshold of depolarization. The possible mechanism of the novocain action and the restorative effect of the direct current anode is discussed.

LITERATURE CITED

1. V. I. Belyaev. In the book: Problems in Lability, Parabiosis, and Inhibition [in Russian], p. 25, Moscow, 1962.
2. A. L. Byzov and M. M. Bongard. Fiziol. zh. SSSR, 1, 110 (1959).
3. A. A. Vishnevskii (A. A. Wischnevsky). Arch. klin. Chir., 1930, Bd. 159, S. 501.
4. A. A. Vishnevskii, and B. I. Khodorov. Éksper. khir., 6, 3 (1959).
5. M. V. Kirzon, O. R. Kol's, and A. M. Tsukerman. Trudy Akad. Med. Nauk SSSR (Moscow) 12, 137 (1951).
6. M. V. Kirzon. In the book: Problems in Lability, Parabiosis, and Inhibition [in Russian], p. 109. Moscow, 1962.
7. R. V. Pravdich-Neminskaya. Trudy Akad. Med. Nauk SSSR (Moscow) 12, 155 (1951).
8. S. P. Protopopov. Pathogenesis and Treatment of Indolent Wounds [in Russian]. Moscow, 1950.
9. F. N. Serkov. Abstracts of Proceedings of a Scientific Conference on the Subject of N. E. Vvedenskii's Concept of Parabiosis [in Russian], p. 91. Leningrad, 1957.
10. S. A. Chepurnov. In the book: Problems in Lability, Parabiosis, and Inhibition [in Russian], p. 248. Moscow, 1962.
11. B. I. Khodorov. Uspekhi sovr. biol. 29, 3, 329 (1950).
12. B. I. Khodorov. In the book: Problems in Lability, Parabiosis, and Inhibition [in Russian], p. 246. Moscow, 1962.
13. A. L. Bennett and K. G. Chinburg, J. Pharmacol. exp. Ther., 1946, Vol. 88, p. 72.
14. J. DelCastillo and L. Stark, J. Physiol. (Lond.), 1952, Vol. 118, p. 207.
15. A. L. Hodgkin, Proc. roy. Soc., 1938, Vol. 126, p. 87.
16. A. Hodgkin and A. Huxley, J. Physiol. (Lond.), 1952, Vol. 116, p. 449.
17. S. Kitamura, Jap. J. Physiol., 1960, Vol. 10, p. 51.
18. P. Mueller, J. gen. Physiol., 1958, Vol. 42, p. 137.
19. J. Posternak and E. Arnold, J. Physiol. (Paris), 1954, Vol. 46, p. 502.
20. A. Shanes, W. Freygang, H. Grundfest, et al., J. gen. Physiol., 1959, Vol. 42, p. 793.
21. I. Tasaki. Conduction of the Nervous Impulse [Russian translation]. Moscow, (1957).
22. R. Taylor, Am. J. Physiol., 1959, Vol. 196, p. 1071.
23. Y. Uechara, Jap. J. Physiol., 1960, Vol. 10, p. 267.