EFFECT OF NOVOCAIN ON ELECTRICAL ACTIVITY OF A SINGLE NODE OF RANVIER IN SOLUTIONS WITH HIGH AND LOW SODIUM ION CONCENTRATIONS

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The work of Crescitelli [11] has shown that a conduction block developing as a result of cocaine on the sciatic nerve in the frog is intensified in the NaCl concentration in the solution is low. These findings were subsequently confirmed by Condouris [10] in experiments on nerve trunks deprived of their perineural membranes. This worker also showed that an increase in the NaCl concentration in solution facilitates the conduction of excitation through the nerve segment modified by cocaine.

Because of the importance of these findings for understanding the mechanism of action of local anesthetics and their clinical application [6], in the present investigation the effect of novocaine was studied on the resting potential (RP), the critical depolarization level (CD), and the amplitude of the action potential (AP) of a single node of Ranvier on a frog's nerve fiber kept in solution with low and high sodium ion concentrations.

EXPERIMENTAL METHOD

The test object consisted of isolated sciatic nerve fibers of the grass frog (Rana temporaria). The single fiber was separated from the nerve trunk by a slight modification of Tasaki's method [18]. The arrangement of the nodes of Ranvier (N_0, N_1, N_2) of the dissected nerve fiber in the experimental chamber is shown in Fig. 1A. The nodes were connected with polarizing (P), stimulating (S), and recording (O) circuits by three nonpolarizing electrodes ($Cu-CuSO_4$). The activity of nodes N_0 and N_2 was inhibited by 0.2% Novocain solution. A highly stable dc amplifier with a cathode follower at the input [5] was used for detecting and recording the potentials of node N_1. The test stimuli were rectangular pulses of direct current, 6-8msec in duration.

Ringer's solution of the following composition was used in the experiment: NaCl—111 mM, KCl—1.34 mM, $CaCl_2$—1.81 mM, $NaHCO_3$—2.4 mM. In the solutions with a low NaCl concentration, isotonicity was maintained by an equivalent amount of sucrose, using the isotonic coefficients of DeVries [7], or with choline chloride. The experiments were carried out at 19-21°.

EXPERIMENTAL RESULTS

Before studying the effect of a low concentration of sodium ions on the Novocain effect, the concentration of Na ions and of the local anesthetic which alone produced only very slight changes in electrical activity of the nodes of Ranvier were chosen. Novocain in a concentration of 10^{-5} g/ml, made up in Ringer's solution of normal salt composition, increases the RP and CD, and slightly reduces the amplitude and the steepness of the ascending phase of the AP (Fig. 1B). Similar changes in RP, CD, and AP of the node take place when the Na ion concentration in solution is lowered to 22 mM (Fig. 1d).

On addition of Novocain in a concentration of 10^{-5} g/ml to a solution with this low sodium concentration the node becomes completely unable to generate an AP; local responses arise to electrical stimuli, and their amplitude increases gradually with an increase in the strength of the current (Fig. 1f). Similar results were obtained in the other experiments.

A decrease in the sodium ion concentration in solution thus intensifies the inhibitory action of Novocain. In other words, the effects of these factors are summated, as a result of which a deeper inhibition of electrical activity takes place than if one of these agents acts alone.

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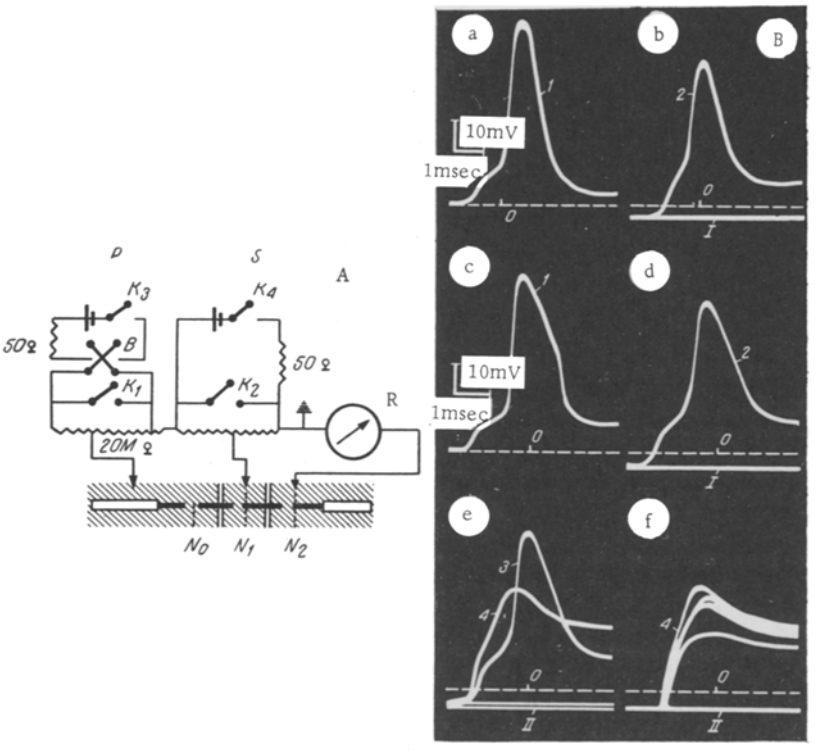

Fig. 1. Scheme of experimental apparatus (A) and curves showing effect of a decrease in sodium ion concentration on electrical activity of a node acted upon by Novocain (B). P) Polarizing circuit; S) stimulating circuit; R) recording circuit; N_0, N_1, N_2) nodes of Ranvier of a single nerve fiber; a, b) action potentials (AP) in Ringer's solutions of normal composition; c) AP in Novocain solution in concentrations of 10^{-5} g/ml; d(2) and e(3)) AP in solution with NaCl in concentration of 220 mM; e(4) and f(4)) local responses in solution containing NaCl in concentration of 220 mM and Novocain 10^{-5} g/ml; 0) initial resting potential (RP); b(i)) change in RP caused by Novocain; d(I)) change in RP caused by removal of Na ions from solution; e(2) and f(2)) resting potentials increased by Novocain and by deficit of Na ions.

A decrease in the sodium ion concentration in solution thus intensifies the inhibitory action of Novocain. In other words, the effects of these factors are summated, as a result of which a deeper inhibition of electrical activity takes place than if one of these agents acts alone.

The same conclusion was reached by Uechara [20], who studied the effect of a general anesthetic (urethane) on the elcctrical activity of a node in a solution with a low concentration of Na ions.

To study the effect of an excess of Na ions on the Novocain effect, the NaCl concentration in the solution was increased only to 220 mM, because the results of our own control experiments and of investigations by other workers [16, 17] show that the increase of osmotic pressure of the solution thus produced has no significant effect on electrical activity of the node.

Records of one of the experiments demonstrating the effect of an increase in sodium ion concentration in the solution on the electrical activity of the node before and after application of Novocain are shown in Fig. 2. These records show that an increase in Na ion concentration to 220 mM causes slight depolarization of the membrane, an increase of CD and an increase in the amplitude of the AP. At the same time the ascending phase becomes steeper, as a result of which the duration of the AP is shortened (Fig. 2B).

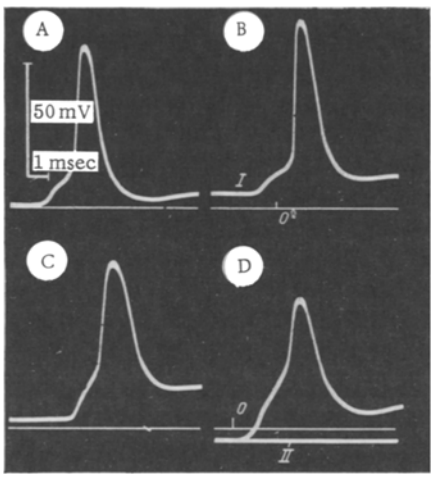

Fig. 2. Effect of an increased sodium ion concentration in solution on electrical activity of a node treated with Novocain. A) Action potential (AP) in Ringer's solution; B) AP in solution with NaCl in concentration of 220 mM, C) the same; but after addition of Novocain to the solution in a concentration of $2 \cdot 10^{-5}$ g/ml; D) AP in solution of Novocain in concentration of $2 \cdot 10^{-5}$ with a normal NaCl concentration; O) initial resting potential (RP); I) change in RP under the influence of an excess of NaCl; II) change in RP under the influence of Novocain.

Addition of Novocain to this solution in a concentration of $5 \cdot 10^{-5}$ g/ml causes only a slight decrease in amplitude of the AP. This is accompanied by an increase in CD and an increase in the duration of the AP, typical of the Novocain effect [3, 4, 8]. This solution with an increased Na ion concentration and with Novocain was then replaced by a solution with a Novocain concentration of $5 \cdot 10^{-5}$ g/ml, but made up in Ringer's solution with a normal NaCl concentration. This causes hyperpolarization of the membrane, a marked increase in CD, and a marked decrease in the amplitude of the AP (Fig. 2F). In other words, the electrical activity is modified, a characteristic feature of the action of Novocain in this concentration.

As the results of the other experiments showed, an increase in the Na ion concentration in solution always weakened the inhibitory action of Novocain.

The results of earlier investigations showed that Novocain lowers the permeability of the membranes of the giant axon of the squid and on the node of Ranvier, and prevents an increase in its permeability during sudden depolarization, i.e., it causes inactivation of P_{Na} [1-4, 8, 9]. From this point of view the results obtained in the present investigation, demonstrating the influence of a low sodium ion concentration in the solution on the Novocain effect, can be clearly understood.

A decrease in the Na ion concentration in the medium is known to reduce the incoming sodium current [12-14]. As a result of this, the AP and CD are increased and the amplitude and steepness of the ascending phase of the AP are lowered. Clearly in the case of a low sodium ion concentration in solution, Novocain, by inactivating P_{Na}, causes deeper inhibition of electrical activity of the node than in a solution with a normal sodium ion concentration.

An increase in the Na ion concentration in the medium has a directly opposite influence on the Novocain effect. Changes in RP and in the amplitude of the AP and its steepness in the ascending phase in a solution with high Na ion concentration are explained by Hodgkin and Huxley [13] on the grounds that in these conditions the equilibrium sodium potential is increased, thereby increasing the inflowing sodium current through the resting and active membrane of the nerve fiber. This leads to a reduction in the RP of the node and to an increase in its AP.

In these conditions inactivation of sodium permeability caused by Novocain in a concentration of $5 \cdot 10^{-5}$ g/ml is insufficient for completely suppressing AP generation. An increase in the Na ion concentration in solution thus facilitates restoration of the amplitude of the AP of the novocainized nerve fiber.

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