COMPARATIVE STUDY OF THE EFFECTS OF PROCAINE, BENCAIN, COCAINE, AND ANESTHESIN ON ELECTRICAL ACTIVITY OF THE NODE OF RANVIER OF SINGLE FROG NERVE FIBERS

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Experiments on single nodes of Ranvier showed that procaine (0.36 mM), bencain (0.38 mM), cocaine (0.29 mM), and anesthesin (benzocaine) (0.3 mM) completely prevent the membrane from generating an action potential (AP) and slightly increased (by 3-6 mV) the initial resting potential. This inhibition is abolished by hyperpolarization of the membrane by 20-25 mV, and AP generation in the nodes is restored. Similar restoration of AP was obtained by increasing the Ca concentration to 36 mM in the solutions of procaine, bencain, and cocaine. An excess of Ca in the anesthesia solution did not restore electrical activity in the node. The restorative effect of the hyperpolarizing current and an excess of Ca in the solution are regarded as the result of abolition of inactivation of the sodium conduction system of the membranes induced by the local anesthetics. It is postulated that the absence of restoration of AP with an increase in the Ca concentration in the solution of anesthesin is due to the unique stereochemical structure of the molecule of this anesthetic and its physicochemical properties. The possible mechanism of interaction between local anesthetics and the membrane at the molecular level is discussed.

The results of investigations on single nodes of Ranvier demonstrate that the inhibitory action of procaine is due to inactivation of the sodium permeability system (P_{Na}) of the membrane [1, 2, 7, 8, 12].

It was accordingly asked whether the inhibitory effects of other local anesthetics (benzocaine), differing in their chemical structure from procaine, can also be explained by inactivation of P_{Na}.

In the investigation described below changes in the electrical activity of the node of Ranvier were studied under the influence of bencain, cocaine, and anesthesin in a medium of normal ionic composition, in solutions with an increased Ca concentration $[Ca]_{in}$, and during hyperpolarization of the membrane. The results of these experiments were compared with changes in electrical activity induced by procaine.

EXPERIMENTAL METHOD

Experiments were carried out on isolated nodes of Ranvier of single nerve fibers isolated [19] from the sciatic nerve of the frog (Rana temporaria). Action potentials (APs) were recorded in a special transparent plastic chamber in which one node was kept in the middle compartment, isolated from neighboring nodes in the side compartments by two insulating air bridges. The stimulating and recording electrical circuits were connected with the nodes of the nerve fiber by means of nonpolarizing $Zn-LnSO_4$ electrodes.

The recording circuit incorporated a cathode follower and two-channel dc amplifier. One channel of the amplifier was intended for recording changes in membrane potential, the other for recording the maximal steepness of the ascending phase of the AP. For this purpose the signal from the output of the first

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TABLE 1. Effect of Local Anesthetics on Parameters of Electrical Activity of Nodes of Ranvier Kept in Solutions with Increased Ca Concentration and during Hyperpolarization of the Membrane (M ± m)

Solution	Number of experiments	Hyperpolarization of membrane by 20-25 mV					Ca concentration increased to 36 mM			After rinsing to remove local anesthetic		
		AP (in mV)	CDL (in mV)	$\dot{V}$ (in V/sec)	AP (in mV)	CDL (in mV)	$\dot{V}$ (in V/sec)	AP (in mV)	CDL (in mV)	$\dot{V}$ (in V/sec)	AP (in mV)	CDL (in mV)	$\dot{V}$ (in V/sec)
Ringer's solution	5	81±33	17,6±0,5	874±26	91,8±1,3	13,8±1,8	1078±54				70,4±2,3	18,8±1,8	798±26
» Procaine 0.36 mM					83±5,6	19,8±1,1	980±34,3	78±1,5	30±2,3	732±31		19±1,4	607±47
Ringer's solution	4	75,2±3,9	16,2±1,2	707±39	91,2±5,4	13,5±2,2	942,5±8,5				69±4,3		
» Bencaine 0.38 mM					85±4,2	14,7±38	847±95					21,2±3,2	665±114
Ringer's solution	5	82,8±3,9	16,6±2,4	812±76	102±4,5	15±2,1	1035±26	76,5±6,9	29,5±4,36	585±77	74±3,8	27,4±2,3	492±69
» Cocaine 0.29 mM					92,8±3,2	23±5,1	795±86						
Ringer's solution	6	81,6±4,2	19,2±33	788±94	95,2±6,4	14,3±3,6	1030±139	78,7±4,7	32,5±3,5	542±51,2	70±5,6		
» Anesthesin 0,3 mM					79,8±78	16,7±2,2	822±144						

Legend: AP) action potential; CDL (critical level of membrane depolarization; $\dot{V}$) maximal steepness of ascending phase of AP.

amplifier was led through a differential RC-circuit with time constant of 10 μsec (100 pF, 100 kΩ) to the input of the second amplifier. Ringer's solutions of the following composition were used: NaCl 110 mM, KCl 1.8 mM, $CaCl_2$ 1.84 mM, tris-HCl buffer, pH 7.3. The experiments were carried out at 19-21°C.

EXPERIMENTAL RESULTS

Table 1 gives the results of 20 experiments carried out with the four local anesthetics: procaine, bencain, cocaine, and anesthesin. They show that the mean amplitude of the AP in Ringer's solution varied in the different series of experiments from 75.2 ± 3.9 to 82.8 ± 3.9 mV. The maximal steepness of the ascending phase of the AP ($\dot{V}$) varied from 707 ± 30 to 874 ± 26 V/sec, and the critical depolarization level (CDL) varied from 16.6 ± 1.2 to 19.2 ± 3.3 mV. All values were obtained without allowing for a short-circuiting factor. Hyperpolarization of the membrane by an anodal current increased the AP amplitude on the average by 13-21% and $\dot{V}$ by 23-33%. Under the influence of anodal hyperpolarization of the membrane CDL was reduced by 19-26% of its initial values. Treatment of the node with procaine, bencain, cocaine, and anesthesin in low concentrations (0.18, 0.19, 0.14, and 0.15 mM, respectively) reduced the amplitude and steepness of the ascending phase of the AP and increased CDL. With an increase in concentration of procaine to 0.36 mM, bencain to 0.38 mM, cocaine to 0.29 mM, and anesthesin to 0.30 mM, the node became unable to generate APs, and only local responses occurred even to strong stimuli. In these concentrations the local anesthetics tested caused a shift of the resting potential by 3-6 mV toward hyperpolarization. The action of a hyperpolarizing current on nodes treated with local anesthetics restored their AP generation. The greatest restoration of the amplitude and steepness of the ascending phase of AP took place in the presence of hyperpolarization of the membrane by 20-25 mV. Under these conditions also, however, the amplitude and steepness of the AP were 9-22% less than initially. After rinsing of the nodes after exposure to the local anesthetics with Ringer's solution their ability to generate APs was restored. The amplitude and $\dot{V}$ were 9-14 and 9-28%, respectively, below the initial values. After rinsing CDL fell but was still higher than before treatment with the local anesthetics.

It is also clear from Table 1 that with an increase in $[Ca]_{in}$ to 36 mM in solutions of procaine (0.36 mM), bencain (0.38 mM), and cocaine (0.29 mM) the nodes of Ranvier regained their ability to generate APs just as during hyperpolarization of the membrane. However, the values of $\dot{V}$ were 17-33% less than initially. Unlike procaine, bencain, and cocaine, the inhibitory action of anesthesin (0.3 mM) was not abolished by an excess of Ca^{++}. In these nodes only local responses appeared even to strong stimuli.

The results indicate that changes in the parameters of electrical activity evoked by the action of bencain, cocaine, and anesthesin on the node membrane are similar to the effect of procaine. This similarity suggests that local anesthetics, like procaine, prevent an increase in the sodium permeability of the membrane (P_{Na}) in response to its depolarization.

Inactivation of P_{Na} ought to be abolished by hyperpolarization of the membrane [15]. In fact, in these experiments a hyperpolarizing current restored AP generation in nodes altered by procaine, bencain, cocaine, and anesthesin. However, hyperpolarization of the membrane is not the only factor abolishing inactivation of P_{Na}. Experiments on the squid giant axon [14] have shown that an increase in $[Ca]_{in}$, like hyperpolarization of the membrane, reduces the degree of P_{Na} inactivation. In experiments with an increase in $[Ca]_{in}$ in solutions of procaine, bencaine, and cocaine restoration of AP generation was observed. Similar restoration of AP with an increase in $[Ca]_{in}$ was observed by other workers in neurons of the cat spinal ganglion [10] and skeletal muscle fibers of the frog [4], treated with procaine. It was concluded from these observations that Ca cations, like the hyperpolarizing current, abolish the inactivation of P_{Na} which lies at the basis of the inhibitory action of the local anesthetics. However, some workers who studied the effect of procaine and xylocaine on ionic currents of nerve fibers consider that local anesthetics cause a decrease in the maximal value of P_{Na} [11, 1, 17, 20]. The causes of the disagreement on this question have been examined by the writer previously [7, 8].

It was pointed out above that the effects of anesthesin, unlike those of procaine, bencain, and cocaine, are not abolished by an excess of Ca. The writer considers that this is connected with the stereochemical structure of the anesthesin molecule and its physicochemical properties.

The anesthesin molecule has no tertiary nitrogen atom in its alkyl chain, which makes it readily soluble in lipids but much less soluble in water [3].

Modern views on the molecular mechanism of the inhibitory action of local anesthetics are based on their ability to penetrate into the lipid layers of excitable membranes [5, 6, 18] and to displace Ca ions competitively from the points of their interaction with lipid groups [9, 13]. This behavior evidently disturbs the organization of the structure of the membrane with the resulting development of P_{Na} inactivation. An increase in $[Ca]_{in}$ brings about the reverse process: displacement of the anesthetics by Ca ions from the membrane lipids. Unlike procaine and the other local anesthetics, anesthesin, with its greater lipophilicity, is probably much more firmly fixed on the membrane lipids. That is why, with an increase in $[Ca]_{in}$, it is not displaced from the lipid structures of the membrane and, consequently, the ability of the node to generate APs is not restored. The hypothesis of the stronger bond between the anesthesin molecules and the lipid complexes of the membrane is perhaps supported by the fact that the activity of the nodes is restored much less readily after exposure to anesthesin than to procaine, bencain, or cocaine.

LITERATURE CITED

1. V. I. Belyaev, Byull. Éksperim. Biol. i Med., No. 8, 24 (1963).
2. V. I. Belyaev, Byull. Éksperim. Biol. i Med., No. 5, 3 (1964).
3. Z. I. Vedeneeva, in: Textbook of Pharmacology [in Russian], Vol. 1, Leningrad (1961), p. 420.
4. E. G. Vornovitskii, Byull. Éksperim. Biol. i Med., No. 10, 11 (1966).
5. N. T. Pryanishnikova, Dokl. Akad. Nauk SSSR, 163, No. 2, 507 (1965).
6. N. T. Pryanishnikova, Trimecaine [in Russian], Moscow (1967).
7. B. I. Khodorov and V. I. Belyaev, Biofizika, 10, 625 (1965).
8. B. I. Khodorov and V. I. Belyaev, Biofizika, 12, 855 (1967).
9. Yu. D. Kholodova and Z. A. Sorokina, in: The Biophysics of Membranes [in Russian], Kaunas (1971), p. 768.
10. J. Aceves and X. Machne, J. Pharmacol. Exp. Ther., 140, 138 (1963).
11. M. P. Blaustein and D. E. Goldman, J. Gen. Physiol., 49, 1043 (1966).
12. T. E. Bloom and G. M. Schoepfle, Am. J. Physiol., 204, 73 (1963).
13. J. I. Feinstein, J. Gen. Physiol., 48, 357 (1961).
14. B. Frankenhaeuser and A. L. Hodgkin, J. Physiol. (London), 137, 218 (1957).
15. A. L. Hodgkin and A. F. Huxley, J. Physiol. (London), 117, 500 (1952).
16. B. Hille, Nature, 210, 1220 (1966).
17. T. Narahashi, J. Cell Physiol., 64, 73 (1964).
18. J. Skom, Acta Pharmacol. Toxicol., 10, 281 (1954).
19. T. Tasaki, Am. J. Physiol., 125, 78 (1939).
20. R. Taylor, Am. J. Physiol., 196, 1071 (1959).