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EFFECT OF ARMIN ON MYELINATED FROG NERVE FIBERS

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Various inhibitors of synaptic cholinesterase are known not only to have a specific action, but also to give rise to other effects [5]. In particular, cholinesterase inhibitors can affect mechanisms of spontaneous and evoked mediator release [2] and the electrogenic membrane of muscle fibers [7]. To understand the mechanism of the blocking of neuromuscular transmission under the influence of anticholinesterase drugs, the effect of these drugs in this manner on the conduction of excitation along fibers must also be evaluated, for it can make a definite contribution to the development of a conduction block.

The object of this investigation was to study the effect of the organophosphorus cholinesterase inhibitor armin (ethyl-p-nitrophenyl ester of ethylphosphinic acid), which has an irreversible anticholinesterase action, on myelinated nerve fibers of the frog *Rana temporaria*.

EXPERIMENTAL METHOD

Potentials of single Ranvier nodes were recorded by the method of Tasaki and Staempfli. The node for testing was perfused with Ringer's solution or with the test solution, and neighboring nodes, separated from the test node by air gaps, were kept in a solution of 120 mM KCl. For stimulation and recording of potentials Ag-AgCl electrodes were used. The electrode connected to the test node was common for the stimulation and recording systems. The node was stimulated by single square pulses of threshold intensity and duration 0.1 msec. To measure the resistance of the nodes, subthreshold pulses with a duration of 20 msec, a strength of 0.1-0.3 nA, in de- and hyperpolarizing directions, were used. A circuit enabling nodes to be polarized by a dc source, maintaining their membrane potentials (MP) at the level of about −100 mV, also was connected to the stimulating circuit. A buffer resistance of 1 GΩ was included in the circuit for stimulation and polarization, which prevented stimulation of the node by the resting current and stabilized the polarizing current. Potentials were recorded by the dc channel of a UBP2-03 amplifier, the input of which was placed a cathode follower with input resistance of 10 GΩ, input capacitance of 0.55 pF, and grid current of $3 \cdot 10^{-12}$ A. The shunting factor in the recording system was 0.6.

The Ringer's solution used had the following composition (in mM): NaCl 110.5, KCl 2.5, $CaCl_2$ 1.9, Tris-buffer 5.0, pH 7.3-7.4. Test solutions of the same salt composition contained $4 \cdot 10^{-8}$, $4 \cdot 10^{-7}$, $4 \cdot 10^{-6}$, and $4 \cdot 10^{-5}$ M armin. A complete change of solution bathing the node could be done in 15-20 sec. Changes in the test parameters were recorded after 3, 6, 10, and 15 min of action of armin. The nodes were not rinsed after the action of armin. The results were subjected to statistical analysis. Mean values and confidence intervals for P = 0.95 are given in Table 1.

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TABLE 1. Background Values of Characteristics of Ranvier Nodes, Their Changes (in % of background values) in Solutions of Armin of Different Concentrations, and Control Changes (in % of background values) during Immersion of Nodes in Ringer's Solution

Parameter	Background value	Armin concentration, M				Control changes
		$4\cdot10^{-8}$	$4\cdot10^{-7}$	$4\cdot10^{-6}$	$4\cdot10^{-5}$	
MP, mV	120,7±21,7	108±4	113±5	106±4	115±8	102±3
Threshold potential, mV	40,7±7,5	108±6	115±7	103±8	114±17	104±6
CDL, mV	78,0±15,1	108±6	106±6	107±6	114±13	103±4
t_r, msec	0,43±0,05	99±3	106±5	115±3	125±9	109±5
A_{sp}, mV	136,6±19,6	106±2	106±4	109±6	116±12	97±4
Steepness of front, V/sec	228,7±30,0	102±5	98±6	95±6	99±18	89±5
t_d, msec	0,82±0,11	110±4	115±6	123±7	148±24	109±5
R_{in}, mΩ	52,1±13,1	101±14	110±10	115±8	130±10	99±7

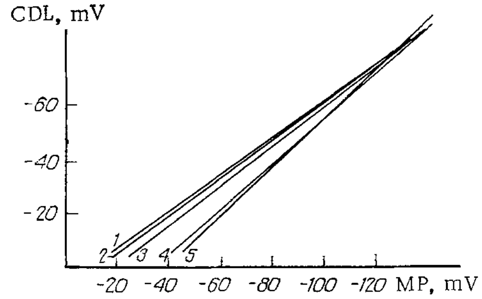

Fig. 1. Dependence of CDL on MP of Ranvier nodes. 1) Regression line for CDL versus MP in Ringer's solution; 2-5) the same in armin solutions of $4\cdot10^{-8}$, $4\cdot10^{-7}$, $4\cdot10^{-6}$, and $4\cdot10^{-5}$ M, respectively.

EXPERIMENTAL RESULTS

Since it was shown previously [1] that armin shortens the duration of integrally recorded action potentials (AP) of motor and sensory groups of fibers of the nerve trunk, in the present investigation the isolated fibers were not identified as being motor or sensory in type. Data on the action of armin in different concentrations are given in Table 1, which also gives the results of the control series of experiments in which changes observed with time after switching from Ringer's solution to Ringer's solution with armin also are shown. It must be pointed out that the value described in the paper as MP corresponds only conventionally to the resting MP, for it is the sum of potentials created by the ionic gradient, by electrogenic transport, and by the polarizing current on the resistance of the node [3]. Under the conditions used changes in MP reflect mainly changes in the input resistance of the nodes (R_{in}) and they serve as the control for determination of resistance on the basis of magnitude of electrotonic shifts.

The main result of the action of armin was an increase in R_{in} of the nodes, and the higher the concentration of armin used, the greater the increase. Under hyperpolarization conditions this led to an increase in MP and to changes in threshold and critical level of depolarization (CDL). The increase in MP also was accompanied by a corresponding increase in amplitude and duration of the leading edge of AP with a constant steepness of rise. Conversely, control experiments showed that if the preparation was kept in Ringer's solution for a time as long as exposure to armin, the steepness of the front of AP decreased. The increase in the half-decay time of AP in armin solutions was within the limits of control changes for an armin concentration of $4\cdot10^{-8}$ M and it significantly exceeded the control values for concentrations of $4\cdot10^{-7}$-$4\cdot10^{-5}$ M.

Regression lines for CDL versus MP, obtained for nodes in Ringer's solution and in solutions of armin, are given in Fig. 1. It will be clear that with an increase in armin concentration there was an increase in the angle of slope of the regression line for CDL on MP. For armin in concentrations of $4\cdot10^{-6}$ to $4\cdot10^{-5}$ M, dependence of CDL on MP differed significantly from the background. During hyperpolarization caused by passage of a current, analysis of these relationships was complicated by the fact that CDL and MP are functions of total ionic conductance (resistance) of the membrane. Nevertheless, changes in the angle of slope of the regression line for CDL on MP in this case is evidence of a change in the velocity of sodium inactivation and potassium activation corresponding to the same change in MP.

In some cases armin caused a sharp drop in potential recorded to 0 despite constancy of the hyperpolarizing current. It can be postulated that this phenomenon is connected with acute demyelination of the fibers. Mustafin [4] described swelling of the Schwann cells during activity of nerve fibers in a solution of armin. Coupled with drying of the internodal segments, this may have led to disturbances of contacts of the axon and Schwann cells in the region of the nodes, and consequently to the appearance of shunts in the polarizing and recording circuits.

Since the response of motor and sensory fibers to armin is the same [1], it seems unlikely that the effects of armin are mediated through changes in relations between axon and Schwann cells, which, at least in motor axons, include a cholinergic stage [3, 8]. The effects of armin are thus the result of its direct action on the electrogenic membrane of nerve fibers. The cause of the increase in R_{in} of the Ranvier nodes in armin solutions described above may therefore be a decrease in leakage conductance, which plays an essential role in post-spike repolarization in frog Ranvier nodes [6]. Evidence that a decrease in leakage conductance is the cause of the increase in R_{in} is given by the prolongation of the decremental phase of the AP in armin. The effect of armin on sodium channels of Ranvier nodes appears to be more complex. On the one hand, the steepness of rise of the AP remains at its initial level in solutions of armin, suggesting that armin may have some disinactivating effect. However, during hyperpolarization of the nodes, maintenance of the steepness of rise of the AP may be the result of an increase in MP with an increase in resistance of the nodes. This explanation is supported by the proportionality between changes in MP, threshold potential, and CDL. On the other hand, analysis of regression lines for CDL versus MP shows that CDL has more positive values in armin solution for the same values of MP than in Ringer's solution. Analysis of data showing a decrease in leakage conduction of the nodes in armin solutions reveals a positive shift of CDL, evidence of the development of sodium inactivation. This type of shift in CDL under the influence of armin, incidentally, has been found in frog muscle fibers also [7]. Under natural conditions this phenomenon, in conjunction with prolongation of post-spike repolarization may determine the conduction block to AP in armin during high-frequency stimulation of the nodes. During infrequent or single stimulation of myelinated axons such a block is unlikely.

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