logical activity of cells and tissues. It was stated in [11] that the mean life span of nematodes, usually 35 days, was increased to 46 days if TP or TPQ was added to the medium in high concentrations.

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LOCATION OF THE BINDING SITE FOR QUATERNARY AJMALINE DERIVATIVES IN THE SODIUM CHANNEL OF THE EXCITABLE MEMBRANE

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KEY WOPDS: ajmaline, sodium channel, cumulative block, axoplasm.

It was shown previously that blockade of sodium channels by the antiarrhythmic N-propylajmaline (NPA; Neogiluritmal) in the nerve fiber [1, 5, 8] and in myocardial cells [2] accumulates during rhythmic membrane depolarization. After the end of stimulation sodium currents (I_{Na}) in response to infrequent testing stimuli are gradually restored, in the course of 15-20 min, to their original level.

NPA is a permanently charged quaternary ammonium compound which can penetrate into the lipid matrix of the membrane because of the hydrophobicity of its C_3H_7 radical, near the charged nitrogen atom. In view of existing ideas on the mechanism of action of quaternary derivatives of local anesthetics on sodium channels [7], the writers postulated that NPA, if applied externally to the membrane, passes through its lipid layer into the cytoplasm, from which it enters the sodium channel when it opens during depolarization [5]. On binding with the receptor site of the inner mouth of the channel, NPA blocks movement of penetrating cations.

To test this hypothesis, in the investigation described below the action of another quaternary derivative of ajmaline, namely N-methylajmaline (NMA), which is less hydrophobic than NPA, on I_{Na} was investigated (see the Scheme, below).

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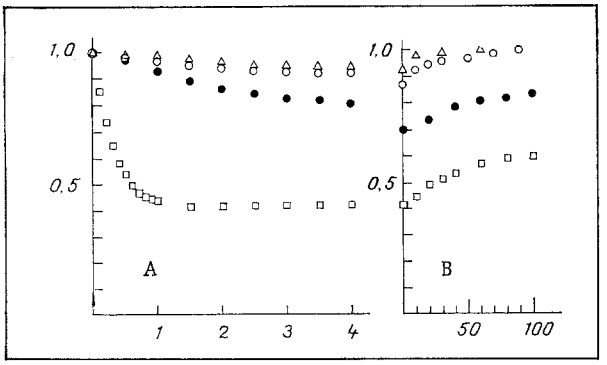
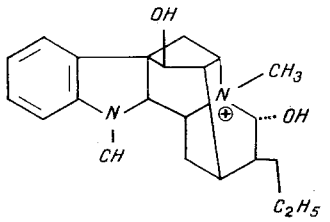


Fig. 1. Cumulative block of sodium currents (I_{Na}) during external and intra-axonal application of NMA. Abscissa, time of stimulation (in sec); ordinate, relative magnitude of I_{Na} in response to n-th stimulus Ci_n/I_1: amplitude to first stimulus I_1 in each rhythmic series taken as unity. A) Time course of reduction in amplitude of I_{Na} during repetitive stimulation of Ranvier node with frequency of 10 Hz; B) recovery of I_{Na} with reduction of frequency of stimulation to 0.1 Hz after 9 sec of stimulation with a frequency of 10 Hz. Stimulation effected with 10-msec depolarizing shifts of potential from —100 mV (holding potential) to 0 mV. Triangles: in Ringer's solution of normal composition (control), empty circles: on addition of 0.1 mM NMA, filled circles: with an increase in NMA concentration in solution to 1 mM, squares: on application of 2 mM NMA inside fiber through divided internodal segments.



EXPERIMENTAL METHOD

Experiments were carried out on the Ranvier node of the frog *Rana ridibunda* by the voltage clamping method [4] with continuous perfusion of the node with control Ringer's solution of the following composition (in mM): NaCl — 112, KCl — 2.5, $NaHCO_3$ — 2, $CaCl_2$ — 2, Tris-buffer 5, pH 7.2. NMA was tested by external application to the membrane, when it was added to the control Ringer's solution, and by intra-axonal injection through the ends of the fiber, divided in a solution of CsF + NMA. The temperature in the experiments varied from 7 to 9°C.

EXPERIMENTAL RESULTS

Like the other quaternary ammonium compounds which block sodium channels NMA induced two dose-dependent types of inhibition of I_{Na}: a "tonic" block [7], arising during the action of NMA on the resting Ranvier node membrane, and a "phasic" (cumulative) block, increasing during repetitive stimulation and decreasing after its end.

For quantitative evaluation of the tonic block I_{Na} was measured in response to a testing stimulus (E = 0 mV), first in the control Ringer's solution, then 5-6 min after addition of NMA to the solution. In the interval between these measurements the membrane was kept in the resting state. The experiments showed that 0.1 mM NMA induced virtually no "tonic" block. With an increase in the NMA concentration to 1 mM the peak value of I_{Na} during this period of exposure fell by about 15% below its initial value. These results must be compared with the effects of external application of tertiary ajmaline [5] and quaternary NPA [1, 5, 8]. In a concentration of 1 mM these substances induce virtually complete blockade of I_{Na}.

The cumulative block was studied by application of a series of 5-msec depolarizing stimuli with a frequency of 10 Hz to the node membrane. The fall in I_{Na} observed under these circumstances was compared with that which developed in the control Ringer's solution in response to the same repetitive stimulation. It will be clear from Fig. 1 that this decrease in I_{Na} in the absence of NMA was very weak: in the experiment under examination, during 9 sec of stimulation it amounted to about 7%. The cause of this cumulative block, as was shown previously [3], is "slow sodium inactivation," induced by K^+ ions present in the Ringer's solution (2.5 mM). Addition of 0.1 mM NMA to the solution induced only a small further increase of the cumulative block: during 9 sec of stimulation I_{Na} decreased by 12% of its initial value. Reducing the frequency of stimulation from 10 to 0.1 Hz led to a gradual increase in I_{Na}, which was completely restored after about 1.5 min.

NMA in a concentration of 1 mM induced a stronger cumulative block, but in this case also, during 9 sec of stimulation I_{Na} fell by only 30% of its initial value. An increase in the amplitude of the depolarizing stimuli to the reversal potential caused temporary deepening of the cumulative block (data not given).

Intra-axonal application of NMA was much more effective. Symmetrical blocking of I_{Na} caused by diffusion of 2 mM NMA from the divided ends of the nerve fiber into the region of the node is illustrated in Fig. 1A. Stimulation began after exposure for 30 min to NMA — long enough for a stationary concentration of the blocker to be produced in the region of the node. The calculated [6] internal NMA concentration was about 1 mM. During 1.5 sec of repetitive stimulation I_{Na} fell by about 60% from its initial value. With a decrease in the frequency of stimulation to 0.1 Hz, I_{Na} began to fall slowly. During external application of ajmaline and NPA to the membrane, incidentally, 0.1 mM was a high enough concentration of these compounds to cause I_{Na} to fall by 60-70% of its original value during repetitive stimulation (10 Hz) [5]. This difference in concentrations is evidence that the hydrophobiciity of the radical near the quaternary nitrogen atom is essential not only for penetration of the compound into the membrane, but also for its interaction with the binding site. The fact that NMA is more effective when applied inside the membrane is evidence that the binding site is located in the region of the axoplasmic end of the sodium channel. The fact that repeated opening of the sodium channels by depolarizing stimuli is essential for the development of a cumulative block justifies the conclusion that this binding site is located inside the channel, and that access to it is obtained through the axoplasm. Temporary strengthening of the cumulative block under the influence of powerful depolarizing shifts of potential indicates that interaction between NMA and the open sodium channel is voltage-dependent.

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