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STIMULUS-DEPENDENT Na-CHANNEL BLOCKADE IN ISOLATED RAT MYOCARDIAL CELLS BY THE ANTIARRHYTHMIC N-PROPYLAJMALINE (NEOGILURITMAL)

Yu. I. Zil'berter, B. I. Khodorov, and B. Schubert

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It was shown previously that blockade of Na currents in nerve fibers by amine local anesthetics [6, 7, 9] and antiarrhythmics [1, 16, 12] can be reversibly potentiated by rhythmic depolarization of the membrane. Analysis of the mechanism of this stimulus-induced cumulative block led to a number of important conclusions regarding the molecular organization of Na channels in the nerve fiber membrane [4]. However, the question of the applicability of these ideas to Na channels of myocardial cells has not been completely settled, because pharmacological analysis of the properties of myocardial Na channels is still only in the initial stages of its development.

In the investigation described below the action of an antiarrhythmic — a quaternary derivative of ajmaline, namely N-propylajmaline (NPA) — on Na currents through a microregion of the membrane (the patch clamp method) of an enzymically isolated rat myocardial cell was studied.

EXPERIMENTAL METHOD

Cells were isolated by the method described in [10]. Full details of the experimental procedure were described previously [2]. The cell selected under the microscope was transferred to a working chamber containing a solution of the following composition (in mM): NaCl 130, KCl 5.4, $MgSO_4$ 1.2, $CaCl_2$ 0.9, glucose 11, MOPS-buffer 20 (pH 7.4). The experiments were carried out at room temperature (20-22°C). By means of a V-shaped polyethylene sucker, with a pore 5-7 μ in diameter, electrodes were connected to a small area of the cell membrane. The control solution in the sucker had the composition indicated above, with the addition of 1 mM $MnCl_2$ and 1 mM 4-aminopyridine to block Ca- and K-channels, respectively. The solutions in the tip of the sucker were changed approximately in the course of 1 sec.

The currents were measured by a "virtual ground" circuit, followed by filtration down to the 3 kHz band. In all experiments the potential was assigned from the level of the cell resting potential (V = 0 mV).

EXPERIMENTAL RESULTS

Replacement of the control solution in the sucker by solution containing 1×10^{-5} to 2×10^{-5} M NPA caused no appreciable changes in amplitude of the Na current (I_{Na}) during the first 2-3 min, provided that the membrane was not stimulated during this time. In conjunction with rhythmic depolarization of the membrane, however, NPA caused progressive decline in I_{Na}.

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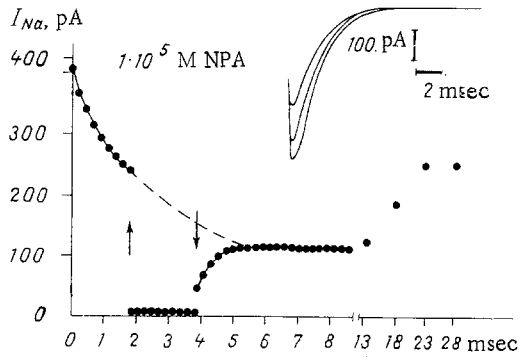

Fig. 1. Stimulus-dependent blockade of Na channels of isolated myocardial cell by 10^{-5} M NPA. Abscissa, time of action of NPA (in min), ordinate, peak amplitude of Na current (in pA) to 10-msec testing stimulus with amplitude V_T from resting potential V = 0 mV. Each testing stimulus was preceded by a hyperpolarizing prepulse. Frequency of stimulation during first 9 min of action of NPA was 5 Hz. From the 9th through the 30th minute recovery of I_{Na} was traced by means of infrequent single stimuli (1 stimulus in 5 min). The amplitude of the stimuli varied: Before the arrow pointing upward, V_T = 60 mV, between the arrows V_T = 120 mV, after the arrow pointing downward, V_T = 60 mV. Points on the graph before the gap on the time axis correspond to the amplitude of I_{Na} to each 15th stimulus. Superposed traces of Na currents to the first, 130th, and 430th, testing stimuli V_T = 60 mV shown in inset. Broken line — extrapolation of time course of blockade of I_{Na} calculated by the equation: $I_{Na} = Ae^{t/\tau} + B$, where A = 290 pA, B = 112.5 pA, and τ = 100 sec.

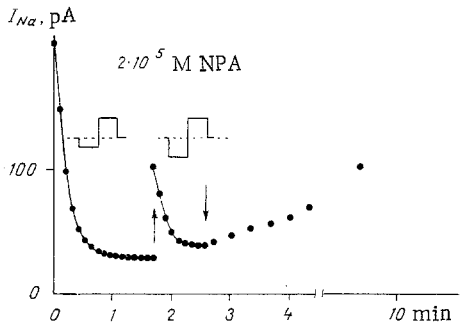

Fig. 2. Effect of NPA, magnitude of hyperpolarizing prepulse, and frequency of stimulation on Na-channel blockade. Abscissa, time of action of NPA (in min), ordinate, peak value of I_{Na} in response to testing stimulus V_T = 60 mV, 10 msec in duration (in pA). Amplitude of hyperpolarizing prepulse: Before arrow pointing upward, V_c = −20 mV; between arrows V_c = −40 mV. Arrow pointing downward indicates time of switching frequency of stimulation from 5 to 1 Hz. Points on graph before arrow pointing downward correspond to amplitude I_{Na} to every 15th stimulus, points after arrow pointing downward correspond to amplitude to every 20th stimulus. Last experimental point corresponds to test stimulus applied 5 min after the end of stimulation. Stimulus program shown schematically in insets.

The results of one of the experiments in which both the NPA concentration and the conditions of membrane simulation were varied are shown in Figs. 1 and 2.

At the beginning of the experiment the action of 10^{-5} M NPA was tested and the amplitude of the test depolarizing stimuli (V_T) was 60 mV; each stimulus was preceded by a hyperpolarizing shift of potential (V_c = −20 mV) lasting 100 msec, which partially abolished the initial inactivation of the Na channels. Rhythmic depolarization (5 Hz) caused a smooth (approximately exponential) reduction in peak values of I_{Na}, and after 2 min (i.e., after 600 stimuli) I_{Na} fell by 40% below its initial value.

Reduction of the I_{Na} peaks under the influence of the blocker was not accompanied by any appreciable changes in the kinetics of the Na currents (see inset to Fig. 1). At the time

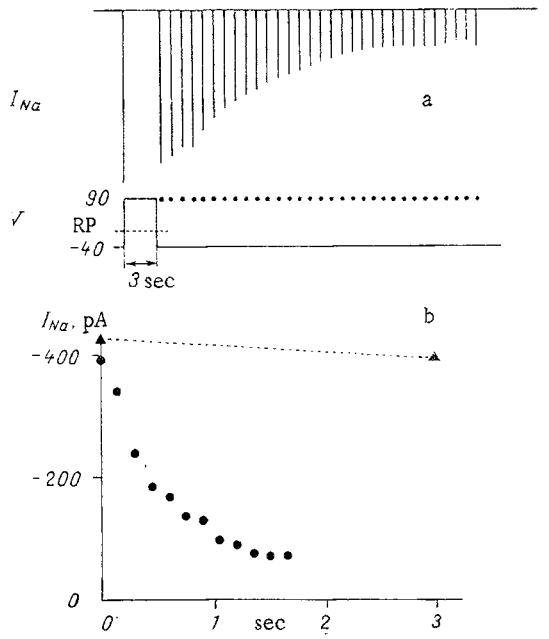

Fig. 3. Comparison of effect of steady and rhythmic membrane depolarization on Na channel blockade by NPA. a) Vertical lines show amplitude of I_{Na} in response to depolarizing shift of potential V_T = 90 mV from resting potential (RP) at V_c = —40 mV. A series of 10-msec depolarizing stimuli with a frequency of 5 Hz followed 0.5 sec after the end of the 3-sec depolarizing step. Stimulus program shown below. Points correspond to every 5th stimulus in series; b) dependence of Na channel blockade on total duration of depolarization based on results of experiment in Fig. 3a. Filled circles denote amplitude of I_{Na} to every 15th pulse in the series. First triangle corresponds to amplitude of I_{Na} in response to 3-sec depolarizing stimulus; second triangle denotes I_{Na} to first stimulus in series. Abscissa, total time of depolarization (in sec).

indicated by the arrow pointing upward the amplitude of the depolarizing stimuli V_T was increased from 60 to 120 mV (V_T = 120 mV corresponded approximately to the reversal potential). Despite the fact that in this experiment complete activation of Na channels (M_∞1.0) took place at V_T = 60 mV, strengthening of depolarization led to potentiation of the block: I_{Na} in response to the first testing stimulus after V_T had returned to its initial value of 60 mV (arrow pointing downward) was reduced compared with the value of I_{Na} extrapolated (on the basis of the preceding kinetics of the cumulative block) to this time (broken line). In the course of subsequent stimulation I_{Na} rose to the steady-state level for V_T = 60 mV.

Stimulation was stopped 9 min after the beginning of action of NPA in order to study the question of reversibility of the block. The process of recovery of I_{Na} was studied with the aid of testing stimuli with a following frequency of once every 5 min. It will be clear from Fig. 1 that liberation of the Na channels from NPA took place very slowly: In 15 min I_{Na} had reached only 62% of its initial value.

In the same experiment we tested the action of 2×10^{-5} M NPA (Fig. 2). The increase in NPA concentration accelerated the drop of the I_{Na} peaks abruptly, and after 1.5 min they reached the steady-state level.

At the time indicated in Fig. 2 by the arrow pointing upward, the hyperpolarizing shift of potential (V_c) was doubled (from —20 to —40 mV) before each testing stimulus. This led to a considerable increase in amplitude of I_{Na} to the first testing stimulus, followed by a rapid fall in I_{Na} to a new, rather higher, steady-state level. It must be pointed out that this second drop of I_{Na} in the rhythmic series had the same kinetics as the first drop. Reducing the frequency of stimulation from 5 to 1 Hz (arrow pointing downward) led to constant recovery of the amplitude of I_{Na}.

There is no doubt that the sharp increase observed in the first I_{Na} in this experiment in response to strengthening of the hyperpolarizing prepulse is the result of an increase in the number of functioning Na channels on account of abolition of the initial Na inactivation,

and that the subsequent drop of I_{Na} is the result of blockade of these channels by NPA in the course of rhythmic depolarization. The conclusion which follows from this experiment is that NPA effectively blocks only open Na channels. Those channels which remained in a state of inactivation at the initital level of the holding potential and $V_c = -20$ mV were not blocked. Their interaction with NPA became possible only after abolition of the inactivation by means of $V_c = -40$ mV.

The conclusion regarding resistance of inactivated Na channels to the action of NPA was confirmed when the effects of steady and rhythmic membrane depolarization on Na channel block by NPA were compared. In the experiment whose results are shown in Fig. 3a, in response to a 3-sec depolarizing step with an amplitude of $V_T = 90$ mV an inward I_{Na} appeared, the value of which was conventionally taken to be 1. The first testing stimulus of the same amplitude ($V_T = 90$ mV), applied 0.5 sec after the end of this long depolarizing step, evoked an I_{Na} whose amplitude was 0.89 of the initial level. During subsequent rhythmic (5 Hz) stimulation with 10-msec depolarizing pulses, I_{Na} fell progressively, and after 150 stimuli it had fallen to 0.19 of its initial value. The total duration of membrane depolarization during this repetitive stimulation was 1.5 sec, i.e., only half the duration of steady-state depolarization, and seven times more effective than the latter. Figure 3b shows the relationship between I_{Na} and the total duration of membrane depolarization under the influence of 2×10^{-5} M NPA.

The results of these experiments are qualitatively similar to those obtained previously in experiments on nerve fibers [1, 13]. This points to common mechanisms of action of NPA on Na channels in these excitable formations: NPA blocks open channels and its interaction with the "receptor site" is potential-dependent in character. These findings are important for the elucidation of mechanisms of the antiarrhythmic action of NPA on the myocardium [5, 8, 11, 12].

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