8. A. M. Kuzin, The Stimulating Action of Ionizing Radiation on Biological Processes. On the Biological Action of Small Doses [in Russian], Moscow (1977).
9. Yu. A. Nesis, in: Extracorporeal Irradiation of Blood [in Russian], Moscow (1980), pp. 141-161.
10. L. S. Reznikova, Complement and Its Importance in Immunologic Reactions [in Russian], Moscow (1967), pp. 36-43.
11. O. V. Smirnova and G. A. Kuz'mina, Zh. Mikrobiol., No. 6, 8 (1966).
12. F. H. Bruns, E. Dünwald, and E. Naltman, Biochem. Z., 330, 497 (1958).
13. G. E. Glock and P. McLean, Biochem. J., 55, 400 (1953).
14. R. C. Kamm and A. G. Smith, Clin. Chem., 18, 519 (1972).
15. J. Mestecky, M. Jilek, and M. Mareckova, Folia Microbiol. (Prague), 11, 179 (1966).

DIFFERENCES IN ACTION OF Ca^{++} IONS ON CUMULATIVE BLOCKADE OF SODIUM CHANNELS INDUCED BY TERTIARY AND QUATERNARY AMINES

L. D. Zaborovskaya

UDC 612.822.1.015.31:546.33].014.46:615.216.2

KEY WORDS: Ranvier node; sodium current; local anesthetic; antiarrhythmic; cumulative blockade.

It was shown previously [1, 4, 7] in experiments on myelinated frog nerve fibers that tertiary amines (procaine and trimecaine) interact chiefly with inactivated Na channels, inducing a state of slow sodium inactivation (SI) in them, which is the cause of cumulative inhibition of the Na current (I_{Na}). An increase in the external Ca^{++} concentration counteracted the development of SI; the proportion of Na channels changed into a state of SI during prolonged (1 sec) membrane depolarization by Ca^{++} was reduced [1, 7]. Calcium ions had a similar action on SI induced by external K^+ [3].

Quaternary derivatives of tertiary amines QX-314 [9], QX-572, QT [1, 7], and N-propylajmaline (NPA) [2, 8, 10] also induced cumulative inhibition of I_{Na}, but under these circumstances they interact with the open Na channel. The fact that SI arises only after application of local anesthetics to the outer surface of the membrane, and that interaction with the open channel requires the presence of a blocker in the axoplasm, suggested that there are "binding sites" in the nerve fiber membrane that are responsible for cumulative blocking of I_{Na} [1, 7]. However, according to Hille's hypothesis [6], all types of cumulative blockade of I_{Na}, whether induced by tertiary or quarternary amines, are due to their interaction with one "receptor," located in the region of the inner opening of the Na channel. Differences in the phenomenology of blockade induced by tertiary and quaternary amines are due entirely to the fact that the former penetrate in the uncharged form into the inner opening of the channel through the lipid bilayer of the membrane, whereas the latter can enter the inner opening only from the axoplasm, after opening of the activation gates of the channel.

According to the single receptor hypothesis for tertiary and quaternary blockers, it would be expected that a change in the external Ca^{++} concentration would have the same effect on frequency-dependent blockade of Na channels induced by these compounds. Data obtained in the present investigation contradict this prediction of Hille's hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on the Ranvier node of isolated nerve fibers of *Rana ridibunda* by the voltage clamp method [5]. The ends of the fiber were divided on either side of the test node in isotonic CsCl solution, which completely blocked K currents. The experiments

Biophysical Research Laboratory, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 31-34, July, 1985. Original article submitted May 23, 1984.

 0007-4888/85/0007-0870$09.50

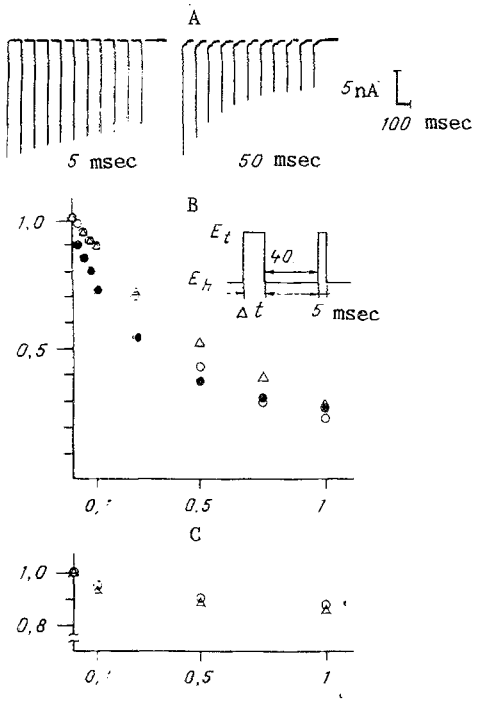

Fig. 1

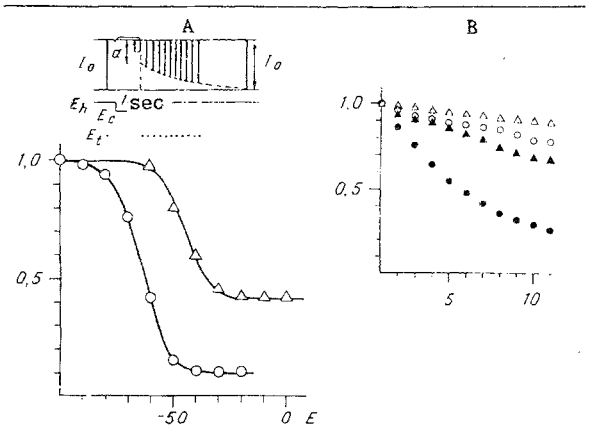

Fig. 2

Fig. 1. Effect of duration of membrane depolarization on phasic blockade of Na channels by tertiary local anesthetics and by the quaternary antiarrhythmic NPA. A) Traces of Na current (I_{Na}) during repetitive (10 Hz) stimulation of Ranvier node with depolarizing pulses of 2 different durations (5 and 50 msec) in presence of 10^{-4} M lidocaine. Holding potential E_h = —105 mV, testing potential shift E_t = —10 mV, 7°C; B) dependence of I_{Na} to testing stimulus on duration of conditioning (Δt) depolarization, in presence of 10^{-4} lidocaine, E_h = —105 mV, 7°C (filled circles), of 10^{-5} M amethocaine, E_h = —110 mV, 8°C (empty circles), and of 10^{-4}M etidocaine, E_h = —95 mV, 7°C (triangles); C) triangles: 10^{-4}M NPA, E_h= —110 mV, 17°C; circles: control Ringer's solution. Abscissa (B, C), duration of conditioning pulse (in sec); ordinate, I_{Na} (in relative units). Value of I_{Na} without conditioning step taken as unity. Order of stimuli shown in inset.

Fig. 2. Effect of increasing Ca^{++} ion concentration on amethocaine-induced blockade of I_{Na}. A) SI induced by amethocaine (10^{-5} M) in two different Ca^{++} ion concentrations: 2 mM (circles) and 20 mM (triangles). Abscissa, membrane potential (in mV); ordinate, ratio of I_{Na} to testing pulse applied immediately after end of I_{Na} with conditioning pulse to I_{Na} without conditioning step. E_h = —110 mV, 8°C. Method of measuring SI shown in inset. I_o) Na current to testing stimulus, a) value of Na curtent extrapolated to end of conditioning step ($S_\infty = a/I_o$); B) blocking of I_{Na} during repetitive stimulation (10 Hz) of node with depolarizing pulse 5 msec (empty circles) and 50 msec (filled circles and triangles) in duration, in presence of 10^{-5} M amethocaine + 2 mM (empty and filled circles) and 20 mM (empty and filled triangles) Ca^{++}. E_h = —110 mV, 8°C. Abscissa, No. of pulse in series; ordinate, I_{Na} (in relative units). Value of I_{Na} to first pulse in series taken as unity.

were conducted at 7-8°C with continuous flow of solution through the compartment of the experimental chamber containing the Ranvier node. The control Ringer's solution contained (in mM): NaCl 114; KCl 2.5, $CaCl_2$ 2, $NaHCO_3$ 2; Tris-HCl 5; pH 7.2. Experiments with tertiary amines were carried out in potassium-free Ringer's solution.

EXPERIMENTAL RESULTS

The previous investigations [1, 7] showed that blockade of I_{Na} induced by procaine and trimecaine during repetitive stimulation is accelerated and deepened with an increase in duration of the depolarizing stimuli. A similar result was obtained in the present study with lidocaine, amethocaine, and etidocaine. Cumulative inhibition of I_{Na} by lidocaine with stimuli of two different durations (5 and 50 msec) is illustrated in Fig. 1A. Lengthening of the stimuli to 50 msec deepened the block of I_{Na} by 35% (with a pulse duration of 5 msec I_{Na} to

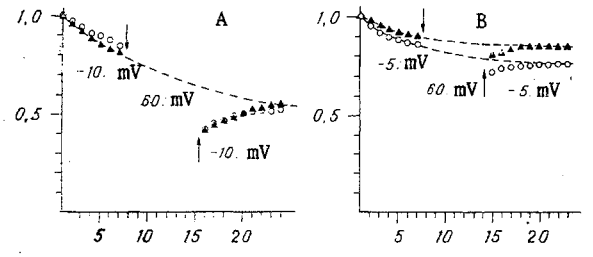

Fig. 3. Comparison of effect of Ca^{++} ions on cumulative blockade of Na channels by NPA (A) and amethocaine (B). A) fall of I_{Na} during repetitive (2 Hz) stimulation of Ranvier node by depolarizing pulses (duration 10 msec) in presence of 10^{-4} M NPA, with two calcium concentrations in solution: 2 mM (circles) and 20 mM (triangles). E_h = −100 mV, 7°C; B) the same as in A, but during action of $2.5 \cdot 10^{-5}$ M amethocaine. E_h = −100 mV, 8°C. Arrows indicate change of amplitude of pulses, voltage of which is shown near curves. Broken lines — extrapolated time course of fall of I_{Na} during repetitive stimulation of node with no increase in stimulus amplitude. Abscissa, No. of pulse in series; ordinate, I_{Na} (in relative units). Value of I_{Na} to first pulse in series taken as unity.

the last pulse of the series was 76% relative to the first, whereas with a duration of 50 msec it was 41%). The cause of this dependence of cumulative blockage of I_{Na} on the duration of the depolarizing stimuli is SI, into which the Na channels are converted in the presence of local anesthetics during the depolarizing stimulus. This conclusion is based on the results of previous investigations [1, 7] and on experimental data shown in Fig. 1B. The testing stimulus (E_t) was applied 40 msec after the end of conditioning depolarization (E_e) of different duration (Δt) in the presence of lidocaine, amethocaine, and etidocaine. The interval of 40 msec between stimuli was chosen because during this time all Na channels are able to recover from fast inactivation. I_{Na} to the testing stimulus, with very short conditioning prepulses, was about equal to the control value, taken as unity. With an increase in duration of the conditioning step, I_{Na} to the testing stimulus progressively decreased, reflecting an increase in the fraction of Na channels transformed into the SI state during the conditioning step.

Increasing the Ca^{++} ion concentration ($[Ca]_o$) in the external solution led to partial abolition of SI: the steady-state fraction of Na channels converted into the SI state was reduced. Figure 2A shows the effect of a tenfold increase in $[Ca]_o$ on the steady-state SI (S_∞) induced by amethocaine. Increasing $[Ca]_o$ to 20 mM caused a shift of the S_∞ versus E curve toward more positive values of E by about 20 mV and, at the same time, considerably raised the minimal level of S_∞^{min}. This effect also was observed in experiments with lidocaine and it was qualitatively similar to that obtained previously in analogous experiments with trimecaine and procaine [1, 7].

Increasing $[Ca]_o$ significantly weakened cumulative inhibition of I_{Na} caused by terriary amines (lidocaine, amethocaine, and etidocaine). The effect of increasing $[Ca]_o$ from 2 to 20 mM on cumulative blockage of I_{Na} induced by amethocaine is shown in Fig. 2B. Increasing the Ca^{++} concentration tenfold, with pulses 5 msec in duration in the series, led to weakening of the blockade of I_{Na} by only 11%, compared with 42% with a pulse duration of 50 msec. This result can be explained on the grounds that Ca^{++} ions prevent interaction of amethocaine with inactivated Na channels, and for that reason the fraction of Na channels transformed into the SI state was reduced by a greater degree during the longer depolarizing pulse.

A completely different result was obtained in experiments with the quaternary adjaline derivative NPA, which interacts with open Na channels [2, 8, 10]. It will be clear from Fig. 1C that SI did not develop during the action of NPA: the fall of I_{Na} after the conditioning depolarizing stimulus in the presence of NPA was indistinguishable from that observed in the control. This small decrease in I_{Na} was due to SI induced by K^+ ions present in the Ringer's solution. Cumulative blockade of I_{Na} developing during repetitive membrane depolarization (pulse duration 5 msec, frequency 2 Hz) in the presence of NPA (10^{-4} M) in Ringer's solution with a normal (2 mM) and increased (up to 20 mM) Ca^{++} ion concentration is shown in Fig. 3A.

It was shown previously [2, 8] that cumulative blockade of I_{Na} induced by NPA is due to entry of molecules of the blocker into the open Na channel. With an increase in amplitude of the depolarizing pulses in the region of potentials in which all Na channels are open, blockade of I_{Na} was potentiated, evidently due to the effect of the electric field either on the molecule of the blocker itself or on its binding site in the membrane. Increasing $[Ca]_o$ from 2 to 20 mM not only did not weaken, but actually potentiated the cumulative blockade of I_{Na} induced by NPA, but was not reflected at all in the voltage-dependent blockade of I_{Na}.

In experiments with the tertiary amines lidocaine, amethocaine, and etidocaine an increase in amplitude of the depolarizing stimuli also led to potentiation of blockade of I_{Na}, but this effect was much weaker than during the action of NPA (Fig. 3B). It can be tentatively suggested that deepening of the cumulative blockade in this case was due to interaction of protonated molecules of the local anesthetic present in the axoplasm with open Na channels. Figure 3 shows that this voltage-dependent blockade of I_{Na} was unchanged, in the experiments with local anesthetics also, with an increase in $[Ca]_o$ from 2 to 20 mM, whereas the cumulative blockage of I_{Na}, due to SI, was abolished in these experimental conditions also.

The results thus indicate that amines can induce two types of cumulative blockade of Na channels: 1) inhibition of I_{Na} due to interaction of molecules of the blocker with open Na channels — it is intensified with an increase in amplitude of the depolarizing stimuli and is not weakened with an increase in Ca^{++} ion concentration; 2) inhibition of I_{Na} due to interaction of molecules of the blocker with Na channels in the inactivated state — it is considerably weakened by an increase in Ca^{++} ion concentration in the external solution (SI). The different action of Ca^{++} ions on these two types of blockade is evidence in support of the previous hypothesis [1, 7] that different "binding sites" exist and are responsible for cumulative blockade of I_{Na} induced by tertiary and quaternary amines.

The author is grateful to Professor B. I. Khodorov for valuable comments during the discussion of the results.

LITERATURE CITED

1. L. D. Zaborovskaya, "Mechanism of action of local anesthetics on the excitable membrane of nerve fibers," Author's Abstract of Candidate's Dissertation, Moscow (1979).
2. L. D. Zaborovskaya and B. I. Khodorov, Byull. Éksp. Biol. Med., No. 5, 578 (1980).
3. E. M. Peganov, B. I. Khodorov, and L. D. Shishkova, Byull. Éksp. Biol. Med., No. 9, 15 (1973).
4. L. D. Shishkova (L. D. Zaborovskaya), Neirofiziologiya, 8, No. 4, 418 (1976).
5. F. Dodge and B. Frankenhaeuser, J. Physiol. (London), 143, 76 (1958).
6. B. Hille, J. Gen. Physiol., 69, 497 (1977).
7. B. Khodorov, L. Shishkova (L. Zaborovskaya), E. Peganov, et al., Biochim. Biophys. Acta, 433, 409 (1976).
8. B. Khodorov and L. Zaborovskaya, Gen. Physiol. Biophys., 2, 233 (1983).
9. G. Strichartz, J. Gen. Physiol., 62, 37 (1973).
10. L. Zaborovskaya and B. Khodorov, J. Molec. Cell. Cardiol., 12, Suppl. 1, 185 (1980).