3. S. N. Golikov and S. I. Loktionov, Byull. Éksp. Biol. Med., No. 4, 61 (1966).
4. A. I. Gromov, Farmakol. Toksikol., No. 2, 145 (1975).
5. P. P. Denisenko, in: The Pharmacology of New Sedatives and Their Clinical Use [in Russian], Moscow (1962), p. 16.
6. V. M. Karasik, in: Proceedings of the 10th Congress of the All-Union Physiological Society [in Russian], Vol. 1, Moscow–Leningrad (1964), p. 63.
7. V. B. Prozorovskii, Farmakol. i. Toksikol., No. 4, 494 (1974).
8. A. T. Selivanova, The Action of Cholinergic Drugs on Higher Nervous Activity [in Russian], Leningrad (1969).
9. R. W. Brimblecome, D. M. Green, T. D. Inch, et al., J. Pharm. Pharmacol., 23, 745 (1971).
10. T. D. Inch, D. M. Green, and P. B. Thompson, J. Pharm. Pharmacol., 25, 359 (1973).
11. D. J. Finney, Probit Analysis, London (1947).
12. M. J. Friedman, J. H. Jaffe, and S. K. Sharpless, J. Pharmacol. Exp. Ther., 167, 45 (1969).
13. A. C. Karczmar and C. L. Scudder, in: Aggressive Behavior, S. Garattini and E. B. Sigg, eds., Amsterdam (1969), p. 209.
14. W. J. Stewart and S. Blain, Psychopharmacologia, 14, 291 (1975).

STIMULUS-DEPENDENT BLOCKING OF SODIUM CHANNELS IN THE RANVIER NODE MEMBRANE BY THE QUATERNARY ANTIARRHYTHMIC DRUG N-PROPYLAIMALIN (NEOGILURYTMAL)

L. D. Zaborovskaya and B. I. Khodorov

UDC 615.22.015.4:612.81

KEY WORDS: sodium channel; Ranvier node; antiarrhythmic drugs; neogilurytmal.

It was shown previously that the blocking action of local anesthetics (tertiary and quaternary amines) on sodium channels in the membrane of nerve [6, 8, 10, 14] and muscle [12] fibers may be reversibly potentiated by application of a series of depolarizing stimuli to the membrane. It was suggested that this stimulus-dependent block of the sodium channels plays an important role in the mechanism not only of the local anesthetic, but also of the antiarrhythmic action of these preparations.

Accordingly, in the investigation described below a study was made of the action of a derivative of aimalin, namely neogilurytmal (NG), one of the most effective antiarrythmic agents for use in medical practice [5, 13], on sodium channels.

EXPERIMENTAL METHOD

Experiments were carried out on the Ranvier node of isolated nerve fibers of the frog *Rana ridibunda* by the voltage-clamp method [7]. The ends of the fiber were cut on either side of the test node in isotonic CsCl solution. Cs^+ ions, diffusing along the axoplasm into the region of the Ranvier node, completely blocked the outward potassium currents. The experiments were carried out under conditions of continuous perfusion of the test node with control Ringer's solution of the following composition (in mM): NaCl 112, KCl 2.5, $NaHCO_3$ 2, $CaCl_2$ 2; pH 7.2, or with the same solution containing NG. The temperature varied in the different experiments from 12 to 15°C.

EXPERIMENTAL RESULTS

NG has no action on resting (closed) sodium channels. This is shown by the fact that exposure of the node for 5-10 min in Ringer's solution containing NG caused no appreciable decrease in the peaks of the inward sodium current (I_{Na}) in any of the experiments (n = 11),

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 S. S. Debov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 5, pp. 578-580, May, 1980. Original article submitted August 1, 1979.

 0007-4888/80/8905-0634$07.50

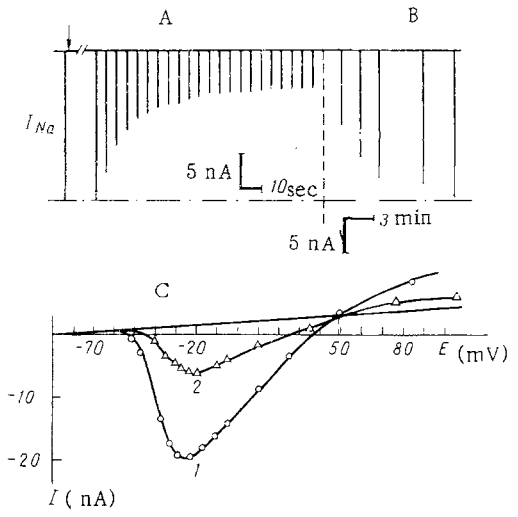

Fig. 1

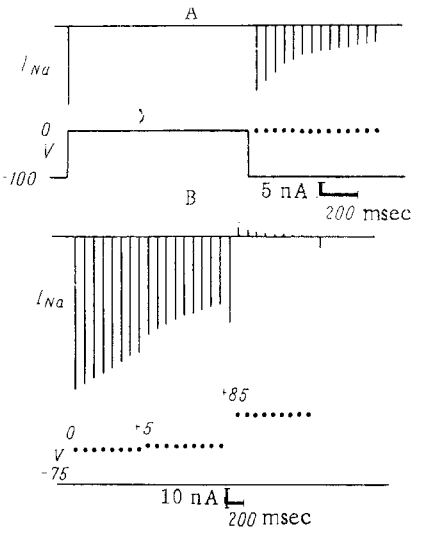

Fig. 2

Fig. 1. Effect of NG on sodium currents in Ranvier node membrane. A) First peak of I_{Na} obtained in control Ringer's solution. Arrow indicates time of application of NG in concentration of 10^{-5} M. Repetitive stimulation with a frequency of 1 Hz began 10 min after application of NG to region of node. Peak values of I_{Na} to each fifth stimulus shown; recovery of I_{Na} after end of repetitive stimulation. Holding potential (E_h) was −100 mV throughout the experiment; amplitude of depolarizing stimuli (V) −100 mV, duration 5 msec. Fiber tested May 21, 1979; C) current–voltage characteristics: 1) in control Ringer's solution, 2) after repetitive stimulation of node (10 Hz) in the presence of NG (10^{-5} M). Fiber tested March 26, 1979.

Fig. 2. Blocking action of NG as a function of duration and amplitude of depolarizing stimuli. Top beam records I_{Na} peaks, bottom beam potential. A) Peak values of I_{Na} in response to depolarizing stimulus (1 sec) and series lasting 5 msec. Fiber tested May 15, 1979; B) effect of change in amplitude of depolarizing stimuli in rhythmic series. Duration of pulses 5 msec. Fiber tested April 20, 1979.

if throughout this period the node was not subjected to testing stimulation (Fig. 1A). However, application of a few short (3-5 msec) depolarizing stimuli to the membrane was sufficient to reveal a marked decrease in I_{Na}. In a rhythmic series this decrease in I_{Na} progressed even if the interval between stimuli was 1 sec (Fig. 1A). It is important to note that under these circumstances only the amplitude of I_{Na} was reduced, and the kinetics of its rise (activation) and fall (inactivation) was virtually unchanged. After the end of repetitive stimulation I_{Na} was restored very slowly (in several tens of minutes). In the experiment whose results are illustrated in Fig. 1, recovery of I_{Na} was practically complete by the 20th minute.

The current–voltage characteristic recorded immediately after the end of repetitive stimulation on the node showed neither a shift of the potential-dependence of sodium activation nor changes in the reversal potential of I_{Na} and of the leakage current (Fig. 1C).

The fall in I_{Na} in a rhythmic series may be the result of interaction between the blocker and either the open [10, 14] or the inactivated channel [10]. To determine in which of these states the sodium channels are blocked by NG, the effect of short (5 msec) repeated depolarizing stimuli was compared with the effect of a single prolonged (1 sec) depolarizing step. The experiments showed that after the end of prolonged depolarization, I_{Na} measured immediately after emergence of the sodium channels from the state of rapid inactivation (50 msec) was reduced by 16%, and to the next short depolarizing stimulus it was reduced by 14%, whereas a series of short depolarizing stimuli (14 stimuli with a total duration of 70 msec) caused a deep decrease in I_{Na} (by 75%). The fact is conclusive evidence that the fraction of sodium channels blocked by NG is determined not by the total duration of depolarization of the membrane, but by the time during which the channels are in the open state. Hence it can be concluded that NG blocks only open sodium channels. In this respect the effect of NG is similar to the action of other quaternary ammonium compounds, both those which are insoluble in lipids and are therefore effective only by intra-axonal administration (QX-314, QX-222, QT) [10, 11,

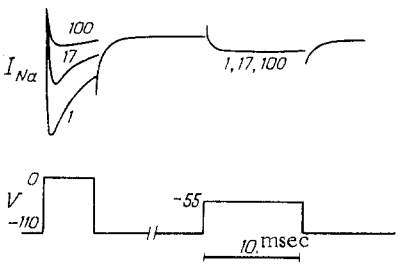

Fig. 3. Action of NG on sodium currents in Ranvier node modified by aconitine. Top beam records I_{Na}, bottom beam potential. Frequency of stimulation of node 20 Hz. Traces of I_{Na} to pulses 1, 17, and 100 are shown. Concentration of aconitine 10^{-5} M, of NG 10^{-5} M. Fiber tested April 2, 1979.

14], and also those soluble in lipids (QX-572) [10, 11] and, therefore, effective by both extra- and intra-axonal application. Judging from the fact that NG, when acting on the outer side of the membrane, can induce I_{Na} blockade, this quaternary ammonium compound may belong to the lipid soluble group.

The higher the amplitude of the depolarizing step, the more marked the blocking of the open sodium channels by NG during repetitive stimulation of the node. This is clear from Fig. 2B, in which the fall in the I_{Na} peak to the testing stimulus (up to E = 0 mV) is compared after nine conditioning pulses with amplitudes of up to E = + 5 mV and E = +85 mV. The fact should be noted that strengthening of the block took place within the zone of potentials in which all sodium channels were in the open state. In special experiments a change in the external (to 30 mM) or inter-axonal (approximately 30 mM) Na^+ ion concentration did not affect the blocking action of NG. This suggests that potentiation of the blocking of I_{Na} in response to an increase in the depolarizing step is associated with the direct effect of the electric field of the membrane on charged NG molecules or on the NG "receptor" in the membrane.

To study the role of inactivation in the mechanism of stimulus-dependent I_{Na} blockade induced by NG, experiments were carried out with aconitine, which abolishes inactivation of sodium channels and, at the same time, causes a change in their activation [1, 3], ionic selectivity, and sensitivity to local anesthetics [2]. Two populations of sodium channels were found in the Ranvier node treated with aconitine: 1) modified channels, without inactivation, and activated at a potential as little as E = −85 mV; 2) normal channels, activated at E = −50 mV. Traces of I_{Na} obtained during repetitive stimulation of the node by paired depolarizing pulses (the first with an amplitude of up to E = 0 mV, the second E = −55 mV) in the presence of NG are illustrated in Fig. 3. In response to the first stimulus a combined inward I_{Na} (normal + modified) appeared, in response to the second, only the modified current. As Fig. 3 shows, during repetitive stimulation the inactivated I_{Na} fell progressively, whereas the modified I_{Na} was virtually unchanged. These data are evidence of the important role of the inactivation mechanism in the development of stimulus-dependent blockade of sodium channels by NG.

In the writers' view these results are interesting on account of the light they shed on the mechanism of the antiarrhythmic action of NG, for sodium channels in the Ranvier node membrane are similar in their biophysical and pharmacological properties to the sodium channels of myocardial cells [4, 9].

The authors are grateful to the firm Giulini Pharma (West Germany) for providing the neogilurytmal, and to É. M. Peganov for help with the work.

LITERATURE CITED

1. G. N. Mozhaeva, A. P. Naumov, and Yu. A. Negulyaev, Neirofiziologiya, No. 2, 152 (1976).
2. Yu. A. Negulyaev and E. D. Nosyreva, Tsitologiya, No. 6, 697 (1979).

3. É. M. Peganov, S. V. Revenko, B. I. Khodorov, et al., in: Molecular Biology [in Russian], No. 15, Kiev (1976), pp. 42-56.
4. B. I. Khodorov, General Physiology of Excitable Membranes [in Russian], Moscow (1975).
5. R. Anttila, R. Tikkanen, and L. Nieminen, Arzneimittel-Forsch., 28, 397 (1978).
6. K. R. Courtney, J. Pharmacol. Exp. Ther., 195, 225 (1975).
7. F. Dodge and B. Frankenhaeuser, J. Physiol. (London), 143, 76 (1958).
8. B. Hille, J. Gen. Physiol., 69, 497 (1977).
9. B. Hille, in: Biophysical Aspects of Cardiac Muscle, New York (1978), pp. 55-74.
10. B. I. Khodorov, L. D. Shishkova, E. M. Peganov, et al., Biochim. Biophys. Acta, 433, 409 (1976).
11. T. Narahashi, I. W. Moore, and R. N. Poston, J. Neurobiol., 1, 3 (1969).
12. W. Schwarz, B. Hille, and P. T. Palade, Biophys. J., 20, 343 (1977).
13. K. Shigenobu, Y. Kasuya, J. Ishike, et al., Chem. Pharm. Bull., 22, 2329 (1974).
14. G. R. Strichartz, J. Gen. Physiol., 62, 37 (1973).

PASSIVE GLYPINE TOLERANCE IN MONKEYS

S. S. Krylov, V. N. Amatuni,
E. K. Georgianova, L. P. Kuznetsova,
and L. P. Tochilkina

UDC 615.217.34.015.46.076.9

KEY WORDS: monkeys; glypine; cholinolytic; tolerance; passive tolerance.

Habituation to therapeutic preparations has been observed for a long time [1], including to drugs with central cholinolytic properties [2, 3]. It is also known that a state of passive tolerance can be created, to morphine for example, by injecting blood serum [4] or brain homogenate [5] obtained from animals actively tolerant to this drug, into intact animals.

The object of this investigation was to attempt to create passive tolerance to the cholinolytic glypine by injecting blood plasma from a tolerant animal into an intact recipient.

EXPERIMENTAL METHOD

Six male baboons *(Papio hamadryas)* were used. The mean age of the baboons was about 7 years and their mean weight about 20 kg. Before the experiments the animals were kept in a group.

Tolerance was produced in two monkeys by daily intramuscular injection of glypine in a dose of 0.05 mg/kg. The experimental scheme is shown in Fig. 1.

The possibility of creation of passive tolerance was studied in two intact monkeys after injection of plasma from tolerant animals into them. Blood was taken from tolerant monkeys 24 h after the last injection of glypine by total exsanguination of the animal under hexobarbital anesthesia. The blood cells were removed by centrifugation. The plasma was injected without further treatment on the same day, intravenously into intact monkeys in a dose of 10 ml/kg body weight. Glypine in a dose of 0.05 mg/kg was injected into the animals 30 min later and the magnitude of the effect of this cholinolytic, if present, was determined. Parallel experiments on two intact monkeys served as the control for the action of glypine. Observations on the animals' behavior continued for 6 h.

Because of the extremely small number of monkeys available, experiments could not be carried out with preliminary injection of intact plasma into control animals. However, similar experiments performed previously on dogs showed that preliminary injection of intact plasma into the animals does not alter their response to subsequent injection of the cholinolytic.

Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulletin' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 5, pp. 580-581, May, 1980. Original article submitted July 2, 1979.

