BLOCKING OF INACTIVATED SODIUM CHANNELS BY THE ANTIARRHYTHMIC CORDARONE

S. V. Revenko, B. I. Khodorov, and Ma. Ya. Avrutskii

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KEY WORDS: antiarrhythmic cordarone; Ranvier node.

Cordarone, or amiodarone hydrochloride (Fig. 1A), is used in clinical cardiology and cardiac surgery for the prevention and control of arrhythmias [2]. There is, as yet, no general agreement regarding the action of cordarone on electrically excitable membranes. In experiments with intracellular recording of action potentials (AP) of a strip of rabbit myocardium, cordarone lengthened the repolarization time without changing either the ascending phase or the amplitude of AP, and on those grounds it was suggested that cordarone blocks membrane permeability for K^+ ions [7]. Other investigations showed that cordarone also leads to a decrease in the amplitude of AP and in the rate of diastolic depolarization of the rabbit sinoauricular node [4].

Considering the closeness of the properties of the ionic channels of nerve and muscle membranes [5], the action of cordarone on ionic currents of the Ranvier node was investigated, and details are given below.

EXPERIMENTAL METHOD

Experiments were carried out on Ranvier nodes of isolated frog nerve fibers by the voltage clamp method [3, 6]. The ends of the fiber were divided in isotonic KCl solution (114 mM). Cordarone was added to the Ringer's solution in a concentration of $7 \cdot 10^{-4}$ M. The experiments were carried out at 12-14°C and pH 7.2 (maintained with the aid of 5 mM Tris). Sodium (I_{Na}) and potassium (I_K) currents were measured from peak and steady-state values of the membrane current obtained in response to a stepwise change of membrane potential (E).

EXPERIMENTAL RESULTS

Replacement of normal Ringer's solution by solution containing cordarone led to a change in the ionic currents. Current-voltage characteristics of I_{Na} and I_K before and 1 min after application of $7 \cdot 10^{-4}$ M cordarone are shown in Fig. 1B. As a result of the application of cordarone, both inward and outward I_{Na} were reduced without any change in their reversal potential, indicating a decrease in sodium permeability. No changes in the kinetics of I_{Na} were observed. The reduction of I_{Na} by cordarone was reversibly potentiated both during repetitive stimulation of the node by a series of depolarizing pulses (each 7 msec in duration), and (and particularly effectively) by prolonged (1 sec) depolarization of the membrane.

The results of a typical experiment to reveal the potential-dependent blocking of sodium channels by cordarone are given in Fig. 2A. The magnitude of this block, developing during a prolonged (1 sec) conditioning shift of potential, was measured by means of a series of short testing stimuli applied at intervals of 300 msec. The duration of these stimuli (3 msec) was chosen to be small enough to ensure that repetitive stimulation by itself did not cause any changes in I_{Na} (Fig. 2, 2). It must be emphasized that an increase in the following frequency of the pulses to 10 sec^{-1} did not lead to any change in I_{Na}. Conditioning depolarization 1 sec in duration (Fig. 2, 3) induced a decrease in I_{Na}, and after the end of this depolarization I_{Na} was restored in accordance with an exponential law with a time constant of 600 msec. Considering that the decrease in the first peak of I_{Na}, measured immediately after the end of conditioning depolarization, is affected mainly by the process of fast sodium inactivation (the H-process), the degree of slow potential-dependent lowering of I_{Na} was estimated by extrapolation of the I_{Na} recovery curve (the broken line in Fig. 2, 3) to the time of ending of conditioning depolarization. It will be clear from Fig. 2, 4 that conditioning hyperpolarization caused a more

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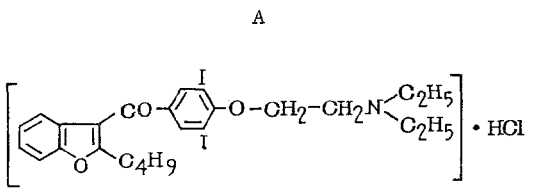

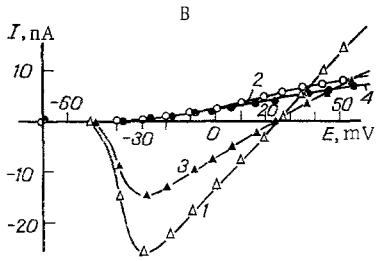

Fig. 1

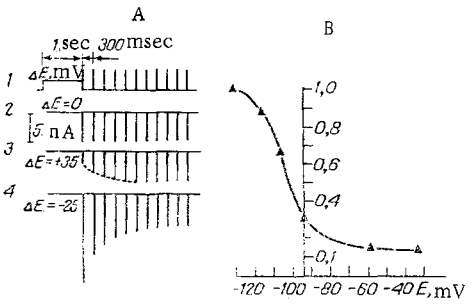

Fig. 2

Fig. 1. Effect of cordarone (A) on I_{Na} and I_K of Ranvier node (B). Current-voltage characteristics of sodium (1, 3) and potassium (2, 4) channels before (1, 2) and after (3, 4) application of $7 \cdot 10^{-4}$M cordarone. Curves 2 and 4 plotted for steady-state level of ionic current, measured at the end of 17 msec of the square pulse. Holding potential −70 mV.

Fig. 2. Potential-dependent blocking of I_{Na} by cordarone. A) Tracings of I_{Na} in response to series of depolarizing pulses (3 msec) applied without a conditioning shift of potential (2), and after depolarizing (3) and hyperpolarizing (4) conditioning shifts of membrane potential. 1) Pulse program. Each pulse of the series depolarized the membrane to the level E = −18 mV. Numbers of traces 2, 3, and 4 show shifts of potential counted from holding potential of −93 mV. B) Dependence of proportion of sodium channels free from slow potential-dependent block (ordinate) on membrane potential E during conditioning shift of potential (abscissa).

than twofold increase in I_{Na}, and it also showed how blocking of the sodium channels developed when the membrane potential returned to the 93 mV level. The time constant of this process (600 msec) is the same as that obtained for trace 3; in other words, the kinetics of the lowering and recovery of I_{Na} was the same at the same potential.

The dependence of the proportion of sodium channels free from slow potential-dependent blocking on membrane potential during a conditioning shift of potential is shown in Fig. 2B (the same experiment; the ratio of the extrapolated value of I_{Na}, measured at the end of the conditioning shift of potential E, to the value of I_{Na} measured without a preliminary shift of potential, is plotted along the ordinate). In different experiments, long (1 sec) hyperpolarizing pulses, shifting the membrane potential by up to 130 mV, were able to restore I_{Na}, when depressed by cordarone, up to 50-90% of its normal value measured before application of cordarone and with a holding potential of 80 mV.

It was shown previously that agents blocking I_{Na} can interact either with open sodium channels [1, 5, 8] or with channels in a state of inactivation [1, 5, 6]. The fact that in the present experiments long (1 sec) depolarization caused a sharp increase in the intensity of blocking of the sodium channels, whereas a series of short (3 msec) pulses did not change the blocking action of cordarone, suggests that this antiarrhythmic interacts with inactivated sodium channels.

No detailed investigation of the effect of cordarone on I_K was undertaken. Under the influence of $7 \cdot 10^{-4}$ M cordarone, I_K was reduced by 15% (Fig. 1B). This result is typical of all the experiments of the series. No changes were observed in the kinetics of I_K.

There is no doubt that the potential-dependent lowering of sodium permeability revealed by these experiments is directly related tothe antiarrhythmic action of cordarone. It can be tentatively suggested that the clinical effect of the drug is enhanced by factors shifting the resting potential of the myocardial cells toward depolarization, e.g., when the K^+ ion concentration in the external solution is increased.

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EFFECT OF KYNURENINE AND ITS METABOLITES ON VASCULAR EFFECTS OF SEROTONIN, NORADRENALIN, AND ACETYLCHOLINE

I. P. Lapin and L. G. Umanskaya

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The kynurenines – metabolites of tryptophan on the kynurenine pathway of intermediate metabolism – possess central and peripheral pharmacological effects [2] and, in particular, they interact with monoamines, their precursors, and psychotropic drugs. Kynurenine and its metabolites [1, 3, 4] lower blood pressure in rats. The effect of kynurenine on the vascular effects of serotonin, noradrenalin, and acetylcholine has been investigated in detail [1]. This paper describes a continuation of the study of its metabolites.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female rats weighing 200-250 g (from the Rappolovo nursery); the animals were anesthetized with urethane (0.7 ml of a 25% solution/100 g body weight, i.e., 1.75 g/kg, intraperitoneally). The systemic blood pressure was recorded by a mercury manometer in the carotid artery. The system of polyethylene tubes of the manometer was filled with heparin solution (1500 units/ml) to prevent the blood from clotting. The original pressure in different experiments varied between 90 and 120 mm Hg. All solutions were made up in fresh physiological saline immediately before injection into the femoral vein in a volume of 0.1 ml in the course of 3 sec. At the beginning of each experiment three or four control injections of physiological saline were given. Fluctuations of pressure caused by them were indistinguishable from those occurring spontaneously. Serotonin sulfate was injected in a dose of 0.5-5 μg, in most experiments in doses of 1 and 2.5 μg (the pressure was lowered by 8-21 mm Hg for 35-60 sec), noradrenalin bitartrate was injected in doses of 0.5-2 μg, in most experiments 0.5 and 1 μg (the pressure was raised by 4-8 mm Hg for 50-180 sec) and acetylcholine was injected in doses of 0.1 and 0.2 μg (the pressure was lowered by 9-14 mm Hg for 25-60 sec). Changes in the effects of serotonin and noradrenalin were determined in each experiment from the difference between the means of the three or four control tests before injection of the kynurenines, taken as 100%, and the mean of two tests after injection of the kynurenines, when the changes (in amplitude of duration) were maximal. The dose of kynurenines chosen in preliminary experiments as most effective for interaction with serotonin and noradrenalin, but not itself changing the blood pressure, was 200 μg. This last feature of the method is very important, for kynurenine and its metabolites [1, 3, 4] lower the blood pressure in rats over a wide range of doses.

Laboratory of Psychopharmacology, V. M. Bekhterev Leningrad Psychoneurological Research Institute.

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