showed little change during the next 2 months. The lower level of mortality in the group of short-sleeping rats than in the long-sleepers evidently reflects the increased sensitivity of the latter to the toxic action of alcohol.

The great similarity between the forms of experimental alcoholism described in rats and forms of chronic alcoholism in man provides a basis for a differential approach to the simulation of this pathology in animals.

LITERATURE CITED

1. E. Bleuler, Textbook of Psychiatry [Russian translation], Berlin (1920).
2. M. E. Burno, Zh. Nevropatol. Psikhiat., No. 4, 585 (1968).
3. N. N. Ivanets, A. L. Igonin, and N. V. Ivanova, Zh. Nevropatol. Psikhiat., No. 2, 237 (1977).
4. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
5. S. V. Shoshina and A. I. Maiskii, Byull. Éksp. Biol. Med., No. 7, 55 (1982).
6. F. D. Sheffield and T. B. Roby, J. Comp. Physiol. Psychol., 43, 471 (1950).
7. F. D. Sheffield, T. B. Roby, and B. A. Campbell, J. Comp. Physiol. Psychol., 47, 349 (1954).

EFFECT OF NONACHLAZINE ON TRANSMEMBRANE IONIC CURRENTS OF ATRIAL TRABECULAE IN FROGS

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Nonachlazine is a new antianginal drug synthesized at the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR. In its chemical structure it is 1,4-diazabicyclo-(4,3,0)-nonanyl-4-propionyl-2-chlorophenothiazine, i.e., it is a 10-aminopropional-2-substituted phenothiazine, a group of compounds characterized by a marked action on the cardiovascular system. Nonachlazine is widely used in medical practice for the treatment of ischemic heart disease (IHD). The drug not only has an antianginal effect, but in some cases it also has an antiarrhythmic action. Under experimental conditions these properties are found on most models of arrhythmia, but they are less marked than those of other compounds (ethmozine, its diethylamino analog) which have a urethane group in position 2 of the phenothiazine ring [1, 2]. The antiarrhythmic properties of the latter are associated mainly with their ability to block the rapid inward sodium current, and this explains their high effectiveness in cardiac arrhythmias connected with the appearance of ectopic foci of excitation in the myocardium [3]. It was interesting to discover with what mechanisms the antiarrhythmic action of nonachlazine is connected. The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

The test object consisted of atrial trabeculae from the frog *Rana esculenta*, about 3 mm long and 0.1 mm in diameter. The trabeculae were placed in a double sucrose gap system to measure transmembrane ionic currents by the voltage clamp method. The apparatus described in [9] was used. Experiments were carried out in a chamber at a constant temperature of 18°C; the strip was stimulated with square pulses of direct current with a frequency of 0.6 Hz.

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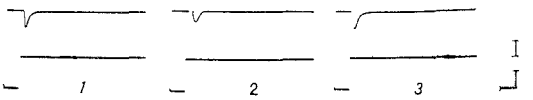

Fig. 1. Reduction of amplitude of maximal inward current by nonachlazine (1.03×10^{-5} M). Membrane potential clamped under double sucrose gap conditions at 70 mV, current activated by a depolarization step of 40 mV from that level. 1) Preparation in Ringer's solution, 2) nonachlazine added, 3) after rinsing preparation with Ringer's solution. Calibration of ionic current (top curves) 1 μA, calibration of potential (bottom curves) 20 mV, time 10 msec.

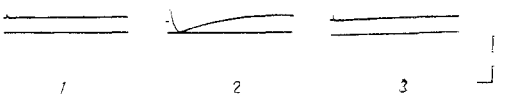

Fig. 2. Depression of slow inward flow of Ca^{++} ions, activated by adrenalin, by nonachlazine. Slow inward current activated in presence of adrenalin by a depolarizing step of 80 mV from holding potential level of 70 mV. Calibration of ionic current (top curves) 1 μA, of potential (bottom curves — 20 mV, of time — 50 msec). 1) Preparation perfused with sodium-free Ringer's solution (NaCl replaced by choline chloride), 2) after addition of adrenalin (4.6×10^{-6} M), 3) the same, after action of nonachlazine (1.03×10^{-5} M).

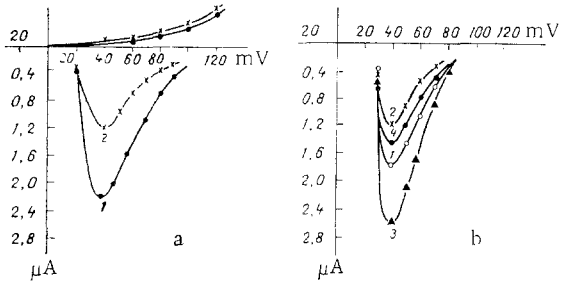

Fig. 3. Effect of nonachlazine (1.03×10^{-5} M) on current—voltage characteristic curves plotted for maximal values of inward and outward currents, in Ringer's solution (a) and in calcium-free solution with addition of prostacycline (b). Abscissa, potential (in mV); ordinate, values of membrane current (in μA). a: 1) Ringer's solution, 2) nonachlazine; b: 1) calcium-free Ringer's solution, 2) nonachlazine, 3) prostacycline (2.7×10^{-7} M), 4) nonachlazine in the presence of prostacycline (2.7×10^{-7} M).

To avoid distortion of the ionic currents introduced under double sucrose gap conditions by the complex structure of heart tissue [4-6], the following techniques were used: Only those fibers with action potential not less than 110 mV were used, and the results of only those experiments in which the control (initial) values of the currents were restored after rinsing the preparation were taken into account. The method was thus used more to assess the direction and power of the effects than to determine absolute physiological values.

The composition of the Ringer's solution was as follows (in mM): NaCl 112, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 1.2. The slow inward current was recorded in Ringer's solution in which NaCl was replaced by choline chloride (113.2 mM) with the addition of 0.1 mM atropine sulfate. Isotonic sucrose solution (from Merck, West Germany), the sodium salt of prostacycline (PGI_2-Na, from Chinoin, Hungary), adrenalin (Tonogen, from Richter, Hungary), and tetrodotoxin (Sankyo) were used in the experiments; the pH of the solutions was 7.4.

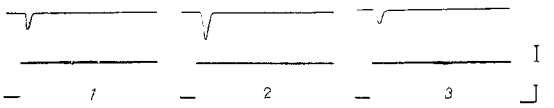

Fig. 4. Action of prostacycline (2.7×10^{-7} M) on maximal inward current during perfusion of preparation with Ringer's solution without Ca^{++} ions (2) and in presence of 1.03×10^{-5} M nonachlazine (3). 1) Maximal inward current during perfusion of preparation with calcium-free Ringer's solution. Calibration of ionic current (top curves) 1 μA, calibration of potential (bottom curve) 20 mV, time 10 sec.

EXPERIMENTAL RESULTS

During perfusion of an atrial trabecula (sinoauricular fiber) with Ringer's solution containing nonachlazine in a concentration of 1.03 ± 10^{-5} M, a decrease in amplitude of the maximal inward current was observed, at all values of membrane potential. For example, depolarization by 20 mV from the level of the holding potential of 70 mV reduced the inward current from 2.17 ± 0.12 μ A in normal Ringer's solution to 1.10 ± 0.29 μA in solution with nonachlazine, i.e., by almost 50% (Fig. 1). Outward currents measured at the end of the depolarizing stimulus, 0.5 sec in duration, were unchanged by nonachlazine. Rinsing the preparation with Ringer's solution completely restored the original values of the transmembrane ionic current. In sodium-free solution nonachlazine, in a concentration of 0.2×10^{-5} M reduced, but in a concentration of 1.03×10^{-5} M abolished the slow inward Ca^{++}-dependent current. Nonachlazine in this concentration prevented the action of adrenalin, which under normal conditions increases conductance of the slow sodium–calcium channel (Fig. 2).

The effect of nonachlazine on current–voltage characteristic curves is shown in Fig. 3a. The writers showed previously that prostacycline PGI_2 increases the fast sodium current in heart cells [7, 8]. It will be clear from Fig. 3b that nonachlazine prevents the stimulating action of prostacycline also. The absence of any action of prostacycline on the inward current after addition of nonachlazine also is shown in Fig. 4.

The data obtained indicate that nonachlazine has a direct effect on myocardial cell membranes and that this effect differs in its electrophysiological mechanism from the action of ethmozine.

These results suggest that the presence of chlorine in position 2 and of a diazabicycloalkanecarboxyl group in position 10 of the phenothiazine ring leads to a change in the electrophysiological mechanism of action of the compounds on myocardial cell membranes. The effect on the slow inward calcium current begins to acquire essential importance in the spectrum of action of these preparations. This provides a basis for a search for substances possessing antifibrillatory properties among compounds similar to nonachlazine in their chemical structure. In addition, data on the electrophysiological mechanisms responsible for the antiarrhythmic action of nonachlazine are interesting from the standpoint of its clinical use in patients with IHD complicated by disturbances of the cardiac rhythm predisposing to the development of fibrillation.

LITERATURE CITED

1. Yu. I. Vikhlyaev, N. V. Kaverina, Z. P. Senova, et al., Farmakol. Toksikol., No. 2, 163 (1971).
2. A. N. Gritsenko, Z. I. Ermakova, and S. V. Zhuravlev, Khim.-farm. Zh., No. 9, 17 (1972).
3. L. V. Rozenshtraukh and V. N. Chikharev, Byull. Éksp. Biol. Med., No. 9, 303 (1980).
4. E. Coraboeuf, Am. J. Physiol., 234, H101 (1978).
5. A. De Hemptinne, Pflüg. Arch. Ges. Physiol., 363, 87 (1976).
6. E. A. Johnson and M. Lieberman, Annu. Rev. Physiol., 33, 479 (1971).
7. V. Kecskeméti, K. Kelemen, and J. Knoll, Acta Biol. Med. Ger., 37, 821 (1978).
8. K. Kelemen, R. Markó, V. Kecskeméti, et al., in: Advances in Pharmacological Research and Practice, Oxford (1980), p. 89.
9. O. Rougier, G. Vassort, D. Garnier, et al., Pflüg. Arch. Ges. Physiol., 308, 91 (1969).