EFFECT OF PYROMECAINE ON IONIC CURRENTS OF ELECTRICALLY EXCITABLE MEMBRANES

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Pyromecaine (N-butylpyrrolidine carboxylic-2 acid-2,4,6-trimethylanilide hydrochloride), synthesized in the Institute of Pharmacology, Academy of Medical Sciences of the USSR [4], has been studied pharmacologically and suggested for use in medical practice as a local anesthetic [5]. Subsequently the antiarrhythmic properties of pyromecaine were discovered [2, 8].

The study of the mechanism of action of pyromecaine revealed that surface activity on the boundary with air, benzene, and solutions of nerve tissue lipoproteins in benzene, lipophilicity, partition coefficient between solid and liquid phases of nerve, ability to undergo absorption in nerve, and its effect on monomolecular layers of lipids (fatty acids and lipids extracted from nerve tissue) are related to the anesthetic activity of pyromecaine and other local anesthetics symbathically [6]. Particularly high correlation was found when anesthetic and antiarrhythmic action was compared with surface activity and with its effect on monomolecular layers of lipids.

Considering the important role of surface phenomena in the action of local anesthetics and antiarrhythmic agents [7], the writers decided to study the effect of pyromecaine on ionic currents in electrically excitable membranes.

EXPERIMENTAL METHOD

Experiments were carried out on a preparation of the guinea pig right ventricle and on isolated perfused neurons from rat spinal ganglia under voltage clamp conditions. Intracellular membrane potentials of myocardial cells were measured by means of a floating glass mi-

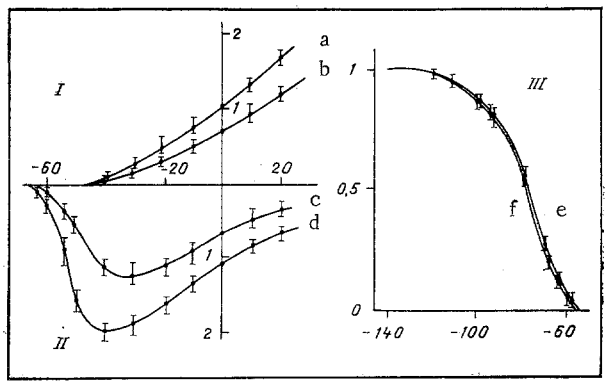

Fig. 1. Effect of pyromecaine on current-voltage characteristic (II) and potential-dependence of steady-state inactivation (III) of fast inward Na currents and current-voltage characteristics (I) of fast outward K currents of rat spinal ganglionic neurons. Abscissa, membrane potential (in mV); ordinate, I, II) strength of current through membrane, $\cdot 10^{-9}$ A, III) Proportion of uninactivated channels.

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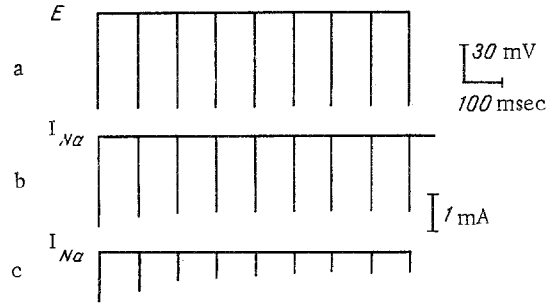

Fig. 2. Effect of pyromecaine on stimulus-dependence of inhibition of fast neuronal Na current: a) program of stimuli, b) control, c) pyromecaine 10^{-5} M.

croelectrode, connected through an Ag-AgCl electrode to the input of a UPT-2 amplifier. Signal differentiation was carried out by an amplifier with parallel feedback, based on the K574DIA microcircuit. The amplitude and shape of the action potential (AP) and the amplitude of the first derivative of AP were recorded with the FOR-2 instrument from the screen of an SI-48B oscilloscope. The duration of the AP plateau was measured at the half resting potential (RP) level. Tyrode solution of the following composition was used (in mM): NaCl 130, $MgCl_2$ 1, $NaHCO_3$ 12, NaH_2PO_4 0.75, glucose 0.55 (pH 7.4). The solution was saturated with carbogen. Ionic currents in the cell membrane of the neurons were measured under voltage clamp conditions during intracellular perfusion by the method in [3]. The composition of the intracellular medium (in mM) was: KF 100, Tris-HF 50 (pH 7.2); the composition of the extracellular medium (in mM) was: NaCl 156, KCl 3, $MgCl_2$ 2.6, Tris-HCl 10 (pH 7.4).

EXPERIMENTAL RESULTS

Pyromecaine reduced the maximal rate of rise of AP (V_{max}). In a concentration of 10^{-5} the compound reduced this value from 185 (178-210) V/sec to 112 (105-125) V/sec. Changes in the shape of AP under the influence of the anesthetic occurred first in the ascending phase. In the presence of 10^{-5} M pyromecaine no significant changes in the level of the AP plateau were observed. The only effect of the action of 10^{-5} M pyromecaine on ventricular myocardial tissue at that stage was elevation of the electrical threshold of excitation on average by 43% (from 32 to 68%, results of five measurements). With an increase in the pyromecaine concentration to 10^{-4} M a significant decrease in duration of the AP plateau was observed. RP of the heart cells was unchanged by pyromecaine even when its concentration was increased to 2 mM. The effects of the local anesthetic on the myocardium described above developed in the course of 1-2 min. The character of the action of pyromecaine on sodium channels was studied on neurons from rat spinal ganglia. In concentrations from $5 \cdot 10^{-6}$ to 10^{-4} M the compound depressed both fast and slow inward Na currents of the neurons. The amplitude of the fast inward Na current was reduced by half by pyromecaine in a concentration of $5 \cdot 10^{-6}$ M (mean of seven measurements). For the slow Na current the value was 10^{-4} (mean of six measurements). Outward K currents of the neurons also were sensitive to the action of pyromecaine. A reduction of the slope of the current-voltage characteristics of the K currents by half was observed in the presence of $5 \cdot 10^{-5}$ M pyromecaine (mean of six measurements; Fig. 1a, b). Considering the leading role of a change in the properties of the fast sodium channels in the pathogenesis of cardiac arrhythmias [9], it was decided to restrict the subsequent analysis to the action of pyromecaine on fast sodium currents of neurons. The amplitudes of the Na current to the first depolarizing stimulus, applied to the neuron membrane 5 min after addition of pyromecaine to the extracellular medium in a concentration of $5 \cdot 10^{-6}$ M was reduced by half compared with the control. The same degree of depression of the fast Na current was obtained during low-frequency (0.5 Hz) activation of the current by depolarizing stimuli. Meanwhile repetitive stimulation of the electrically excitable cell membranes with a frequency of 10 Hz led to stimulus-dependent blocking of Na currents (Fig. 2). An increase in the amplitude of depolarizing stimuli from 60 to 110 mV with a holding potential of −100 mV caused no significant intensification of stimulus-dependent blockade. Introduction of a long depolarizing prepulse before each testing stimulus, in the presence of pyromecaine, delayed reactivation of the Na current (Fig. 3a, b). In a concentration with greatly reduced the amplitude of fast Na currents, pyromecaine did not change the potential-

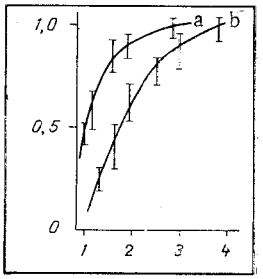

Fig. 3. Effect of pyromecaine on reactivation of fast Na currents of rat neurons after prolonged depolarizing prepulse. Abscissa, time between end of depolarizing prepulse and beginning of test pulse t log t, msec; ordinate, ratio I_t/I_∞. a) Control, b) pyromecaine 10^{-5} M. Duration of depolarizing prepulse 1 sec, amplitude 20 mV. Testing pulse 5 msec, +30 mV.

dependence of their steady-state activation and inactivation (Fig. 1c, f). The effects of pyromecaine on the inward Na currents described above developed in the course of 1-2 min after extracellular application. The compound did not change the characteristics of the fast Na currents after intracellular application in concentrations of up to $5 \cdot 10^{-4}$ M.

The rate of change of membrane potential in the initial phase of AP and at the AP plateau level is known to be connected with subsequent activation of the fast Na channels and slow Ca channels respectively of the cardiomyocyte sarcolemma. The predominant action of pyromecaine on the rate of change of membrane potential in the initial phase of AP means that the element of the electrically excitable membrane of the cardiomyocyte which is most sensitive to the action of this local anesthetic is the fast inward sodium channels. This is confirmed by elevation of the electrical threshold of excitation of the myocardial tissue. The same conclusion can be drawn from comparative analysis of the action of pyromecaine on fast and slow inward channels of rat spinal ganglionic neurons. Inhibition of fast Na currents by the compound was more than an order of magnitude stronger than inhibition of slow Na currents. A comparative study of the effects of pyromecaine on electrically excitable membranes of cells with different kinds of organization thus showed that fast sodium channels are the most sensitive element of these membranes to the action of the local anesthetic.

Pyromecaine did not affect potential-dependence of steady-state activation and inactivation of fast sodium channels (Fig. 1c, f). On the basis of a formal description of sodium currents [1], and considering that during intracellular perfusion the equilibrium potential for Na^+ remains unchanged, it can be concluded that inhibition of the fast sodium current by pyromecaine, whereas dependence of activation and inactivation of the sodium currents on membrane potential remains unchanged, means that molecules of the anesthetic reduce maximal cationic conductance of these channels. Pyromecaine blocks both activated and resting (unactivated) channels. This conclusion follows from the fact that inhibition of the sodium currents develops independently of whether the sodium channels are activated or not after addition of pyromecaine to the extracellular medium. Like many other local anesthetics [10] pyromecaine has a stimulus-dependent action on sodium channels (Fig. 2). Analysis of potential-dependence of steady-state activation of sodium currents shows that during membrane depolarization to −40 mV only 40% of the total number of channels are activated. On depolarization to +10 mV all sodium channels are activated. The absence of potentiation of the stimulus-dependent action of pyromecaine with an increase in membrane depolarization from −40 mV (depolarizing stimulus 60 mV) to +10 mV (depolarizing stimulus 110 mV) shows that this local anesthetic does not interact with open channels. At the same time, potentiation of the stimulus-dependent action of pyromecaine on the addition of a subthreshold depolarizing prepulse, causing an increase in the number of channels in the inactivated state, shows that pyromecaine molecules are bound mainly with inactivated channels. This conclusion is in agreement with the description of the action of local anesthetics on sodium channels of the electrically excitable Ranvier node membrane given previously [11, 12].

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EFFECT OF GABA AND ITS AGONISTS AND ANTAGONISTS ON UTERINE SMOOTH MUSCLE

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The increase in excitability and contractile activity of the uterus arising as a result of the action of certain etiologic and pathogenetic factors is one cause of abortion [5]. The study of potential physiological antagonists of endogenous factors in uterine hyperactivity may provide a basis for the search for effective antiabortion agents in this case. Hence the importance of a study of endogenous biologically active substances which inhibit the receptor systems of the uterus.

In the last few years reports have been published of the effect of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter of the CNS, and its derivatives on the smooth muscle of peripheral organs, which is realized through a GABA-receptor mechanism [7, 15].

This paper describes the study of the action of GABA and some of its agonists and antagonists on contractile activity of uterine smooth muscle.

EXPERIMENTAL METHOD

Experiments were carried out *in vitro* on 176 segments of the uterine cornua of 24 ovariectomized rabbits (with respect to receptor composition and response to neurotransmitters, sex hormones, and drugs the rabbit uterus closely resembles the human uterus [6]). The animals were killed on the 15th day after ovariectomy. An isolated segment of the uterine cornu, 1.5-2 cm long, was fixed to the floor of a small beaker containing Ringer–Locke or Krebs' nutrient solution. The free end of the segment was tied by a ligature to an Engelmann's lever. The temperature during the experiments was maintained at 38°C by means of a mercury contact thermometer. The nutrient solution was aerated by means of an AÉN-2 microcompressor. Contractions of the uterine segments were recorded by pens of an ink-writing

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