EFFECT OF THE MACROCYCLIC POLYESTER 15-CROWN-5 ON IONIC PERMEABILITY OF EXCITABLE MEMBRANES

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The parmacology and biophysics of macrocyclic polyester complexones have been at the focus of attention of research workers in recent years [9, 16]. The use of these compounds as modulators of ionic permeability of excitable membranes is particularly interesting. It was shown in [16] that 15-crown-5 has a marked chronotropic and inotropic action on the isolated frog heart, which provides the basis for a search for new cardiotropic drugs in the macrocyclic polyester series.

The aim of this investigation was to study the mechanism of action of 15-crown-5 on biological and model membranes.

EXPERIMENTAL METHOD

The strength and frequency of contraction of the isolated guinea pig atrium were recorded by means of a strain-gauge transducer, Topaz-2 amplifier, and N-3020-3 automatic writer. The velocity of contraction was estimated by means of a piezoelectric transducer. The signal from the transducer was recorded photographically from the oscilloscope screen. The refractory period of atrial contractions was determined by the method described previously [8]. Ionic currents of the electrically excitable membrane of isolated rat spinal ganglionic neurons were measured under intracellular dialysis and voltage clamp conditions by the method in [7]. The effect of 15-crown-5 on the surface potential of biomembranes was studied on a bimolecular lipid membrane (BLM) by a potentiodynamic method [1]. BLM was formed on the mouth of a small Teflon jar from a solution of phosphatidylcholine in decane (40 mg/ml).

EXPERIMENTAL RESULTS

Polyester 15-crown-5 in a concentration of $5 \cdot 10^{-5}$-10^{-3} M did not affect the force of contraction of the isolated rat heart but reduced the frequency of its contractions. A similar effect was observed on the isolated guinea pig atrium (Fig. 1). The negative chronotropic action of the polyester on the atrium was combined with lengthening of the refractory period of its contractions (Fig. 1) and a decrease in the velocity of the contractions. In a concentration of 10^{-3} M it reduced the velocity by 48%. In the same concentrations 15-crown-5 did not affect the chronotropic reactions of the heart to acetylcholine and adrenalin and its action was unchanged in the presence of atropine and Inderal (propranolol).

As a first approximation the action of 15-crown-5 on the heart of warm-blooded animals can thus be reduced to a change in the parameters of excitability of the sinuatrial node.

Because of the high stability constant of complexes of 15-crown-5 with ions of the alkaline earth metals in water [11], it can be postulated that some of the effects of the compound described above on the atrium may be sensitive to Ca^{++} concentration. In fact 15-crown-5, in a concentration of $2 \cdot 10^{-4}$ M, abolished the action of Ca^{++} on the refractory period of contractions of the guinea pig atrium. Simple calculations show that the decrease in the Ca^{++} concentration in physiological saline on account of complex formation with 15-crown-5 did not exceed 5%. Judging from the relationship between the action of Ca^{++} and its

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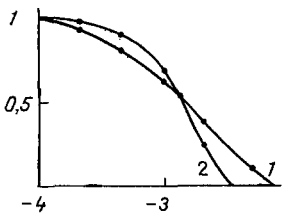

Fig. 1

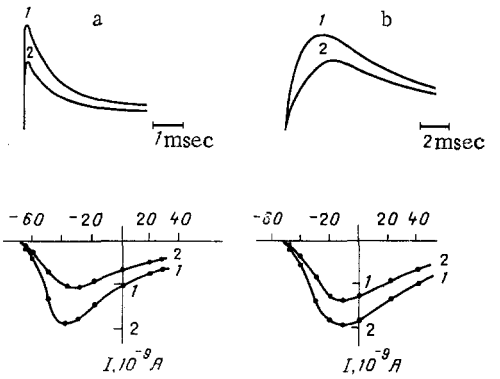

Fig. 2

Fig. 1. Effect of 15-crown-5 on frequency of spontaneous contrations (1) and highest bound frequency (2) of contractions of isolated guinea pig atrium. Abscissa log of concentration of 15-crown-5 (in M); ordinate, ratio of spontaneous (1) or highest bound (2) frequency of contractions in experiment to corresponding values in control (in relative units).

Fig. 2. Change in current-voltage characteristics and kinetics of fast (a) and slow (b) sodium currents of rat neurons under the influence of 15-crown-5. Examples of traces of currents shown above, current-voltage characteristic curves below. 1) Control; 2) 15-crown-5.

concentration, this decrease in the concentration of these ions cannot explain the effect of 15-crown-5 on the refractory period of contractions of the isolated atrium.

The rapid (10-30 sec) development of the action of 15-crown-5 on the isolated atrium is perhaps attributable to changes in the parameters of electrical excitability of the cardiomyocyte membrane. A leading role in determination of the frequency of the cardiac pacemaker and amplitude of atrial contractions is known to be played by slow tetrodotoxin (TTX)-resistant sodium-calcium channels of the inward current [5, 14], and the duration of the refractory period of the electrically excitable membrane of cardiomyocytes depends on the rate of reaction of TTX-resistant and TTX-sensitive channels of the inward current [10]. Neurons of the rat spinal ganglia have two types of inward current channels — slow and fast. The properties of the former are very similar to those of the slow-sodium-calcium channels of the sinuatrial node and myocardium. For instance, these types of channels are resistant to the action of TTX, they are blocked by Mn and Co ions and by verapamil and D-600, antagonists of Ca^{++}, and the potential-dependence of their activation is shifted toward lower potentials with an increase in the external Ca^{++} concentration [4, 5]. There is also some similarity in the properties of the fast TTX-sensitive currents of neurons and cardiomyocytes [3, 6]. The present investigation was limited to analysis of the inward currents of rat neurons.

Polyester 15-crown-5 inhibited slow inward currents in a concentration of 10^{-2} M and fast currents in a concentration of $2 \cdot 10^{-3}$ M, without changing their reversal potentials (Fig. 2). Slowing of the rate of activation of channels of the slow inward current (Fig. 2b) was probably responsible for the slowing of contractions of the guinea pig atrium under the influence of the crown ester. The ability of 15-crown-5 to block Na and Ca channels of the electrical excitable membranes of rat and molluscan neurons and electrically and chemically excitable membranes of smooth-muscle cells perhaps extends also to cardiomyocyte plasma membranes. This would explain depression of the frog myocardium under the influence of 15-crown-5 [16]. The force of contractions of the frog's heart, by contrast with the heart of warm-blooded animals, is determined mainly by permeability of the cell membrane to Ca^{++}. This could explain the difference between the action of 15-crown-5 on contraction of the heart observed in [2, 16].

Inhibition of inward currents of rat neurons was not accompanied by any change in potential dependence of steady-state activation and inactivation. This is evidence that 15-crown-5 does not disturb the function of activation and inactivation mechanisms of the inward

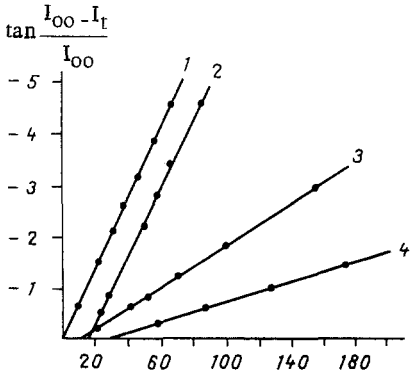

Fig. 3

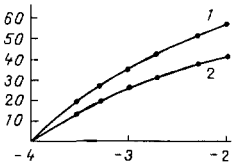

Fig. 4

Fig. 3. Effect of 15-crown-5 on reactivation of inward sodium current of rat neurons: 1, 2) control; 3, 4) 10^{-3} M 15-crown-5. Ca^{++} concentration: 1, 3) 2.6 mM; 2, 4) 1 mM. Abscissa, time (in msec).

Fig. 4. Dependence of action of Ca^{++} on surface potential of BLM on its concentration. 1) Control; 2) 10^{-3} M 15-crown-5. Abscissa, log of concentration (in M); ordinate, potential (in mV).

current channels of neurons. It follows from the equation describing the inward current of rat spinal ganglionic neurons [2] that inhibition of inward currents by 15-crown-5 can be explained only by a decrease in maximal conductance of the corresponding channels. The probable cause of this decrease may be steric hindrances arising during entry of the 15-crown-5—Na^{+} complex into the mouth of the channel. This view is confirmed indirectly by the absence of any effect of 15-crown-5 on the amplitude of inward currents when applied intracellularly. Inhibition of slow TTX-resistant currents in the sinuatrial node by verapamil, D-600, Mn, and Co leads to a decrease in frequency of the endogenous pacemater [6].

It follows from the data described above that the specific character of the physiological activity of 15-crown-5 is similar in many respects to the spectrum of cardiotropic action of Ca channel blockers. On this basis it can be postulated that the likely cause of the negative chronotropic action of 15-crown-5 is a mixed effect of depression of conductance through slow sodium-calcium channels of sinuatrial node cells and also through slow and fast inward current channels in atrial cells.

To study the causes of lengthening of the refractory period of atrial contractions, it was assumed that reactivation of the inward current channels of the cardiomyocytes makes the decisive contribution to the determination of its value. Considering the similarity in the properties of inward current channels of rat spinal ganglionic neurons and cardiomyocytes noted above, it was decided to investigate the effect of 15-crown-5 on reactivation processes in the electrically excitable neuron membrane.

Polyester 15-crown-5 in a concentration of 10^{-3} M significantly delayed reactivation of the Na current, and this action was Ca-dependent (Fig. 3). For instance, reducing the Ca^{++} concentration in the external solution from 2.6 to 1 mM, in the presence of 15-crown-5 led to an increase in the time constant of reactivation from 50 to 103 msec. The same changes in the Ca^{++} concentration in the control delayed the beginning of reactivation of Na currents by 15 msc without any effect on the time constant of reactivation. The presence of 15-crown-5 against the background of 2.6 mM Ca^{++} also caused delay of 10 msec at the beginning of the reactivation process, accompanied by an increase in the time constant of reactivation from 15 to 50 msec. Reducing the Ca^{++} concentration to 1 mM potentiated the action of the polyester on reactivation of the Na current, delayed the beginning of the reactivation process by 25 msec, and increased the time constant to 103 msec.

It can be concluded from the data described above that the effect of 15-crown-5 on the recovery of Na channels from inactivation can be reduced to two phenomena: delay of the beginning and slowing of the rate of reactivation. The first of these phenomena is similar in form to a fall in the Ca^{++} concentration in the juxtamembranous layer. One of the consequences of a reduction in the density of membrane-bound calcium is known to be a disturbance of the physicochemical characteristics of protein-lipid interactions, leading to changes in the parameters of activity of membrane enzymes, receptors, and channels [15].

A study of the effect of 15-crown-5 on the Ca-induced potential of BLM showed that the polyester can in fact prevent the action of Ca^{++} on a lipid membrane (Fig. 4).

It was shown in [13] that the high stability constant of complexes of 15-crown-5 with alkaline earth metals is due to the formation of double complexes with 2:1 stoichiometry. The formation of mixed 15-crown-5—Ca^{++}—carbonyl or phosphodiester complexes of the oxygen atom of phospholipid molecules can be proposed, having regard to the high affinity of Ca^{++} for these groups [12]. The formation of such complexes must undoubtedly lead to weakening of the membranotropic action of Ca^{++}.

A possible cause of the effect of 15-crown-5 on the refractory period of the inward current channels may thus be a disturbance of the Ca-dependence of packing of the lipids of the lipoprotein matrix of the membrane.

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