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Cardiotropic properties of membrane-active crown-ethers

Właściwości kardiotropowe błonoaktywnych eterów koronowych

U.T. SHARAFUTDINOVA, U.B. TOYIROV,
O.V. YARISHKIN, U.Z. MIRKHODJAEV

Streszczenie. Jednym z głównych obszarów badawczych w dziedzinie aktywności biologicznej związków koronowych jest określanie mechanizmów odpowiedzialnych za wpływ tych związków na błony biologiczne. Celem takich badań jest znalezienie nowych leków nasercowych oraz środków psychotropowych wpływających na wegetatywny układ nerwowy. W niniejszej pracy opisano dwie pochodne acylowe DB18C6, tj. 4',4"(5")-diacetylo-DB18C6 i 4',4"(5")-diwalerylo-DB18C6 oraz pseudocykliczny analog DB18C6, które wywierały efekty kardiotropowe w izolowanym sercu żaby. Wydaje się, że podstawą tych efektów jest wpływ związków koronowych na homeostazę jonów wapnia w komórkach serca.

Słowa kluczowe: etery koronowe, błony biologiczne, mięsień sercowy.

Sharafutdinova U.T., Toyirov U.B., Yarishkin O.V., Mirkhodjaev U.Z., *Department of Biophysics, National University of Uzbekistan, 700174 Vuzgorodok, Tashkent, Uzbekistan*

INTRODUCTION

One of the main directions in the research field of biology active crown compounds is investigation of the mechanisms responsible for their influence on excitable membranes with the purpose of creating new cardio- and psychotropic agents as well as those influencing on the vegetative nervous system. First modeling investigations, which showed the capability of crown compounds to affect excitable tissues, were conducted as described in some papers (Gunther et al., 1979; Bogatsky et al., 1985). Later

it was demonstrated on organs of animals. So, 15C5, 3,7- diazanonane - l,9-diamine and 1,4,8,11-tetra-aza-cyclo-tetradecane were shown to change the force and frequency of the heart's contractions (Bogatsky et al., 1985, Bogatsky, 1983). Those effects of crown compounds were not abolished by modulators of the adrenergic and cholinergic systems of the heart's activity regulation. The authors came to the conclusion that a direct cardiotropic action of 15C5 and nitrogen-containing crown compounds could underlie the effects. This action consists in changing the electric potential sensitivity and permeability of the cardiac myocyte membrane. Ca^{2+} complex-formers (Mirkhodjaev et al., 1986) and especially Ca^{2+}-ionophores (Mirkhodjaev et al., 1984) having been found, a basis was formed for investigations of their influence on the excitable biological systems in functioning of which Ca^{2+} ions play one of key roles. Necessity of Ca^{2+} ions for prolonged realization of hearts contractions has been known for a long time (Jukov, 1969). Ca^{2+} influx through Ca^{2+} channels is thought to be the most, important for the process of excitation-contraction coupling (Evans et all, 1997). The process is initiated by the cell membrane depolarization resulting in opening of voltage gated Na^{+} and Ca^{2+} channels. In cardiomyocytes transition from relaxation with low [Ca^{2+}] to contraction is provided by a small amount of Ca^{2+} passing through the sarcolemma. This incoming Ca^{2+} induces the mass release of Ca^{2+} from its intracellular stores (Fabiato, 1985). The released Ca^{2+}, in turn, initiates a contraction act by binding to contractive proteins. Ca^{2+}~ homeostasis in the cardiac myocytes functioning is of great importance for at least the following reasons: firstly, cardiac myocytes must maintain constant level of internal Ca^{2+} ([Ca],) <200 p.M. [Ca]o being ImM (Huser et al., 1996), such a low [Ca], level has to exist under conditions of 5000-fold Ca^{2+} gradient. Secondly, the excitation-contraction coupling includes a complex of interconnected electric processes in membrane, which are produced by specific ionic channels of sarcolemma. This realizes itself in release of Ca^{2+} ions from intracellular stores through Ca^{2+} channels with the following removal of Ca^{2+} ions from the cytosol. The main principle of this process is the maintenance of steady-state [Ca], homeostasis: a quantity of Ca^{2+} ions entering cytosol during every single contraction must be removed before the next one (Barry et al., 1993, Holda et al., 1998) Thirdly, the contractile force of myocardium is regulated by changes in the rate of Ca^{2+} transport into cytosol. Compounds capable of modifying [Ca], homeostasis can significantly change the contractile force of single myocytes and a whole intact heart (Ishide, 1996).

Two diacyl-derivatives of DB18C6: 4',4"(5")-diacetyl-DB18C6, 4',4"(5")-divaleryl--DB18C6 and pseudocyclic analog of DB18C6 were found out to have cardiotropic effects on the isolated frog's heart. The interference of the compounds in Ca^{2+} homeostasis of cardiac cells is supposed to underlie some of these effects.

MATERIALS AND METHODS

The isolated frog's heart preparations were made according to Buldakova et al. (1985). The preparations were perfused with a Ringer's solution containing (in g/l): 6.50 NaCI, 0.14 KCl, 0.12 CaCl2, 0.20 NaHC03.

In the work the following compounds were studied: 4',4"(5")-diacetyl-DB18C6, 4',4"(5")-divaleryl-DB18C6, pseudocyclic analog of DB18C6 (Fig. l). Crown-ethers were synthesized and kindly provided by Tashmukhamedova A.K. Absolute ethanol solutions of the compounds were used for adding to perfused system.

The hearts contractions were registered using a mechanotrone 6MX1B. The mechanotrone transferred mechanical signals to proportionally amplified electrical ones, which were registered by a X-Y recorder.

RESULTS AND DISCUSSION

COMPLEX-FORMING AND TRANSPORT PROPERTIES OF DIACYL-DERIVATIVES OF DB18C6

Earlier, when studying complex-forming properties of varies substituted DB18C6 (Mirkhodjaev et al., 1984), we investigated the ability of some diacyl-derivatives of DB18C6, differing in the substituent's carbonic chain length, to form complexes with Ca^{2+} ions. It was also of interest to assay a Ca^{2+} complexing ability, if any, of a pseudocyclic analog of DB18C6. All the investigated compounds of the acyl – substituted homology row from 4',4"(5")-diacetyl-DB18C6 to 4',4"(5")-dioctanoil-DB18C6 as well as the pseudocyclic analog of DB18C6 were found out to form complexes with Ca^{2+} ions in ethanol (Table 1). From the obtained data it is obvious that an increase in the length of carbonic chain of acyl-substituents is accompanied by an increase in the stability constants of complexes. The most stable complexes are formed by 4',4"(5")-dipropionyl-DB18C6. Further lengthening of the chain leads to a decrease in the complex stability, which might be resulted from increasing spatial obstacles for complexes to be formed.

Diacyl-derivatives of DB18C6 are known to induce the permeability of model (Mirkhodjaev et al., 1984) and natural (Tashmukhamedov et al., 1979) membranes to divalent cations, Ca^{2+}/Mg^{2+} selectivity of these compounds being significant. Among DB18C6 diacyl-derivatives the highest ionophoric activity was shown by dibutyryl--DB18C6 and divaleryl-DB18C6, i.e. the compounds whose side substituents contain 4–5 carbonic atoms (Tashmukhamedov et al., 1987). Some crown compounds and their analogs are able to transport Ca^{2+} ions by a mechanism different from mobile carrier

transport. They are shown to induce formation of Ca^{2+} permeable ionic channels in bilayer membranes (Mirkhodjaev, 1989). Such a channel-forming activity was, in particular, demonstrated by a pseudocyclic analog of DB18C6 (Mirkhodjaev et al., 1989).

Table 1. The stability constants (IgK) of Ca^{2+} complexes of diacyl-derivatives of DB18C6 and of the pseudocyclic analog of DB18C6 in ethanol

Tabela 1. Stałe stabilności (IgK) kompleksów Ca^{2+} z diacylopochodnymi DB18C6 oraz pseudocyklicznym analogiem DB18C6 w etanolu

№	Compound	IgK
1	4',4"(5")-diacetyl-DBl8C6	6.83
2	4',4"(5")-dipropionyl-DB18C6	6.89
3	4',4"(5")-dibutyryl-DB18C6	6.87
4	4',4"(5")-divaleryl-DB18C6	6.74
5	4',4"(5")-dihexanoil-DB18C6	6.31
6	4',4"(5")-diheptanoil-DB18C6	5.72
7	4',4"(5")-dioctanoil-DB18C6	5.36
8	pseudocyclic analog of DB18C6	5.10

EXPERIMENTS WITH THE ISOLATED FROG'S HEART

The results of above-described investigations of complex formation of DB18C6 diacyl-derivatives and the pseudocyclic analog of DB18C6 with Ca^{2+} ions as well as Ca^{2+} transporting properties of these compounds made us study influence diacyl--derivatives of DB18C6: 4',4"(5")-diacetyl-DB18C6 (strong Ca^{2+}-complex-former), 4',4"(5")-divaleryl-DB18C6 (Ca^{2+}-ionophore) and the pseudocyclic analog of DB18C6 (Ca^{2+}-channelformer) on the contractility function of the isolated frog's heart.

Ventricle

When studying influence of 4',4"(5")-diacetyl-DB18C6 on the amplitude of the ventricle contractions we found out that an increase in concentrations of the compound was followed by reduction of the amplitude and frequency of the ventricle contractions. In a concentration of 4', 4"(5")-diacetyl-DB18C equal to 1–10 Ma full stop of contractions in the phase of diastole was observed. After 5–6 washings with a Ringer's solution the ventricle recovered completely its original contractility function.

Experiments on influence of 4',4"(5")-divaleryl-DB18C6 on the ventricle contractions showed that the compound decreased the amplitude of contractions, the frequency of contractions being diminished in relatively higher concentrations. In a concentration of the compound equal to $2–10^{-4}$ M a full stop of contractions in the diastole phase occurred. After being washed with a Ringer's solution the ventricle failed to recover completely its original contraction activity, arrhythmia of contractions being observed.

The pseudocyclic analog of DB18C6 influenced on the amplitude and frequency of the ventricle contractions similarly to 4"(5")-diacetyl-DB18C. In a concentration of the compound equal to 10–4 M we registered a full stop of contractions in the diastole phase. After washing with a Ringer's solution the ventricle recovered completely its original contractility function.

Based on a comparative analysis of dependence of the contractions amplitude and frequency on concentrations of the investigated compounds the corresponding plots were made (Fig. 2 and 3).

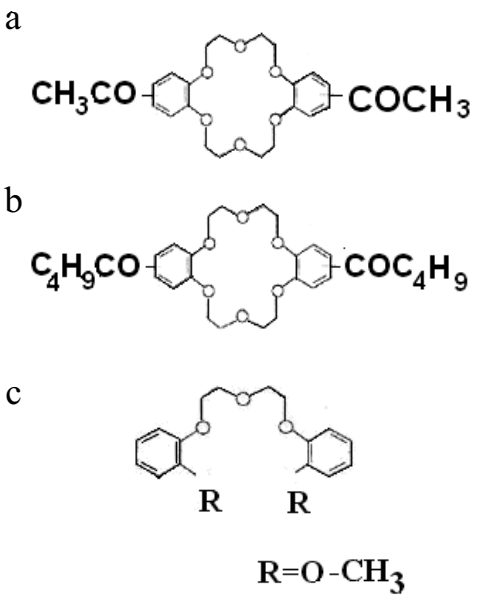

Fig. l. The investigated crown compounds: a) 4',4"(5")-diacetyl-DB18C6, b) 4',4"(5")-divaleryl--DB18C6, c) pseudocyclic analog of DB18C6

Rys. 1. Struktura badanych związków konorowych: a) 4',4"(5")-diacetylo-DB18C6, b) 4',4"(5")-diwalerylo-DB18C6, c) pseudocykliczny analog DB18C6

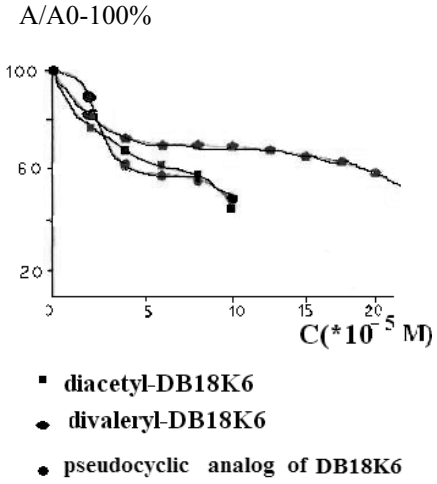

Fig. 2 Amplitude – concentration relationships in ventricle. Ao – the contraction amplitude before administration of the compounds

Rys 2. Zależność amplituda – stężenie w komorze serca. Ao – amplituda skurczu przed podaniem związku

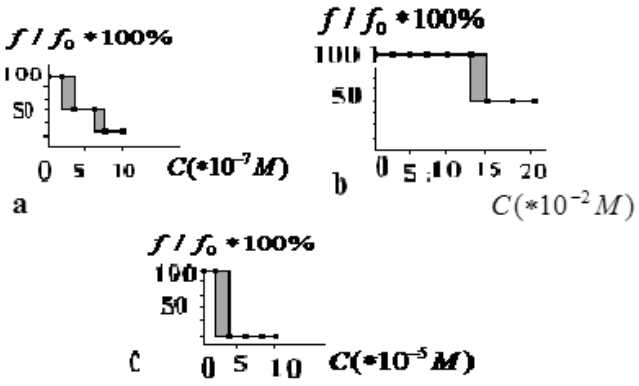

Fig. 3. Frequency – concentration relationships in ventricle for (a) 4"(5")-diacetyl-DB18C, (b) 4',4"(5")-divaleryl-DB18C6 and (c) the pseudocyclic analog of DB18C6. fo – the contraction frequency before administration of the compounds. The shaded spaces are used to designate the ranges of concentrations in which discrete changes in the heart beats frequency occur

Rys 3. Zależność stężenie – częstotliwość w komorze dla (a) 4"(5")-diacetylo-DB18C, (b) 4',4"(5")-diwalerylo-DB18C6 i (c) pseudocykliczny analog DB18C6. fo – częstotliwość skurczu przed podaniem związku. Zacienione pola odpowiadają zakresom stężeń, przy których pojawiają się nieciągłości w biciu serca

Auricle

On the next stage of our work we studied influence of 4"(5")-diacetyl--DB18C and 4',4"(5")-divaleryl-DB18C6 $_{G}$n the amplitude and frequency of the

auricle contractility function. Figures 4 and 5 illustrate the results of this series of experiments.

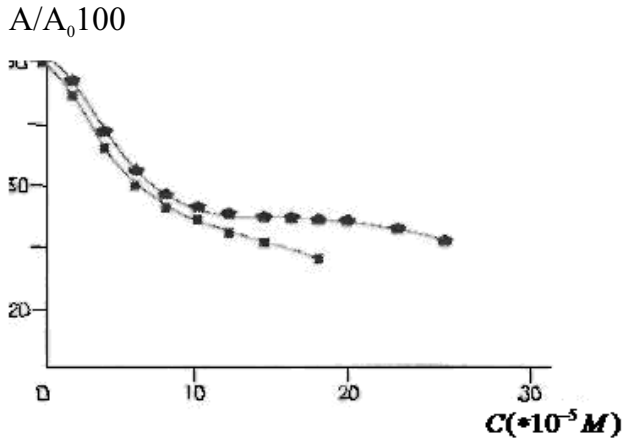

Fig. 4. Amplitude – concentration relationships in auricle. Ao – the contraction amplitude before administration of the compounds

Rys. 4. Zależność amplituda – stężenie w przedsionku. Ao – amplituda skurczu przed podaniem związku

As seen from the figures the action of the compounds on the auricle contractility is similar to their action on ventricle. A washing of preparations with a Ringer's solution showed that the contractility function of auricle recovered completely after application of 4"(5")-diacetyl-DB18C and only partly after application of 4',4"(5")-divaleryl-DB18C6. A full stop of contractions occurred in a concentration of 4"(5")-diacetyl-DB18C equal to $1.8*10^{-4}M$. This concentration is somewhat higher than that sufficient to stop the ventricle contractions.

In contrast to 4"(5")-diacetyl-DB18C and the pseudocyclic analog of DB18C6, 4',4"(5")-divaleryl-DB18C6 was not washed away and the preparations did not recovered their original contraction activity. This gives evidence for irreversible incorporation of 4',4"(5")-divaleryl-DB18C6 into the membranes of muscle fibers. An analysis of changes in the contraction activity of the ventricle caused by administration of 4',4"(5")-divaleryl-DB18C6 shows the presence of marked arrhythmia of contractions (Fig. 6). No arrhythmia was observed when the compound was administered to the auricle. This may suggest that block of conductance caused by 4',4"(5")-divaleryl-DB18C6 in specialized conducting fibers takes place.

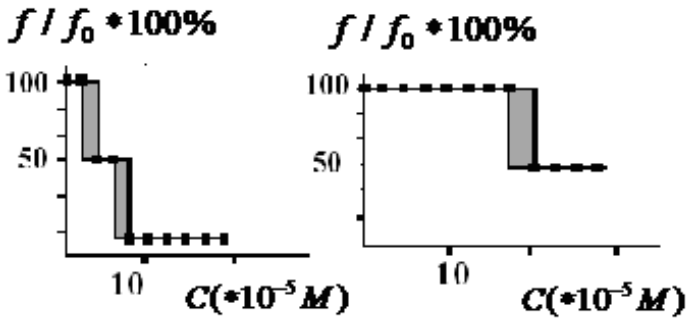

Fig. 5. Frequency – concentration relationships in auricle for (a) 4"(5")-diacetyl-DB18C and (b) 4',4"(5")-divaleryl-DB18C6. fo – the contraction frequency before administration of the compounds. The shaded spaces are used to designate the ranges of concentrations in which discrete changes in the heart beats frequency occur

Rys. 5. Zależność stężenie – częstotliwość w przedsionku dla (a) 4"(5")-diacetylo-DB18C and (b) 4',4"(5")-diwalerylo-DB18C6. fo – częstotliwość skurczu przed podaniem związku. Zacienione pola odpowiadają zakresom stężeń, przy których pojawiają się nieciągłości w biciu serca

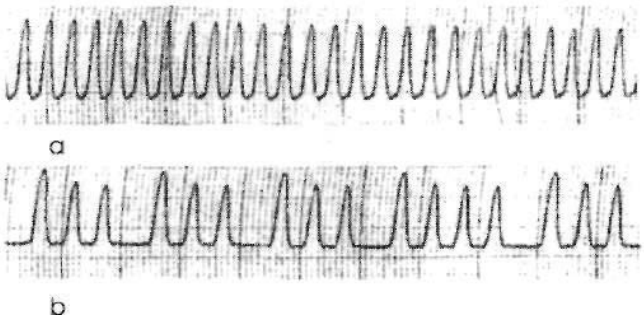

Fig. 6. Registration of the contractile activity of a ventricle before (a) and after administration and then washing away of 4',4"(5")-divaleryl-DB18C6 (b)

Rys. 6. Zapis aktywności skurczowej komory przed (a) i po podaniu oraz wypłukaniu 4',4"(5")-diwalerylo-DB18C6 (b)

The stop of the auricle and ventricle contractions occurred in diastole, which can be explained by a decrease in Ca^{2+} ions in the perfusion solution owing to their binding by crown-ethers.

An analysis of amplitude-concentration relationships (Fig. 2 and 4) shows that the relationships are not monotonous. The initial running down phase of the curves can reflect the process of complex formation and a further increase

in concentrations of the compounds would be expected to decrease free Ca^{2+} ions and, as a result, reduction of the amplitude of contractions. However, from the amplitude-concentration relationship for 4',4''(5'')-divaleryl-DB18C6 and the pseudocyclic analog of DB18C6 it is seen that in certain intervals of concentrations the amplitude values remain invariable. This fact can evidence for induction of Ca^{2+} transport into muscle cells by the compounds.

The multiphase dependence of the dynamics of frequency-concentration relationships observed in experiments with the ventricle and auricle (Fig. 3 and 5) might well suggest that the investigated compounds influence on processes of impulse generation in cardiac pacemakers cells. There are at least three factors determining the discharge rate of a pacemaker cell: 1) the steepness of a spontaneous depolarization from the membrane resting potential level to the threshold level, 2) the level of the resting potential and 3) the level of the threshold potential. A change in the heart-beats frequency of a whole intact heart can occur owing to another cause: if the activity of the main pacemaker slows down too much latent pacemakers and then specialized fibers take on the function of impulse generation. Changes in the discharge frequency of the main pacemaker caused by alterations of the above listed factors are known to account for slight adapting changes in the rhythm rate of heart beats. But considerable changes in the contractions frequency of a whole heart occur almost solely due to depression of the main and switching on another pacemaker latent before. A more accurate definition of the mechanisms responsible for the influence of the investigated compounds on the frequency of the heart's contractions is a subject for further investigations. Here we can speculate that there might be a direct interaction between the compounds and ionic channels regulating the membrane potential of pacemaker cells, which leads to reduction of the excitability of the pacemaker cells membrane and, as a result, inhibition of the pacemaker's excitation function. Sinus node localized the main pacemaker having been depressed, other specialized tissues begin to act as the pacemaker.

CONCLUSIONS

To resume the results of this study it can be concluded that 4',4''(5'')-diacetyl-DB18C6 (known to be a strong Ca^{2+}-complex-former), 4',4''(5'')-divaleryl--DB18C6 (Ca^{2+}-ionophore) and pseudocyclic analog of DB18C6 (Ca^{2+}-channel-former) are capable of reducing the amplitude and frequency of the ventricle and auricle contractions. In certain concentrations the compounds caused a full stop of contractions. The influence of the compounds on the contraction amplitude

might well be connected with their interference in the cell Ca^{2+} homeostasis, presumably, by lowering $[Ca^{2+}]o$ as a result of complex formation. The diminution of the contraction frequency can be caused by the compounds induced depression of the main pacemaker localized in sinus node of the frog's heart, which results in the pacemaker function is taken on by other specialized fibers.

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