one receptor for this lectin. Far fewer human serum proteins interact with PHA. Consequently, TBG and α_2-GP are among the few serum proteins which possess at least two receptors to react with PHA, and this phenomenon can be used to isolate them in the pure form.

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EFFECT OF CALCIUM CHANNEL BLOCKERS ON THE POSITIVE INOTROPIC EFFECT OF IONOPHORE A23187 IN THE MYOCARDIUM

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KEY WORDS: ionophore A23187; calcium antagonists; force of contraction; myocardium

The carboxyl ionophore A23187 increases the force of contraction and Ca-current in the myocardium [2, 5]. The action of ionophore A23187 is due to its ability to form complexes with Ca^{++} ions [4, 9]. It has been shown on artificial media that ionophore A23187 forms complexes not only with Ca^{++} ions, but also with other bivalent cations; ions which block the Ca-current in the myocardium, moreover, form more stable complexes with the ionophore than with Ca^{++} [9]. Ionophore A23187 also binds with a number of organic compounds, including the well-known calcium antagonists verapamil and its derivative D-600 [8].

The aim of this investigation was to study the ability of Zn^{++} and Mn^{++} ions and organic compounds fenigidine and D-600 to inhibit the positive inotropic effect of ionophore A23187 and also to compare the ability of calcium antagonists to inhibit myocardium contraction and the effect of the ionophore.

EXPERIMENTAL METHOD

Experiments were carried out on atrial strips of Rana ridibunda. The technique of recording mechanical activity and the conditions of stimulation were described previously [1]. The original physiological saline

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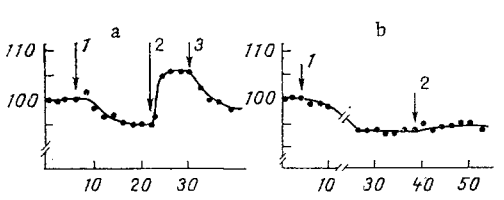

Fig. 1

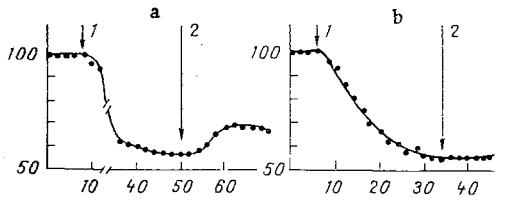

Fig. 2

Fig. 1. Action of ionophore A23187 on force of contraction of atrial strip in the presence of two concentrations of Zn^{++} ions. Abscissa, time (in min); ordinate, ratio of force of contraction in presence of blocker and normal force of contraction (in %). a: 1) 10^{-5} M Zn^{++}, 2) 10^{-5} M Zn^{++} and 10^{-5} M A23187; 3) normal physiological saline. b: 1) $2 \cdot 10^{-5}$ M Zn^{++}; 2) $2 \cdot 10^{-5}$ M Zn^{++} and 10^{-5} M A23187. a and b relate to different experiments.

Fig. 2. Action of ionophore A23187 on force of contraction of atrial strip in presence of two concentrations of D-600. a: 1) 10^{-7} M D-600; 2) 10^{-7} M D-600 and 10^{-5} M A23187. b: 1) $5 \cdot 10^{-7}$ M D-600, 2) $5 \cdot 10^{-7}$ M D-600 and 10^{-5} M A23187. Remainder of legend as in Fig. 1.

TABLE 1. Dependence of Positive Inotropic Effect of Ionophore A23187 on Concentration of Calcium Channel Blockers

Test substance and its concentration (in M)	No. of experiments	Force of contraction during action of blocker, %	Increase in force of contraction during action of A23187, %
Control	20	100	27±4,76
Zn^{2+}:			
10^{-5}	3	92,1±4,9	9,7±6,8
$2 \cdot 10^{-5}$	4	91,7±5,7	1,1±2,5
Mn^{2} , $2 \cdot 10^{-4}$	6	82,8±4,6	1,8±1,5
D-600:			
10^{-7}	3	62,5±14,1	15,5±9,4
$5 \cdot 10^{-7}$	4	46,7±16,8	0,75±1,47
Fenigidine:			
10^{-6}	3	39,4±11,0	38.2±14,8
10^{-5}	4	20,1±6,8	7,4±6,5
10^{-5}*	2	20,1±6,8	9,3

Legend. *) Fenigidine not present in solution with ionophore. For all tests P = 0.95.

contained (in mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.8, glucose 5.5, Tris-Cl 10; pH 7.6-7.7 (except solutions with Zn^{++} ions, for which pH 7.0). D-600 (from Knoll, West Germany), fenigidine (resynthesized in the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR), and ionophore A23187 (from Calbiochem-Behring, USA) were dissolved in dimethyl sulfoxide (DMSO), after which a small quantity of distilled water was added, and working solutions were prepared from the aqueous suspension thus formed by dilution. The corresponding concentration of DMSO was added to the control solutions. The concentration of A23187 in all experiments was 10^{-5} M.

EXPERIMENTAL RESULTS

Ionophore A23187 (10^{-5} M), added to the perfusion solution, increased the force of contraction of the atrial strip on average by 27 ± 4.76+ (n = 20).

A particular feature of the action of the ionophore was that rinsing out in normal physiological saline followed by repeated treatment with the ionophore did not produce any increase in the force of contraction. Comparison of the action of the ionophore under normal conditions and in the presence of the blocker was thus based on results obtained on different strips.

A scheme of the experiment and an example of the effect of Zn^{++} ions in concentrations of 10^{-5} and $2 \cdot 10^{-5}$ M on the force of contraction and the positive inotropic effect of A23187 are illustrated in Fig. 1. A similar example showing the effect of two concentrations of D-600 (10^{-7} and $5 \cdot 10^{-7}$ M) is given in Fig. 2. Averaged results of the action of Zn^{++}, Mn^{++}, D-600, and fenigidine are given in Table 1.

As the results show, addition of D-600 ($5 \cdot 10^{-7}$ M), Zn^{++} ($2 \cdot 10^{-5}$ M), and Mn^{++} ($2 \cdot 10^{-4}$ M) to the physiological saline blocked the positive inotropic effect of A23187 virtually completely.

Experiments in artificial organic media showed that the dissociation constant of the complex of A23187 with Zn^{++} ions is an order of magnitude lower than the dissociation constant of the complex of the ionophore with Mn^{++} ions and almost four orders of magnitude lower than that of the complex with Ca^{++} ions [9]. The results indicate that affinity of the ionophore for bivalent cations is similar also in biological membranes. For instance, Zn^{++} and Mn^{++} ions, in concentrations much lower than the calcium concentration in the perfusion solution, completely inhibited the action of the ionophore on the force of contraction. Meanwhile the blocking concentration of Zn^{++} ions was an order of magnitude lower than the concentration of Mn^{++} ions. Depending on their ability to block the positive inotropic action of A23187, the calcium antagonists tested can therefore be arranged in the following order: D-600 > Zn^{++} > Mn^{++}.

These calcium antagonists, in concentrations abolishing the effect of the ionophore, reduced the force of contraction of the myocardium by different degrees: Zn^{++} and Mn^{++} by about 10 and 20% respectively, D-600 by more than 50%. Since the action of these antagonists on the force of contraction is mainly due to the Ca-channels of the cell membrane [3, 6, 7], it can be concluded that the affinity of Zn^{++}, Mn^{++}, and D-600 for the channel is lower than with the ionophore A23187. Although D-600 depresses the force of myocardial contraction and the positive inotropic effect of the ionophore in a concentration much below that of Zn^{++} and Mn^{++}, the selectivity of the blocking effect of D-600 on the ionophore is lower.

As Table 1 shows, fenigidine had no selective blocking effect on the action of the ionophore. For instance, in a concentration of 10^{-6} M fenigidine did not reduce the effect of the ionophore, whereas it blocked the force of contraction by about 50%. An increase in the concentration of the blocker to 10^{-5} M led to irreversible depression of the force of contraction by 80%. In this case the increase in the force of contraction due to the action of the ionophore was reduced to about 7% of the normal value, regardless of whether fenigidine was present in the solution with the ionophore or had been removed from it before addition of the ionophore. If it is accepted that diminution of the effect of the ionophore was due to the formation of fenigidine–ionophore complexes, this means that an adequate amount of fenigidine, which is a lipophilic agent, is preserved in the cell membrane, where it binds with ionophore A23187.

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