DEPENDENCE OF CALCIUM EFFLUX FROM THE MYOCARDIUM ON THE CONCENTRATION OF CALCIUM CHANNEL BLOCKERS

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A number of substances which act as calcium channel blockers or calcium antagonists reduce the force of myocardial contraction [10]. It is generally considered that the force of contraction is reduced through blocking of calcium channels [9]. However, calcium antagonists are known to lower the intracellular Ca^{2+} concentration in strips of heart muscle in the absence of stimulation [5].

The aim of this investigation was to study dependence of Ca^{2+} efflux from the resting ventricle on the concentration of verapamil (izoptin), nifedipine (fenigidine, adalate, BAY 1040), and nicardipine, and also to study the effect of the above-mentioned calcium antagonists on calcium efflux from the tissue in the presence of a reduced Na^{+} concentration and an increased K^{+} concentration in the external solution.

EXPERIMENTAL METHOD

Experiments were carried out on the ventricle of a large frog (*Rana ridibunda*), weighing 350-400 g. After dissection the ventricle was washed free from blood, cut into several strips, the surface wiped dry, after which the strips were weighed and placed for adaptation for 30 min in cuvettes containing oxygenated Ringer's solution (NaCl 110 mM; KCl 2.5 mM; $CaCl_2$ 1.1 mM; Tris-HCl 10 mM; glucose 5.5 mM; pH 7.3-7.4) at 18-20°C. During the adaptation period and later, the strips were not stimulated. The infrequent spontaneous activity of the strips (observed visually) always disappeared during adaptation. After adaptation the strips were transferred to the test solution for 30 min, one strip being used as the control. Ca^{2+} exchange was recorded in the solution by means of Ca^{2+}-selective (Ca) electrodes, made by the Leningrad "Burevestnik" Research-Production Combine, and incorporated into a differential measuring circuit. Two Ca electrodes, lowered into two cuvettes, were used in this circuit. The cuvettes were connected by a glass bridge, filled with 110 mM NaCl solution in agar-agar. After the "zero" had been established in the working solution in the two cuvettes, the difference in Ca^{2+} concentration in the test solution, in which the strip had been placed previously, and in the working solution was measured. To test any possible effect of a change in the concentrations of Ca^{2+} and of K, Ca-antagonists in the external solution on sensitivity of the Ca-electrode, their reaction to standard additions of Ca^{2+} to the solution used was measured. The Ca-electrode responded to these additions in all experiments in the same way as in Ringer's solution. This means that the substances used do not affect the sensitivity of the Ca-electrode. The Ca^{2+} concentration thus established in the solution (in mM) was expressed per kilogram wet weight of tissue. The experimental results were subjected to statistical analysis by Student's test ($P < 0.05$). The following calcium antagonists were used: verapamil (from LEK, Yugoslavia), nifedipine (fenigidine, resynthesized in the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR), and nicardipine (from Yamanouchi Chemical, Japan).

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TABLE 1. Changes in Ca^{2+} Concentration (in millimoles/kg wet weight of tissue) in Presence of Ca Antagonists (10^{-5} M) and with Reduced Na^{+} Concentration or Increased K^{+} Concentration in External Solution

Ca antagonists	Change in Ca^{++} exchange in tissue of ventricle ($\pm\Delta Ca$)			
	scheme 1	scheme 2		50 mM KCl + Ca antagonist
	60 mM NaCl + Ca antagonist	60 mM NaCl	60 mM NaCl + Ca antagonist	
		Ca^{++} influx	Ca^{++} efflux	Ca^{++} influx
Verapamil	≃0 (4)	0,24±0,05 (4)	0,24±0,09 (4)	0,26±0,08 (5)
Nifedipine	≃0 (4)	0,25±0,05 (3)	0,24±0,08 (4)	0,32±0,09 (6)
Nicardipine	≃0 (4)	0,24±0,07 (4)	0,23±0,15 (4)	0,25±0,14 (5)
Control (solution without Ca antagonist), Ca^{++} influx	0,28±0,02 (10)	0,245±0,06 (14)		0,3±0,08 (9)

Legend. Number of experiments shown in parentheses.

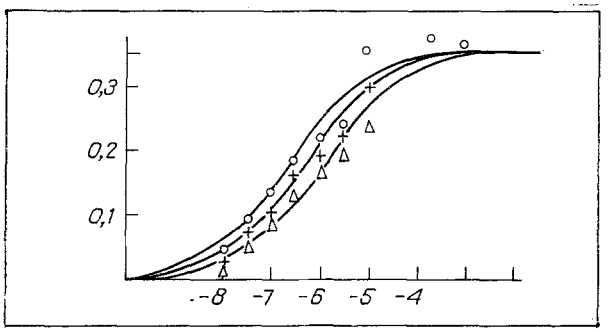

Fig. 1. Dependence of Ca^{2+} efflux from myocardial tissue (ordinate, in millimoles/kg wet weight of tissue) on concentration of Ca antagonists (abscissa, in log B). Circles) verapamil, crosses) nifedipine, triangles) nicardipine.

EXPERIMENTAL RESULTS

After adaptation of the strip of ventricle for 30 min verapamil, nifedipine, or nicardipine was added to the Ringer's solution. An increase in the Ca^{2+} concentration in the measuring cuvette was recorded 30 min later. The points in Fig. 1 represent experimental data for the relationship between calcium efflux from the tissue and concentration of calcium antagonists. Analysis of the results shows that, as a first approximation, they can be described by the equation:

$$\Delta Ca = \Delta Ca_{Max} \frac{1}{1 + \sqrt{K/B}},$$

where ΔCa denotes Ca^{2+} efflux (in mM/kg wet weight of tissue), ΔCa_{max} the greatest possible Ca^{2+} efflux from the tissue when $B \to \infty$, K is a constant, and B the concentration of calcium antagonists; $\Delta Ca_{max} = 0.35 \cdot 10^{-3}$ M.

For verapamil $K = 2.2 \cdot 10^{-7}$ M, for nifedipine $K = 8.1 \cdot 10^{-7}$ M, and for nicardipine $K = 10^{-6}$ M. The continuous curves between the experimental points in Fig. 1 were drawn according to the equation with the above-mentioned constants.

Incidentally, values of the constant in the equation were similar to corresponding values obtained by studying the dependence of force of contraction on concentration of calcium channel blockers: for nifedipine $K = 5 \cdot 10^{-7}$ M [1] and for nicardipine $K = 10^{-6}$ M [2].

Dependence of Ca^{2+} efflux from the strips and the concentration of calcium antagonists can be explained on the assumption that the Ca antagonists mainly block Ca^{2+} influx into the cell and do not reduce the intensity of its "evacuation" from the cell. Calcium influx into the cell is determined by: the calcium channel [3], the passive leakage channel [7], and the sodium-calcium exchange system [4, 8].

Since the tests were carried out in the absence of external stimulation and of spontaneous activity it is unlikely that the Ca^{2+} influx into the cell was limited by the calcium

channel. Calcium antagonists may perhaps block Ca^{2+} influx via the remaining channels, but it could not be determined by these experiments which channel is responsible.

Evacuation of Ca^{2+} from the cell is effected by the sodium-calcium exchange system and the Ca-ATPase pump. The first system is sensitive to Na^+ concentration and depends on membrane potential. With diminution of the Na gradient and depolarization of the cell the intensity of Ca^{2+} evacuation is reduced. The second system is insensitive to these parameters [6]. Which of these systems is responsible for Ca^{2+} evacuation under the present experimental conditions? To answer this question, the effect of Na^+ and of cell depolarization on Ca^{2+} efflux from the tissue was investigated.

Participation of Na^+ in Ca^{2+} evacuation from the cell under the influence of Ca antagonists was studied by two methods. In the first case (scheme 1) the Ca^{2+} concentration was measured after the strips had been transferred from Ringer's solution into solution with low sodium concentration (60 mM, replaced by sucrose) with one of the calcium antagonists. As Table 1 shows, the antagonists studied under these experimental conditions did not lead to evacuation of Ca^{2+} from the strip of ventricle. In the second case (scheme 2) the ventricle was transferred in the following order: Ringer's solution—low-sodium solution—low sodium solution with one calcium antagonist (10^{-5} M). The results in Table 1 show that in experiments conducted according to scheme 2, addition of Ca antagonists led to efflux of Ca^{2+} from the strip.

The cell membrane was depolarized by KCl (50 mM). It will be clear from Table 1 that addition of one calcium antagonist (10^{-5} M) did not cause evacuation of Ca^{2+}.

In the writers' view, evacuation of Ca^{2+} from the cell takes place with the participation of the sodium-calcium exchange system, for Ca^{2+} efflux was stopped when the external Na^+ concentration was lowered and during depolarization of the cell. It must also be noted that evacuation depends not only on the Na^+ concentration, but also on the intracellular Ca^{2+} concentration.

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