Dependence of Ca Outflow and Depression of Frog Myocardium Contraction on Ryodipine Concentration

E. NARUŠEVIČIUS[1], V. GENDVILIENĖ[1], R. MAČIANSKIENĖ[1], G. HMELJ-DUNAI[1], A. VELENA[2] and G. DUBURS[2]

1 *Z. Januškevičius Institute of Cardiovascular Physiology and Pathology, Gvardiečiu 17, 233007, Kaunas, Lithuanian SSR*
2 *Institute of Organic Synthesis: Latvian SSR, Academy of Sciences, Aizkraukles 21, 226006, Riga, Latvian SSR, USSR*

Abstract. The effect of ryodipine on calcium outflow from tissues, on contraction force, the duration of action potentials and the relaxation phase time-constant in the contraction cycles of myocardial strips was studied using frog heart preparations. It was found that calcium outflow (ΔCa) as a function on ryodipine concentration can be represented as:

$$\Delta Ca = (\Delta Ca)_{max} \cdot \frac{1}{1 + \sqrt{\frac{2 \times 10^{-7}}{B}}}$$

A linear correlation exists between Ca^{2+}, contraction blocking and the shortening of the action potential in the presence of various ryodipine concentrations. Ryodipine (10^{-5} mol/l) decreased the relaxation time-constant by about 20% as compared to controls. It was concluded that calcium outflow from myocardial tissues in response to ryodipine is due to blockade of calcium entry into the cells and their output through the Na^{+}—Ca^{2+} exchange system. Frog heart myocardial contractions are essentially under the control of calcium entry through sarcolemmal calcium channels.

Key words: Calcium uptake — Ryodipine — Contraction force — Duration of action potentials — Relaxation phase time-constant

Introduction

Blockers of calcium channels are also known to depress heart contraction force (Morgan et al. 1983) and to promote Ca^{2+} outflow from heart tissues

(Hmelj—Dunai and Naruševičius 1985). The aim of this work was to investigate whether depletion of calcium ions, caused by calcium channel blockers has any effect on heart contraction parameters. In this context we examined the effects of ryodipine (ryosidine, foridone, 4-*o*-difluoromethoxy-phenyl-2,6-dimethyl-3,5--dimethoxycarbonyl-1,4-dihydropyridine: used to block the outflow of calcium ions from calcium channels in frog heart tissues), on contraction force, the duration of action potentials and on the time constant of their relaxation phase.

Materials and Methods

The experiments were performed on frog heart preparations of *Rana ridibunda*.

The dependence of calcium outflow from heart tissues on ryodipine concentration was studied in the following manner. After its extraction the heart was cut into strips and washed to remove blood. The surface of the heart strips was lightly dried and after wet weight determination the preparations were placed in oxygenated Ringer' solution (mmol/l): NaCl-2.5, $CaCl_2$-1.1, Tris HCl-10, glucose-5, pH 7.3—7.4 for 30 min at 18—20°C. Then the strips were immersed in the test solution for 30 min in a 1.0 ml chamber. Ca^{2+} concentration was measured with Ca^{2+}-selective electrodes employing a differential measurement configuration under resting heart conditions.

Ryodipine, the structural analogue of nifedipine, having a 2-$OCHF_2$ group in the 4-phenyl substituent of 1.4-dihydropyridine ring, was synthesised in the Institute of Organic Synthesis (see Kastron et al. 1982).

For preparation of physiological saline with 10^{-5} mol/l ryodipine 3.6 mg ryodipine was dissolved in 0.3 ml ethanol and diluted with physiological saline to one l. In order to obtain the required concentrations of the drug were examined (mol/l): 10^{-9}, 10^{-8}, 3×10^{-8}, 10^{-7}, 2×10^{-7}, 10^{-6}, 10^{-5}. Successive solutions of different concentration were used starting from the lowest concentration. Measurements were performed after a suitable period to allow for concentration equilibrium to be reached.

The action of ryodipine on contraction force and on the duration of action potentials was examined on 3—5 mm long frog ventricle strips, placed in a 10 ml flow-through chamber. Mechanical activity was measured isometrically. For electrical stimulation, square wave stimuli of 10—15 ms duration at 0.25—0.3 Hz were applied. The amplitude of the stimuli was 3—4 fold higher that of the estimated threshold value.

The amplitude of contraction force (P) and the duration of action potentials, (T) at the corresponding ryodipine concentrations were normalized to the outgoing values. The duration of action potentials was registered at halfmaximal of amplitude. The time-constant of the relaxation phase of isometrical contractions (τ) was determined as follows: the relaxation process could be described with the equation below (Isakov et al. 1981).

$$P_t = P_{max} \cdot \exp - (t/\tau)^2 \qquad (1)$$

where (P_t) is contraction force at a given moment, P_{max} is maximum of contraction force, t is time of discharge counted from P_{max}, τ is relaxation time constant. This constant was determined by measuring the time of relaxation at the $P_t = 0.36\, P_{max}$ level.

Confidence limits were estimated for $P = 0.95$ (i.e. $\alpha = 0.05$).

Results

The experimental data relating changes in heart ΔCa^{2+}, contraction force P/P_0, and the duration of action potentials T/T_0 with ryodipine concentration are given in Table 1.

Analysis of our results obtained by studying Ca^{2+} outflow from heart tissues in response to various ryodipine concentrations (Table 1) shows that

$$\frac{\Delta Ca}{\Delta Ca_{max}} = \frac{1}{1 + \sqrt{K/B}} \tag{2}$$

where ΔCa^{2+} stands for Ca^{2+} outflow at a given ryodipine concentration, ΔCa^{2+}_{max} is maximally possible Ca^{2+} outflow from heart tissues at $B \to \infty$, B is ryodipine concentration, K is a constant value equal to 2×10^{-7} mol/l.

Fig. 1 illustrates experimental Ca^{2+} values normalized relative to $\Delta Ca^{2+}_{max} = 0.32$ mmol/l kg wet weight. The solid line in Fig. 1 was plotted as in (2) complete with constants.

The dependence of Ca^{2+} outflow from myocardial cells at various ryodipine concentrations can be explained if we assume, that ryodipine favours Ca^{2+} outflow by affecting Na^+—Ca^{2+} exchange and blocking Ca^{2+} entry in to the cell through leakage of Ca^{2+}-channels (Hmelj—Dunai and Naruševičius 1985).

The effect of the blocking activity of ryodipine on contraction force can be predicted by the equation below:

$$B = (1 - P/P_0) \tag{3}$$

where P is contraction force at a given ryodipine concentration, P_0 — is contraction force uside control conditions (i.e. without ryodipine).

The dependence of B on ryodipine concentration R using the data from Table 1 is shown in Fig. 1. It can be seen that this dependence agrees perfectly with analogous findings concerning Ca^{2+} outflow from myocardial tissues after ryodipine treatment.

Furthermore, a linear correlation exists between contraction force and the duration of action potentials (see Table 1).

$$\frac{P}{P_0} = k \quad \frac{T - T^0}{T_0} \tag{4}$$

where T is the duration of action potentials at given ryodipine concentration, T_0 — is the duration of action potentials in the absence of ryodipine, T^0 — is the duration of action potentials independent of calcium current (see Fig. 2).

$$k = 2.2; \quad T^0/T_0 = 0.55$$

Table 1. Dependence of contraction force, duration of action potentials blocking activity and calcium outflow from frog heart on ryodipine concentration

C(mol/l)	10^{-9}	10^{-8}	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}	$3+10^{-6}$	10^{-5}
Ca outflow from tissues mM/kg w.w.	—	0.05 ± 0.02 $n = 9$	0.09 ± 0.01 $n = 4$	0.14 ± 0.02 $n = 9$	0.18 ± 0.01 $n = 4$	0.23 ± 0.06 $n = 9$	0.25 ± 0.09 $n = 4$	0.30 ± 0.04 $n = 5$
P/P_0	0.88 ± 0.04 $n = 6$	0.86 ± 0.09 $n = 6$	—	0.62 ± 0.19 $n = 6$	—	0.24 ± 0.08 $n = 6$	—	0.07 ± 0.01 $n = 6$
Blocking activity after $B = (I - P/P_0)$	0.12	0.14	—	0.28	—	0.76	—	0.93
T/T_0	0.95 ± 0.24 $n = 3$	0.94 ± 0.06 $n = 3$	—	0.86 ± 0.05 $n = 3$	—	0.72 ± 0.02 $n = 3$	—	0.60 ± 0.01 $n = 3$
Calculated from: $\frac{P}{P_0} = 2.2\left(\frac{T}{T} - 0.55\right)$	0.88	0.86	—	0.68	—	0.34	—	0.11

n = number of experiments

Consequently (from equation 4), contraction force is proportional to the duration of the component action potential, which is dependent on calcium current ($T - T^0$) (see Fig. 2).

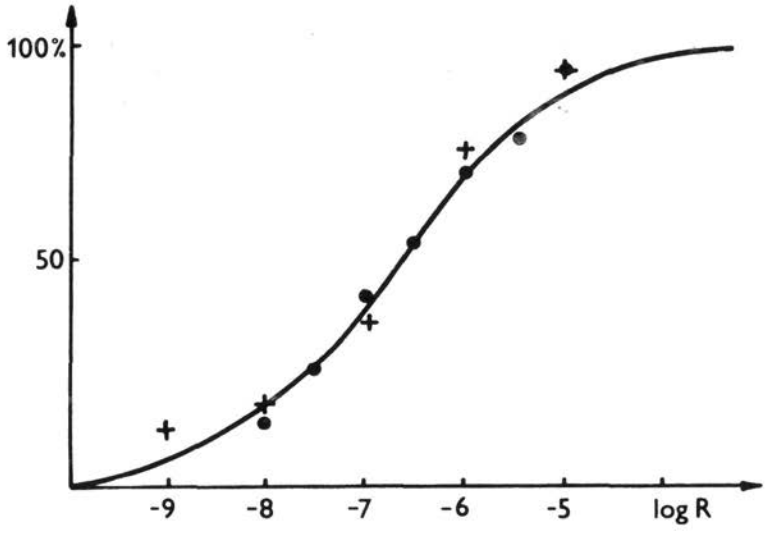

Fig. 1. Dependence of calcium outflow from frog heart $\left(\frac{\Delta Ca}{\Delta Ca_{max}}\right)$ (●) and blocking activity ($1 - P/P_0$) (×) on ryodipine concentration (R). The solid line was constructed according to formula (2) (see text) with $K = 2 \times 10^{-7}$ mol/l. Abscissa: ryodipine (R) log mol/l concentration. Ordinate: calcium outflow and contraction force as a % of the basal level.

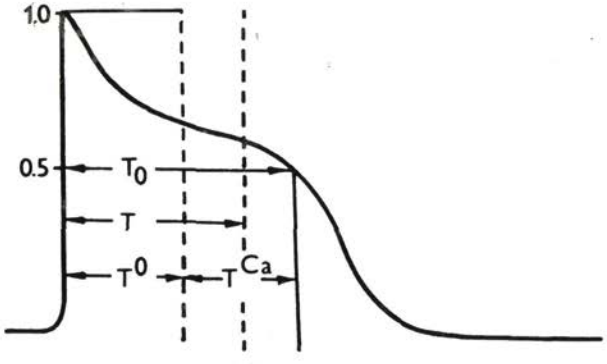

Fig. 2. Schematic representation of a frog myocardium action potential (AP). The measurements of AP duration and designations of their components are indicated with arrows. T_0 — duration of AP without ryodipine, T — duration of AP at a given ryodipine concentration, T^0 — duration of AP, independent of Ca^{2+} channels, T^{Ca} — duration of AP directed through calcium channels.

Considering that the calcium ions carried through open calcium channels are sufficient for activation of contractions (Pytkowski et al. 1983) it may be concluded from the results obtained that ryodipine, like other calcium antagonists, depresses contraction force predominantly by acting as a blocking agent for sarcolemmal calcium channels.

Does ryodipine exert an influence on the rate of relaxation phase of isometrical contraction? Fig. 3 provides an example of a single contraction cycle and the duration of an action potential recorded under control conditions and in the presence of ryodipine (10^{-5} mmol/l).

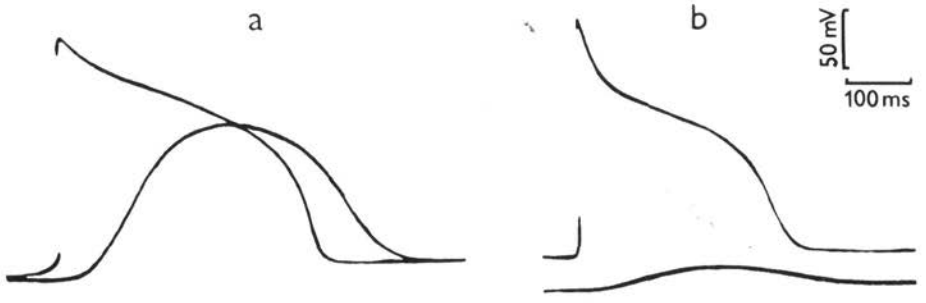

Fig. 3. An illustrative example of experimental recordings of a single contraction cycle and action potential under control conditions (*a*) and following ryodipine administration (*b*) (10^{-5} mol/l).

The mean values of the relaxation phase time constant (τ/τ_0) measured under control conditions (τ_0) and in the presence of 2×10^{-7} mol/l and 10^{-5} mol/l ryodipine (τ) are 1; 0.8 ± 0.08; 0.77 ± 0.13 respectively. Ryodipine decreased the relaxation phase time constant about 20% compared to the control value. At the same time, no appreciable difference between the τ/τ_0 values in the presence of 2×10^{-7} mol/l and at 10^{-5} mol/l ryodipine was noted.

The shortening of the single contraction cycle at the expense of the relaxation phase following nifedipine, diltiazem, D-600, verapamil and perhexiline administration has also been reported by other investigators (Morgan et al. 1983).

Since calcium is already excluded from the medium around myofibrillae at the beginning of the relaxation phase (see experiments with equorine by Morgan et al. 1983) it may be concluded that the observed decrease in the time constant of the relaxation phase in the presence of ryodipine cannot be explained by the involvement of Na^+—Ca^{2+} exchange during myocardial relaxation. It is possible, however, that relaxation time-constant shortening is caused by blockade of Ca^{2+} leaky channels.

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