Effect of Slow Calcium Channel Blockers on the Electromechanical Activity of Frog Moycardium in the Presence of Epinephrine

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Abstract. The calcium channels blockers fenihidine (3.5×10^{-5} mol/l), ryosidine (10^{-5} mol/l), D-600 (10^{-5} mol/l) and Mn ions (2×10^{-3} mol/l or 5×10^{-3} mol/l) block contraction force and shorten the duration of action potentials of the frog myocardial ventricle strand under normal conditions. When contraction force and the duration of action potentials were restored by epinephrine (10^{-5} mol/l), these agents were unable to supress these parameters. The increase in both contraction force and the duration of action potentials induced by epinephrine were blocked by acetylcholine. Recording by voltage clamp of inward calcium current (I_{Ca}) of the frog atrial trabeculae it was found that fenihidine decreases I_{Ca} activated by epinephrine to a smaller extent than observed at normal conditions. Let as assume that epinephrine increases I_{Ca} by means of increasing number of calcium channels so these data support the proposed existence of as many as two calcium channel fractions in frog myocardium, which differ in the sensitivity to calcium channels blockers.

Key words: Frog myocardium electrophysiology — Calcium channels blockers — Epinephrine

Introduction

Increase in the contractile strength of myocardium induced by catecholamines is due to a rise in calcium current (Reuter 1974). It is assumed that the effect of rising calcium current primarily determined by the appearence of new calcium channels whose number varies in parallel with the cAMP level in the cell (Sperelakis and Schneider 1976). Reuter and Scholz (1977) have demonstrated that the kinetics of calcium current induced by epinephrine do not differ from that under normal conditions, the selectivity of calcium channels also remaining unaffected. Similar data were obtained by Lazarev et al. (1980). It was concluded that the calcium channels activated by catecholamines are with respect to their kinetic properties,

identical to the calcium channels activated under normal conditions. Nevertheless there have been reports suggesting that the epinephrine-induced calcium current shows higher sensitivity to acetylcholine action as compared to the initial calcium current (Josephson and Sperelakis 1982). It is of interest therefore to determine whether there are differences in sensitivity with respect to specific blockers of inward calcium current between epinephrine-activated Ca^{2+} channels and those operating in normal conditions.

The aim of this study was to examine, in a frog myocardium preparation activated by epinephrine, the suppression of contractile force; duration of action potentials; and inward calcium current induced by established calcium channel blockers, such as fenihidine (nifedipine), ryosidine, D-600 and manganese ions.

Materials and Methods

Electromechanical activity of myocardium was examined on a strip of frog ventricle 3—5 mm in length placed in a 3 ml flow-type chamber. Contractile activity was registered isometrically with a 6M × 2B mechanotron. Electric impulses of 10—15 ms duration and a frequency of 0.25—0.3 Hz were used for stimulation. The amplitude of the stimuli was 3—4 times that of the threshold value.

Intracellular action potentials were recorded with glass microelectrodes containing 2.5 mol/l KCl. The duration of action potentials was measured at 50% of their amplitude. Physiological saline was (in mmol/l): NaCl — 110, KCl — 2.5, $CaCl_2$ — 1.08, tris Cl — 10, glucose — 5.5, pH 7.4—7.5.

Ionic currents were registered on atrial trabeculae isolated from the frog heart. The preparation (⌀ 90—130 μm, 3—5 mm long) was placed in a perfusion chamber with a double sucrose gap. For potential fixation and to register ionic current, CEZ-1100 and MEZ-7101 assemblies of amplifiers (Nihon Kohden, Japan) were employed. The described saline was perfused through the testing section of the chamber. Sucrose bridge compartments were perfused with 0.24 mol/l sucrose solution in double-distilled water, and the marginal sections of the chamber were filled with 0.12 mol/l KCl solution. Prior to registration of the slow inward current, the cardiac trabecula was treated with tetrodotoxin (3×10^{-6} mol/l) (Sankyo, Japan) to block the fast inward current.

The inward calcium current was measured from the outward current level. The outward current value at the maximal inward current was determined by extrapolation the outward current during long step (800 ms) of membrane depolarization. The resting potential value was registered as change of potential measurable after substitution of physiological solution to KCl solution in the section of the chamber where recording electrode was placed. Potential determined by this way was $-70 \div -80$ mV.

The calcium antagonists used in the study were fenihidine (nifedipine, adalat, BAY' a 1040) — 3.5×10^{-5} mol/l (Bayer and Ehara 1978; Kohlhardt and Fleckenstein 1977) resynthesized in the Institute of Organic Synthesis; ryosidine (10^{-5} mol/l) (original 1,4-dihydropyridine drug synthesized in the same Institute by Kastron et al. 1979); D-600 (methoxyverapamil) (10^{-5} mol/l) (Knoll, Federal Republic of Germany); manganese chloride (2×10^{-3} mol/l, 5×10^{-3} mol/l); acetylcholine iodide (10^{-6} mol/l) (Chemapol, Czechoslovakia); and epinephrine or epinephrine hydrochloride (10^{-5} mol/l). To prevent epinephrine selfoxidation, ascorbic acid (5×10^{-5} mol/l) was added to the solutions.

Figure 1 depicts the structural formulae of organic substances used in our study as calcium channel inhibitors.

The effect of the above calcium antagonists on the electromechanical responses of myocardial strip from frog atrium was studied as described below. After the myocardial strip had reached a plateau or stationary level of contractile activity (approximately after one hour) one of the calcium antagonists

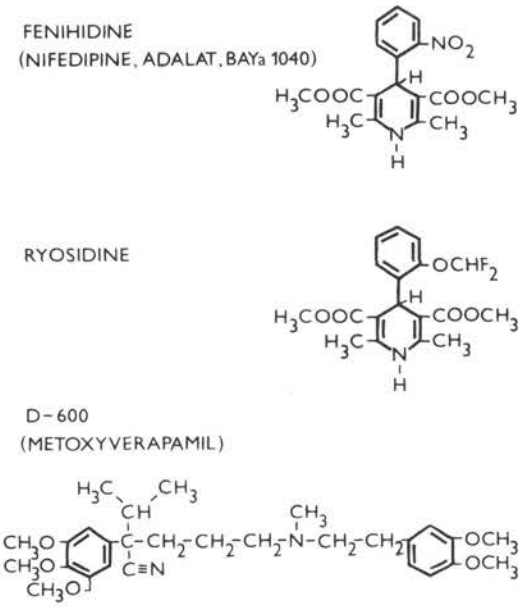

Fig. 1. Structural formulae of calcium channel blockers.

capable of lowering contraction force to a new stationary level was added to the initial solution. After attaining a new stationary level of contraction force the blocking agents acting irreversibly (fenihidine, ryosidine, D-600) were omitted from the solution and epinephrine was added. In the case of manganese ions, acting reversibly, epinephrine was added to the Mn^{2+} — containing solution. Subsequently, the same concentrations of organic blockers (concentration of manganese ions was changed from 2×10^{-3} mol/l to 5×10^{-3} mol/l) were applied in order to examine their influence on the effects of epinephrine on contraction force and on the duration of action potentials.

The influence of fenihidine on epinephrine-activated Ca^{2+} inward current in frog atrium trabeculae was investigated in a similar manner. Steady state values of tension and action potential duration in Tables 1 and 2 are given as mean values ± standard deviations.

Results

Figure 2 illustrates a typical case of the effect of the fenihidine on contraction force (curve 1) and on the duration of action potentials (curve 2) in response to epinephrine (following a prior irreversible suppression of electromechanical activity of frog myocardium by fenihidine). It may be seen that fenihidine (3.5×10^{-5} mol/l) over a period of 30—40 min decreases contraction strength and the duration of action potentials by 95% and 48%, respectively. Epinephrine (10^{-5} mol/l) added to the perfusion solution from which fenihidine had been excluded resulted in a restoration of the contraction force and of the duration of action potentials by approximately 30%. Subsequent repeated exposure to fenihi-

Table 1. Effect of organic calcium channel blockers, epinephrine, and acetylcholine on the contractile responses and the duration of action potentials of frog myocardium

Antagonists used	Solutions				
	Physiol. saline + antagonist	Physiol. saline + epinephrine (10^{-5} mol/l)	Physiol. saline + epinephrine + antagonist	Physiol. saline + epinephrine + acetylcholine (10^{-6} mol/l)	Physiol. saline + (washing for 15 min)
Fenihidine 3.5×10^{-5} mol/l					
$P \pm \sigma$	10.7 ± 2.1	33.2 ± 4.8	33.2 ± 4.9	15.0 ± 2.3	13.0 ± 3.4
$APD \pm \sigma$	62.3 ± 4.6	83.7 ± 5.6	81.6 ± 5.5	63.0 ± 7.3	63.7 ± 4.7
	($n = 9$)	($n = 9$)	($n = 9$)	($n = 6$)	($n = 4$)
Ryosidine 10^{-5} mol/l					
$P \pm \sigma$	7.7 ± 1.5	25.5 ± 4.4	24.8 ± 4.2	4.1 ± 2.2	9.4 ± 3.1
$APD \pm \sigma$	63.0 ± 5.1	94.9 ± 7.3	94.7 ± 7.7	49.1 ± 1.2	54.9 ± 7.0
	($n = 8$)	($n = 8$)	($n = 8$)	($n = 3$)	($n = 5$)
D-600 10^{-5} mol/l					
$P \pm \sigma$	9.4 ± 3.9	30.2 ± 13.9	27.0 ± 10	—	9.2 ± 3.4
$APD \pm \sigma$	56.1 ± 6.0	109.4 ± 6.6	112.8 ± 4.3	—	60.6 ± 5.9
	($n = 3$)	($n = 3$)	($n = 3$)		($n = 3$)

P, *APD* — percentual stand for contraction force and duration of action potentials, respectively (initial level was taken as 100%); *n* — is number of experiments, σ — standard deviations

dine did not abolish the effect of epinephrine. After washing the preparation with physiological saline, contraction force and the duration of action potentials return to approximately the same level as that observed at 3.5×10^{-5} mol/l of fenihidine. The averaged results of 9 experiments are displayed in Table 1.

Equally, under conditions of irreversible calcium channel blockade with ryosidine (10^{-5} mol/l) the average contraction force and action potential-duration responses to epinephrine were 18% and 31%, respectively (Table 1). Ryosidine added to a solution containing epinephrine failed to affect these two parameters. After washing with physiological saline the contraction force and duration of action potentials were restored to approximately the level observed at 10^{-5} mol/l ryosidine. Data obtained in one experiment are illustrated in Fig. 3. The averaged results are displayed in Table 1.

A similar pattern of changes was also observed when D-600 and manganese ions were used as calcium channel antagonists.

Table 2. Effect of manganese ions and epinephrine on contraction force and the duration of action potentials of frog myocardium

Antagonist	Solution				
	Physiol. saline + 2×10^{-3} mol/l Mn^{2+}	Physiol. saline + 2×10^{-3} mol/l Mn^{2+} + 10^{-5} mol/l epinephrine	Physiol. saline + 2×10^{-3} mol/l Mn^{2+}	Physiol. saline + 5×10^{-3} mol/l Mn^{2+} + 10^{-5} mol/l epinephrine	Physiol. saline + 5×10^{-3} mol/l Mn^{2+}
Mn^{2+}					
$P\pm\sigma$	11.4 ± 1.9	38.7 ± 8.6	14.3 ± 3.3	44.3 ± 7.4	14.4 ± 2.0
$APD\pm\sigma$	46.6 ± 7.3	92.1 ± 7.6	41.8 ± 7.5	92.4 ± 7.8	52.6 ± 4.6
	$(n=5)$	$(n=5)$	$(n=5)$	$(n=7)$	$(n=5)$

P, *APD* — stand for contractile response and duration of action potentials, respectively (initial level was taken as 100%); *n* — is number of experiments, σ — standard deviations

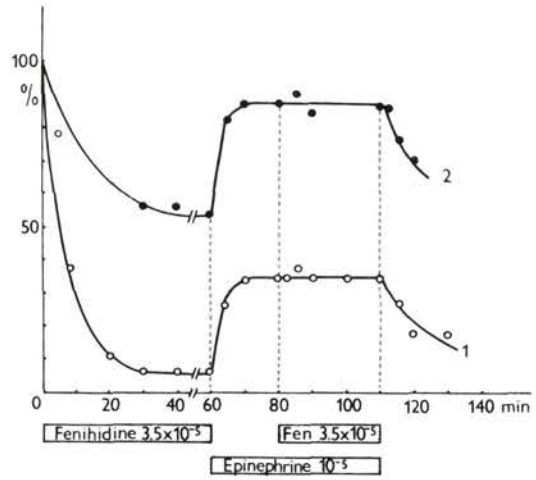

Fig. 2. Effect of fenihidine on contraction force and the duration of action potentials of a strip of frog heart ventricle under normal conditions and in the presence of epinephrine. Curve 1 — contraction force, curve 2 — duration of action potentials. Abscissa: perfusion time (min). Ordinate: alteration of contraction force and the duration of action potentials (initial level is taken as 100%). Intervals between exposures and concentrations (in mol/l) of test agents are indicated below.

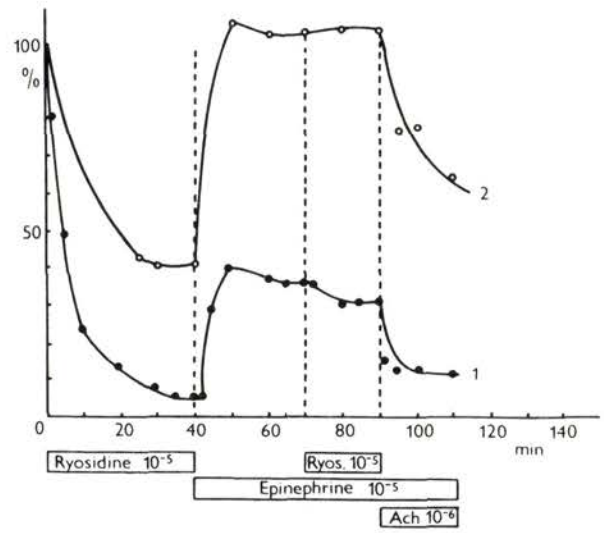

Fig. 3. Effect of ryosidine on the contraction force and the duration of action potentials of a strip of frog heart ventricle under normal conditions and in the presence of epinephrine. Curve 1 — contraction force, curve 2 — duration of action potentials. Further details as in Fig. 2.

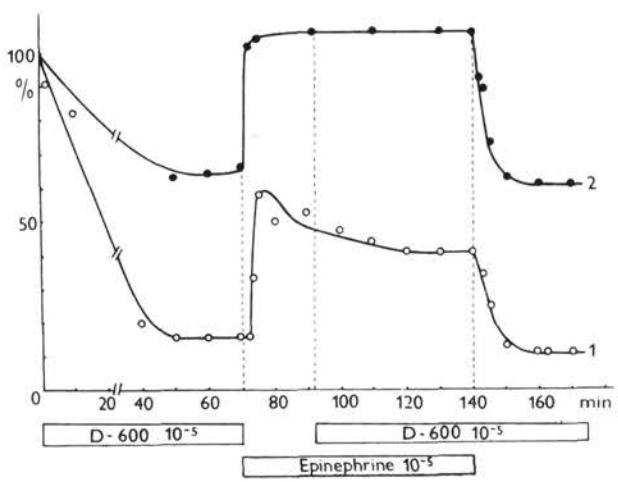

Fig. 4. Effect of D-600 (methoxyverapamil) on contraction force and the duration of action potentials of a strip of frog heart ventricle under normal conditions and in the presence of epinephrine. Curve 1 — contraction force, curve 2 — duration of action potentials. Further details as in Fig. 2.

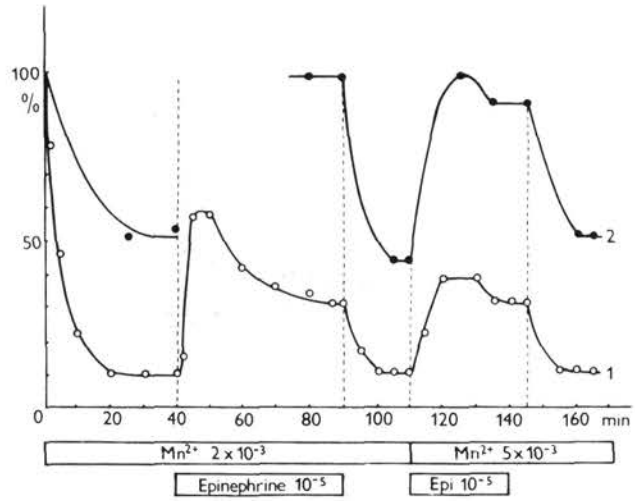

Fig. 5. Effect of manganese ions on contraction force and the duration of action potentials of a strip of frog heart ventricle under normal conditions and in the presence of epinephrine. Curve 1 — contraction force, curve 2 — duration of action potentials. Further details as in Fig. 2.

Figure 4 illustrates the effect of D-600 on contraction force (curve 1) and the duration of action potentials (curve 2) under normal conditions and in the presence of epinephrine; Table 1 summarizes the results of 3 experiments. Like fenihidine and ryosidine, D-600 (10^{-5} mol/l) virtually failed to affect the contractile tension and the duration of action potentials in response to epinephrine.

As follows from Fig. 5, and from the averaged results listed in Table 2, the changes in contraction force and in the duration of action potentials induced by epinephrine could not be abolished by manganese ions either. It should be emphasized that higher Mn^{2+} concentrations (5×10^{-5} mol/l) exerted no appreciable influence on the effect of epinephrine on the above parameters.

Direct measurements of inward calcium current conducted on frog atrium trabeculae indicate that fenihidine (3.5×10^{-5} mol/l), leading depression of the calcium current in normal conditions by 70%—90%, either diminished to a considerably smaller extent the epinephrine-activated calcium current or was completely ineffective. For instance, in 4 out of 6 experiments epinephrine-activated calcium current dropped by 50% (as shown in Fig. 6), in the other two experiments calcium transport was not affected by fenihidine.

Josephson and Sperelakis (1982) have found that calcium current activated by isoproterenol in chick embryo ventricular cell cultures is suppressed by acetylcholine (10^{-6} mol/l), although the same acetylcholine concentration failed to affect the background initial calcium current. Our own studies revealed that the increased

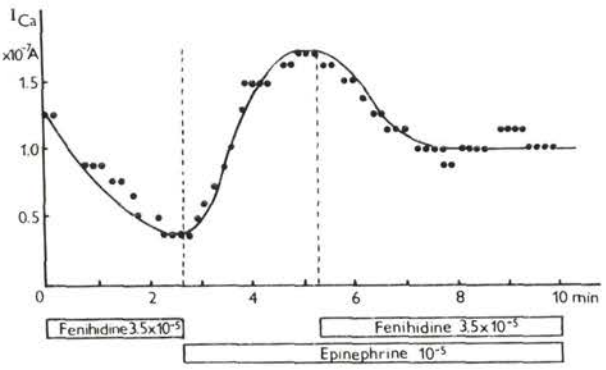

Fig. 6. Effect of fenihidine on inward calcium current under normal conditions and in the presence of epinephrine. The fast sodium influx is blocked by pretreament with 3×10^{-6} mol/l of tetrodotoxin. Abscissa: perfusion time (min). Ordinate: inward calcium current registered during membrane depolarization with 70 mV potential steps; resting potential was -75 mV. Further details as in Fig. 2.

contractile tension and increased duration of action potentials in response to epinephrine was resistent to the influence of the blocking agents used but was almost completely reversed by acetylcholine (10^{-6} mol/l) (Table 1, Fig. 3). It must be pointed out that in normal conditions this acetylcholine concentration brings about a reduction in contraction force and duration of action potentials by $30 \pm 7\%$ and $37 \pm 7\%$, respectively.

Discussion

Our findings demonstrate that inhibitors of slow calcium channels, such as fenihidine, ryosidine, D-600 and Mn^{2+} are effective in suppressing contractile tension and diminish the duration of action potentials in normal isolated frog myocardial preparations, but fail to affect the increase in the parameters in response to epinephrine. The reaction to epinephrine was blocked by acetylcholine. It should be noted that the registered changes in contraction force and in the duration of action potentials in response to epinephrine normally amount to $30 \pm 8\%$ and $30 \pm 10\%$, $n = 7$, respectively, i. e. they are, within the limits of experimental error, close to those in the presence of the blocking agents used.

Let us assume that epinephrine increases I_{Ca} by means of increasing number of calcium channels so our data support the proposed existence of as many as two calcium channel fractions in frog myocardium which can be identified by using epinephrine. In frog myocardium these two fraction differ in their sensitivity to calcium to channel blockers: the epinephrine-induced fraction being less sensitive to the specific blocking agents fenihidine, ryosidine, D-600 and manganese ions.

Some literature data confirm the existence of two calcium channel fractions.

The blocking effect of acetylcholine on isoproterenol-activated calcium transport in chick embryo ventricular cell-cultures studied by Josephson and Sperelakis (1982) allows to postulate the existence of two types of calcium channels: cAMP-dependent blocked by acetylcholine and cAMP-independent, resistent to acetylcholine blockade. These two fractions on types of channels are, however, inhibited by verapamil.

Ochi (1981) shows that acetylcholine diminished I_{Ca} conductance of guinea-pig papillary muscle without affecting the gating and ion-selective properties of I_{Ca} channels. The efficiency inhibition was greater in the presence of epinephrine.

Gőrlitz et al. (1975) have reported that in isolated guinea-pig atria both D-600 and nifedipine (10^{-7} mol/l) normally inhibit contraction force by 55%, but have no effect on this parameter increased by norepinephrine (10^{-5} mol/l).

Direct registration of calcium influx on frog atrium trabeculae using the voltage-clamp technique demonstrates that epinephrine-activated calcium inward current is less sensitive to fenihidine than initial calcium current (Kshutashvili et al. 1980). If we accept Reuter's et al. (1983) assertion, that catecholamines caused an increase in the probability of the channel opening (p) but not in their number, so our data can explain the uniformity of calcium channels because in this case calcium current $I_{Ca} = Npi$, where N = number of non-blocked channel, p — probability of the channel opening, i — estimated single channel current. Our experiments did not allow to differentiate these suppositions.

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