Pharmacological blockade of sarcoplasmic reticulum induces a negative lusitropic effect

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Abstract. The relaxation and the inter-beat mechanical tension are termed lusitropic functions. It is generally assumed that they are primarily determined by Ca^{2+} homeostasis of cardiac cell and by interactions of Ca^{2+} with the contractile machinery. In the present study we studied the effects of various pharmacological interventions on the excitation-contraction coupling in right ventricular papillary muscles of adult rabbits at various stimulation rates. The maximal force of isometric contraction (MG, a.u.), the time to peak of isometric contraction (TTP, ms), the maximal speed of relaxation (dF/dt_{relax}), the diastolic tension (DT, a.u.) and the total tension (MG+DT, a.u.) were measured. To affect excitation-contraction coupling, caffeine (5 mmol·l^{-1}), ryanodine (1 μmol·l^{-1}) and dantrolene sodium (50 μmol·l^{-1}) were used. Whereas caffeine and ryanodine elicited a pronounced negative lusitropic effect, the effect of dantrolene was less dramatic with preserved frequency dependence. The results indicate that the key element for affecting the lusitropic functions is the ryanodine receptor of the sarcoplasmic reticulum (SR). The lusitropic effects of dantrolene, that affects cardiac excitation-contraction coupling but only minimally the ryanodine receptors of SR, were considerably less pronounced. The findings agree with the assumption that the lusitropic disturbances are closely related to the defects of SR ryanodine receptors of cardiac myocytes.

Key words: Lusitropy — Excitation-contraction coupling — Caffeine — Ryanodine — Dantrolene

Introduction

The lusitropic functions are diastolic functions. There are two kinds of events. First, the processes that determine the time course of relaxation, and second, processes that determine the resting (diastolic, inter-beat) tension of the myocardium. The rate of relaxation, especially of ventricular muscle, and the decrease in resting tension are supremely important for the optimal filling of cardiac cavity with the blood. Consequently, they determine the capability of ejection in subsequent systolic period (Yellin et al. 1990; Gaasch and LeWinter 1994). The contraction/relaxation cycle of cardiomyocytes is primarily controlled by changes in intracellular concentration of Ca^{2+} (Ca^{2+}_i). The resting Ca^{2+}_i is usually below 10^{-7} mol·l^{-1}. The contraction is induced by an increase in Ca^{2+}_i up to 10^{-5} mol·l^{-1}. Analogously, the relaxation is primarily determined by a decrease in Ca^{2+}_i. In the adult mammalian myocardium, the main source of the contraction activator (Ca^{2+}) is the sarcoplasmic reticulum (SR). Dynamics of relaxation and the level of resting tension depend on the rate of decline of Ca^{2+}_i, which is mainly due to the active transport of Ca^{2+} back to SR. The mechanisms of cellular Ca^{2+} homeostasis show considerable inter-species differences (Bers 1997) as well as differences due to postnatal ontogeny or senescence (Brutsaert and Sys 1989; Haddock et al. 1998; Mandinov et al. 2000).

As evidenced by the whole range of both experimental and clinical studies by the group of Judith Gwathmey, the lusitropic functions represent both general and early symptom of cardiac failure (Davidoff and Gwathmey 1994). In detail, they analysed the importance of diastolic functions in congestive heart failure (Gwathmey and Ingwall 1995).

The initiation, time course and force of contraction (inotropic functions) of the adult mammalian myocardium are largely determined by the release of Ca^{2+} from the SR (Bers 1991; Shuba and McDonald 1995). The relaxation, its speed

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and completness and other lusitropic processes are mainly due to the reuptake of Ca^{2+} from the contractile apparatus to the SR (Edes et al. 2001). In part, $Ca^{2+}{}_i$ is lowered by activity of the Na^+/Ca^{2+} exchanger (Štengl and Pučelík 2000; Kimura 2001).

So far, just a few studies addressed the dysfunction of the ryanodine receptor in the context of cardiac lusitropy. The present study, therefore, focuses on the analysis of lusitropic functions of the right ventricular myocardium of adult rabbit during pharmacological interventions that influence the ryanodine receptor (RyR) and the excitation-contraction coupling: caffeine, ryanodine, dantrolene.

Materials and Methods

The experiments were performed using papillary muscles (length of 4–6 mm, diameter up to 0.8 mm) from right ventricles of young adult rabbits of either sex (age 6–9 months, average weight 1.72 ± 0.13 kg). At this age the maturation of excitation-contraction coupling is completed, SR is both morphologically and functionally mature including RyR and the cardiac muscle exhibit optimal mechanical properties.

After receiving heparine (1000 IU/kg of body weight), rabbits were killed by an overdose of pentobarbital (60 mg/kg of body weight) applied to the ear vein. The heart was quickly excised and further preparation took place in a warm, and oxygenated solution. After opening the right ventricle, suitable papillary muscles were excised with an adjacent portion of the ventricular wall. Only papillary muscles that did not show the spontaneous electro-mechanical activity were used for the experiments. The papillary muscles were placed into a two-chamber stimulation bath and with a thread (Ethicon) they were attached to the mechano-electrical transducer (Hugo Sachs Isometric Force Transducer, F 30, Germany).

The bath was constantly perfused with an oxygenated, warm (36°C) modified Tyrode solution (mmol·l^{-1}): NaCl 137; KCl 4.5; $MgCl_2$ 1; $CaCl_2$ 2; glucose 10; HEPES 5 (pH 7.4 with NaOH), at a speed of 4–5 ml/min.

The preparation was electrically stimulated using rectangular pulses (duration of 0.1–1 ms, stimulation frequency of 1 Hz) produced by stimulator Pulsmaster 300 (WPI, USA) together with stimulus isolator A 360 (WPI, USA). After an initial adaptation period (20 min), during which the muscle was only modestly stretched, the muscle was further elongated untill the contraction force was 90% of the maximum. After the second adaptation period (20 min), the muscle length was again adjusted to compensate for the additional diastolic slackening due to the previous elongation. After this final setting, the resting tension and the maximal contraction force were followed in the final phase of stabilization (20 min). If the parameters during this final phase did not change, the preparation was used for the experiment.

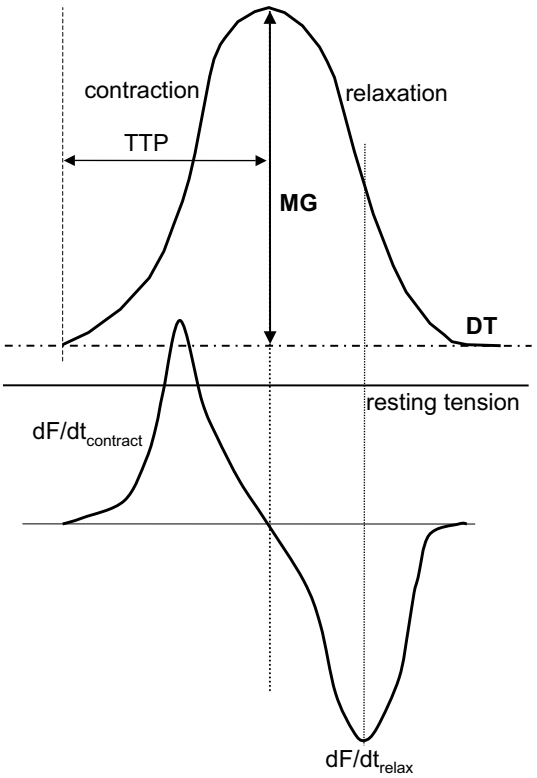

Figure 1. Schematic representation of the measured parameters. Upper part: the isometric tension recording (in a.u.). Bottom part: its first-order derivative. DT, diastolic tension (tension between beats); MG, isometric force during the contraction cycle; dF/dt_{relax}, maximal speed of relaxation; TTP, time to peak of contraction (ms); $dF/dt_{contract}$, maximal speed of contraction; resting tension, tension to which DT decreases after a long period of rest (approximately 60 s).

To modify the Ca^{2+} homeostasis, caffeine, ryanodine and dantrolene were used. Caffeine (5 mmol·l^{-1}, Sigma) was added to the Tyrode solution. Stock solution of ryanodine (Sigma) was prepared in dimethylsulfoxide (DMSO) and this stock solution was added to the experimental Tyrode solution (1 μmol·l^{-1}). The concentration of the vehicle was always below 0.05% and it did not have any significant effects. Stock solution of dantrolene (Sigma or Tocris Cookson, Ltd.) was made in DMSO and added to the experimental solution (50 μmol·l^{-1}).

The cycle length (CL) of the stimulation varied from 200 ms to 2 s (stimulation frequency of 5–0.5 Hz). The parameters were measured after reaching steady-state conditions. Only the experiments, in which both baseline and intervention measurements were successfully obtained, were included for the analysis.

The mechanical manifestations were registered, stored and analysed. From the recordings the following parameters were measured (Fig. 1): 1. MG, maximal value of the contrac-

tion force reached during contraction expressed in arbitrary units (a.u.); 2. TTP, time to the peak of contraction (ms); 3. dF/dt_{relax}, the maximal speed of relaxation expressed in a.u.; 4. DT, diastolic tension expressed in a.u.

Data were presented as mean ± S.E.M., *n* preparations were always given in the text or in the legends. Statistical analysis was performed with Student *t*-test at the level of significance $p < 0.05$.

Results

The magnitudes of contraction force (MG, relative units) at various stimulation frequencies in control (open squares) and in the presence of pharmacological interventions are shown in Fig. 2. The pharmacological interventions influence RyR and they include caffeine (5 mmol·l^{-1}), ryanodine (1 μmol·l^{-1}) and sodium dantrolene (50 μmol·l^{-1}). In control, the dependence of MG on the CL was well pronounced and inversely proportional: the bigger CL, the smaller MG. In the range from 200 to 2000 ms, the contraction force decreased approximately four times. With caffeine (Fig. 2, left panel), the isometric contraction force was significantly reduced and the dependence on the CL was lost. Ryanodine (Fig. 2, middle panel) also reduced MG. The dependence of MG on CL in the presence of ryanodine was bell-shaped with maximal MG at CL of 500 ms. Dantrolene (Fig. 2, right panel) again decreased MG. The dependence of MG on CL remained preserved as in control, although less pronounced.

TTP in control increased with increasing CL (Fig. 3). Caffeine shortened TTP at all CLs tested (Fig. 3, left panel) with preserved dependence on CL. Ryanodine did not influence TTP significantly (Fig. 3, middle panel). Dantrolene decreased TTP significantly only at long CLs (Fig. 3, right panel).

Fig. 4 shows dF/dt_{relax} in control and in the presence of pharmacological interventions at various CLs. The relationship between dF/dt_{relax} and CL was inversely proportional: dF/dt_{relax} decreased with increasing CL (Fig. 4). Caffeine reduced dF/dt_{relax} considerably and the dependence on CL was completely lost (Fig. 4, left panel). Ryanodine also diminished dF/dt_{relax} and a bell-shaped dependence on CL appeared (Fig. 4, middle panel). Dantrolene decreased dF/dt_{relax} to certain extent, the dependence of dF/dt_{relax} on CL, however, was preserved (Fig. 4, right panel).

DT in control varied approximately between –10 AU to + 10 AU (DT at 1 Hz was always taken as 0) and it increased with increasing CL (Fig. 5). Application of caffeine (Fig. 5, left panel) or ryanodine (Fig. 5, middle panel) induced a similar response: increase in DT and the dependence of DT on CL became inversely proportional. In the presence of ryanodine, however, the inversely proportional dependence of DT on CL was less pronounced than in the presence of caffeine. In contrast with previous interventions, dantrolene (Fig. 5, right panel) did not influence DT significantly.

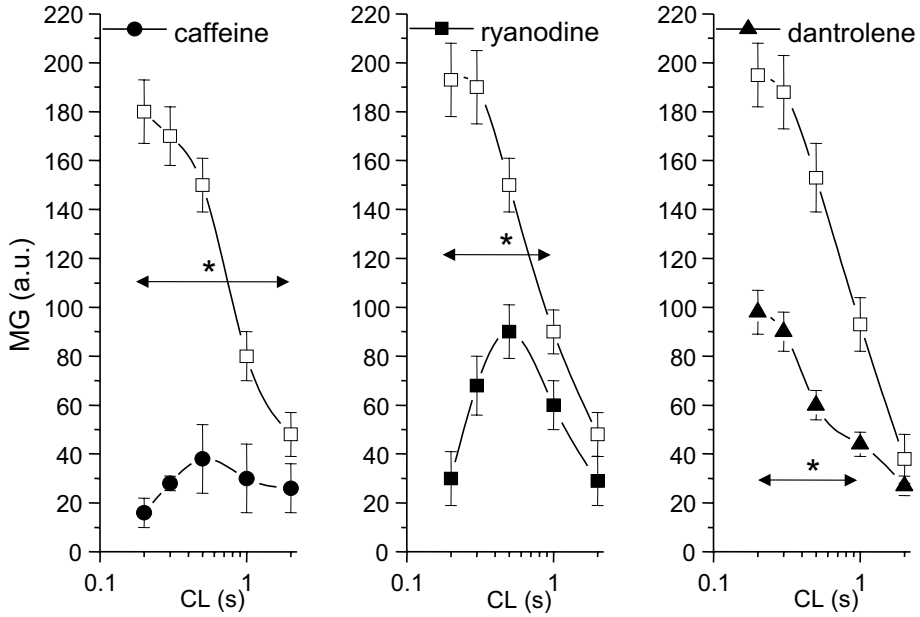

Figure 2. Maximal force of isometric contraction (MG). Open squares, control. Filled symbols, interventions. Left panel: effects of caffeine ($n = 11$). Middle panel, effects of ryanodine ($n = 9$). Right panel, effects of dantrolene ($n = 10$). Only preparation, in which both control measurement and measurement in the presence of intervention were successfully performed, were used for the analysis. Data are presented as mean ± SEM; CL, cycle length (s); * $p < 0.05$.

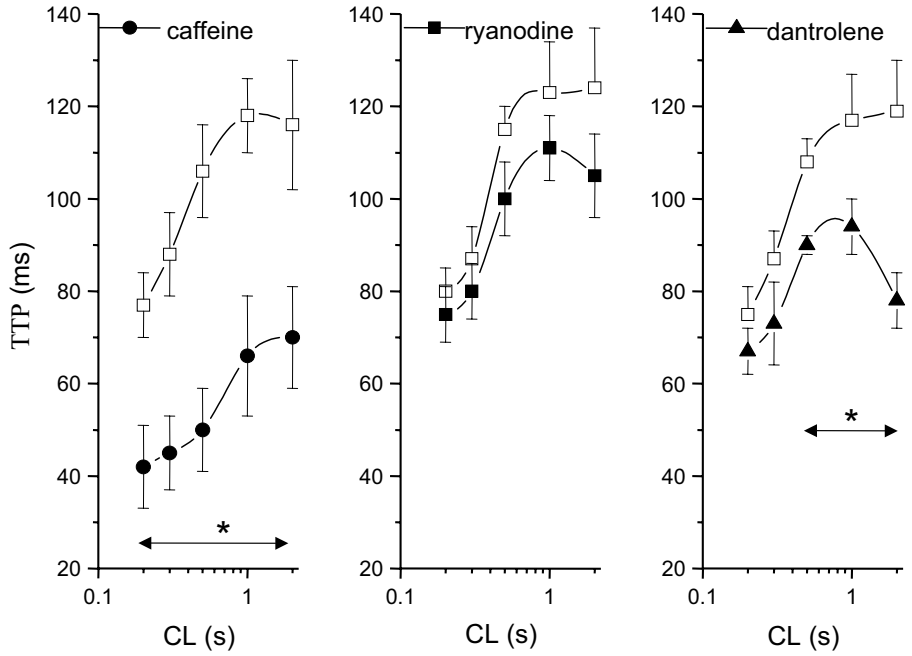

Figure 3. Time to peak of contraction (TTP, ms). Time from the beginning of action potential (not shown) to the peak of contraction. The same order of panels and the same symbols as in Fig. 2. $n = 8$ (caffeine, left panel), $n = 9$ (ryanodine, middle panel), $n = 10$ (dantrolene, right panel), * $p < 0.05$.

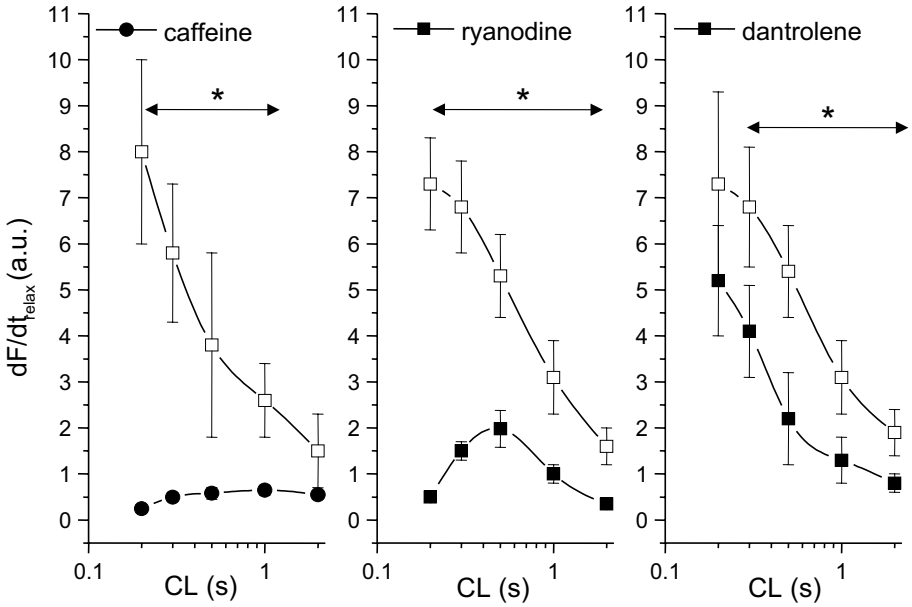

Figure 4. Maximal force decreasing during relaxation (dF/dt_{relax}). The same order of panels and the same symbols as in Fig. 2. $n = 10$ (caffeine, left panel), $n = 10$ (ryanodine, middle panel), $n = 9$ (dantrolene, right panel), * $p < 0.05$.

In Fig. 6, the total tension (TT, i.e. the sum of MG and DT) is shown at various CLs in control and in the presence of various pharmacological interventions. In control, a steep inversely proportional dependence of TT on CL was observed. Caffeine (Fig. 6, left panel) and dantrolene (Fig. 6, right panel) show a similar effect on TT: reduction, which

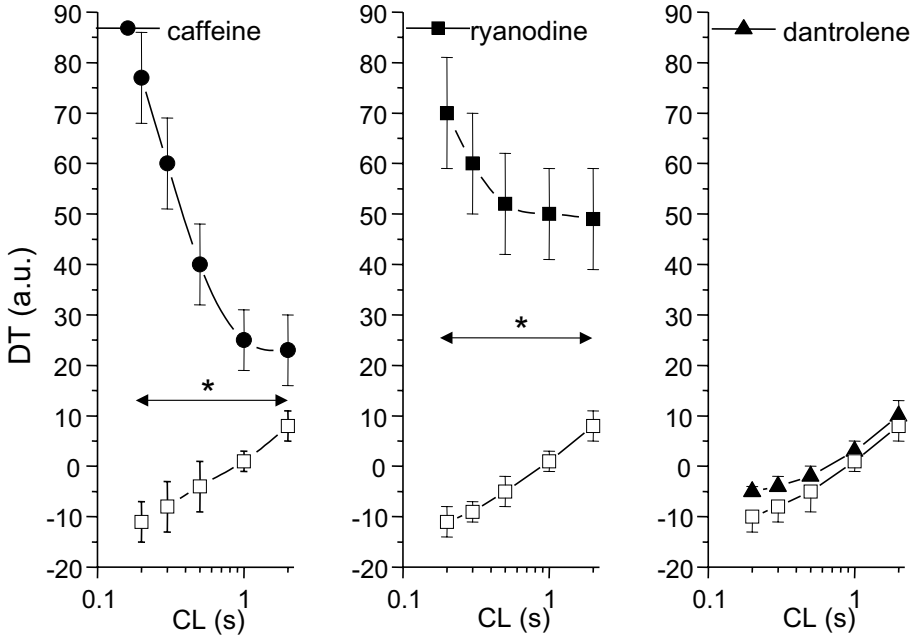

Figure 5. Diastolic tension (DT). The same order of panels and the same symbols as in Fig. 2. $n = 9$ (caffeine, left panel), $n = 10$ (ryanodine, middle panel), $n = 11$ (dantrolene, right panel), * $p < 0.05$.

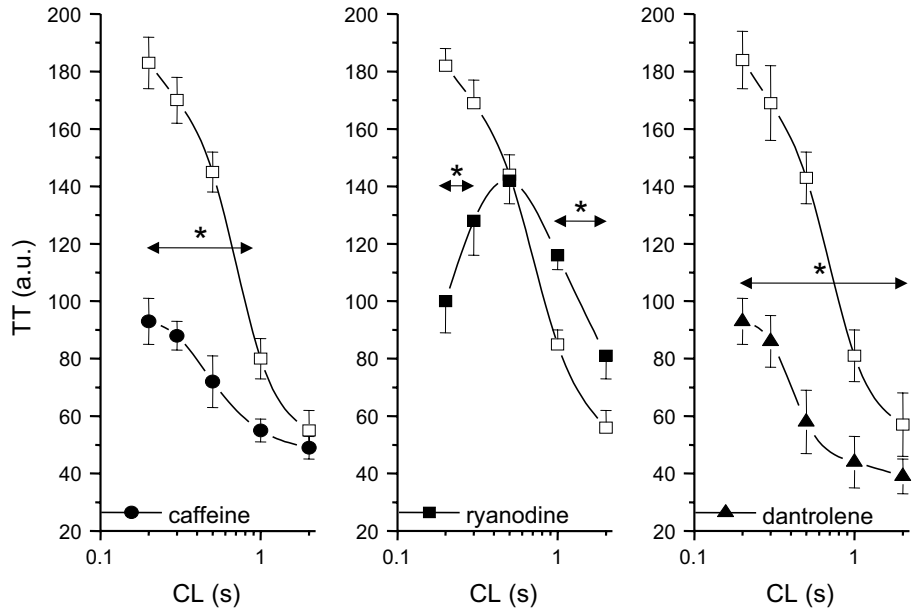

Figure 6. Total tension (TT = MG+DT). The same order of panels and the same symbols as in Fig. 2. $n = 9$ (caffeine, left panel), $n = 9$ (ryanodine, middle panel), $n = 10$ (dantrolene, right panel). * $p < 0.05$.

was more pronounced at short CLs. The dependence of TT on CL in the presence of caffeine or dantrolene remained inversely proportional. It should be emphasized that although the effects of caffeine and of dantrolene on TT were similar, the components of TT (MG and DT) were affected by these interventions differently (Figs. 2 and 5). In the presence of ryanodine (Fig. 6, middle panel) the dependence of TT on CL was bell-shaped with maximal value at CL of 500 ms. At short CLs, ryanodine decreased TT; at long CLs there was a tendency to increase TT.

Discussion

The results showed that DT in control increased with increasing CL. In the presence of caffeine and ryanodine the highest DT occured at short CLs and it decreased with prolonging CL. The effect of dantrolene on DT was negligible. MG in control reached maximal value at the shortest CL, with prolonging CL it steeply decreased. Caffeine diminished the dependence of MG on CL and reduced MG below minimal control values. In the presence of ryanodine, maximal MG occured at CL of 500 ms, at both longer and shorter CLs MG decreased. TTP in control increased from approximately 80 to 120 ms with prolonging CL (from 200 ms to 2 s). In the presence of caffeine, the dependence on CL remained similar, however there was a shift to smaller values (from 40 up to 70 ms). Ryanodine did not influence TTP significantly. Dantrolene exerted a significant shortening of TTP only at long CLs. dF/dt_{relax} in control decreased with increasing CL. Caffeine completely diminished the CL dependence and at short CLs even reversed it. Also ryanodine reduced the CL dependence of dF/dt_{relax}. Dantrolene shifted the curve to smaller values, but the CL dependence was preserved.

The shortening of TTP in the presence of caffeine was rather surprising. It is likely that the caffeine-dependent reduction in TTP is due to the fact that the contraction in the presence of caffeine starts from substantially higher levels of resting tension than in control. The addition of active contraction on top of this background tension is therefore less pronounced.

Weber (1968) demonstrated for the first time that caffeine leads to a release of Ca^{2+} from SR vesicles. In rat cardiomyocytes saturated with indo-1 caffeine decreased Ca^{2+} transients in a concentration-dependent manner (Negretti et al. 1993). This effect is due to activation of RyR of SR (Rousseau and Meissner 1989). Caffeine and theophylline also directly increase the sensitivity of myofilaments for Ca^{2+} (Fabiato A. and Fabiato F. 1973) and by that they magnify the negative lusitropic effect due to depletion of Ca^{2+} from SR (Vittone et al. 1994).

Ryanodine modifies the conductance and gating of RyR channel. At nano- to micromolar concentrations it induces subconductive channel states, at higher concentrations the RyR channel is closed completely (Xu et al. 1998). RyRs in mammalian ventricular myocardium are anatomically close to the L-type Ca^{2+} channels, which are largely localised in the sarcolemma of T-tubule. The narrow gap between RyR and L-type Ca^{2+} channel is easily bridged by diffusion of Ca^{2+} ions, which bind to RyRs and induce a release of Ca^{2+} from SR (Ca^{2+}-induced Ca^{2+}-release, CICR, Fabiato 1985) and consequent contraction.

Some authors consider that dantrolene could suppress CICR in myocardium. In rat papillary muscles, dantrolene (50 μm) showed a mild negative inotropic effect and it reduced aequorine Ca^{2+} transients. In papillary muscles with Ca^{2+}, overload dantrolene diminished diastolic oscillations of Ca^{2+}_i, consequently spontaneous contractions were inhibited and DT decreased (Meissner et al. 1996). In hamster left ventricular myocardium, dantrolene partially suppressed mechanical activity. The effects were much more pronounced in conditions of Ca^{2+} overload than in normal conditions (Satoh et al. 1996).

The relaxation of the cardiac muscle is auxotonic, i.e. the decrease in force and the lengthening of fibers proceed simultaneously (Chemla et al. 2000). With regard to the quantification of lusitropic mechanisms we have eliminated one variable (length of the preparation) by performing the measurements in isometric mode. The changes in the contraction force then reflect Ca^{2+}_i and metabolic state of preparation. Another argument in favor of isometric mode is the fact that changes in the length of muscle fiber can significantly influence the sensitivity of thin filaments for Ca^{2+}, and consequently the lusitropic characteristics (Solaro 2001).

Cardiac contraction was analysed in detail in many research papers, reviews and monographs (e.g. Bers 1991; Brady 1995). In contrast, the cardiac lusitropic functions, despite their theoretical attractiveness and clinical importance, were so far studied only unsystematically, usually with regard to direct clinical outcomes (Brutsaert and Sys 1989; Chemla et al. 2000). The reasons of this state are both historical and methodical since detailed understanding of processes involved in cardiac relaxation and DT regulation required transition from the whole-organ studies to the exploration of subcellular and molecular processes. The consensus opinion that starts to prevail, is that the diastolic functions of healthy heart are determined by the end-systolic volume and by the lusitropic functions. This aproach explains the independence of relaxation speed on the load in healthy myocardium and it is supported by the following arguments. According to Chemla et al. (2000), the systolic depot of potential energy and the intrinsic myofilament properties depend mainly on the lengths of myofibrils and only slightly on the load. The effect of the end-systolic length of myocardial fibers (i.e. end-systolic volume) on the speed and completness of relaxation is similar between mammals, including human (Courtois et al. 1992). The effect of load on these parameters in healthy human myocardium is negligible (Eichorn et al. 1992) and this was confirmed also in various animal models (Gaasch et al. 1980; Brutsaert and Sys 1989; Gillebert 1997). The intracardiac pressure starts to decrease and the fibers start to prolong when end-systolic length of fibers is reached. To clarify whether this view is more correct than the interpretation based on the effects of load, new experiments aimed especially at stereo-conformational and molecular aspects of sarcomere will be necessary.

Research of the interplay between cardiac systolic and diastolic processes clearly shows that the lusitropic func-

tions are in most cardiac diseases impaired earlier and more dramatically that the inotropic functions (Gaasch and Le Winter 1994). Slow and incomplete relaxation (increased resting tension) negatively influences cardiac filling, especially in combination with decreased compliance of cardiac cavities and shorter duration of diastole (due to increased heart rate). Heart failure on basis of lusitropic dysfunction in combination with hypertension represents in older patients an important clinical disorder (Mandinov et al. 2000).

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