Effect of inotropic interventions on contraction and Ca\textsuperscript{2+} transients in the human heart

KLARA BRIXIUS, MARCUS PIETSC, SUSANNE HOISCHEN, JOCHEM MÜLLER-EHMSEN, AND ROBERT H. G. SCHWINGER
Medizinische Klinik III, Universität zu Köln, D-50924 Cologne, Germany

Effect of inotropic interventions on contraction and on Ca\textsuperscript{2+} transients in the human heart. J. Appl. Physiol. 83(2): 652–660, 1997.—The present study investigated the influences of inotropic intervention on the intracellular Ca\textsuperscript{2+} transient [intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}i])] and contractile twitch. Isometric twitch and [Ca\textsuperscript{2+}], (fura 2 ratio method) were measured simultaneously (1 Hz, 37°C) after stimulation with Ca\textsuperscript{2+} (0.9–3.2 mM), the cardiac glycoside ouabain (Oua; 0.1 μM), the β₁- and β₂-adrenoceptor-agonist isoprenaline (Iso; 1–10 nM), and the Ca\textsuperscript{2+} sensitizer EMD-57033 (30 μM) by using isolated human nonfailing right auricular trabeculae (n = 19). Inotropic interventions increased force of contraction and peak rate of tension rise (+T) significantly. Only Iso stimulated peak rate of tension decay (−T) higher than +T (P < 0.05), thereby reducing time of contraction (T\text{switch}). EMD-57033 increased +T more effectively than −T and prolonged T\text{switch} (P < 0.05). Oua, Iso, and Oua, and Iso, but not EMD-57033, increased systolic Ca\textsuperscript{2+}. Diastolic Ca\textsuperscript{2+} increased after stimulation with Oua or Ca\textsuperscript{2+}, but not in the presence of EMD-57033. Iso shortened the Ca\textsuperscript{2+} transient and did not influence diastolic Ca\textsuperscript{2+}. In conclusion, positive inotropic agents differently affect force and [Ca\textsuperscript{2+}], depending on their mode of action. Inotropic interventions influence diastolic Ca\textsuperscript{2+} and thus may be less advantageous in a situation with altered intracellular Ca\textsuperscript{2+} homeostasis (e.g., heart failure due to dilated cardiomyopathy).

fura 2; heart failure; isoprenaline; ouabain; EMD-57033

Intracellular Ca\textsuperscript{2+} homeostasis plays a central role in regulating excitation-contraction coupling in the mammalian heart. The contractile state of the heart is dependent on the availability of intracellular free Ca\textsuperscript{2+} to the myofilaments. Ca\textsuperscript{2+} enters the cytosolic compartment through the sarcolemma from the extracellular space or through the sarcoplasmic reticulum (SR) from intracellular compartments, i.e., Ca\textsuperscript{2+}-triggered Ca\textsuperscript{2+} release (19). Increasing the systolic intracellular free Ca\textsuperscript{2+} concentration runs parallel to changes in maximal force development (13). Even in the terminally failing human myocardium, elevation of the concentration of intracellular Ca\textsuperscript{2+} results in an increase in force of contraction (FOC) similar to that in nonfailing tissue (12, 22). Therefore, inotropic enhancement has been suggested to still be an effective means of increasing force maximally even in diseased human hearts, i.e., terminally failing myocardium due to dilated cardiomyopathy (12, 22).

The high-systolic Ca\textsuperscript{2+} concentrations may be decreased to low-diastolic Ca\textsuperscript{2+} levels by three main mechanisms: 1) Ca\textsuperscript{2+} will be taken up into the SR by activation of SR or endoplasmic reticulum Ca\textsuperscript{2+}-adenosinetriphosphatase (SERCA); 2) activation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger will lead to Ca\textsuperscript{2+} extrusion out of the cytoplasm; and 3) Ca\textsuperscript{2+} buffer proteins will bind free Ca\textsuperscript{2+} ions. Interventions that lead to an identical increase in force after augmentation of intracellular Ca\textsuperscript{2+} concentration may affect parameters of contraction and relaxation in a different mode depending on their actions on the intracellular Ca\textsuperscript{2+}-lowering systems. This mode of action of an inotropic compound has to be kept in mind, especially when the compound is being used to increase force in diseased human myocardium with an already increased diastolic Ca\textsuperscript{2+} level (6).

Force development and maximal systolic free Ca\textsuperscript{2+} concentration have been extensively studied in numerous excellent investigations by using the Ca\textsuperscript{2+} indicators aequorin (12, 18), quin 2 (14) and indomethacin 1 (27). Because alterations of contraction-coupling may be largely influenced by changes of diastolic Ca\textsuperscript{2+}, Ca\textsuperscript{2+} indicators focusing on changes in low-diastolic Ca\textsuperscript{2+} levels have been developed. Fura 2 is an example (11). Because of its low dissociation constant (228 nM), fura 2 is a Ca\textsuperscript{2+} fluorescence indicator that has been reported to be more suitable in measuring changes of diastolic Ca\textsuperscript{2+} than aequorin (11). Use of fura 2 has shown that peak Ca\textsuperscript{2+} transients were reduced, diastolic Ca\textsuperscript{2+} levels were increased, and the diastolic Ca\textsuperscript{2+} decay was significantly prolonged in cardiomyocytes from patients with terminal heart failure due to dilated cardiomyopathy (6). In addition, the fura 2 ratio method has been used as a suitable tool for studying intracellular Ca\textsuperscript{2+} transients and force in isolated muscle strip preparations of rat myocardium (3).

In the present study, intracellular free Ca\textsuperscript{2+} concentrations were measured simultaneously with parameters of FOC by using the fura 2 ratio method after inotropic interventions. Because different inotropic interventions may influence force development and intracellular Ca\textsuperscript{2+} homeostasis, depending on their mode of action, the purpose of the present study is to investigate the effect of inotropic stimulation on force development and intracellular Ca\textsuperscript{2+} simultaneously. Parameters of contraction and the intracellular Ca\textsuperscript{2+} transient were measured after inotropic stimulation with 1) adenosine 3',5'-cyclic monophosphate (cAMP)-independent inotropes [the cardiac glycoside ouabain (Oua)] or elevation of extracellular Ca\textsuperscript{2+}; 2) the cAMP-dependent inotrope, the β-adrenoceptor-agonist isoprenaline (Iso); and 3) the Ca\textsuperscript{2+} sensitizer EMD-57033.

MATERIALS AND METHODS

Preparation of isolated auricular trabeculae. Right atrial tissue was taken from patients undergoing aortocoronary

652 0161-7567/97 $5.00 Copyright © 1997 the American Physiological Society http://www.jap.org
bypass surgery \( n = 19, 17 \text{ men and 2 women; age } 57.8 \pm 2.6 \text{ yr (range } 41-71 \text{ yr)} \) without clinical signs of cardiac failure as measured by heart catheterization (normal ejection fraction, end-diastolic volume, and stroke volume) and by echocardiography. None of the patients had received either cardiac glycosides, Ca\(^{2+}\)-channel antagonists, or Ca\(^{2+}\)-channel agonists within 7 days of surgery or \( \beta \)-adrenoceptor agonists 48 h before surgery. Patients gave written informed consent before the operation. Drugs used for general anesthesia were flunitrazepam, fentanyl, and pancuronium bromide with isoflurane. The tissue was delivered to the laboratory within 10 min in ice-cold preoxygenated Bretschneider solution of the following composition (in mM): 15 NaCl, 10 KCl, 4 MgCl\(_2\), 180 histidine, 2 tryptoophan, 30 mannitol, and 1 potassium dihydrogen oxoglutarate. From each native myocardial tissue sample, auricular trabeculae 0.6–0.8 mm wide and 6–8 mm long were selected under microscopic observation (Axiovert 100, Zeiss, Oberkochen, Germany) and carefully prepared to avoid cutting injury.

Fura 2 loading. Intracellular Ca\(^{2+}\) was measured by the fluorescence indicator fura 2 (10). To facilitate cell loading, fura 2 was used as acetoxymethyl (AM) ester. These AM esters passively cross the plasma membrane and, once inside the cell, are cleaved to cell-impermeant products by intracellular esterases (10). For the initial control measurement of FOC, one end of the muscle strips was clamped at a muscle holder, and the other end was attached to a force transducer (SI, Heidelberg, Germany). The muscle fibers were superfused with an oxygenated (95% O\(_2\)-5% CO\(_2\)) Tyrode solution (in mM): 119.8 NaCl, 5.4 KCl, 1.05 MgCl\(_2\), 0.9 CaCl\(_2\), 22.6 NaHCO\(_3\), 0.42 NaHPO\(_4\), 0.28 ascorbic acid, and 0.05 disodium EDTA (37°C, pH 7.40). The muscles were stretched until an optimal force was generated while they were stimulated by a pulse generator (Föhr Medical Instruments, Egelsbach, Germany) with a square-wave pulse (field stimulation) of 10-ms duration 10% above threshold voltage at a frequency of 1 Hz. Muscle strips with adequate mechanical performance were incubated for 4 h in darkness to avoid photobleaching of the dye in an oxygenated (95% O\(_2\)-5% CO\(_2\)) Ringer solution (in mM): 147 NaCl, 4 KCl, and 2.2 CaCl\(_2\) (22°C, pH 7.4) containing 5 µM of fura 2-AM. The nontoxic detergent cremophor EL (0.5%) was added to this incubation solution to increase the permeability of the sarcolemma of the muscle cells to fura 2-AM. Experiments were performed as previously described (29).

Ca\(^{2+}\) and force measurement. After fura 2 loading, the muscles were rinsed with oxygenated Tyrode solution for 15 min. Afterward, the muscle strips were again fixed at both ends between the muscle holder and the force transducer. Fibers were only used for further experiments when isometric force developed after fura 2 loading was at least 90% of the initial control value before loading. The force transducer was connected by an analog-to-digital converter to a personal computer. For online data analysis, special software was used (SI).

Fura 2 fluorescence was measured by using a dual-wavelength fluorometer equipped with an inverted microscope as shown in Fig. 1 (SI). Light was emitted through a mercury arc lamp (USH-IO2DH, Ushio, Tokyo, Japan). A rotating filter wheel allowed alternating excitation at wavelengths of either 340 or 380 nm with a frequency of alteration of 125 Hz. The emitted fluorescent light resulting from the excitation with one of these wavelengths was recorded at 510 nm and sorted in the respective channels of the photomultiplier. Ca\(^{2+}\) transients were digitized, and the fluorescence ratio was stored in a personal computer. A shutter was placed between the mercury arc lamp and the filter wheel to avoid photobleaching of fura 2 by continuous exposure to the excitation light. The shutter could be manually opened and closed, respectively. In this way, the trabeculae were only exposed to the excitation light at definite time points during the experiments. Every 10 min, for measurement of the inotropic effect of ouabain or EMD-57033 or at the time when the maximal inotropic effect after application of Iso or Ca\(^{2+}\) was reached, fura 2 light emission was recorded. The FOC was continuously recorded by an oscilloscope. Control experiments were performed to measure fura 2 fluorescence under the same experimental conditions in muscle strips from the same heart samples without drug addition. Fura 2 fluorescence did not change during the experimental conditions used.

The twitch of contraction and the intracellular Ca\(^{2+}\) signal were simultaneously monitored after stimulation with 0.9–3.2 mM Ca\(^{2+}\), 0.1 µM ouabain, 1–10 nM Iso, and 30 µM EMD-57033. The following parameters were determined: FOC (in mN); time to peak tension (TPT; in ms); time to half

![Schematic representation of experimental design for intracellular Ca\(^{2+}\) measurement.](image-url)
Materials. Fura 2-AM was obtained from Molecular Probes (Eugene, OR). Iso and Triton X were purchased from Sigma Chemical (Deisenhofen, Germany). Oua was generously provided by Herbert Pharma (Wiesbaden-Bierstadt, Germany). EMD-57033 was a gift of Dr. I. Lues (Merck, Darmstadt, Germany). All other chemicals were of analytic grade or the best grade commercially available. A stock solution of 10 mM fura 2-AM was dissolved in dimethyl sulfoxide (DMSO) and stored at –20°C as previously described (29). For studies with isolated cardiac preparations, stock solutions were prepared daily in twice-distilled water. EMD-57033 was dissolved in 100% DMSO. The final concentration of DMSO in the perfusion solution never exceeded 0.05%.

Statistical analysis. Data are means ± SE. For comparison within one group, the paired t-test was applied. Otherwise, statistical significance was analyzed with Student’s t-test for unpaired observations or by analysis of variance. A value of P < 0.05 was considered significant. In the experiments, the inotropic effect of the drug in each muscle strip was compared with the control, drug-free situation of the very same preparation. Statistical analysis was performed according to Wallenstein et al. (30). Statistical evaluation was confirmed by the “Medizinisches Rechenzentrum” of the University of Cologne.

RESULTS

Isometric twitch and Ca²⁺ transient. Changes in the intracellular Ca²⁺ transient and contractile twitch were studied simultaneously in isolated right auricular trabeculae of human nonfailing myocardium (n = 19). Figure 3 shows original tracings of the contractile twitch and the Ca²⁺ transient (A) and the relationship between the time parameters of the fura 2 fluorescence signal and the contraction (B). Isometric twitch and Ca²⁺ transient started at nearly the same time, but the maximum of the Ca²⁺ transient was reached before the peak of contractile twitch. In contrast, the relaxation of the isometric twitch was finished before the fura 2 signal had reached its basal value. Parameters for force development and Ca²⁺ transient are shown in Table 1. Thus the time course of the Ca²⁺ transient as measured by fura 2 was longer than T twitch, as a result of the slow decline of the high systolic Ca²⁺ levels measured by fura 2. By measurement of the intracellular Ca²⁺ transient with fura 2, a fast systolic Ca²⁺ increase and a prolonged diastolic Ca²⁺ decrease can be monitored in human nonfailing right auricular trabeculae.

Inotropic stimulation with Ca²⁺ and ouabain. Figure 4 shows original tracings taken at control conditions...
A

\[
\begin{align*}
R340/380_{Ca} & \quad 0.94 \pm 0.15 \\
RPR & \quad 52.5 \pm 3.1 \\
R. R & \quad 177.5 \pm 9.0^f \\
T_{Ca} & \quad 539.1 \pm 31.0^f 
\end{align*}
\]

Values are means ± SE; n = 24. FOC, force of contraction; TPT, time to peak tension; T‰, time to half peak relaxation; +T, maximal rate of tension increase; −T, maximal rate of tension decrease; T\text{twitch}, duration of contraction; R340/380_{Ca}, peak Ca\textsuperscript{2+}; RPR, time to peak Ca\textsuperscript{2+}; R. R, time to half peak Ca\textsuperscript{2+} relaxation; T\text{Ca}, duration of Ca\textsuperscript{2+} transient. *P < 0.05 vs. Ca\textsuperscript{2+} transient. †Signal was not quantified.

Table 1. Parameters of contractile twitch and of Ca\textsuperscript{2+} transient of isolated right auricular muscle strip preparations from nonfailing human myocardium (0.9 mM Ca\textsuperscript{2+}, 1 Hz, pH 7.4)

Table 2. Effects of inotropic interventions on contractile force and Ca\textsuperscript{2+} transient

\[
\begin{align*}
\text{Ca\textsuperscript{2+}} & \quad \text{Oua} & \quad \text{Iso} & \quad \text{EMD-57033} \\
\text{FOC} & \quad \uparrow & \quad \uparrow & \quad \uparrow & \quad \uparrow \\
TPT & \quad = & \quad = & \quad = & \quad = \\
T‰ & \quad \uparrow & \quad \uparrow & \quad \uparrow & \quad = \\
+T & \quad \uparrow & \quad \uparrow & \quad \uparrow & \quad = \\
−T & \quad = & \quad = & \quad = & \quad = \\
T_{\text{twitch}} & \quad = & \quad = & \quad = & \quad = \\
\text{Ca\textsuperscript{2+} transient} & \quad & \quad & \quad & \\
R340/380_{Ca} & \quad \uparrow & \quad \uparrow & \quad \uparrow & \quad = \\
RPR & \quad = & \quad = & \quad = & \quad = \\
R. R & \quad = & \quad = & \quad = & \quad = \\
R340/380_{sys} & \quad = & \quad = & \quad = & \quad = \\
R340/380_{ED} & \quad = & \quad = & \quad = & \quad = \\
T_{Ca} & \quad = & \quad = & \quad = & \quad = \\
\end{align*}
\]

(0.9 mM Ca\textsuperscript{2+}, 1 Hz, 37°C) and after the application of 3.2 mM Ca\textsuperscript{2+} (Fig. 4A) or 0.1 µM Oua (Fig. 4B; see also Table 2) to stimulate isometric FOC.

An elevation of extracellular Ca\textsuperscript{2+} concentration (0.9–3.2 mM) resulted in a significant increase in FOC from

Fig. 4. Original tracings of alterations of isometric twitch and intracellular Ca\textsuperscript{2+} transient during elevation of extracellular Ca\textsuperscript{2+} concentration (A) and application of 0.1 µM ouabain (B) in human myocardium. Both substances enhanced FOC and end-diastolic and systolic Ca\textsuperscript{2+}. 
2.1 ± 0.5 to 4.2 ± 0.7 mN and in +T from 25.5 ± 5.1 to 62.1 ± 9.3 mN/s, respectively. The corresponding values for −T were 11.2 ± 3.2 and 24.4 ± 2.6 mN/s. No significant alterations in TPT (117.1 ± 5.9 vs. 113.3 ± 5.8 ms), T1/T (114.4 ± 10.5 vs. 120.6 ± 9.0 ms), and To_w (451.1 ± 24.6 vs. 503.3 ± 30.0 ms) were observed (Fig. 5). Elevation of the extracellular Ca2+ concentration from 0.9 to 3.2 mM enhanced FOC, +T, and −T to a similar degree (FOC: 221.1 ± 41.9%; +T: 233.0 ± 34.9%; −T: 231.4 ± 36.8%).

The cardiac glycoside Oua (0.1 µM) significantly increased FOC (12.0 ± 3.3 to 2.8 ± 0.7 mN), +T (17.8 ± 4.1 to 43.4 ± 12.5 mN/s), and −T (9.2 ± 2.2 to 20.0 ± 5.4 mN/s). The application of 0.1 µM Oua did not influence TPT (115.0 ± 9.7 vs. 127.5 ± 13.0 ms), T1/T (110.8 ± 13.0 vs. 130.8 ± 10.8 ms), and To_w (463.3 ± 44.5 vs. 476.7 ± 47.3 ms) significantly. In addition, 0.1 µM Oua increased FOC, +T, and −T to a similar extent (FOC: 221.1 ± 12.6%; +T: 230.9 ± 15.0%; −T: 213.9 ± 10.0%) (Fig. 5).

Figure 6 illustrates the concentration- and time-dependent effects, respectively, of the cAMP-dependent inotropes Ca2+ and Oua on FOC and intracellular Ca2+ concentration. In the presence of Oua or after elevation of extracellular Ca2+, an increase in the systolic Ca2+ transient (Fig. 6, C and D) was accompanied by an increase in FOC. Both Ca2+ and Oua significantly increased maximal systolic Ca2+ (Ca2+: 188.7 ± 24.7% Oua: 143.4 ± 16.6%) and end-diastolic Ca2+ (R340/380ED Ca2+: 60 ± 20% of the basal fura 2 amplitude; R340/380ED Oua: 56 ± 17% of the basal fura 2 amplitude). In the presence of 3.2 mM Ca2+, or 0.1 µM Oua, time parameters of the Ca2+ transient were not changed compared with basal condition.

Inotropic stimulation with Iso. In contrast to Ca2+ and Oua, Iso mediates its positive inotropic effect by increasing the concentration of intracellular cAMP. Figure 7 represents two original tracings of the course of contraction and Ca2+ transient under control conditions and in the presence of 10 nM Iso. Iso significantly increased the parameters of cardiac contractility (for FOC, basal: 2.4 ± 0.4 mN, 10 nM Iso: 3.8 ± 0.6 mN; +T, basal: 41.6 ± 10.6 mN/s, 10 nM Iso: 58.0 ± 10.2 mN/s) and relaxation (−T, basal: 20.7 ± 5.1 mN/s, 10 nM Iso: 35.5 ± 6.7 mN/s). The increase in cardiac relaxation was more pronounced (−T, basal: 211.0 ± 20.9%) compared with the increase in the parameters of cardiac contraction (FOC, 164.6 ± 17.3% basal; +T 142.0 ± 14.5% basal). Iso had no influence on TPT but significantly decreased T1/T (basal: 89.4 ± 7.6 ms, 10 nM Iso: 78.8 ± 6.6 ms), thus reducing To_w (basal: 405.6 ± 27.6 ms, 10 nM Iso: 356.1 ± 24.5 ms; Fig. 8). Figure 9 illustrates the effect of increasing concentrations of Iso on FOC (A and B) and changes in intracellular Ca2+ (C and D) in human myocardium. Iso concentration dependently increased FOC and intracellular Ca2+. Iso did not change end-diastolic Ca2+ levels (R340/380ED Ca2+: −2.8 ± 7% of the basal fura 2 amplitude). Iso significantly reduced R1/2R (basal: 185.0 ± 20.0 ms, 10 nM Iso: 158.3 ± 19.6 ms) and TCa (basal: 628.0 ± 73.8 ms, 10 nM Iso: 574.0 ± 69.4 ms).
Inotropic stimulation with EMD-57033. An original tracing of the experiments under control conditions and after the application of 30 µM EMD-57033 is given in Fig. 10. EMD-57033 significantly increased FOC (1.5 ± 0.5 vs. 2.6 ± 0.9 mN) and +T (17.9 ± 8.6 vs. 28.2 ± 13.4 mN). In contrast, EMD-57033 failed to increase −T. EMD-57033 had no significant influence on TPT (106.0 ± 6.8 vs. 116.0 ± 6.8 ms) but significantly prolonged TₛT (114.0 ± 13.2 vs. 144.0 ± 15.0 ms) and TₛTw (450.0 ± 41.0 vs. 672.0 ± 44.7 ms; Fig. 8). EMD-57033 did not influence systolic Ca²⁺ (R₃₄₀/₃₈₀Ca: 76 ± 10%) or end-diastolic Ca²⁺ levels (R₃₄₀/₃₈₀ED: −15 ± 9% of basal fura 2 fluorescence amplitude). Time parameters of Ca²⁺ transient were unaffected after stimulation of FOC with EMD-57033 as well (Fig. 8). Figure 9 compares the concentration-dependent effects of Iso and EMD-57033 on FOC and intracellular Ca²⁺. Both EMD-57033 and Iso increased FOC. However, only the inotropic effect of Iso, and not of EMD-57033, was accompanied by an increase in intracellular Ca²⁺.

DISCUSSION

Abnormalities in intracellular Ca²⁺ handling may become exaggerated under stimulated conditions, i.e., after inotropic stimulation of FOC with agents increasing systolic Ca²⁺. Thus it seems worthwhile to simultaneously study the influence of various inotropic interventions on the Ca²⁺ transient (systolic and diastolic Ca²⁺) and the contractile twitch in human myocardium. In the present study, inotropic stimulation was achieved by an elevation of extracellular Ca²⁺ concentration, by application of the cardiac glycoside ouabain, the β-adrenoceptor-agonist Iso, and EMD-57033, which has been suggested to increase the Ca²⁺ sensitivity of the contractile filaments. Because attention has to be
focused on diastolic Ca^{2+} changes, the Ca^{2+} indicator fura 2 was used. Fura 2 was shown to be suitable to measure changes of low Ca^{2+} concentrations (8, 11).

Under basal conditions (1 Hz, 37°C), the T_{Ca} as measured by fura 2, was significantly prolonged compared with T_{twitch}. These findings are in contrast with observations obtained with the indicator aequorin in right ventricular myocardium of failing and nonfailing human hearts (12, 18). In these studies the Ca^{2+} transient declined toward baseline before the mechanical event was finished. However, differences might exist in Ca^{2+} handling and sensitivity depending on the origin of the muscle, e.g., right vs. left ventricle or atrium (23). Furthermore, there are important differences in the experimental conditions from those in the studies of Morgan (18) and Gwathmey et al. (12). In the present study the isolated right auricular trabeculae were perfused at a temperature of 37°C and electrically stimu-
lated at a frequency of 1Hz. The experiments of Morgan (18) and Gwathmey et al. (12) were performed at a temperature of 30°C and a stimulation frequency of 0.33 Hz. Frequency-dependent changes of FOC depend on experimental temperature and frequency range investigated (7). In addition, intracellular Ca²⁺ transients may change in a frequency-dependent mode as well (21).

The Ca²⁺ transients in the studies of Morgan (18) and Gwathmey et al. (12) were measured by the Ca²⁺ indicator aequorin. In the present study fura 2 was used as Ca²⁺ indicator. Fura 2 has a high affinity for Ca²⁺, with an in vitro dissociation constant close to 200 nM (11). The binding kinetics of fura 2 have been calculated to be much slower in the myofibrillar environment (23 s⁻¹) (4) than in salt solutions (84 s⁻¹) (15). In addition, the Ca²⁺ indicator aequorin is very sensitive to changes in peak systolic Ca²⁺ changes but less sensitive in the low-Ca²⁺ concentration range during diastole (8). The magnitude of the Ca²⁺ transient measured by fura 2 (or other indicators) can only be evaluated after calibration of the signals (5). The present study demonstrates the prolongation of the duration of the fura 2 signal compared with $T_{\text{switch}}$, which is in agreement with the study of Baylor and Hollingwood (4).

The various inotropic agents used (Ca²⁺, ouabain, Iso, EMD-57033) increased contractile force but differently affected the contractile cycle and the intracellular Ca²⁺ transient (Table 2). Ouabain and Ca²⁺ increased systolic and diastolic intracellular Ca²⁺ in parallel to force development. Iso increased intracellular Ca²⁺, but not diastolic Ca²⁺, and initiated a fast decline of the high systolic Ca²⁺ levels. The Ca²⁺ sensitizer EMD-57033 significantly increased force but had no influence on parameters of the intracellular Ca²⁺ transient.

The Ca²⁺-induced increase of intracellular Ca²⁺ concentration in human myocardium confirms the findings of Tatsukawa et al. (28) and Frampton et al. (10) by using fura 2 in isolated rat ventricular myocytes. It is also consistent with data observed by using the bioluminescent aequorin in quiescent ferret ventricular muscle preparation (1) or in cultured chick embryonic heart cells by using indomethacin 1 as Ca²⁺ indicator (20). Therefore, an increase in extracellular Ca²⁺ will increase the systolic as well as the diastolic Ca²⁺ as measured by the fura 2 ratio method by using multielement or single-cell preparations.

The cardiac glycoside ouabain influenced the time course of contraction similarly to Ca²⁺. Ouabain increased systolic and diastolic Ca²⁺ without effect on the time parameters of the Ca²⁺ transient. These results are confirmed by studies of cultured rat ventricular cells (16, 28).

In contrast to Ca²⁺ and ouabain, Iso accelerated cardiac relaxation more than contraction. This increase of relaxation was paralleled by an increase in the decay of the intracellular Ca²⁺ transient. The β-adrenoceptor agonist Iso stimulates adenylyl cyclase, leading to an elevation of intracellular cAMP, which activates intracellular protein kinases phosphorylating a number of subcellular sites. Phosphorylation of the voltage-dependent Ca²⁺ channel increases intracellular Ca²⁺ influx during each depolarization and thereby the amount of Ca²⁺ in the cell. As a result, systolic force is increased. Because cAMP-dependent protein kinase also leads to phosphorylation of troponin I, the Ca²⁺ sensitivity of the contractile apparatus will be reduced. Thus β-adrenoceptor agonists should increase intracellular Ca²⁺ concentration to a greater extent than force to initiate the same effectiveness as an elevation of the extracellular Ca²⁺ concentration. Phosphorylation of phospholamban, the regulatory subunit of the Ca²⁺ pump of the SR, enhances the rate of resequestration of intracellular Ca²⁺ during diastole, thus also leading to an increased Ca²⁺ uptake and relaxation during diastole.

A new class of inotropic compounds has been developed that enhances the sensitivity of the myofilaments to Ca²⁺, rather than increasing the Ca²⁺ availability to the myofilaments. EMD-57033 is the (+) enantiomer of the inotropic agent EMD-53998 (23). It has been found to possess potent Ca²⁺-sensitizing action with little effect on phosphodiesterases (26). The mechanism by which EMD-57033 increases the Ca²⁺ sensitivity of the myofilaments is unknown. Preliminary reports have indicated that EMD-57033 has no effect on Ca²⁺ binding to troponin C and is unlikely to affect thin-filament interactions, suggesting that it affects the cross-bridge cycling mechanism itself. In the human myocardium EMD-57033 increased FOC (26) and slowed cardiac relax-
changes in diastolic Ca^{2+} concentrations, as shown in the present study. The various inotropic interventions examined in this study differentially influenced diastolic Ca^{2+}. At low concentrations the CAMP-dependent inotrop I so accelerated relaxation (24) and did not enhance diastolic Ca^{2+} levels or diastolic tension. Diastolic Ca^{2+} increased after stimulation with ouabain or Ca^{2+}, but not in the presence of EMD-57033. In human heart failure, changes in diastolic Ca^{2+} have been demonstrated, which have been suggested to be at least partly responsible for the altered force-frequency relationship. Low concentrations of I so reversed the negative force-frequency relationship to a positive one (24). This has been suggested to be due to an enhanced activity of SERCA

After stimulation with ouabain or Ca^{2+}, relaxation but did not affect systolic or diastolic intracellular Ca^{2+} concentrations, as shown in the present study.

The cellular basis of the force-frequency relationship to a positive one (24). This suggests that a new activity has been demonstrated, which has been suggested to be at least partly responsible for the altered force-frequency relationship. Low concentrations of I so reversed the negative force-frequency relationship to a positive one (24). This has been suggested to be due to an enhanced activity of SERCA

The authors thank A. Herber, A. Gross, and T. Schewior for excellent technical help.

This work was supported by the Deutsche Forschungsgemeinschaft (Fo¨rderkennzeichen01KS9502), and the Graduiertenkolleg Molekular-T. S. Schewior for excellent technical help.

We are indebted to all colleagues in the Department of the CellCalcium.


REFERENCES


