Effect of Cyclopiazonic Acid on the Force-Frequency Relationship in Human Nonfailing Myocardium

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ABSTRACT

The present study investigated the functional role of the sarcoplasmatic reticulum Ca$^{2+}$-ATPase in contraction and relaxation, intracellular Ca$^{2+}$-transients, as well as on the force-frequency relationship in human myocardium. The Ca$^{2+}$-ATPase activity of membrane vesicles isolated from sarcoplasmatic reticulum (SR) obtained from nonfailing donor hearts (n = 7) was measured in the presence of cyclopiazonic acid (CPA, 0–30 μM), a highly specific inhibitor of the Ca$^{2+}$-ATPase of the SR (SERCA). The effects of CPA on parameters of contraction and relaxation, force-frequency relationship and [Ca$^{2+}$], transients (with fura-2) were studied on isolated left ventricular muscle strips from human nonfailing myocardium. CPA concentration-dependently inhibited SERCA activity of isolated SR vesicles. In the presence of CPA (30 μM) the former positive force-frequency relationship in human left ventricular nonfailing myocardium became negative. Especially at high frequencies of stimulation, CPA decreased developed tension, peak rate of tension rise and systolic fura-2-light emission, whereas time to peak tension, time to peak [Ca$^{2+}$], time to 95% relaxation, diastolic tension and diastolic Ca$^{2+}$ levels were increased. Peak rate of tension decay and time to half-relaxation and half-decay of [Ca$^{2+}$] were not altered significantly after treatment with CPA. These findings provide evidence that the SERCA plays a functional role in the frequency-dependent increase in force of contraction in human myocardium. Because an impaired function of the SERCA is predominantly followed by alterations of inotropic and to a lesser degree of lusitropic function, other important factors to lower [Ca$^{2+}$], and influence relaxation may be present in human myocardium to compensate for the reduced SERCA activity, e.g., Na$^{-}$-Ca$^{2+}$ exchanger.

The frequency-dependent changes in force of contraction of isolated myocardium is present in most mammalian species, but also seems to be species dependent (Buckley et al., 1972). Whereas in specimens like guinea-pig, rabbit and human myocardium an increase of the frequency of stimulation is followed by an increase in developed tension, also called positive FFR or “Bowditch-Treppe,” a negative FFR is present in the rat. It is widely accepted that the FFR and the kinetics of the myocardial contraction cycle strongly depend on the intracellular Ca$^{2+}$ homeostasis (Gwathmey et al., 1990; Pieske et al., 1995). The Ca$^{2+}$-ATPase of the sarcoplasmic reticulum and the Na$^{+}$-Ca$^{2+}$ exchanger of the sarcolemma seem to be the most important mechanisms to extrude Ca$^{2+}$ from the cytosol during diastole and lower high systolic intracellular Ca$^{2+}$ concentrations initiating an effective relaxation (Bassani et al., 1994). The contribution of the SR-Ca$^{2+}$-ATPase and the Na$^{+}$-Ca$^{2+}$ exchanger in the regulation of the intracellular Ca$^{2+}$ during contraction and relaxation is species-dependent. In rat myocardium, the SR-Ca$^{2+}$-ATPase predominates in the function of lowering intracellular Ca$^{2+}$, whereas the contribution of the Na$^{+}$-Ca$^{2+}$ exchanger seems to be negligible. In contrast, in rabbit myocardium, Ca$^{2+}$ extrusion via the Na$^{+}$-Ca$^{2+}$ exchanger has a more important contribution on Ca$^{2+}$ extrusion out of the cytosol besides the function of the SR-Ca$^{2+}$-ATPase compared with rat myocardium (Bassani et al., 1994). The relative contribution of the SR-Ca$^{2+}$-ATPase to the provision of the Ca$^{2+}$ involved in contractile activation and relaxation in human myocardium is still a matter of debate. Only indirect data are presently available (Hasenfuss et al., 1994).

To evaluate the role of the SR-Ca$^{2+}$-ATPase for the regulation of contraction-twitch intracellular Ca$^{2+}$-transients

ABBREVIATIONS: +T, peak rate of tension rise; −T, peak rate of tension decay; TPT, time to peak tension; T1/2T, time to half relaxation; T95T, time to 95% relaxation; SR, sarcoplasmic reticulum; SERCA, SR-Ca$^{2+}$-ATPase; CPA, cyclopiazonic acid; FFR, force-frequency relationship; I1/2I, time at half decay of Ca$^{2+}$; Isysp, systolic light emission; IPI, time to peak Ca$^{2+}$; Na$^{+}$EDTA, ethylenedinitrilotetraacetic acid disodium salt dihydrate; AM, acetoxyethyl; EGTA, ethyleneglycol-bis(2-aminoethoxy)ether)-N,N',N'-tetraacetic acid.
and the force-frequency relationship in human myocardium, we used the highly specific inhibitor of the SR-Ca\(^{2+}\)-ATPase CPA. CPA, a myotoxic produced by various fungi of Aspergillus and Penicillium species, has been reported to be a selective inhibitor of the SR-Ca\(^{2+}\)-ATPase expressed in fast-twitch muscle (Seidler et al., 1989), smooth muscle (Uyama et al., 1992) and also cardiac muscle (Takahashi et al., 1995; Yard et al., 1994). It has been reported to be without effect on F-type ATPase, such as mitochondrial H\(^+\)K\(^-\)-ATPase and on several other P-type ATPases, such as Na\(^+\)K\(^-\)-ATPase of the kidney and brain and the Ca\(^{2+}\)-ATPase of the plasma membrane (Seidler et al., 1989). Even at high concentrations, CPA has no effect on Ca\(^{2+}\)-sensitivity of the contractile apparatus (Takahashi et al., 1995), Ca\(^{2+}\) currents (Bonnet et al., 1994; Badawi et al., 1995) and the Na\(^+\)Ca\(^{2+}\) exchanger (Yard et al., 1994) beside its inhibitory action on the SR-Ca\(^{2+}\)-ATPase in cardiac muscle. Thus, because of its high specificity CPA is a useful pharmacological tool to investigate the predominant contribution of the SR-Ca\(^{2+}\)-ATPase to the intracellular Ca\(^{2+}\) homeostasis and the kinetics of contraction at different frequencies of stimulation in human myocardium.

**Materials and Methods**

**Human myocardium.** Nonfailing human myocardium was obtained from seven donors who were brain dead as a result of traumatic injury. These nonfailing hearts could not be used for transplantation for technical reasons. Patient history of the organ donors (age: 45 ± 6 years) revealed no evidence of heart disease.

**Cardiac muscle strip preparation and measurement of force of contraction.** Immediately after excision, the papillary muscles were placed in ice-cold preaerated Tyrode's solution (for composition see below) and delivered to the laboratory within 10 min. From each native myocardial tissue sample papillary muscle strips were prepared (less than 0.8 mm width and 8–10 mm length) with muscle fibers running in parallel to the length of the strips. Connective tissue was carefully trimmed away. The muscles were suspended in an organ bath (25 ml) at 37°C containing a modified Tyrode's solution of the following composition (in mM): NaCl, 119.8; KCl, 5.4; MgCl\(_2\), 1.05; CaCl\(_2\), 1.8; NaHCO\(_3\), 22.6; NaH\(_2\)PO\(_4\), 0.42; glucose, 5.05; ascorbic acid, 0.28; Na\(_2\)EDTA, 0.05. The bathing solution was continuously aerated with 95% O\(_2\) and 5% CO\(_2\). The muscles were stimulated by two platinum electrodes by field stimulation from a Grass S 88 stimulator (frequency 1 Hz; duration 5 ms; intensity 10–20% above threshold). Preparations were allowed to equilibrate for at least 90 min, with the bathing solution being changed once after 45 min. Isometric force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, Germany or Föhr Medical Instruments GmbH, Egelsbach, Germany) attached to a Hellige Helco scriptor (Hellige, Freiburg, Germany) or Gould recorder (Gould Inc., Cleveland, OH). Concentration-dependent mechanical effects were obtained, i.e., developed tension, +T, −T, TPT, T1/T2 and T9/T5. After complete mechanical stabilization, the force-frequency relationship was studied starting with a rate of 0.5 Hz up to 3 Hz. Control strips showed no changes in base-line isometric tension during the time necessary to complete pharmacological testing. Experiments were performed as described previously in detail (Schwinger et al., 1993).

**Isolation of vesicles from the SR.** The SR was prepared according to the method of Sitsapesan and Williams (1990). The preparation was carried out at 4°C. Myocardial tissue was chilled in ice-cold homogenization buffer with the following composition (in mM): sucrose, 300; phenylmethylsulfonyl fluoride, 1; piperoxide-N,N'-bis(2-ethanesulfonic acid) (PIPERES), 20, pH 7.4. Connective tissue was trimmed away, and myocardial tissue was homogenized with a motor-driven homogenizer (Braun, Berlin, Germany). The homogenate was spun at 8,000 rpm (Beckman JA20, Beckman, Munich, Germany) for 20 min. The supernatant was filtered through four layers of gauze, and the pellet was discarded. The supernatant was centrifuged at 35,000 rpm (Sorvall A 641, Sorvall, Bad Homburg, Germany). The pellet containing SR-membrane vesicles was resuspended in a buffer containing (in mM): sucrose, 400; (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 5; 5-amino-2-(hydroxy-methyl)-1,3-propanoldiol (TRIS), 5, pH 7.2, and were frozen fractionated in liquid nitrogen and stored at −80°C until use. Protein concentration was measured according to Lowry et al. (1951).

**Measurement of Ca\(^{2+}\)-ATPase activity.** The reaction was carried out as described previously (Schwinger et al., 1995) based on the following coupled enzyme reactions:

1. ATP → ADP + P (This reaction was catalyzed by the SR-Ca\(^{2+}\)-ATPase.)
2. ADP + phosphoenolpyruvate → ATP + pyruvate (The reaction was catalyzed by the pyruvate kinase.)
3. Pyruvate + NADH → lactate + NAD\(^{+}\) (The reaction was catalyzed by the lactate dehydrogenase.)

The oxidation of NADH was continuously monitored by the decreased absorbance at 340 nm with a spectrophotometer (Beckman DU 640, Beckman, Munich, Germany). The reaction was carried out in a volume of 1 ml at 37°C. The SR vesicles (final concentration, 50 μg/ml) were suspended in the reaction mixture with the following composition (in mM): 3-[N-morpholino]propanesulfonic acid (MOPS), 21; Na\(_2\)HPO\(_4\), 4.9; EGTA, 0.06; KCl, 100; MgCl\(_2\), 3; Ca\(^{2+}\)-Ionophore A 23187, 0.003; phosphoenolpyruvate, 1; NADH, 0.2; pyruvate kinase/lactate dehydrogenase enzyme mixture, 8.4/12 U. The free calcium concentration in the reaction mixture was 35 μM and was calculated according to Fabiato (1988). The reaction was started with ATP (1 mM) and was constant for at least 5 min. The basal activity was measured in the absence of Ca\(^{2+}\) and the presence of EGTA (4 mM) simultaneously. All experiments were carried out in triplicate. The activity of the SR-Ca\(^{2+}\)-ATPase was given in nanomoles of ATP per milligram of protein × min.

**Measurement of the Ca\(^{2+}\)-transient in electrically stimulated multicellular muscle preparations.** Electrically driven muscle strips from human nonfailing myocardium (left ventricular papillary muscle strips, n = 3) were used to study the influence of CPA (30 μM) on Ca\(^{2+}\)-transients simultaneously with force generation by use of the fura-2 ratio method (Grynkiewicz et al., 1985).

Intracellular Ca\(^{2+}\) was measured by the fluorescence indicator fura-2 (Grynkiewicz et al., 1985). To facilitate cell loading fura-2 was used as AM ester. These AM esters passively cross the plasma membrane, and once inside the cell, they are cleaved to cell-impermeant products by intracellular esterases. For the initial control measurement of force of contraction, one end of the muscle strips was clamped at a muscle holder and the other end was attached to a force transducer (Scientific Instruments, Heidelberg, Germany). The muscle fibers were superfused with an oxygenated (95% O\(_2\), 5% CO\(_2\)) Tyrode's solution (in mM): NaCl, 119.8; KCl, 5.4; MgCl\(_2\), 1.05; CaCl\(_2\), 0.9; NaHCO\(_3\), 22.6; NaH\(_2\)PO\(_4\), 0.42; glucose, 5.05; ascorbic acid, 0.28; Na\(_2\)EDTA, 0.05; 37°C, pH 7.4). The muscles were stimulated by a pulse generator (Föhr Medical Instruments GmbH, Egelsbach, Germany) with a square wave pulse of 10 ms duration 10% above threshold voltage at a frequency of 1 Hz. Muscle strips with an adequate mechanical performance were incubated for 4 hr in darkness to avoid photobleaching of the dye in an oxygenated (95% O\(_2\), 5% CO\(_2\)) Ringer's solution (in mM: NaCl, 147; KCl, 4; CaCl\(_2\), 2) at 22°C, pH 7.4, containing 5 μM/l of the fura-2-AM. Experiments were performed as described previously (Vahl et al., 1994).

After fura-2 loading, the muscles were rinsed with oxygenated Tyrode's 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 AD-converter to a personal computer. For on-line data analysis a special software was used (Scientific Instruments, Heidelberg, Germany).

Fura-2 fluorescence was measured with a dual wavelength fluorometer equipped with an inverted microscope. Light was emitted through a mercury arc-lamp (USH - IO2DH, Ushio, Tokyo, Japan). A rotating filter wheel (chop frequency, 125 Hz) allowed alternating excitation at wavelengths of either 380 nm (for the Ca\(^{2+}\)-free fura-2) or 340 nm (fura-2-Ca\(^{2+}\) complex). The emitted fluorescence light resulting from the excitation with one of these wavelengths was recorded at 510 nm and sorted in the respective channels of the photomultiplier. The ratio of both fura-2 fluorescence signals (R\(_{380/360}\)) was continuously recorded (Scientific Instruments, Heidelberg, Germany), digitalized and displayed on a storage oscilloscope. At the same time the fluorescence ratio was stored at a personal computer and data as systolic light emission, time to peak Ca\(^{2+}\) (I1/2I), and diastolic light emission were determined, as well.

**Materials.** ATP, pyruvate kinase/factate dehydrogenase mixture and phosphoenolpyruvate were obtained from Boehringer (Mannheim, Germany). Isoprenaline, phenylmethylsulfonyl fluoride, Ca\(^{2+}\)-Ionophore 23187 and CPA were purchased from Sigma (Deisenhofen, Germany). Fura-2-AM was obtained from Molecular Probes (Eugene, OR). A stock solution of fura-2-AM (10 mM) was dissolved in dimethyl sulfoxide and stored at \(-20^\circ\)C as described by Vahl et al. (1994). All other chemicals were of analytical grade or the best grade commercially available. Only deionized and double-distilled water was used throughout.

**Statistics.** The data shown are means ± S.E.M. For comparison within one group, the paired t test was applied. Otherwise, statistical significance was analyzed by use of the Student's t test for unpaired observations or by ANOVA; P < .05 was considered significant.

**Results**

**Effect of CPA on Ca\(^{2+}\)-ATPase activity.** To demonstrate that CPA is able to inhibit SR-Ca\(^{2+}\)-ATPase specifically in human nonfailing myocardium, the SR-Ca\(^{2+}\)-ATPase activity was measured with use of isolated vesicle preparations from nonfailing human myocardium (n = 7). CPA (0.01–10 \(\mu\)M) concentration-dependently inhibited Ca\(^{2+}\)-ATPase activity as shown in figure 1. The activity of the SR-Ca\(^{2+}\)-ATPase in nonfailing human myocardium in the absence of CPA was 242 ± 20 nmol/mg protein × min (+ A 23187; free Ca\(^{2+}\), 35 \(\mu\)M). The EC\(_{50}\) value for the inhibitory effect of CPA on SR-Ca\(^{2+}\)-ATPase was 0.14 \(\mu\)M (95% confidence limits, 0.12–0.16 \(\mu\)M). Therefore, CPA inhibited SR-Ca\(^{2+}\)-ATPase activity in isolated vesicles from human nonfailing myocardium concentration-dependently.

**Effects of CPA on the force-frequency relationship.** Figure 2 shows the influence of the SR-Ca\(^{2+}\)-ATPase inhibitor CPA (30 \(\mu\)M) on the force-frequency relationship (0.5–3 Hz) in electrically driven left ventricular papillary muscle strips from nonfailing human hearts (n = 7). In the absence of CPA (control) an increase in the frequency of stimulation was followed by a significant increase in the developed tension from 0.5 Hz up to 2 Hz (4.1 ± 0.7 mN vs. 5.4 ± 0.6 mN; 6.3 ± 0.7 mN/m² vs. 8.3 ± 0.3 mN/m² after normalization of developed force to cross-sectional area of the muscle strips, P < .05, fig. 2A). In the presence of CPA the developed tension at 0.5 Hz was decreased compared with control (3.2 ± 0.7 mN; 5.0 ± 1.0 mN/m²). In contrast to control, the increase in the frequency of stimulation in the presence of CPA (30 \(\mu\)M) was not accompanied by a significant increase

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![Fig. 1](image-url)  
**Fig. 1.** Effect of CPA (0.01–10 \(\mu\)M/l) on SR Ca\(^{2+}\)-ATPase in vesicles of SR from nonfailing human myocardium (n = 7). CPA concentration-dependently inhibits SR-Ca\(^{2+}\)-ATPase activity. Ca\(^{2+}\)-ATPase activity in the absence of CPA was 242 ± 20 nmol/mg protein per min. Changes of Ca\(^{2+}\)-ATPase activity are shown as percent of the initial value.

![Fig. 2](image-url)  
**Fig. 2.** Force-frequency relationship (0.5–3 Hz) of human nonfailing myocardium (electrically driven papillary muscle strips) in the absence and presence of 30 \(\mu\)mol/l CPA (n = 7). (A) Ordinate, change in force of contraction in mN/mm²; abscissa, frequency in Hz. In the presence of CPA (30 \(\mu\)mol/l) the former positive force-frequency relationship worsened especially at frequencies above 2 Hz. Significant differences of increase in force of contraction between control condition and CPA treatment were indicated (*control vs. CPA, P < .05). (B) Ordinate, diastolic tension in mN/mm²; abscissa, frequency in Hz.
in force of contraction (0.5 Hz: 3.2 ± 0.7 mN vs. 2 Hz: 3.5 ± 0.6 mN; 5.0 ± 1.0 mN/mm² vs. 5.4 ± 0.6 mN/mm²). Especially at higher frequencies of stimulation (above 2 Hz), the increase in force of contraction was significantly depressed after inhibition of the SR-Ca²⁺-ATPase by CPA (30 μM) compared with control, as marked in figure 2. Therefore, in the presence of CPA an increase in frequency of stimulation was not accompanied by an increase in force of contraction.

Increasing frequency of stimulation was accompanied by an increase in diastolic tension (fig. 2 B). In the presence of CPA diastolic tension increased to a greater extent at frequencies of stimulation above 1.5 Hz. Increasing the frequency of stimulation from 0.5 Hz to 2.0 Hz, the increase in diastolic tension was 1.0 ± 0.3 mN/mm² at control conditions and 2.6 ± 0.8 mN/mm² after treatment with 30 μM CPA, indicating that CPA inhibited relaxation in nonfailing human muscle strip preparation at stimulation rates higher than 90 beats per minute.

**Inotropic and lusitropic effects of CPA.** Figure 3A compares time to peak tension at 0.5 Hz and 2 Hz under control conditions and in the presence of CPA (30 μmol/l). Under both conditions, control and CPA, time to peak tension was diminished significantly at 2 Hz compared with 0.5 Hz (control: 167 ± 4 ms vs. 208 ± 13 ms, CPA: 190 ± 7 ms vs. 226 ± 15 ms; P < .05). However, CPA increased TPT tension significantly compared with control conditions at the low (0.5 Hz) and the high rate (2 Hz) of stimulation (P < .05). Similarly, T95T was diminished while increasing frequency from 0.5 to 2 Hz in the control (430 ± 54 ms vs. 244 ± 7 ms, P < .05) and the CPA group (484 ± 58 ms vs. 273 ± 7 ms, P < .05) (fig. 3B). At 0.5 Hz CPA had no additional influence on T95T as compared with control (484 ± 58 vs. 430 ± 54 ms), whereas at 2 Hz CPA significantly prolonged T95T compared with control (273 ± 7 vs. 244 ± 7 ms). In contrast, T1/2T was not significantly prolonged in the presence of CPA, neither at 0.5 Hz (control: 143 ± 5 ms vs. CPA: 137 ± 12 ms) nor at 2 Hz (control: 112 ± 6 ms vs. CPA: 127 ± 11 ms).

Peak rate of tension rise and peak rate of tension decay, as described previously, strongly depend on the developed force (Schwinger et al., 1992). To evaluate changes of these parameters after the inhibition of the SRCA by CPA, which are not caused by changes of force of contraction, the +T and −T were normalized to developed force. At control conditions, but not in the presence of CPA, the +T and −T increased significantly after changing the frequency of stimulation from 0.5 Hz to 2 Hz. At low frequencies of stimulation (0.5 Hz), there was no difference of +T and −T between control and CPA. At higher frequencies of stimulation, +T was significantly lower after CPA treatment compared with control. There was also a reduction of −T after CPA treatment, but this alteration was not significant.

**Effects of CPA on the intracellular Ca²⁺-transient.** Figure 4 gives an original tracing of simultaneous measure-
ments of the Ca\(^{2+}\)-transient with the fura-2 ratio method and the twitch of contraction with isolated papillary muscle strips from nonfailing human myocardium to demonstrate the effects of CPA on Ca\(^{2+}\)-transients. In the presence of CPA the developed tension and the systolic light emission (\(R_{\text{340/380}}\)) was decreased by 45.68 \(\pm\) 3.73% and 28.80 \(\pm\) 6.90% compared with control. TPT and IPI were prolonged in the presence of CPA by 26% and 45% compared with control, but CPA has only small effects on parameters of relaxation such as T1/2T and I1/2I, which were increased in the presence of CPA. Additionally, CPA increased the diastolic tension as well as the diastolic light emission as shown in figure 4.

**Discussion**

The role of the SR-Ca\(^{2+}\)-ATPase in initiating a positive force-frequency relation in human myocardium can be concluded only from the indirect data available (Hasenfuss et al., 1994). Thus, direct evidence on the contribution of the SERCA on parameters of contraction and relaxation, [Ca\(^{2+}\)]\(_i\), transients and the FFR in human myocardium are still lacking. In addition, the function of the SERCA does not necessarily correlate with reduced protein or mRNA levels (Schwinger et al., 1995). Furthermore, the reduced activity of the SERCA described in failing human hearts is not followed by the expected prolongation of the parameters of relaxation or an increase in diastolic tension in measurements of isolated myocardial tissue, even at higher frequencies of stimulation (Schwinger et al., 1993; Hasenfuss et al., 1994), as well. Because species differences may exist, the role of the SERCA in contraction and relaxation must be studied in human myocardium. The contribution of the SERCA or the Na\(^+\)/Ca\(^{2+}\) exchanger to initiate the decline of the [Ca\(^{2+}\)]\(_i\) transient is 92% and 7% in rat myocardium, but 70% to 28% in rabbit myocardium, respectively (Bassani et al., 1994). The high specificity of CPA on inhibition of the SERCA in skeletal muscle (Seidler et al., 1989; Goeger and Riley, 1989) and smooth muscle (Uyama et al., 1992) has been confirmed in cardiac muscle as well (Takahashi et al., 1995, Bonnet et al., 1994, Badaoui et al., 1995, Agata et al., 1993; Yard et al., 1994). Thus, CPA was used in this study to characterize the importance of the SERCA activity on regulation of contraction and relaxation (twitch kinetics, [Ca\(^{2+}\)]\(_i\), transients, force-frequency relationship) in human myocardium.

As shown in figure 1 CPA concentration-dependently inhibits the activity of the SERCA in myocardial vesicle preparations. Inhibition is complete at a concentration of 10 \(\mu\)M. As described by Baudet et al. (1993), a complete inhibition of the SERCA by CPA in multicellular preparations is not seen, even at high concentrations. This requirement of higher concentrations of CPA to achieve effects on contractility (compared with effects on isolated SR vesicles) may be caused by compartmentalization (Langer, 1992) or diffusion (higher diffusion distance, intracellular target) of CPA in multicellular preparations. To achieve the inhibitory action of CPA on SERCA, a concentration of 30 \(\mu\)M was used. As described by several authors, even at concentrations up to 30 \(\mu\)M, CPA has no effect on the Ca\(^{2+}\) sensitivity of the myofilaments (Takahashi et al., 1995; Bonnet et al., 1994), on Ca\(^{2+}\) currents (Bonnet et al., 1994; Badaoui et al., 1995), on the peak inward and steady state membrane currents (Takahashi et al., 1995) and on Na\(^+\)/Ca\(^{2+}\) exchange in cardiac muscle (Yard et al., 1994).

As shown in this study, inhibition of the SERCA by CPA is predominantly followed by alterations in the parameters of muscle contraction. The time to peak tension was significantly prolonged in the presence of CPA at low and high frequencies of stimulation; the developed tension and peak rate of tension rise were significantly reduced by CPA at high frequencies of stimulation. Additionally, the maximal amplitude of the [Ca\(^{2+}\)]\(_i\) transient was reduced and time to the peak [Ca\(^{2+}\)]\(_i\) transient was prolonged in the presence of CPA. In contrast, the effects of CPA on muscle relaxation were less pronounced than its effects on muscle contraction. CPA did not have an effect on the peak rate of tension decay or the time to half-relaxation. These findings were independent from the stimulation frequency used. In contrast, the time to 95% relaxation and the increase in diastolic tension was augmented at higher frequencies in the presence of CPA compared with control. Consistent with these effects on muscle contraction and relaxation, the decay of intracellular Ca\(^{2+}\) was not affected. However, some increase of diastolic Ca\(^{2+}\) levels occurred.

It seems possible that the degree of SERCA inhibition in the multicellular preparations is lower than that observed in the isolated SR preparations of the same hearts. A small inhibitory action on the SERCA activity might lead to a reduced force of contraction because of a stronger competition of the Na\(^+\)/Ca\(^{2+}\) exchanger with the SERCA. This may lead to a reduced force generation but could be less effective in slowing relaxation. Thus, the impaired SERCA activity in the present study is followed predominantly by alterations of inotropic and, to a lesser extent, lusitropic function. This may be supported by the finding that thapsigargin (another specific inhibitor of SERCA activity) caused remarkably slow and incomplete SR Ca\(^{2+}\) depletion in multicellular preparations of rabbit myocardium (Baudet et al., 1993) in contrast to observations in single myocytes. With consistent use of rapidly cooling contractures to assess the SR load in multicellular rabbit papillary muscle strip preparations, CPA was only effective in decreasing SR Ca\(^{2+}\) content by 59% (Baudet et al., 1993) and reducing twitch force by 40%. These authors concluded that complete blockade of SR Ca\(^{2+}\) uptake by CPA or thapsigargin in multicellular muscle preparations cannot be assumed (in rabbit myocardium). Both agents seemed to shift the balance of Ca\(^{2+}\) fluxes in favor of Ca\(^{2+}\) extrusion by the sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchanger (Baudet et al., 1993). It seems that a modest decline in SR Ca\(^{2+}\) content can have a disproportionately large effect on the twitch amplitude and SR Ca\(^{2+}\) release (Bassani et al., 1995) (e.g., measured as caffeine-induced Ca\(^{2+}\) release). Thus, slowing of relaxation by CPA may be modest (because of incomplete blockade of the SERCA in multicellular preparations) in the present experiments even though force development is decreased significantly. However, the inhibitory action of CPA on SERCA activity largely affects the frequency-dependent force generation in humans.

The negative inotropic effect of CPA may be explained by an increased Ca\(^{2+}\) extrusion by the sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchanger and a reduced SR Ca\(^{2+}\) load. At higher frequencies of stimulation the negative inotropic effects by inhibition of the SERCA (e.g., by CPA) may be augmented as the time of diastole is shortened. The effects of CPA on parameters of
relaxation (lusitropy) are present consistently at high frequencies of stimulation. CPA prolonged time to 95% relaxation and increased diastolic tension. Thus, the Na+/Ca2+ exchanger cannot completely compensate the increased diastolic Ca2+ levels after the CPA inhibitory action on SERCA activity at high frequencies of stimulation. On the other hand, the Na+/Ca2+ exchanger works most effectively when intracellular Ca2+ is high. Thus, as intracellular Ca2+ declines during relaxation this system is less able to extrude Ca2+, especially with frequency-dependent rising intracellular Na+. In addition, the inhibited SR Ca2+ pump also cannot lower intracellular Ca2+ very rapidly when blocked by CPA. Consequently, CPA may still create a diastolic as well as systolic dysfunction without significantly slowing relaxation.

As discussed by Langer (1992), the Na+/Ca2+ exchanger may reduce intracellular Ca2+ load. A fast relaxation can be arranged by action of the Na+-Ca2+ exchanger and the SERCA in rabbit myocardium (Bers and Bridge, 1989). In human myocardium an increased activity and protein expression of the Na+/Ca2+ exchanger has been reported in heart failure (Studer et al., 1994; Reinecke et al., 1996; Flesch et al., 1996) and has been discussed to be compensatory for the altered SERCA activity. This might be supported by the finding that parameters of relaxation (Schwinger et al., 1993) and diastolic tension (Hasenfuss et al., 1994) are not altered in failing compared with nonfailing myocardium. However, this holds true only for low frequencies of stimulation. Intracellular Na+ has been reported to increase with increasing frequency of stimulation. In various species Na+/Ca2+ exchange has been shown to play a role in the frequency-dependent increase in force of contraction by inhibition of Ca2+ extrusion secondary to an increase in Na+. (Cohen et al., 1982; Frampton et al., 1991; Shouten and Ter Keurs, 1991). In addition, Bassani et al. (1995) conclude from their findings that a rise in SR Ca2+ at higher intracellular Na+ concentration could sensitize the Ca2+ release mechanism, enhancing the efficacy of Ca2+ entry via the Na+/Ca2+ exchanger as a potential activator of SR Ca2+ release. This mechanism may potentiate changes on Ca2+ handling after increased intracellular Na+. In human heart failure, i.e., in a condition with reduced SERCA activity, as well as after blockade of the SERCA by CPA, the force-frequency relationship is negative. Thus, the altered SERCA activity affects the SR Ca2+ load and the interaction of SERCA activity and Na+/Ca2+ exchange. Because the direction of Ca2+ movement through Na+/Ca2+ exchanger depends on the membrane potential, an elevation of average intracellular Ca2+ could increase the operation of the Na+/Ca2+ exchanger in the net Ca2+ efflux mode because of a less positive Ca2+ equilibrium potential (Langer, 1992). However, this assumption needs to be proven.

In summary, the present study provides evidence that an altered SR-Ca2+-ATPase function in human myocardium may lead to an impaired inotropic and lusitropic function and to a negative force-frequency relationship in humans. Thus the SR-Ca2+-ATPase activity is important in initiating the activation and relaxation of human muscle contraction. Further studies are needed to focus on the complex interaction of SR-Ca2+-ATPase and Na+/Ca2+ exchanger on the regulation of contraction, relaxation and the force-frequency relationship, especially in humans.


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