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Effect of cyclopiazonic acid, an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, on the frequency-dependence of the contraction-relaxation cycle of the guinea-pig isolated atrium

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1 The relevance of a functional sarcoplasmic reticulum (SR) membrane system to the contraction-relaxation cycle and to the force-frequency relationship of guinea-pig atrial tissue was investigated. Cyclopiazonic acid (CPA) was used to inhibit selectively the activity of the SR Ca^{2+}-ATPase. IC_{50} values of 0.2 μM or 1.0 μM were measured in guinea-pig isolated SR membranes in the absence or presence of millimolar ATP, respectively. CPA (0.3–30 μM) did not inhibit the activity of the sarcolemmal Na^+-Ca^{2+}-exchanger as measured in isolated cardiac cell membrane preparations.

2 In guinea-pig isolated left atrium paced at 2.5 Hz (30°C), CPA (1–100 μM) produced a concentration-dependent reduction in developed tension and a fall in the maximum rate of tension increase ($+dT/dt_{max}$) and decrease ($-dT/dt_{max}$). The twitch duration was markedly increased due to a prolongation of the time to peak tension, and in particular, the relaxation phase.

3 The contraction-relaxation cycle of the left atrium showed a marked dependence on the frequency of stimulation. The developed tension and $+dT/dt_{max}$ showed a progressive increase from 0.5 Hz, reaching peak values at a stimulation rate of 1.5–2.5 Hz, the positive staircase phenomenon. Higher frequencies of stimulation caused a fall in these parameters. Resting tension was unaffected. The time-course of the contraction-relaxation cycle was also frequency-dependent, with both time to peak tension and relaxation time showing a progressive fall from 2.0–3.5 Hz.

4 The addition of CPA (30 μM) caused marked alterations in the frequency-dependence of the contraction-relaxation cycle. The frequency-dependence of developed tension, $+dT/dt_{max}$ and $-dT/dt_{max}$, was shifted downwards, particularly at higher frequencies, and the frequency at which peak values of $+dT/dt_{max}$ and $-dT/dt_{max}$ were reached was shifted leftwards. The resting tension of the tissues in the presence of 30 μM CPA was increased markedly at frequencies greater than 2 Hz. The time-course of the contraction-relaxation cycle was markedly prolonged between 1.0 and 3.5 Hz, due to an effect on both time to peak tension and relaxation time.

5 In conclusion, these results show that CPA is a highly selective inhibitor of the cardiac SR Ca^{2+}-ATPase, without effect on the sarcolemmal Na^+-Ca^{2+}-exchanger, and suggest that a functional SR Ca^{2+}-ATPase is necessary for the normal contraction-relaxation cycle of guinea-pig cardiac tissue. Additionally, the results suggest an increasing dependence of tension development on SR Ca^{2+}-ATPase with increasing frequency, which may reflect either a frequency-dependent activation of this enzyme or the diminished contribution of the Na^+-Ca^{2+} exchanger. These results also provide novel support for the mechanism of the depressed force-frequency relation found in cardiac tissue of heart failure patients, in which there is a reduced expression of Ca^{2+}-ATPase.

Keywords: Ca^{2+}-ATPase; force-frequency relation; cyclopiazonic acid; contraction-relaxation cycle; sarcoplasmic reticulum

Introduction

The developed tension and time-course of the contraction-relaxation cycle of the isolated myocardium of the majority of mammalian species is dependent on the frequency of stimulation (Buckley *et al.*, 1972). For example, the developed tension of human myocardium increases with the increase in stimulation frequency (positive staircase or treppe effect), while twitch duration decreases (Gwathmey *et al.*, 1990; Mulieri *et al.*, 1992). These changes have been directly related to an increase in the magnitude of the cytosolic calcium transient as well as a shortening of its duration (Wier & Yue, 1986; Gwathmey *et al.*, 1990). In adult mammalian myocardium the peak concentration of cytosolic calcium is principally determined by the amount of calcium released from the sarcoplasmic reticulum (SR) which in turn is related to the amount of calcium present in the SR (Hilgemann & Noble, 1987; Lewartowski & Pytkowski, 1987). One possible explanation for the staircase phenomemon is that there is a frequency-dependent activation of calcium uptake by the SR, but this has so far been difficult to test due to the lack of a selective and specific inhibitor of the Ca^{2+}-ATPase.

Cyclopiazonic acid, a mycotoxin produced by *Aspergillus penicillium*, was originally described as an inhibitor of the SR Ca^{2+}-ATPase expressed in fast twitch muscle from the rabbit (Seidler *et al.*, 1989) and the rat (Goeger & Riley, 1989) and of smooth muscle (Deng & Kwan, 1991; Bourreau *et al.*, 1991; Suzuki *et al.*, 1992). It is reported to be without effect on F-type ATPases, such as the mitochondrial H^+-ATPase and on several other P-type ATPases, such as the gastric H^+,K^+ ATPase, the kidney and brain Na^+,K^+-ATPases, and the Ca^{2+}-ATPase of the plasma membrane (Seidler *et al.*, 1989). In the present paper CPA is shown to be a potent inhibitor also of the slow-twitch muscle isoform of the SR Ca^{2+}-ATPase expressed in guinea-pig heart without effect on the Na^+-Ca^{2+} exchanger. The effects of this selective SR inhibitor on the twitches and on the frequency-dependence of the contraction-relaxation cycle of the guinea-pig isolated left atria were therefore examined. Guinea-pig left atrial tissue was chosen for the present investigation because of the similarity of its response (Reiter, 1966; Penefsky *et al.*, 1972;

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Lewartowski & Pytkowski, 1987), to that of human ventricular tissue (Feldman *et al.*, 1988; Mulieri *et al.*, 1992) with increasing frequency. A preliminary account of these results has recently been presented to the British Pharmacological Society (Yard & Ball, 1993).

Methods

SR preparation

Guinea-pig SR vesicles were prepared from the left ventricle as previously described for canine ventricular SR (Chamberlain *et al.*, 1983). The SR fraction was suspended in 250 mM sucrose, 10 mM imidazole, pH 7.0, 0.5 mM dithiothreitol, and 0.1 mM phenylmethyl-sulphony fluoride at a protein concentration of 15 mg ml^{-1}. Aliquots were quickly frozen in liquid nitrogen and stored at − 70°C.

Preparation of cardiac sarcolemmal (SL) vesicles

SL vesicles were prepared from calf ventricle by the sucrose gradient centrifugation method of Jones (1988) except that 50 μM PMSF was added to all solutions. The final vesicle pellet was suspended in 10 mM histidine, pH 7.0, 0.25 mM sucrose, 50 μM PMSF. Aliquots were stored at − 70°C until used. Protein concentration was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Ca^{2+}-uptake measurements

Calcium uptake by SR membranes was measured at 37°C by using the Millipore filtration technique (0.22 μm filters type GS) and ^{45}Ca basically according to Chiesi & Inesi (1979). The initial rate of Ca-uptake was determined in a medium containing (in mM); KCl 100, sucrose 100, MOPS (pH 6.8) 50, $MgCl_2$ 5, NaN_3 5, potassium oxalate 3, ATP 2, EGTA 0.5, and $CaCl_2$ 0.15 (free Ca concentration was 0.47 μM). SR membranes were preincubated with various concentrations of CPA for 10 min with the uptake medium lacking either ATP or $CaCl_2$. Accordingly, the uptake reaction was then started by adding either $CaCl_2$ or ATP.

Measurement of Na^+-Ca^{2+} exchange activity

Na_i^+-dependent Ca^{2+} uptake activity was measured essentially as described by Seiler & Fleischer (1988). The sarcolemmal vesicles were incubated 4 h on ice in 160 mM NaCl, 40 mM 3-(N-morpholino)propanesulphonic acid (MOPS), pH 7.4 at a concentration of 0.2 mg ml^{-1}, sedimented at 70000 *g* for 1 h and suspended in a small volume of the same buffer; 3 μl of vesicles were than rapidly diluted into 60 μl of a buffer containing 160 mM KCl, 40 mM MOPS-Tris pH 7.4, 1 μM valinomycin, 0.3 μCi of $^{45}CaCl_2$ ml^{-1}, 0.2 mM EGTA and 0.188 mM $CaCl_2$ (yielding an approximate free Ca^{2+} concentration of 1 μM). The uptake reaction was stopped by rapid filtration of the sample and extensive washing of the filters (4 times with 2 ml portions of quenching buffer containing 160 mM KCl, 40 mM MOPS, pH 7.4, 1 mM $LaCl_3$). The control samples were diluted into a buffer containing 160 mM NaCl instead of 160 mM KCl.

Tension measurements

Male guinea-pigs (300–450 g) were anaesthetized with Pentothal (100 mg kg^{-1}, i.p.). The heart was rapidly removed and placed in Krebs-Henseleit solution continuously gassed with 95% O_2 and 5% CO_2. The composition was as follows (in mM): NaCl 120, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.53, $NaHCO_3$ 25; KH_2PO_4 1.17 and glucose 10. The left atrium was dissected and mounted between plastic micro tissue clamps in a 100 ml tissue bath containing Krebs-Henseleit solution at 30°C, pH 7.45, and gassed as above. (−)-Propranolol hydrochloride (1 μM) was present in the bathing solution throughout the experiment.

The tissue was directly stimulated by unipolar square wave pulses of 5 ms duration with a voltage 15–20% greater than threshold by a Grass stimulator (Grass Instruments, Quincey, MA, U.S.A.) Isometric force was measured by a force transducer (Hugo Sachs Electronic, March-Hugstetten, Germany) The tissues were stretched to a length (l_{max}) at which the developed tension was maximal, by application of a resting tension of approximately 0.5 g.

Concentration-dependent effects, and time-course, of CPA on the contraction-relaxation cycle

The left atria were stimulated continuously at a rate of 2.5 Hz for a 2 h stabilization period. Cumulative concentration-response relationships were constructed for cyclopiazonic acid, 1–100 μM (0.007–0.7% dimethylsulphoxide (DMSO) allowing an equilibration time of 1 h at each concentration. The time-course of effect of 30 μM CPA or vehicle (0.2% DMSO) was examined in a further set of preparations over a 120 min period.

Effect of CPA on the frequency-dependence of the contraction-relaxation cycle

Left atria were stimulated continuously at a rate of 1 Hz for a 2 h equilibration period. Baseline parameter-frequency relations were obtained following stimulation at each frequency for 4 min (pilot experiments showing this to be sufficient time for stable values) starting at 0.5 Hz and increasing in 0.5 Hz increments to 3.5 Hz (data not shown). Then either CPA (final concentration of 30 μM) or an equivalent volume of the vehicle (0.2% DMSO) was added. Following a 1 h equilibrium period a further parameter-frequency relation was obtained.

Drugs and chemicals

CPA and (−)-propranolol hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), Pentothal from Abbott (Cham, Switzerland). CPA was prepared in DMSO immediately prior to use. All other chemicals were of the highest analytical grade commerically available.

Data analysis

All isolated tissue parameters were recorded on a commercially available data-acquisition software computer package (PT-1; Po-Ne-Mah, Storrs, CT, U.S.A.). The developed tension (total tension − resting tension), resting tension, time to peak tension, time to 50% or 95% relaxation (time from peak tension to point of 50% or 95% of change in developed tension, respectively), $+dT/dt_{max}$ (maximum rate of increase of developed tension), $-dT/dt_{max}$ (maximum rate of decrease of developed tension), and twitch duration (time to peak tension + time to 95% relaxation) were automatically computed. All data are expressed as mean ± standard error of the mean (s.e.mean). Parameter-frequency relations following DMSO or CPA were compared by analysis of covariance, the baseline relations being used as the covariate.

Results

Effects of CPA on the Ca^{2+}-uptake activity of cardiac SR and on the Na^+-Ca^{2+}-exchanger of cardiac sarcolemma

Cyclopiazonic acid has been reported to be a potent and selective inhibitor of the Ca^{2+}-ATPase activity of the skeletal muscle SR (Seidler *et al.*, 1989; Goeger & Riley, 1989) and of smooth muscle (Deng & Kwan, 1991, Bourreau *et al.*, 1991;

Suzuki *et al.*, 1992). In the present study we investigated the compound on SR membranes isolated from guinea-pig ventricle and observed that it completely inhibited the Ca^{2+}-uptake activity with an IC_{50} value of 0.2 μM (Figure 1, filled squares). Interestingly, the presence of millimolar ATP protected ATPase from inhibition and shifted the IC_{50} value up to 1.0 μM (see Figure 1, open squares).

The lack of activity of CPA on various other ATPase systems has already been reported (Seidler *et al.*, 1989). Because of the potential importance of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger, in the present study the effect of CPA was determined. No significant inhibition of the Na^+-Ca^{2+}-exchanger was observed with CPA at concentrations as high as 30 μM (Table 1).

Effect of CPA on the contractile activity of guinea-pig atria paced at 2.5 Hz

CPA caused a marked and concentration-dependent reduction in developed tension, $+dT/dt_{max}$ and $-dT/dt_{max}$ (Figure 2a and Table 2). With 30 μM CPA, for example, there was a 40% reduction in developed tension. The duration of the contraction-relaxation cycle was clearly prolonged by CPA as shown in Figure 2b where all traces have been normalized to 100% to clarify this effect. This prolongation was principally due to a marked increase in the relaxation time (eg., 42% prolongation of 95% relaxation time, with 30 μM CPA). There was less prolongation of the contractile phase (Table 2, time to peak tension), with the maximum effect occurring at 30 μM. At a frequency of 2.5 Hz there was no effect on the resting tension even at the highest CPA concentration (100 μM) tested (Figure 2a and Table 2).

The time-course of action of 30 μM CPA was investigated in further detail (Figure 3): All parameters reached equilibrium within 1 h, defining the equilibrium time used for the parameter-frequency relations.

Frequency-dependence of the contraction-relaxation cycle

Several parameters of the contraction-relaxation cycle showed a marked dependence on the frequency of stimulation. Developed tension (Figure 4a, open symbols) showed a progressive increase from 0.5 to 1.5 Hz reaching a peak tension of 216% (as a percent of the value at 0.5 Hz). Between 2–3.5 Hz the developed tension decreased slightly. The maximum rate of developed tension, $+dT/dt_{max}$ (Figure 4b, open symbols), showed a similar frequency-dependence to that of developed tension, peaking at 2.0 Hz. This was in contrast to $-dT/dt_{max}$ which continued to increase over the frequency-range 0.5–3.5 Hz. Resting tension (Figure 4d, open symbols) was unaffected even at the highest frequency of 3.5 Hz. The time-course of the contraction-relaxation cycle was also frequency-dependent, with both time to peak tension (Figure 4e, open symbols) and 95% relaxation time (Figure 4f, open squares) showing a progressive shortening from 1.0 to 3.5 Hz, such that the calculated total twitch

Table 1 Effect of cyclopiazonic acid (CPA) on the Na^+-Ca^{2+} exchange activity of isolated cardiac cell membranes

	CPA [μM]			
	Control	0.3	3	30
Na^+-Ca^{2+} exchange activity (%)	100	91 ± 7	98 ± 11	93 ± 3

The Na^+-dependent Ca^{2+}-uptake reaction was carried out as described in the methods section. The values (mean ± s.e.mean of 3 preparations) are expressed relative to the activity of the control reaction which was set to 100%.

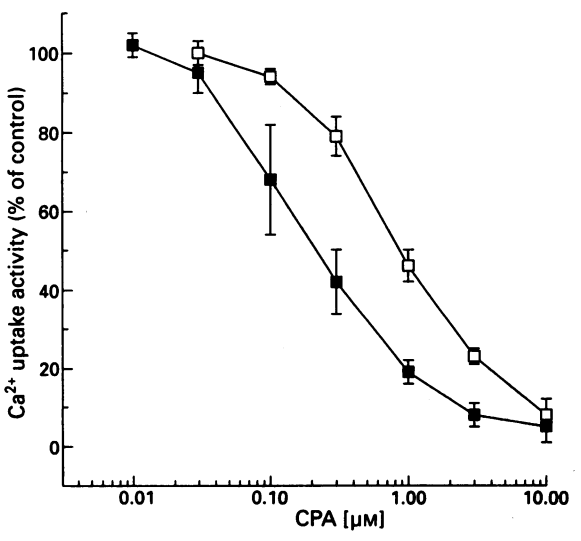

Figure 1 Effect of cyclopiazonic acid (CPA) on the Ca^{2+}-uptake activity of guinea-pig isolated myocardial SR membranes. The uptake reactions were started either by the addition of ATP (■) or $CaCl_2$ (□), details of which are given in the Methods section. Data are represented as mean ± s.e.mean of 5 preparations.

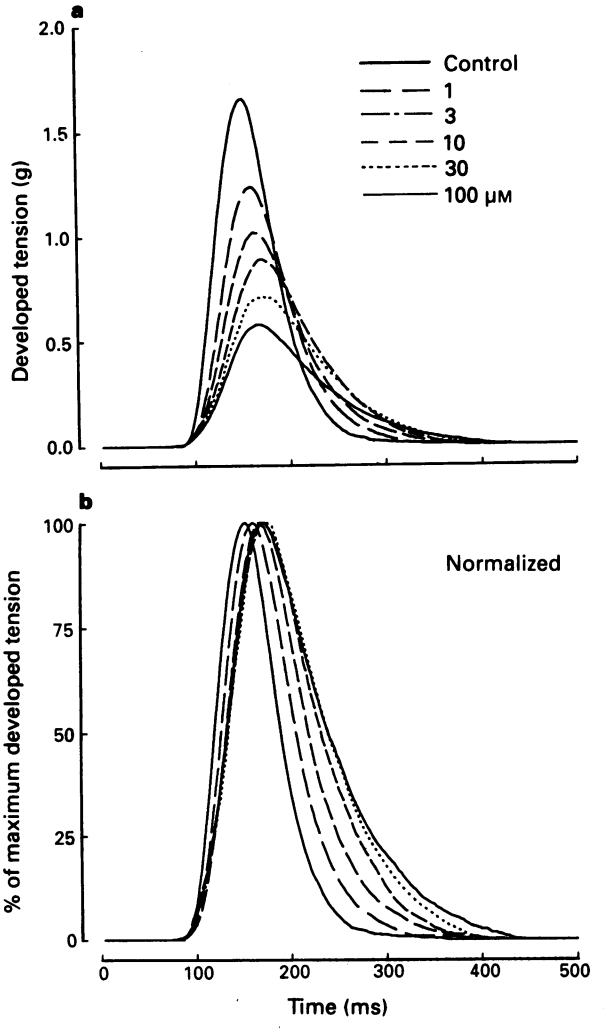

Figure 2 Representative trace (a) showing the concentration-dependent effects of cyclopiazonic acid (CPA) on the contraction-relaxation cycle of the guinea-pig left atrium measured at 2.5 Hz. Data shown in the lower panel (b) have been normalized for developed tension to clarify the effects of CPA on relaxation.

Table 2 Concentration-dependent effects of cyclopiazonic acid (CPA) on guinea-pig isolated left atria stimulated at 2.5 Hz

CPA (μM)	*Developed tension* (g)	*Resting tension* (g)	*Time to peak* (ms)	*Time to 50% relaxation* (ms)	*Time to 95% relaxation* (ms)	$+dT/dt_{max}$ (g s^{-1})	$+dT/dt_{max}$ (g s^{-1})	*Twitch duration* (ms)
0	1.82 ± 0.28	0.53 ± 0.02	81 ± 3	52 ± 2	151 ± 10	42.2 ± 8.0	25.0 ± 3.8	232 ± 12
10	1.28 ± 0.16	0.56 ± 0.02	101 ± 3	70 ± 4	201 ± 5	24.0 ± 2.7	13.3 ± 0.8	303 ± 5
30	1.08 ± 0.16	0.57 ± 0.02	103 ± 4	73 ± 5	215 ± 7	20.0 ± 2.8	11.0 ± 0.8	319 ± 5
100	0.68 ± 0.16	0.59 ± 0.05	96 ± 7	76 ± 7	223 ± 5	13.6 ± 2.8	8.3 ± 1.2	320 ± 4

Data are presented as mean ± s.e.mean ($n = 6$).

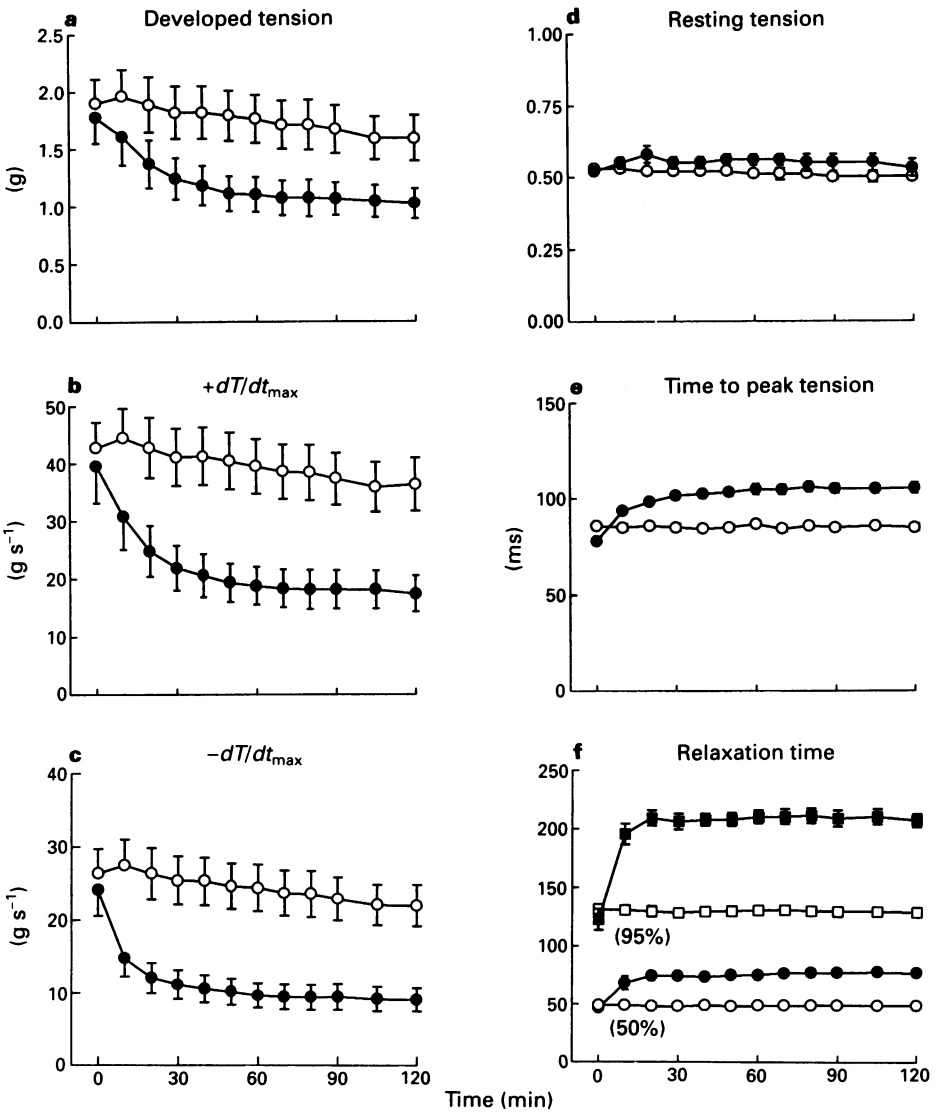

Figure 3 Time-course of the effects of 30 μM cyclopiazonic acid CPA on the contraction-relaxation cycle of the guinea-pig left atria measured at 2.5 Hz: (a) developed tension, (b) $+dT/dt_{max}$, (c) $-dT/dt_{max}$, (d) resting tension, (e) time to peak tension, and (f) time to 50% (circle) and 95% (squares) relaxation. The control group (0.2% DMSO) are represented by open symbols and those in the presence of 30 μM CPA by closed symbols. Each point is the mean ± s.e.mean of 6 tissues.

duration decreased from 247 ± 14 to 186 ± 14 ms ($P < 0.001$).

Effect of CPA on the frequency-dependence of the contraction-relaxation cycle

The addition of CPA (30 μM) caused marked alterations in the frequency-dependence of the contraction-relaxation cycle (Figure 4, closed symbols). The developed tension-frequency relation (Figure 4a) was shifted downwards, particularly at the higher frequencies (e.g., 63% reduction at 3.5 Hz, $P < 0.001$). The frequency-dependence of both $+dT/dt_{max}$ and $-dT/dt_{max}$ (Figure 4b,c) were similarly affected, with a marked reduction particularly at the higher frequencies (e.g., $+dT/dt_{max}$ being reduced by 70% at 3.5 Hz ($P < 0.001$) compared to 40% at 1.5 Hz ($P < 0.001$). In addition, the frequency at which the peak values of these parameters occurred displayed a clear leftward shift (e.g., from 2.0 to 1.5 Hz for $+dT/dt_{max}$).

The resting tension of the tissues in the presence of 30 μM CPA (Figure 4d, closed symbols) was significantly increased at frequencies greater than 2 Hz (e.g., 34% increase at 3.5 Hz; $P < 0.05$) in contrast to those tissues not treated.

The time-course of the contraction-relaxation cycle was

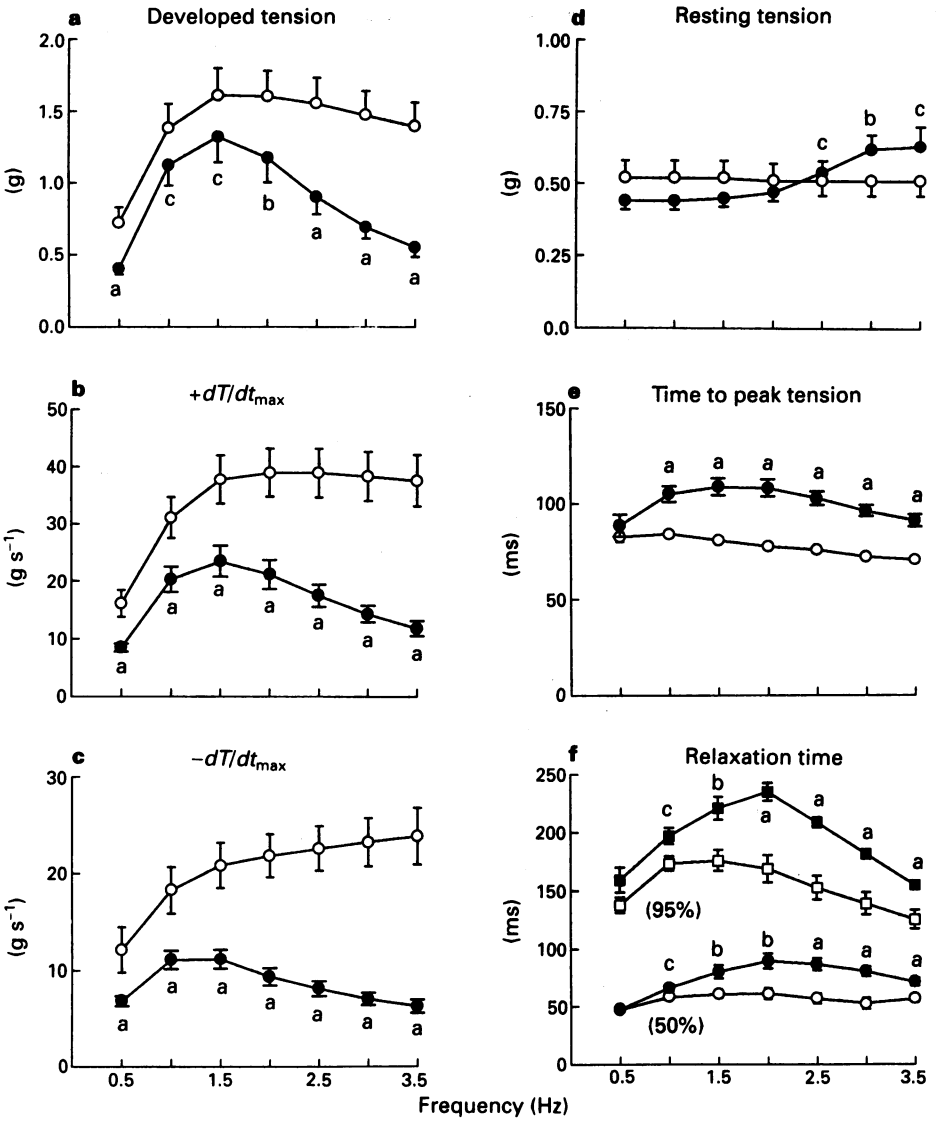

Figure 4 Effect of 30 μM cyclopiazonic acid (CPA) on the frequency-dependence of the contraction-relaxation cycle of the guinea-pig isolated atria: (a) developed tension, (b) $+dT/dt_{max}$, (c) $-dT/dt_{max}$, (d) resting tension, (e) time to peak tension, and (f) time to 50% (○, ●) and 95% (□, ■) relaxation. The control group (0.2% DMSO) are represented by open symbols and those in the presence of 30 μM CPA by closed symbols. Each point is the mean ± s.e.mean of 11 tissues. Statistically significant differences from the control group at the corresponding frequency are denoted by: $^aP<0.001$; $^bP<0.01$; and $^cP<0.05$.

also markedly affected by CPA. Specifically there was a marked prolongation of the twitch duration of the contraction-relaxation cycle between 1.0 and 3.5 Hz, due to a prolongation of time to peak tension (Figure 4e, closed symbols), as well as relaxation time (Figure 4f, closed symbols).

There was no significant effect of a 1 h equilibration with DMSO (0.2%) on the frequency-dependent relations of any of the measured parameters (data not shown).

Discussion

Role of calcium and the SR pump in contraction-relaxation cycle

The rapid cycling of the free Ca^{2+} concentration in adult mammalian cardiac cells is mainly under the control of the activity of the SR membranal system. The cisternal compartments of the SR are the source of most of the excitatory Ca^{2+} ions and the Ca^{2+}-pumping ATPase of the SR is the major mechanism concerned in lowering the free Ca^{2+} concentration to resting levels. Other Ca^{2+}-translocating entities, however, are required to ensure the contractile function of the myocardium. During each contraction-relaxation cycle there is a specific amount of Ca^{2+} entering the cell which is necessary for contraction. This Ca^{2+} of extracellular origin must be ejected during the recovery phase to avoid a Ca^{2+}-overload. Ca^{2+} ions entering the cell via the activation of the voltage-dependent Ca^{2+}-channels are necessary to induce the massive Ca^{2+} release from the SR cisternal compartments. The Na^+-Ca^{2+} exchange activity of the plasma membrane also plays a significant role and could contribute to Ca^{2+}-entry during the plateau phase of the action potential (Nuss & Houser, 1992). These Ca^{2+} ions could either participate directly in the stimulation of the myofilaments or could be used to refill rapidly the SR compartment. Another major role of the Na^+-Ca^{2+} exchanger is to extrude Ca^{2+} ions from the cytosol thus contributing to the relaxation phase (Carafoli, 1987; Philipson, 1990).

CPA has been reported to be a fairly specific inhibitor of the SR Ca^{2+}-ATPase activity (Seidler *et al.*, 1989 and this study). The effects of CPA on the contractile characteristics of guinea-pig atria can most simply be explained by this inhibitory mechanism. Even though SR preparations from

guinea-pig ventricle have been utilized in this study, the same Ca^{2+}-ATPase isoform is also expressed in the atria (Brandl *et al.*, 1986). Therefore, the assumption is made that the atrial SR Ca^{2+} -ATPase is equally affected by CPA. An inhibited SR Ca^{2+}-ATPase would result in a slower removal of intracellular free Ca^{2+} ions, leading to slower relaxation rates (see Figure 2 and Table 2). The slower relaxation kinetics could eventually lead to an increase in resting tension, in particular at the higher frequencies of stimulation (Figure 4d). Inhibition of the SR Ca^{2+} pump would lead to a gradual emptying of the SR compartment and thus to a reduced amount of Ca^{2+} being available for release with the next action potential, decreasing the developed tension (Figures 2, 3a and 4a).

CPA had only a minor, and non dose-related, effect on the contractile phase (time to peak, Table 2), the explanation for which is not clear. The effects of CPA on contractility become clearly evident only at relatively high concentrations (above 10 μM, see Figure 2), while, on the isolated SR system, Ca^{2+}-ATPase inhibition can already be detected in the submicromolar range (see Figure 1). The higher concentration required in the tissue system could be due to compartmentalisation problems (the target of CPA is intracellular) and, in particular, to the presence in the cytosol of the myocytes of millimolar concentrations of ATP, which protect the ATPase from inhibition in a competitive manner (see Figure 1). A similar effect has been previously reported (Seidler *et al.*, 1989) using fast twitch muscle preparations, where ATP protected the Ca^{2+}-ATPase against CPA inhibition in a competitive fashion.

Due to its complex involvement in the contraction-relaxation cycle, a reduction of the Na^+-Ca^{2+} exchange activity could also result in negative inotropic and lusitropic effects on the myocardium. It was therefore mandatory to exclude a possible involvement of the Na^+-Ca^{2+} exchanger in the action of CPA. No inhibition of the exchange reaction of calf heart plasma membrane could be detected. Calf hearts (ventricle) were chosen as the biological material since large amounts of tissue were needed to obtain a sufficient amount of acceptably pure plasma membranes. So far, cardiac Na^+-Ca^{2+} exchanger has been cloned from dog (Nicoll *et al.*, 1990) and calf (Aceto *et al.*, 1992) and found to be highly homologous. Therefore, it is likely that the results obtained with the calf Na^+-Ca^{2+} exchanger can be extrapolated to the guinea-pig atria. Based on such an assumption, the inhibitory effects of CPA on the contractile performance of intact guinea-pig atria could not have resulted from an inhibition of the Na^+-Ca^{2+}-exchanger (Table 1).

Frequency-dependence of contraction-relaxation cycle and effects of CPA

The developed tension and time-course of the contraction-relaxation cycle of the guinea-pig cardiac tissue, like the majority of other mammalian species, is highly dependent on the frequency of stimulation (Figure 4) as has been described for other mammalian cardiac tissues (Buckley *et al.*, 1972). For example, the developed tension of human ventricular myocardium increases with increasing stimulation frequency (positive staircase or treppe effect), while twitch duration decreases (Gwathmey *et al.*, 1990; Mulieri *et al.*, 1992). Such changes have been directly related to an increase in the magnitude of the cytosolic calcium transient as well as a shortening of its duration (Wier & Yue, 1986; Gwathmey *et al.*, 1990). The present findings of a shortening of twitch duration (due to a reduction in both time to peak tension and relaxation time) are consistent with the explanation of a frequency dependent activation of calcium uptake (leading to a reduction in relaxation time) and subsequent release (increase in developed tension) by the SR. The finding that attenuation of the force-frequency relation by CPA is more marked at higher frequencies further supports this hypothesis: because of the limiting contribution of the Na^+-Ca^{2+} exchanger to the *rapid* regulation of intracellular Ca^{2+}, the contraction-relaxation cycle becomes increasingly dependent on the performance of the SR system at higher frequencies.

Potential significance of altered force-frequency relation following Ca^{2+}-ATPase inhibition and that of the failing myocardium

The present results bear several points of similarity to findings obtained with cardiac tissue from heart failure patients where the force-frequency relation is either attenuated (Feldman *et al.*, 1988) or reversed (Mulieri *et al.*, 1992). It has been suggested that this is due to abnormalities in calcium handling with prolongation of Ca^{2+} transients (Gwathmey *et al.*, 1987; Morgan *et al.*, 1990; Beuckelmann *et al.*, 1992) due to an estimated 50% reduction in calcium uptake function (Hasenfuss *et al.*, 1992). The reduction in the number of calcium pumps was shown to be due to a reduced expression of mRNA for Ca^{2+}-ATPase (Mercadier *et al.*, 1990; Arai *et al.*, 1993).

The similarity between the force-frequency relation reported here with CPA and that found in failing heart tissue is of particular interest since both a chronic decrease in Ca^{2+}-ATPase pump expression (heart failure) or acute pump inhibition (CPA), lead to impaired calcium uptake and release, i.e., they are *functionally equivalent*. In this respect, the present results provide novel support for the role of the SR Ca^{2+}-ATPase in the attenuated force-frequency relation of cardiac tissue obtained from heart failure patients. One potential difference however lies in the effects on relaxation. While there was a marked prolongation of relaxation following CPA, the relaxation of failing heart tissue is reported to be prolonged in some cases (Gwathmey *et al.*, 1990) but not in others (Mulieri *et al.*, 1992). However, the recent finding that the expression of the Na^+-Ca^{2+} exchanger is increased in myopathic tissue of at least some patients (Reinecke *et al.*, 1992) might explain the discrepancy: an increased Na^+-Ca^{2+} exchange activity would be expected to improve relaxation and therefore compensate, at least in part, for the down-regulation of the SR Ca^{2+}-ATPase. A further implication of these studies is that agents which could directly stimulate the remaining Ca^{2+}-ATPase of failing cardiac tissue would be expected to normalize the force-frequency relation, and improve cardiac pump function.

In summary, these results suggest that a functional SR Ca^{2+}-ATPase is necessary for the normal contraction-relaxation cycle. Additionally, the results suggest an increasing dependence of tension development on SR Ca^{2+}-ATPase at increasing frequency, which may reflect either a frequency-dependent activation of this enzyme or the diminished contribution of the Na^+-Ca^{2+} exchanger. These results also provide novel support for the mechanism of the depressed force-frequency relation found in cardiac tissue of heart failure patients, in which there is a reduced expression of Ca^{2+}-ATPase.

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