Influence of cyclosporine A on contractile function, calcium handling, and energetics in isolated human and rabbit myocardium

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Abstract

Objective: The immunosuppressive drug Cyclosporine A (CsA) is a key substance in pharmacological therapy following solid organ transplantation and has been suggested to prevent cardiac hypertrophy. We investigated the direct effects of CsA on myocardial function, because these are largely unknown. Methods: In multicellular cardiac muscle preparations from end-stage failing and non-failing human hearts as well as from non-failing rabbit hearts we investigated the effects of CsA on contractile performance, sarcoplasmic reticulum (SR) Ca\(^{2+}\)-load, cytosolic calcium transients, calcium sensitivity of the myofilaments, and myocardial oxygen consumption. Results: In failing human muscle preparations there was a concentration dependent decrease in contractile force; the maximal effect amounted to 55.6±6.4% of control while EC\(_{50}\) was reached at 1.0±0.3 nM (n=6). These concentrations are at and even below the therapeutic plasma levels. CsA decreased the aequorin light signal in human failing trabeculae to 71.5±5.9% (n=5), indicating decreased calcium transients. Estimation of the SR calcium load via measurement of rapid cooling contractures revealed a decrease to 84.4±6.5% in failing human preparations (n=6). Measurements of both decreased SR calcium load and force development in presence of CsA were also observed in four non-failing human muscle preparations. In rabbit muscle preparations (n=8), developed force decreased to 50.2±7.7% (n=8, EC\(_{50}\): 1.9±0.4 nM) and rapid cooling contractures to 74.0±7.4% of control at 100 nmol/l CsA. No direct effects were observed on myofilament calcium sensitivity nor on maximal force development of permeabilized preparations from the rabbit (n=7). Oxygen consumption measurements showed that CsA decreased the economy of contraction to 76.4±7.9% in rabbit preparations (n=8). Conclusions: CsA causes a direct cardio-depressive effect at clinically relevant concentrations, most likely due to altered handling of Ca\(^{2+}\) by the SR.

Keywords: Calcium (cellular); Contractile function; e–c Coupling; Heart failure; Immunology; Oxygen consumption; SR (function)

1. Introduction

Cyclosporine A (CsA) is one of the most commonly used immunosuppressive drugs as part of pharmacological therapy following solid organ transplantation and various autoimmune diseases. CsA therapy is associated with severe side effects like arterial hypertony, atherosclerosis, and thromboembolic and renal complications [1]. In addition, there are studies indicating a cardiotoxic potential of CsA [2,3] and chronic effects of CsA on contractile function [4–9]. CsA is a potent inhibitor of the calcium-calmodulin-dependent phosphatase calcineurin resulting in decreased expression of T-Cell responsive genes, such as the IL-2 gene [10]. Moreover, because calcineurin signalling may play a relevant role in regulation of cardiac hypertrophy, CsA was used as a tool to study this pathway and was even suggested to control cardiac hypertrophy [11,12]. However, animal studies regarding the effect of

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CsA to prevent hypertrophy have yielded conflicting results [13,14]. Moreover, increased susceptibility to decompensation and failure has been reported [15].

Although CsA is frequently used as part of the integral therapy following organ transplantation and as a tool, the acute effects on calcium cycling and myocardial contractility are not known. In this study we investigated the direct effect of CsA on contractility of multicellular muscle preparations dissected from rabbit hearts, end-stage failing, and non-failing human hearts.

2. Methods

2.1. Muscle preparation

Human multicellular muscle preparations (n=23) were dissected from end-stage failing hearts (ischemic cardiomyopathy, n=5, or dilated cardiomyopathy n=9) that were obtained from patients undergoing cardiac transplantation. Four additional preparations were obtained from two non-failing donor hearts that could not be transplanted for technical reasons. All but two non-failing preparations were taken from the right ventricles. Patient characteristics (five female, nine male) were (average±SD, range); age 56±9, 42–70 years; weight 75±13, 60–107 kg; LV-ejection fraction 29±8, 15–39%; pulmonary capillary wedge pressure 23±11, 8–43 mmHg; and cardiac index 2.3±1.0 L/min/m². Medication (number of patients) included digitalis (11), diuretics (10), β-receptor antagonists (8), ACE-inhibitors (9), statins (4), calcium channel antagonists (2), and PDE-inhibitors (1).

Hearts were transported in a modified, ice-cold Krebs–Henseleit (K–H) solution containing (in mM): 120 NaCl, 5.0 KCl, 2.0 MgSO₄, 1.2 NaH₂PO₄, 20 NaHCO₃, 0.25 CaCl₂, with the addition of 20 mM 2,3-butanedione monoxime (BDM) as a cardioprotective agent.

Female White New-Zealand rabbits weighing 1.5–2.5 kg were anaesthetized with thiopental (50 mg/kg) via the ear vein after heparinization (1000 IU). Hearts were rapidly dissected and retrogradely perfused through the aorta with a Krebs–Henseleit solution and right ventricular trabeculae were dissected as previously described [16,17]. Outlines of the study were designed and carried out in accordance with institutional guidelines regarding care and use of animals.

All muscle preparations were dissected with the aid of a stereo microscope. Muscles were carefully dissected and dimensions were measured at 40X magnification (resolving power ~10 µm). In the protocols with intact muscles, preparations were mounted in the experimental set-up in the BDM-containing K–H solution, which was immediately switched to a K–H solution without BDM. All K–H solutions were kept at equilibrium with 95% O₂/5% CO₂, resulting in a pH of 7.4. Intact rabbit muscles were discarded (n=2, out of 29) when either developed force at 2 Hz stimulation frequency was <10 mN/mm² or run-down during the experiment exceeded 15% per hour. Human muscle preparations were discarded when developed force was <3 mN/mm² or run-down during the experiment exceeded 15% per hour (n=2). Average dimensions (width×thickness×length, in µm) were 423±40, 361±37, and 2868±159 (human failing, n=23), 575±73, 538±43, and 2625±191 (human non-failing, n=4), and 294±24, 251±21, 2763±225 (rabbit, n=27).

2.2. Mechanical measurements

Muscles were mounted using two blocks of ventricular or valvar tissue in the experimental set-up between a basket-shaped extension [18,19] of a force transducer and a hook connected to a micro-displacement device. Following mounting of the muscles, superfusion with K–H (at 37°C) was started and the calcium concentration was raised from 0.25 to 1.75 mM in steps of 0.25 mM every 2–5 min. When the [Ca²⁺] of 1.0 mM concentration was reached, stimulation was started through 5 ms asymmetric pulses at 20% above threshold voltage (typically 2–4 V) at 1.0 Hz. At 1.75 mM [Ca²⁺], the muscle was carefully stretched in several steps until diastolic force (Fₘₜ₉₈) was about 10–20% of active developed force (Fₐᵥₑₙ). This reflects a sarcomere length of about 2.1–2.2 µm [18,19]. The muscles were left contracting under these conditions for at least an additional hour to equilibrate. The following mechanical parameters were measured: active developed force (Fₐᵥₑₙ, in mN/mm²), diastolic force (Fₘₜ₉₈, in mN/mm²), normalized maximum and minimum of the first derivative of force (±dF/dt/F and −dF/dt/F, in s⁻¹), time from stimulation to peak tension (TTP, in ms), to 50% relaxation (TT50%, in ms), and to 90% relaxation (TT90%). Concentration response curves (between 10⁻¹⁰ and 10⁻⁷ M CsA) were measured in eight rabbit and six failing human muscle preparations.

CsA was dissolved in ethanol. As a control to test for the impact of the vehicle and time-dependent deterioration of the muscle preparations, in eight rabbit and two human preparations the equivalent amounts of ethanol were given at the same time-course. The cumulative amount of ethanol added throughout an entire experiment was <0.12% of the superfusate volume.

2.3. Aequorin measurements

In five human preparations from failing hearts, the concentration–response curve was repeated, while cytosolic calcium transient measurements were performed. Therefore the preparations were loaded with aequorin as previously described [20,21]. Peak systolic light emission was taken as indicator of the peak systolic calcium transient.
2.4. Rapid-cooling measurements

To estimate the sarcoplasmic reticulum (SR) calcium content, measurements of rapid cooling contractures (RCCs) were performed in a set-up modified after Bers [22], Bridge [23] and Pieske and coworkers [24]. The set-up was modified regarding muscle attachment to reduce compliance of the preparation and regarding the superfusate pathway. In this protocol 10 human (6 failing and 4 non-failing) and 8 rabbit muscle preparations were included. Muscles were mounted and equilibrated identically to those in the concentration response curve group. Rapid cooling contractures were measured by switching off stimulation and simultaneously cooling the superfusate to about 0.5–1.5°C within 1–2 s by rapidly cooling the superfusate inflow tract with custom designed heat/cold-exchangers. The amplitude of the developed contracture was taken as an index of SR Ca2+-content. CsA was added to a final concentration of 10−7 M. After force had stabilized, RCCs were again measured. Repetitive measurements were used to quantify stability of the preparation and for possible correction for time-dependent run-down of the preparation.

2.5. Oxygen consumption measurements

To measure the impact of CsA on the total economy of the contracting muscle we measured oxygen consumption in a separate set of experiments. In total eight rabbit muscle preparations and three failing human muscle preparations were studied. In a measurement chamber equipped with an oxygen-sensor, muscles were mounted and equilibrated as described above. Oxygen consumption measurements were performed with a stopped-flow protocol where the decrease of partial oxygen pressure was measured to calculate the amount of oxygen consumption as described previously [25] in absence and in presence of CsA. Economy of contraction was taken as the slope of the relationship between the force–time integral (FTI) and myocardial oxygen consumption. To test whether an increased basal metabolism was independent of an effect on the activation process, BDM was used. In presence of 30 mM BDM, basal metabolism oxygen consumption was measured in presence and in absence of 100 nM CsA.

2.6. Myofilament calcium responsiveness of rabbit preparations

In seven thin, skinned rabbit muscle preparations, we measured calcium sensitivity, slope of the force–pCa relationship, and maximal force development in the absence and in the presence of 10−7 M CsA. After dissection, suitable muscles were immediately placed in a relaxing solution containing 1% v/v Triton X-100 and stored at 4°C for 15–20 h prior to use. Average dimensions were 172±32 μm in width, 148±32 μm in thickness, and 3057±258 μm in length. Composition of all solutions was calculated using methods modified after Fabiato and Fabiato [26], and was identical to those previously described [27]. Muscles were attached via miniature aluminium clips to hooks connected to a force-transducer and a micro-displacement device in a muscle bath (volume 80 μl) that was kept at 15.0±0.2°C throughout the experiment [27] After 3 maximal activations during which diastolic force was set to about 10% of developed force, a force–pCa (pCa=−log(Ca2+)) relationship was measured. Between each measurement muscles were relaxed for at least 2 min followed by at least 2 min of pre-activation. This protocol ensures stability of the preparation as is reflected by the very low amount of run-down of the preparations (<10% in each individual preparation) over the 3 h timespan of an experiment. After the highest calcium concentration, an additional maximal activation was measured followed by two maximal activations in solutions containing 10−7 M CsA (to measure the effect on maximal developed force). CsA was also present in the relaxing and pre-activating solution. pCa50% (calcium concentration where developed force is half-maximal) was taken as an indication of calcium sensitivity. Slope of the curve at pCa50% (nHill) was taken as an indicator of cooperativity of the myofilaments.

2.7. Data analysis and statistics

In the protocols where dose-response, rapid cooling contractures, and myofilament responsiveness were measured, data were both collected and analyzed off-line with custom-designed data-acquisition programs (LabView, National Instruments). All programs contained an on-line analysis mode to quantify contractile parameters during the experiment. Concentration response curves were fitted with a modified Hill-equation and half-maximal (EC50) and maximal effect were calculated for each individual experiment. Hill-fit parameters were treated statistically as if they were obtained by direct measurement. Data collection of oxygen consumption measurements and calcium transients was done with commercially available software and analyzed off-line. Statistical significance was determined by Students’ t-test for paired or unpaired data where applicable. Data are expressed as means (S.E.M. unless stated otherwise. Two-tailed values of P<0.05 were accepted as significant.

3. Results

3.1. Effects of CsA on concentration response measurements

We observed a concentration dependent decrease of maximal active developed twitch force (Fdev) in all muscle preparations. In Fig. 1 the concentration response curve for
both rabbit and human muscle preparations is given. In failing human myocardium (n=6) half-maximal effect of twitch force was reached at 1.01±0.31×10⁻⁷ M CsA. The maximal effect was reached at 10⁻⁷ mol/l and amounted to a reduction in F_{dev} to 55.6±6.4% of control. Mechanical twitch parameters of control (before concentration response curve) and at 10⁻⁷ M CsA are given in Table 1. The decrease in F_{dev} was paralleled by a decrease in times to peak tension, and times to 50% and 90% relaxation. In rabbit myocardial preparations (n=8), half-maximal effect was calculated to be reached at 1.88±0.38×10⁻⁹ M CsA. At 10⁻⁷ M CsA maximal effect was reached which amounted to a decrease in F_{dev} to 50.2±7.7% compared to control. To rule out that the measured effects of CsA on mechanical parameters were caused by deterioration of the preparations due to the solvent ethanol and/or with time, in eight rabbit and two human preparations from failing hearts, the same concentration response curve was measured, using vehicle only (Maximal amount of ethanol added during one experiment was 0.12% v/v). On average, in rabbit myocardium there was small (12±3%) decrease in F_{dev}. This decrease incorporates both the effect of ethanol alone and the time-dependent rundown of the preparation. In a previous study [16] we observed under similar conditions a time dependent rundown of F_{dev} over periods comparable to these experiments of about 2–6%. Thus, the effect of ethanol alone on F_{dev} is maximally about 10%, and is quantitatively comparable to values previously observed in different species. [28,29]. In the two human preparations there was a decrease of 2% and 7% over the entire protocol. Time to complete a concentration response curve was about 60–75 min. In a separate control experiment no change was observed in the pH of the K–H solution used in our studies before, and after addition of 10⁻⁷ M CsA (0.1% v/v ethanol). Moreover, in three experiments we measured a concentration response curve (10⁻¹⁰ to 10⁻⁵ M) of FK-506 in rabbit myocardium. In sharp contrast to CsA, F_{dev} decreased marginally (likely due to the solvent ethanol and deterioration) to 92±4% of control at 10⁻⁷ M, and then even rose to 104±4% of control at 10⁻⁵ M FK-506.

3.2. Effects of CsA on aequorin light transients

Aequorin light transients (Fig. 2) were measured in five muscle preparations from end-stage failing human hearts. In these preparations, the application of 10⁻⁷ M CsA resulted in a decrease in F_{dev} to 82.7±4.2% of control (Fig. 2b). This was paralleled by a decrease in amplitude of the aequorin light transient to 71.5±5.9% of control, indicating a decrease in the amplitude of the calcium transient amplitude (Fig. 2a).

3.3. Sarcoplasmic reticulum calcium load

To study whether altered calcium content of the SR could explain the reduction in contractility after addition of CsA, SR calcium content was estimated by rapid cooling

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Rabbit (n=8)</th>
<th>Control</th>
<th>CsA</th>
<th>Failing Human (n=6)</th>
<th>Control</th>
<th>CsA</th>
</tr>
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<tr>
<td>F_{dev} (mN/mm²)</td>
<td>7.98±2.44</td>
<td>4.23±1.23 *</td>
<td>5.76±2.12</td>
<td>3.37±1.4 *</td>
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<tr>
<td>F_{0} (mN/mm²)</td>
<td>5.12±1.70</td>
<td>4.64±1.54</td>
<td>3.13±0.54</td>
<td>2.94±0.67</td>
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<tr>
<td>TTP (ms)</td>
<td>117±5</td>
<td>105±4 *</td>
<td>199±7</td>
<td>182±10 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT50% (ms)</td>
<td>178±7</td>
<td>157±6 *</td>
<td>344±18</td>
<td>330±28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT90% (ms)</td>
<td>228±7</td>
<td>208±7 *</td>
<td>543±42</td>
<td>544±60</td>
<td></td>
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<tr>
<td>+dF/dt/F (s⁻¹)</td>
<td>15.2±0.6</td>
<td>16.5±0.6 *</td>
<td>8.6±0.3</td>
<td>9.3±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−dF/dt/F (s⁻¹)</td>
<td>−12.5±0.4</td>
<td>−14.1±0.5 *</td>
<td>−5.0±0.4</td>
<td>−5.1±0.6</td>
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</tbody>
</table>

* Analysis of twitch timing and contractile parameters at 10⁻⁷ M CsA compared to control. Data represent n=8 for rabbit and n=6 for failing human muscle preparations. F_{dev}: developed force, F_{0}: diastolic force, TTP: time from stimulation to peak tension, TT50% (TT90%): time from stimulation to 50% (90%) relaxation, +dF/dt/F: maximal rate of force development, normalized, −dF/dt/F: maximal rate of relaxation, normalized. * denotes a significant difference (P<0.05) compared to control.
contractures (RCCs). At the basic stimulation frequency of 1 Hz, \( F_{dev} \) and RCCs were measured before and after addition of \( 10^{-7} \) M CsA. A typical experiment with a human preparation is given in Fig. 3a. Original recordings of force and temperature before (left panel) and 15 min after addition of \( 10^{-7} \) M CsA are given. As can be seen, both isometric twitch force and the amplitude of the rapid cooling contracture were decreased in the presence of CsA. The decrease in RCC amplitude indicated a diminished SR calcium load following application of CsA.

In failing human preparations RCC-amplitude decreased from \( 6.07 \pm 1.49 \) to \( 4.59 \pm 1.06 \) mN/mm\(^2\) (83.0\( \pm \)3.1%, \( P < 0.05 \)). In non-failing human preparations (\( n=4 \)), RCC amplitude also decreased after addition of \( 10^{-7} \) M CsA, from \( 17.6 \pm 6.1 \) to \( 13.8 \pm 4.1 \) mN/mm\(^2\) (80.5\( \pm \)8.4%, \( P < 0.05 \)). Additionally, we observed that in the non-failing human preparations, force development (8.0 \( \pm \)1.2 mN/mm\(^2\)) was higher compared to failing preparations (5.3\( \pm \)1.3 mN/mm\(^2\), \( P < 0.05 \)). In Fig. 3b, \( F_{dev} \) and RCC amplitude are given as percentage of control (as measured

Fig. 2. Cytosolic calcium transients. Aequorin light signals (A) and twitch force development (B) under control conditions and in presence of \( 10^{-7} \) M CsA. Diameter of the preparation was 600 \( \mu \)m; preparations were stimulated at 1 Hz, 37°C, pH 7.4, and 1.75 mM Ca\(^{2+}\).

Fig. 3. Rapid cooling contractures. A: Original registration of force and temperature of a non-failing human muscle before and 15 min after addition of \( 10^{-7} \) M CsA. B: Left bars: average effect of \( 10^{-7} \) M CsA on developed force in rabbit (R, \( n=8 \)), non-failing human (NF, \( n=4 \)), and failing human myocardium (F, \( n=6 \)), represented as the fraction of pre-CsA values. Right bars: average effect of CsA on rapid cooling contractures of the same muscles. * denotes a significant difference (\( P < 0.05 \)) compared to control.
right before addition of CsA) for rabbit, non-failing human, and failing human muscle preparations.

In all eight trabeculae from the rabbit we observed a decrease in the amplitude of the RCC (from 9.6±2.4 to 7.6±1.9 mN/mm², P<0.05) after administration of CsA. F_{dev} was also decreased (from 6.9±1.1 to 4.6±0.8 mN/mm², P<0.05) as expected, although slightly less than in the concentration response measurements.

3.4. Myofilament calcium responsiveness

Although the results of the RCC experiments indicate the mechanism of action to be on SR calcium handling/SR calcium load, interpretation of the data from RCCs could be complicated by altered calcium sensitivity of the myofilaments. This would over- or under-estimate the SR calcium content. Thus, measurements of myofilament responsiveness serves a dual purpose: (a) to validate our finding of altered SR calcium load, and (b) to test for a possible mechanism of action on the actin–myosin matrix per se. Average data of the skinned fibre experiments from rabbit myocardium (n=7) scaled to their respective maximum are given in Fig. 4a. Mean contractile parameters calculated from the individual force–pCa curves are given in Fig. 4b. In skinned rabbit myocardium, CsA had no effect on any parameter measured, indicating that the effect of CsA is not mediated by a direct impact on calcium sensitivity (EC_{50}), cooperativity (nHill), or maximal force generating capacity (F_{max}) of the myofilaments (data in Fig. 4b). This implies that the changes in the amplitude of the RCC experiments are solely caused by an effect on SR calcium load.

3.5. Oxygen consumption

To investigate whether the decrease in developed tension would be accompanied by an equivalent decrease in oxygen consumption we measured changes in partial oxygen pressure to calculate myocardial economy. At 2.5 mM Ca^{2+} and at 1 Hz the rabbit preparations used 2.56±1.58 ml O_{2}/min. per 100 g of muscle volume. Despite decreased force development after addition of 10^{-7} M CsA (from 6.91±1.6 to 3.51±0.58 mN/mm², P<0.05), O_{2} consumption still amounted to 2.56±1.91 ml O_{2}/min. per 100 g of muscle volume. In Fig. 5a a typical experiment is depicted. At similar force–time integral (FTI), O_{2} consumption is higher in the presence of CsA. On average, economy of contraction (inverted slope of the relationship between O_{2}-consumption/FTI) was decreased from the control value of 13.68±2.69 to 10.40±2.0 N s m/ml O_{2} in presence of 10^{-7} M CsA (P<0.05). This reflects a decrease in economy to 76.4 ±7.9% in rabbit muscle preparations (Fig. 5b). That CsA increased the basal metabolism independent of an effect on the activation process was tested by using BDM. In the presence of 30 mM BDM, oxygen consumption was significantly higher in presence than in absence of CsA (Fig. 5c). Alternatively, when basal metabolism was calculated as the intercept of the ordinate (i.e. extrapolation to zero FTI) similar results were obtained; this intercept was higher in the presence than in absence of CsA (0.55±0.13 vs. 0.97±0.15 ml O_{2}/min. per 100 g muscle tissue, P<0.05) again suggesting that basal oxygen consumption had increased.

Similar results (average economy of 69.5% in presence
of 10⁻⁷ M CsA) were obtained with muscle preparations from failing human myocardium (n=3, not shown).

4. Discussion

In the present study we observed a concentration-dependent cardio-depressive effect of CsA in human and rabbit myocardium with an IC₅₀ of 1–2 nM and a maximal negative inotropic effect of 30–50% occurring at 100 nM. The sarcoplasmic Ca²⁺ content was lowered in the presence of CsA, which was associated with decreased cytosolic calcium transients. CsA did not affect myofilament calcium responsiveness. Despite decreased mechanical performance, myocardial oxygen consumption was unchanged, indicating a decreased economy of contraction with CsA.

4.1. Mechanism

There are different possibilities to explain the mechanisms by which CsA influences calcium cycling and consequent mechanical performance of the myocardium. Decreased SR calcium load could result from reduced transsarcolemmal calcium influx or from an increased leak of calcium from the SR due to altered function of the ryanodine receptor (RyR), or another mechanism. No definitive answer regarding the mechanism by which CsA decreases SR calcium load can be derived from the present study. However, from our oxygen-consumption measurements we would like to derive the hypothesis that the effects of CsA result from increased SR calcium leak. This hypothesis would be in agreement with a report that CsA interacts or modifies SR proteins; in chronically CsA-treated animals, an alteration of the SR calcium release channel was observed [30].

Despite the depressed contractility after addition of CsA, oxygen consumption was not reduced. When isometric FTI, which has been shown to be a major determinant of myocardial O₂-consumption [25], was plotted in relation to O₂-consumption, we found that in presence of CsA the intercept and slope were significantly higher. This indicates higher energy consumption of the basal, non-force producing muscle and a further over-proportional increase of O₂-consumption compared to the increase in mechanical activity. Also, inhibition of the activation process by BDM underlined that the increase in basal oxygen consumption in presence of CsA did not originate in activation processes. The findings would be in line with the hypothesis that CsA induces a calcium leak in the SR with subsequent increased energy consumption for calcium cycling. A similar observation has been proposed after application of Ryanodine [31].

4.2. Implications

The present study has important implications regarding (i) the experimental use of CsA to evaluate signalling of hypertrophic response and (ii) the clinical use of CsA as an immunosuppressive agent.

The experimental use of CsA has been propagated for differentiation between calcineurin-dependent and independent signalling of hypertrophic response because CsA would specifically inhibit calcineurin without direct effects on calcium cycling [10]. Moreover, CsA was used to evaluate the effects of calcineurin inhibition on the development of hypertrophy and cardiac failure in transgenic animal models of cardiac hypertrophy [12] and animal models of pressure overload hypertrophy [13,14]. Because CsA has direct effects on calcium cycling and myocardial contractility, results of these studies must be interpreted with caution. The calcineurin-independent effects of CsA
may in part be responsible for the discrepancy in these findings regarding prevention of hypertrophy after application of CsA and regarding the observation of reduced myocardial function and increased susceptibility to decompensation and heart failure in animals treated with CsA [15]. Of note, the effects of CsA on mechanial function and calcium cycling does not seem to be related to its effect on calcineurin because FK-506 which is also an inhibitor of calcineurin did not negatively influence mechanical parameters of the myocardium. At high concentrations of FK-506 even a small positive inotropic effect was observed, this in agreement with a study of FK-506 on unloaded shortening of isolated myocytes [32].

The clinical use of CsA stems from its ability to prevent graft rejection following organ transplantation, and exerts its major therapeutic effect by inhibiting activation of the T-cells through inhibition of calcineurin phosphatase activity [33,34]. Cardio-depressive actions of chronic CsA treatment have been previously described in clinical and pre-clinical settings [2,9] but the direct acute effects of CsA on cardiac muscle contraction are not known. In our experiments we observed the maximum negative inotropic effect of CsA at 100 nM. This is within, or even below the therapeutically applied range (80–160 nM) reflecting plasma levels of 100–200 ng/ml [1]. Thus, it is likely that cardiac contractility may be decreased following the application of CsA in patients. A negative inotropic effect of CsA could be clinically relevant in patients with pre-existing cardiac dysfunction, receiving immunosuppressant therapy with CsA for autoimmune diseases or after organ transplantation. Clinical studies are warranted to investigate the acute effects of CsA on cardiac function.

4.3. Limitations

Most of the preparations that were obtained from the human hearts were from end-stage failing hearts, thereby not necessarily reflecting the physiological situation. Although a more extensive study of non-failing human myocardium would be of potential importance, the availability of viable, truly non-failing myocardium is extremely rare and therefore precludes larger study designs. Also, measurements of the absolute diastolic calcium may be transcribed pathway for cardiac hypertrophy. Cell 1998;93:215–227.

Limitations

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4.4. Summary

In summary, CsA exhibits a direct negative inotropic effect in human and rabbit cardiac muscle preparations, most likely due to a sustained Ca$^{2+}$ leakage from the SR.

Acknowledgements

This work was partially supported by research grants of the Deutsche Forschungs-gemeinschaft to G.H., and by a research development grant to P.M.L.J.

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