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# FK506 does not affect cardiac contractility and adrenergic response in vitro

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**Abstract**

FK506 (tacrolimus) is a new immunosuppressant being used in cardiac allograft transplantation. While cyclosporine A has been shown to exert an acute negative inotropic effect on isolated heart muscle preparations, little is known of the inotropic influence of FK506. The $Ca^{2+}$ release channel of human skeletal muscle and cardiac muscle is associated with FK506 binding proteins (FKBP), FKBP12 and FKBP12.6, respectively. FKBPs can be dissociated by treatment with FK506. As a consequence of FK506 exposure, isolated skeletal muscle and cardiac muscle ryanodine receptors show altered gating characteristics. Therefore, we analyzed the direct inotropic effect of FK506 exposure to isolated, intact heart muscle preparations from the human and rabbits. Experiments were performed on isolated, electrically stimulated right atrial auricular muscle strips obtained from human myocardium during elective open heart surgery and on intact right ventricular trabeculae from rabbit hearts. The human preparations were exposed to concentrations of $8 \times 10^{-9}$, $8 \times 10^{-8}$ and $8 \times 10^{-6}$ M FK506 followed by a cumulative dose–response curve with isoprenaline as a non-selective β-adrenoceptor agonist. Our data suggest that FK506 does not exert any positive or negative inotropic effect in either human or rabbit myocardium. 

*Keywords:* FK506; Immunophilin; Ryanodine receptor; Excitation–contraction coupling; FK506 binding protein (FKBP)

## 1. Introduction

FK506 (tacrolimus) is an immunosuppressant, isolated in 1984 from *Streptomyces tsukubaensis*, and is claimed to be an effective drug against allograft rejection (Winkler and Christians, 1995). Like cyclosporine A and rapamycin, FK506 belongs to a family of immunosuppressant drugs suppressing T-cell activity, interleukin 2-mediated activation of cytotoxic T-cells or interleukin 2 induction, respectively. All three drugs bind to immunophilins, which are the intracellular receptors of these drugs. FK506 binds specifically to the immunophilin FK506 binding proteins (FKBP), which are ubiquitously expressed proteins with *cis-trans* peptidyl-prolyl isomerase activity (Groth, 1995; Harding et al., 1989). The FK506–FKBP12 complex binds to the A-subunit of the $Ca^{2+}$-calmodulin-dependent serine/threonine phosphatase, calcineurin, inhibits its enzyme activity (Bierer et al., 1990; Griffith et al., 1995) and hence the translocation of NF-AT4 to the nucleus, which is regarded as the key step in the initiation of interleukin 2 transcription and subsequent T-cell activation (Shibasaki et al., 1996).

Physiological intracellular targets of FKBPs are $Ca^{2+}$ release channels such as the cardiac and skeletal muscle ryanodine receptor and the inositol-(1,4,5)-trisphosphate receptor (Cameron et al., 1995a,b; Lam et al., 1995b; Timerman et al., 1993, 1994). The ryanodine receptor represents the intracellular $Ca^{2+}$ release channel involved in excitation–contraction coupling in striated muscle cells located on the sarcoplasmic side of the triadic structure

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(Block et al., 1988; Caterall, 1991; McPherson and Campbell, 1993; Otsu et al., 1990; Zorzato et al., 1990). At least two different isoforms are expressed in striated muscles, which are specific for cardiac or skeletal muscle, respectively. The skeletal ryanodine receptor is tightly associated with FKBP12, whereas the cardiac ryanodine receptor binds to FKBP12.6 (Kaftan et al., 1996; Lam et al., 1995a,b; Timerman et al., 1996; Xin et al., 1999).

The binding of FK506 to FKBPs causes dissociation of the complex from both the cardiac and the skeletal ryanodine receptor (Lam et al., 1995b; Timerman et al., 1993, 1995). FK506 binding to native vesicles of terminal cisternae alters the gating characteristics of the $Ca^{2+}$ release channel in rabbit skeletal muscle (Ahern et al., 1994) incorporated in bilayers by increasing mean open time and $Ca^{2+}$ peak release (Brillantes et al., 1994). In cardiac muscle, however, the incubation with FK506 does not influence the gating characteristics of single-channel preparations although FKBP12 and FKBP12.6 are present in cardiac cytosol (Timerman et al., 1996). On the other hand, in rat heart cells, FK506 had effects on the duration of spontaneous and depolarization-evoked $Ca^{2+}$ sparks (Xiao et al., 1997). An increased open probability of the cardiac ryanodine receptor was also recently reported for FKBP12-deficient mice (Shou et al., 1998).

Recently, binding of FKBP12.6 to the cardiac ryanodine receptor has been shown to be regulated by protein kinase A phosphorylation of the channel subunits. Protein kinase A phosphorylation of the cardiac ryanodine receptor dissociates FKBP12.6 and alters channel characteristics (Marx et al., 2000).

It is noteworthy that cyclosporine A causes a direct cardio-depressive effect at clinically relevant concentrations. Cyclosporine A depresses contractility of isolated human heart and rabbit heart preparations by up to 50%, most likely due to alterations in sarcoplasmic reticulum $Ca^{2+}$-handling (Janssen et al., 2000).

These findings do not allow an interaction of FK506 with the contractile state of the human myocardium to be excluded. In this study, we have therefore examined the influence of FK506 on contractile behaviour and its putative influence on adrenergic regulation of excitation–contraction coupling in isolated human cardiac muscle trabeculae obtained during elective open heart surgery, and on right ventricular trabeculae from rabbit hearts.

## 2. Material and methods

### 2.1. Human atrial experiments

Experiments were performed on isolated, electrically stimulated right atrial auricular muscle strips obtained from human myocardium during elective open heart surgery. The use of human cardiac tissue was approved by the local ethics committee and after informed consent of the patients. Preparation of atrial tissue began within 5–10 min of surgical removal in oxygenated Krebs–Henseleit solution at room temperature. The right atrial appendages were dissected to yield trabecular strips (4–5 mm in length and 1 mm or less in diameter) without endocardial damage and with fibers running parallel to the length.

Human preparations were mounted in 10-ml organ baths containing Krebs–Henseleit solution of the following composition [mM]: 118.2 NaCl, 4.8 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 $NaH_2PO_4$, 24.9 $NaHCO_3$, 10 glucose, pH 7.4; equilibrated with 95% $O_2$/5% $CO_2$ at 37 °C. Myocardial strips were electrically stimulated by square wave pulses about 20% above threshold (3–8 V) at a frequency of stimulation of 1 Hz (stimulator, Hugo Sachs Elektronik, March Hustetten, Germany). Developed tension of the preparation (maintained under a resting tension of 5 mN) was recorded via a strain gauge on a chart recorder (Hellige, Freiburg, Germany). Preparations were allowed to equilibrate for 2 h.

After equilibration, FK506 (Fujisawa Japan) was added to the bathing solution. FK506 was dissolved in 100% ethanol. When FK506 was added, the control preparations were exposed to the same concentration of ethanol. The ethanol concentration did not exceed 0.06% (v/v). Muscle strips were incubated for 30 min with concentrations of $8 \times 10^{-9}$, $8 \times 10^{-8}$ and $8 \times 10^{-6}$ M FK506, followed by a cumulative dose–response curve with isoprenaline as a non-selective β-adrenoceptor agonist. Concentration–response curves were monitored and compared to those from control samples from the same cardiac donor and not exposed to FK506.

### 2.2. Rabbit ventricular experiments

Right ventricular cardiac trabeculae were obtained from adult chinchilla-bastard rabbits, weighing between 1.5 and 2.5 kg after sodium thiopental anaesthesia and heparinization via the ear vein. Protocols for care and use of animals conformed to institutional guidelines. Preparations were stimulated at 1 Hz and perfused with a Krebs–Henseleit solution as described in detail previously (Janssen et al., 2000).

Trabeculae were mounted between a KG-4 force transducer (Scientific Instruments, Heidelberg, Germany) and a micro-displacement device (for details, see Janssen et al., 2000). FK506 (maximal in 0.05% v/v ethanol) concentration–response curves were made by successively increasing the concentration from $10^{-10}$ to $10^{-5}$ M in logarithmic steps. A series of control experiments was performed to assess the effect of ethanol; however, no inotropic effects were observed at the maximal ethanol concentration used in the study. These control experiments were also used to evaluate deterioration of the preparations over time to possibly differentiate between active negative inotropic effect and experimentally induced muscle fatigue.

### 2.3. Calcineurin bioassay

Rabbit ventricular trabeculae were incubated as described above. Muscles were homogenised and protein extracts were used in a cellular calcineurin phosphatase assay kit according to the manufacturers' instructions (Biomol, Germany). The assay uses the RII phosphopeptide as substrate and the released free phosphate is detected.

### 2.4. Data analysis and statistics

Human data were analyzed from chart paper recordings. Rabbit data were recorded and analyzed both on- and off-line, using custom-written software in LabView (National Instruments, TX, USA). Muscles that had excessive rundown during the equilibration period were discarded. Student's *t*-test for paired or unpaired data was used where applicable. A two-tailed value for $P < 0.05$ was taken as significant. Data are represented as means ± S.E.M. unless stated otherwise.

## 3. Results

We tested myocardial samples of patients from various New York Heart Association (NYHA) classifications. In no case could we observe a FK506-induced change in basal contractile force compared to that of the control tissue even at the highest concentration, $8 \times 10^{-6}$ M (Fig. 1), and there was also no significant difference in basal contractility depending on the NYHA classification of the patient. Thus, neither a positive nor a negative inotropic effect of FK506 was observed in human atrial strips.

In order to examine whether FK506 modulates β-adrenergic effects on excitation–contraction coupling, we recorded cumulative concentration–response curves of isoprenaline during FK506 exposure of human atrial myocardium. The isoprenaline-stimulated force did not show any differences between control and FK506-exposed muscle strips (Fig. 2).

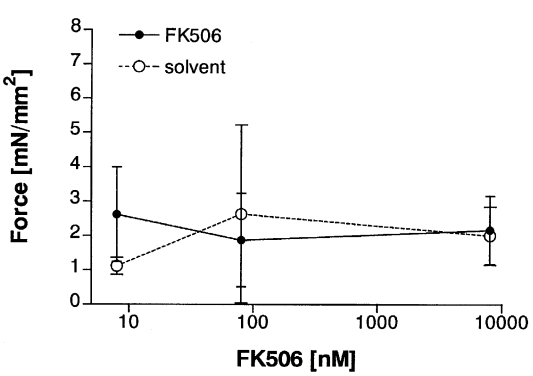


Fig. 1. Changes in force of contraction of human atrial auricular tissue exposed to different concentrations of FK506 for 30 min (mean ± S.D.).

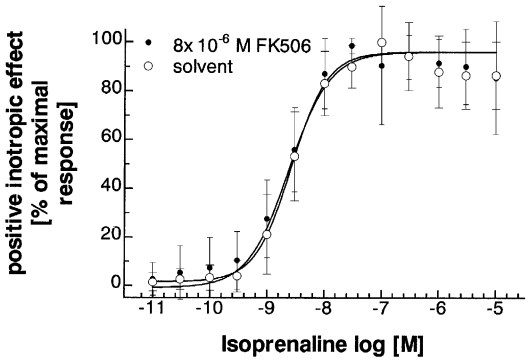


Fig. 2. Isoprenaline concentration response curve of human atrial trabeculae incubated with $8 \times 10^{-6}$ M FK506 for 30 min [●] ($n = 5$). There was no effect on β-adrenergic response compared with control tissue exposed to solvent [○] ($n = 6$; basal contractility 2 mN/mm$^2$, maximal developed force after isoprenaline stimulation was up to 14 mN/mm$^2$).

To assess the quality of our preparations, we calculated the amount of force production per cross-section area in a subset of human atrial preparations by dividing the absolute developed force by the apparent cross-sectional area. During inotropic stimulation, this amounted to about 14 mN/mm$^2$, comparable to previous observations in human atrial muscle tissue.

These results were confirmed for rabbit muscle preparations. No inotropic effect of FK506 was observed between $10^{-10}$ and $10^{-6}$ M. However, at the extremely high concentration of $10^{-5}$ M, FK506 displayed a slight positive inotropic effect (Fig. 3). Compared to control muscle preparations that had lost 10% of their initial force at this time during the protocol, developed force rose from 92% (at $10^{-6}$ M) to 104% of the initial values at $10^{-5}$ M. Thus, the positive inotropic effect of FK506 at this concentration was of the order of 10%. Twitch timing was not influenced by FK506. Time from stimulation to peak tension was 128 ± 4 ms for the control and 127 ± 4 with $10^{-5}$ M FK506, while time from peak tension to 50% relaxation was 49 ± 4 ms for the control and 48 ± 4 ms with $10^{-5}$ M FK506.

In contrast, cyclosporine A exerted a pronounced negative inotropic effect at $10^{-7}$ M. Contractile force decreased by 45% compared to that in control preparations (Fig. 3). Even after washout of the drug, a statistically significant reduction in force development persisted.

The lack of an inotropic effect of FK506 up to a concentration of $10^{-6}$ M prompted us to examine whether FK506 and cyclosporine A exerted any functional effect on intact rabbit muscle preparations. Indeed, $10^{-7}$ M FK506 inhibited cellular calcineurin phosphatase activity by 53% when compared to control preparations, whereas cyclosporine A ($10^{-7}$ M) inhibited calcineurin by 30% ($n = 2$ each; data not shown). These experiments showed that both immunosuppressive drugs were taken up by the muscle preparations from the organ bad solution. Moreover, FK506 appears to inhibit calcineurin more effectively

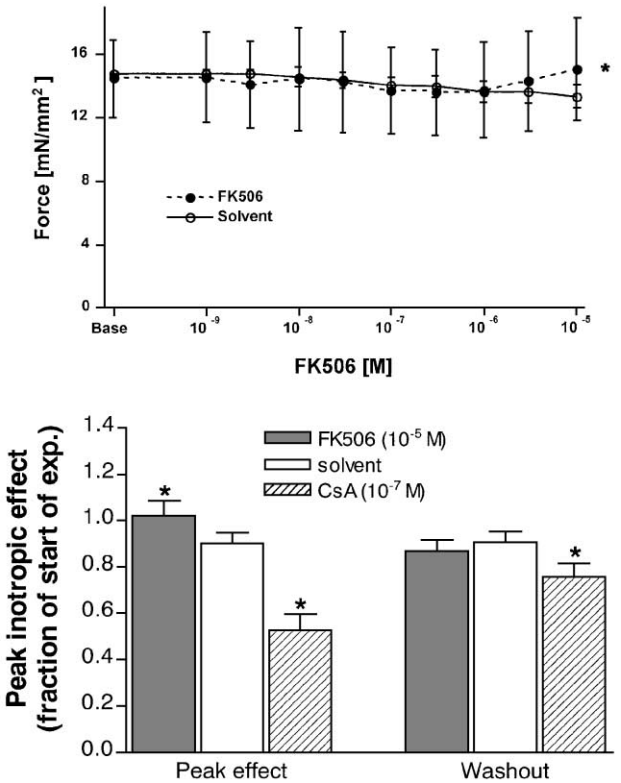


Fig. 3. (Upper panel) Concentration–response curve in healthy rabbit cardiac trabeculae for FK506 ($n = 3$, solid circles) versus solvent ($n = 5$, open circles). Only at the highest concentration was a slight positive inotropic effect observed. (Lower panel) Peak inotropic effect of FK506, solvent (control) and cyclosporine A. Whereas FK506 displays a small, fully reversible positive inotropic effect at $10^{-5}$ M, cyclosporine A at $10^{-7}$ M shows an acute negative inotropic effect, which persisted somewhat after washout (cyclosporine A data partially adapted from Janssen et al., 2000). $^{*}P < 0.05$ versus solvent (unpaired Student's *t*-test).

than cyclosporine A, which is in agreement with the clinical use of different target concentrations in heart-transplanted patients (cyclosporine A, 125–208 nM; FK506, 6–18 nM). Thus, although FK506 appears to have a higher specific inhibitory effect on calcineurin A activity, it has less influence on the inotropy of cardiac muscle preparations than does cyclosporine A.

## 4. Discussion

In this study, we have observed no direct inotropic effect of FK506 on human atrial or rabbit ventricular muscle preparations at physiologically relevant concentrations of FK506. Only at the very high concentration of $10^{-5}$ M, was a slight positive inotropic effect observed in rabbit ventricular muscles. This positive inotropic effect is in close agreement with results of a previous study on isolated rabbit myocytes, where a small positive inotropic effect was also recorded (McCall et al., 1996).

FK506 is a highly hydrophobic substance. It is therefore poorly absorbed after oral administration. The oral bioavailability ranges between 5% and 67%, depending on the patient, with peak plasma concentrations ranging from $0.5 \times 10^{-9}$ to $7 \times 10^{-9}$ M (0.4–5.6 μg/l) after a single oral dose of 0.15 mg/kg. The steady state plasma concentrations of FK506 in patients undergoing hepatic transplantation were described to be in the range of $5.6 \times 10^{-9}$–$17.9 \times 10^{-9}$ M (4.5–14.4 μg/l) (Jain et al., 1993; Peters et al., 1993). However, the largest portion of FK506 is transported in the blood via erythrocytes (75–80%). Therefore, plasma concentrations of FK506 are suggested to be 10–30 times below that of whole blood samples. In the plasma fraction, 88% of FK506 is bound to proteins of the non-lipid fraction, especially to albumin and α-1 acid glycoprotein (Peters et al., 1993).

In order to choose concentrations reflecting the situation in patients undergoing orthotopic heart transplantation, we tested three different concentrations distributed over a broad range, reflecting the situation in plasma ($8 \times 10^{-9}$ M), or in whole blood ($8 \times 10^{-8}$ M), and a concentration far exceeding these concentrations ($8 \times 10^{-6}$ M) (Peters et al., 1993; Timerman et al., 1993).

The lack of any effect on contractility might reflect the low bioavailability of FK506 to the myocardial tissue sample from the solution in the organ bath. On the other hand, we tested FK506 in a concentration of up to $8 \times 10^{-6}$ M, which is $10^3$ times more than we would expect to be the peak concentration in whole blood samples of patients and is in the range of what is used to remove FKBP from the isolated ryanodine receptor (Timerman et al., 1993). Because a small positive inotropic effect was only observed at the extremely high concentration of $10^{-5}$ M with rabbit ventricular muscle preparations, this finding, although intriguing, most likely has no functional or clinical relevance.

Our results suggest that human cardiac contractility and excitation–contraction coupling is not changed by short-term FK506 exposure. Therefore, the native human cardiac ryanodine receptor seems not to be influenced by therapeutic FK506 dosages, which is in agreement with the data for isolated cardiac ryanodine receptors (Timerman et al., 1996). From these data, we would not expect negative effects of FK506 immunosuppression on the contractile status and adrenergic response of orthotopically transplanted human hearts. This is in sharp contrast to the recent findings of a direct negative inotropic effect of cyclosporine A in end-stage failing human myocardium as well as in rabbit myocardium (Janssen et al., 2000).

On the other hand, there is a recent report that FKBP12-knockout mice exhibited an altered open probability of cardiac ryanodine receptors but showed no effect on the skeletal muscle ones. These data remain an enigma because the cardiac ryanodine receptor was found to bind FKBP12.6 but not FKBP12, although this isoform is present in cardiac cytoplasm (Timerman et al., 1996). More-

over, these mice showed dilated ventricles and an increased incidence of ventricle septum defects (Shou et al., 1998), which might be comparable to what was observed in FK506-immunosuppressed children after orthotopic heart transplantation (Atkison et al., 1995). The incubation of terminal cisternae vesicles with FK506 leads to dissociation of FKBP12 from the skeletal ryanodine receptors (Timerman et al., 1993). Therefore, whether this animal model reflects the genetic counterpart of the "pharmacologically FKBP12-depleted" cardiac ryanodine receptor needs to be investigated. Moreover, the observed hyperphosphorylation of the cardiac ryanodine receptor in failing human hearts was suggested as a possible underlying mechanism for cardiac dysfunction in heart failure, because phosphorylation of the cardiac ryanodine receptor by protein kinase A results in dissociation of FKBP12.6 and destabilization of the channel (Marx et al., 2000). The lack of any effect of FK506 could thus also be due to the fact that the drug, which enters the cell, is completely buffered by the soluble FKBP12 pool in the cytosol.

In summary, unlike cyclosporine A, FK506 exerts no negative inotropic effect on myocardial contractility on a short-term basis although it tends to have higher specific inhibitory effects on calcineurin activity when compared to cyclosporine A in our muscle preparations. From these data, it can be concluded that the negative inotropic effect of cyclosporine A is not due to inhibition of calcineurin, but uses different pathways. Cyclosporine A binds to cyclophilin A and the cyclosporine A–cyclophilin A complex, like the FK506–FKBP complex, inhibits the phosphatase activity of calcineurin. Calcineurin is thought to represent the molecular sensor that couples altered $Ca^{2+}$ handling with cardiac hypertrophy and heart failure (Molkentin et al., 1998; Olson and Williams, 2000). The use of FK506 to obtain immunosuppression in cardiac transplantation thus appears to be safe even with marginal myocardial function as observed in severe rejection episodes.

## References


Ahen, G.P., Junankar, P.R., Dulhunty, A.F., 1994. Single channel activity of the ryanodine receptor calcium release channel is modulated by FK-506. FEBS Lett. 352, 369–374.

Atkison, P., Joubert, G., Barron, A., Grant, D., Paradis, K., Seidman, E., Wall, W., Rosenberg, H., Howard, J., Williams, S. et al., 1995. Hypertrophic cardiomyopathy associated with tacrolimus in paediatric transplant patients. Lancet 345, 894–896.

Bierer, B.E., Somers, P.K., Wandless, T.J., Burakoff, S.J., Schreiber, S.L., 1990. Probing immunosuppressant action with a nonnatural immunophilin ligand. Science 250, 556–559.

Block, B.A., Imagawa, T., Campbell, K.P., Franzini-Armstrong, C., 1988. Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. J. Cell Biol. 107, 2587–2600.

Brillantes, A.B., Ondrias, K., Scott, A., Kobrinsky, E., Ondriasova, E., Moschella, M.C., Jayaraman, T., Landers, M., Ehrlich, B.E., Marks, A.R., 1994. Stabilization of calcium release channel (ryanodine receptor) function by FK50-binding protein. Cell 77, 513–523.

Cameron, A.M., Steiner, J.P., Roskams, A.J., Ali, S.M., Ronnett, G.V., Snyder, S.H., 1995a. Calcineurin associated with the inositol 1,4,5-trisphosphate receptor–FKBP12 complex modulates $Ca^{2+}$ flux. Cell 83, 463–472.

Cameron, A.M., Steiner, J.P., Sabatini, D.M., Kaplin, A.I., Walensky, L.D., Snyder, S.H., 1995b. Immunophilin FK506 binding protein associated with inositol 1,4,5-trisphosphate receptor modulates calcium flux. Proc. Natl. Acad. Sci. U. S. A. 92, 1784–1788.

Caterall, W.A., 1991. Excitation–contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels. Cell 64, 871–874.

Griffith, J.P., Kim, J.L., Kim, E.E., Sintchak, M.D., Thomson, J.A., Fitzgibbon, M.J., Fleming, M.A., Caron, P.R., Hsiao, K., Navia, M.A., 1995. X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12–FK506 complex. Cell 82, 507–522.

Groth, C.G., 1995. Immunosuppressive regimens of tomorrow. Transplant. Proc. 27, 2971–2973.

Harding, M.W., Galat, A., Uehling, D.E., Schreiber, S.L., 1989. A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. Nature 341, 758–760.

Jain, A.B., Abu Elmagd, K., Abdallah, H., Warty, V., Fung, J., Todo, S., Starzl, T.E., Venkataramanan, R., 1993. Pharmacokinetics of FK506 in liver transplant recipients after continuous intravenous infusion. J. Clin. Pharmacol. 33, 606–611.

Janssen, P.M., Zeitz, O., Keweloh, B., Siegel, U., Maier, L.S., Barckhausen, P., Pieske, B., Prestle, J., Lehnart, S.E., Hasenfuss, G., 2000. Influence of cyclosporine A on contractile function, calcium handling, and energetics in isolated human and rabbit myocardium. Cardiovasc. Res. 47, 99–107.

Kaftan, E., Marks, A.R., Ehrlich, B.E., 1996. Effects of rapamycin on ryanodine receptor $Ca^{2+}$ release channels from cardiac muscle. Circ. Res. 78, 990–997.

Lam, E., Martin, M., Wiederrecht, G., 1995a. Isolation of a cDNA encoding a novel human FK506-binding protein homolog containing leucine zipper and tetratricopeptide repeat motifs. Gene 160, 297–302.

Lam, E., Martin, M.M., Timerman, A.P., Sabers, C., Fleischer, S., Lukas, T., Abraham, R.T., O'Keefe, S.J., O'Neill, E.A., Wiederrecht, G.J., 1995b. A novel FK506 binding protein can mediate the immunosuppressive effects of FK506 and is associated with the cardiac ryanodine receptor. J. Biol. Chem. 270, 26511–26522.

Marx, S.O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosemblit, N., Marks, A.R., 2000. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell 101, 365–376.

McCall, E., Li, L., Satoh, H., Shannon, T.R., Blatter, L.A., Bers, D.M., 1996. Effects of FK-506 on contraction and $Ca^{2+}$ transients in rat cardiac myocytes. Circ. Res. 79, 1110–1121.

McPherson, P.S., Campbell, K.P., 1993. The ryanodine receptor/$Ca^{2+}$ release channel. J. Biol. Chem. 268, 13765–13768.

Molkentin, J.D., Lu, J.R., Antos, C.L., Markham, B., Richardson, J., Robbins, J., Grant, S.R., Olson, E.N., 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 93, 215–228.

Olson, E.N., Williams, R.S., 2000. Calcineurin signaling and muscle remodeling. Cell 101, 689–692.

Otsu, K., Willard, H.F., Khanna, V.K., Zorzato, F., Green, N.M., MacLennan, D.H., 1990. Molecular cloning of cDNA encoding the $Ca^{2+}$ release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. J. Biol. Chem. 265, 13472–13483.

Peters, D.H., Fitton, A., Plosker, G.L., Faulds, D., 1993. Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drugs 46, 746–794.

Shibasaki, F., Price, E.R., Milan, D., McKeon, F., 1996. Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. Nature 382, 370–373.

Shou, W., Aghdasi, B., Armstrong, D.L., Guo, Q., Bao, S., Charng,

M.-J., Mathews, L.M., Schneider, M.D., Hamilton, S.L., Matzuk, M.M., 1998. Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. Nature 391, 489–492.

Timerman, A.P., Ogunbumni, E., Freund, E., Wiederrecht, G., Marks, A.R., Fleischer, S., 1993. The calcium release channel of sarcoplasmic reticulum is modulated by FK-506-binding protein. Dissociation and reconstitution of FKBP-12 to the calcium release channel of skeletal muscle sarcoplasmic reticulum. J. Biol. Chem. 268, 22992–22999.

Timerman, A.P., Jayaraman, T., Wiederrecht, G., Onoue, H., Marks, A.R., Fleischer, S., 1994. The ryanodine receptor from canine heart sarcoplasmic reticulum is associated with a novel FK-506 binding protein. Biochem. Biophys. Res. Comm. 198, 701–706.

Timerman, A.P., Wiederrecht, G., Marcy, A., Fleischer, S., 1995. Characterization of an exchange reaction between soluble FKBP-12 and the FKBP.ryanodine receptor complex. Modulation by FKBP mutants deficient in peptidyl-prolyl isomerase activity. J. Biol. Chem. 270, 2451–2459.

Timerman, A.P., Onoue, H., Xin, H.B., Barg, S., Copello, J., Wiederrecht, G., Fleischer, S., 1996. Selective binding of FKBP12.6 by the cardiac ryanodine receptor. J. Biol. Chem. 271, 20385–20391.

Winkler, M., Christians, U., 1995. A risk-benefit assessment of tacrolimus in transplantation. Drug Saf. 12, 348–357.

Xiao, R.P., Valdivia, H.H., Bogdanov, K., Valdivia, C., Lakatta, E.G., Cheng, H., 1997. The immunophilin FK506-binding protein modulates $Ca^{2+}$ release channel closure in rat heart. J. Physiol. (London) 500, 343–354.

Xin, H.B., Rogers, K., Qi, Y., Kanematsu, T., Fleischer, S., 1999. Three amino acid residues determine selective binding of FK506-binding protein 12.6 to the cardiac ryanodine receptor. J. Biol. Chem. 274, 15315–15319.

Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N.M., Lai, F.A., Meissner, G., MacLennan, D.H., 1990. Molecular cloning of cDNA encoding human and rabbit forms of the Ca release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. J. Biol. Chem. 265, 2244–2256.