Calcineurin-independent inhibition of \(K_{v}1.3\) by FK-506 (tacrolimus): a novel pharmacological property

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FK-506 (tacrolimus) is a potent immunosuppressant that is widely used to prevent rejection after organ transplantation and in the treatment of various autoimmune diseases (13, 26). FK-506 binds to FK-506 binding protein (FKBP), a cytosolic protein, and the resulting FK-506-FKBP complex inhibits the action of calcineurin, a \(Ca^{2+}\)-/calmodulin-dependent phosphatase 2B, which subsequently prevents calcineurin-dependent interleukin (IL)-2 transcription and T lymphocyte activation (24, 36, 45). Thus the mechanism of immunosuppressive action in these cells is thought to be mediated by a calcineurin-dependent mechanism.

Kv1.3, a member of the Shaker family of voltage-gated \(K^+\) channels, is a delayed-rectifier channel (41). Kv1.3 currents display characteristic gating kinetics: under whole cell patch-clamp conditions, Kv1.3 is activated within a few milliseconds in response to depolarizing pulses and undergoes a slow C-type inactivation during prolonged depolarization (9). Because Kv1.3 recovers from inactivation extremely slowly, cumulative inactivation occurs after a train of repetitive pulses (25). Kv1.3 is found in many tissues, specifically in lymphocytes and the brain (41). In human T lymphocytes, it sets the cell membrane potential and controls the efflux of \(K^+\) necessary to maintain \(Ca^{2+}\) influx (3). Therefore, Kv1.3 is a primary regulator of \(T\) lymphocyte activation and is widely recognized as a potential target for immunotherapy (14).

Calcineurin is known to have a variety of cellular functions in different types of cells such as neurotransmitter release, the activity of several ion channels, synaptic plasticity, and nerve regeneration (37, 47). Immunosuppressants such as FK-506 and cyclosporin A (CsA), calcineurin inhibitors, also regulate the activity of ion channels. For example, FK-506 and CsA block high-voltage-activated \(Ca^{2+}\) channels in hippocampal neurons via a calcineurin-dependent mechanism (27). It has been reported that both drugs act in a similar manner on voltage-activated \(Ca^{2+}\) channels in hippocampal neurons and coronary arterial smooth muscle cells (29, 48). In addition, the inhibition of calcineurin by FK-506 and CsA causes the upregulation of cell surface functional \(Na^+\) channels in adrenal chromaffin cells (35). In contrast, CsA reduces the functional expression of \(K_{v}2.1\) \(K^+\) channels and nicotinic and 5-HT\(_3\) receptors to a significant extent (4, 16, 17). These effects are mediated by inhibition of the activation of the calcineurin-dependent pathway. However, several studies have alluded to the possibility that FK-506 has a direct and nonspecific effect on membrane structures and function in different cells. FK-506 inhibits outward \(K^+\) currents that are responsible for the repolarization of the action potentials in rat ventricular myocytes (10, 11) and modulates the single-channel activity of \(Ca^{2+}\)-dependent \(K^+\) channels via a calcineurin-independent mechanism (44). In addition, FK-506 blocks the voltage-gated \(K^+\) current, resulting in a time-dependent and rapid membrane depolarization in human T lymphocytes by a yet-to-be defined mechanism (31). These results also raise the possibility that FK-506 modulates ion channel activity via mechanisms that do not involve calcineurin-dependent inhibition.
not involve effects on calcineurin. Although these observations suggest the possibility of the direct blocking action of FK-506 on voltage-gated K⁺ channels, the mechanism responsible and the effect on the kinetics of the channels are not well understood. Therefore, in the present study, we examined the effects of FK-506 on the cloned K⁺ channel Kv1.3 to investigate the direct action and detailed kinetics between the drug and the channel.

**EXPERIMENTAL PROCEDURES**

**Stable transfection and cell culture.** Chinese hamster ovary (CHO) cells (American Type Culture Collection, Rockville, MD) were maintained in Iscove’s modified Dulbecco’s medium (IMDM; Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum, 0.1 mM hypoxanthine, and 0.01 mM thymidine in a humidified 5% CO₂ incubator at 37°C. The CHO cells used stably expressed Kv1.3, Kv1.5, or Kv4.3 channels as previously described (5, 7, 28). The cultures were exchanged at 2- to 3-day intervals with fresh IMDM containing 0.3 mg/ml of genetin (Invitrogen) and passed every 2–3 days with the use of a brief trypsin-EDTA treatment. The trypsin-EDTA-treated cells were seeded onto glass coverslips (diameter 12 mm, Fisher Scientific, Pittsburgh, PA) in a petri dish 24 h before use. For electrophysiologic recordings, coverslips with attached cells were transferred to a continually perfused recording chamber (RC-13, Warner Instrument, Hamden, CT).

**Electrophysiological recordings.** Currents were recorded at room temperature (22–24°C) with the whole cell and inside-out configuration of the patch-clamp technique with an Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA). Micropipettes were pulled from PG10165-4 glass capillary tubing (World Precision Instruments, Sarasota, FL) and had resistances of 2–3 MΩ when filled with internal pipette solution. The liquid junction potentials between the external and pipette solution were offset before the pipette made contact with the cell. The micropipettes were gently lowered onto the cells, and gigaohm seal formation was achieved by applying suction. After pipette capacitance compensation, the cells were ruptured by application of a brief additional suction. Seal resistances were in the range of 4–10 GΩ. Therefore, whole cell capacitive currents were compensated with analog compensation without leakage compensation. In the whole cell configuration, series resistances were ~4–8 MΩ. The effective series resistances were usually compensated by 80% if the current exceeded 1 nA. The sampling frequency was 5 kHz, and the currents were filtered at 2 kHz (4-pole Bessel filter) before being digitized. Data acquisition and analysis were performed on an IBM Pentium computer with pCLAMP 9.0 software (Molecular Devices).

**Solutions and drugs.** The bath solution contained (mM) 140 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, and 10 HEPES and was adjusted to pH 7.3 with NaOH. This bath solution was used as the internal pipette solution for the inside-out recordings. The internal pipette solution contained (in mM) 140 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 EGTA and was adjusted to pH 7.3 with KOH. This pipette solution was slowly inactivated while the depolarizing pulse was maintained, as described previously (7, 25). When applied to the external bath solution, FK-506 (1, 3, 10, and 30 μM) not only reduced the peak amplitude of the current but also altered the external bath solution, FK-506 (1, 3, 10, and 30 μM) not only reduced the peak amplitude of the current but also altered the time course for current decay, increasing the rate of current decay during depolarization at the concentrations used. Under control conditions, the current decay of Kv1.3 was well fitted to a single exponential function with a time constant of 166.3 ± 9.5 ms (n = 11). After the addition of FK-506, the apparent inactivation of Kv1.3 was accelerated with time constants of 141.5 ± 7.5, 114.5 ± 8.6, 75.5 ± 7.6, and 38.8 ± 4.3 ms (n = 11) for 1, 3, 10, and 30 μM, respectively. Thus the peak amplitude of the current was affected much less than the steady-state current amplitude at the end of the 200-ms depolarizing pulse. The current amplitude measured at the end of the 200-ms depolarizing pulse to +40 mV was used as an index of the inhibition. A nonlinear least-squares fit of the Hill equation to the concentration-response data yielded an IC₅₀ value of 5.6 ± 0.7 μM and nᵣ of 1.5 ± 0.1 (n = 11) for FK-506 at +40 mV.

We further examined the effects of another immunosuppressant, rapamycin, on Kv1.3 currents (Fig. 1B). Similar to FK-506, rapamycin enhanced the rate of current decay, resulting in a decrease in current amplitude at the end of a 200-ms depolarizing pulse. Figure 1B shows the concentration dependence for the rapamycin-induced inhibition of Kv1.3 measured at the end of the 200-ms depolarizing pulse. A nonlinear least-squares fit of the Hill equation to the concentration-response data yielded an IC₅₀ value of 6.7 ± 0.8 μM and nᵣ of 1.3 ± 0.1 (n = 6) at +40 mV. Thus the potency of rapamycin was similar to that of FK-506 in inhibiting the Kv1.3 current.
To assess the reversibility of the effect of the drug, the single depolarizing pulse was repeated while 5 μM FK-506 was applied. As shown in Fig. 2, when solutions were switched to solutions containing the drug, steady-state inhibition of KV1.3 was reached within 2 min. The washout of FK-506 by perfusion with a drug-free solution was also complete within 3 min. The currents recovered to 93.1 ± 2.4% (n = 9) of the control from the steady-state inhibition by FK-506. Therefore, the inhibition was reversible on washout, with little rundown in the current being observed under these conditions.

Effects of calcineurin inhibitors on inhibition of KV1.3 by FK-506. The KV1.3 channel can be phosphorylated and dephosphorylated (8, 32, 40), and FK-506 has been reported to function as a phosphatase inhibitor (45). To determine whether calcineurin is involved in the FK-506-induced inhibition of KV1.3, we investigated the effects of other calcineurin inhibitors, such as cypermethrin in the bath solution and calcineurin autoinhibitory peptide in the pipette solution. Figure 3A shows the effects of cypermethrin on the inhibition of KV1.3 by FK-506. After a 30-min preincubation with 40 nM cypermethrin, 5 μM FK-506 inhibited the steady-state current of KV1.3, measured at the end of a depolarizing pulse of +40 mV, by 59.3 ± 0.9% (n = 4). Figure 3B shows the effects of 100 μM calcineurin autoinhibitory peptide in the pipette solution. Over a 10-min period after membrane rupture, cell dialysis with a pipette solution containing calcineurin autoinhibitory peptide produced no effect on the amplitude or kinetics of KV1.3 compared with the control measured immediately after membrane rupture. With addition of 5 μM FK-506 to the bath solution, the steady-state current of KV1.3 was decreased by 50.7 ± 1.2% (n = 7). The lack of effect of cypermethrin and calcineurin autoinhibitory peptide on the FK-506-induced inhibition of KV1.3 strongly indicates that FK-506 directly inhibits KV1.3 via a calcineurin-independent mechanism.

Voltage-dependent inhibition of KV1.3 by FK-506. Figure 4 shows the effect of FK-506 on the current-voltage (I–V) relationship. Under control conditions, the I–V relationship was almost linear for depolarizing pulses between −40 and +40 mV (Fig. 4, A and C). In the presence of 5 μM FK-506, the steady-state currents were inhibited over the entire voltage range in which KV1.3 was activated, as shown in Fig. 4, B and C. In a plot of the relative current (I_{FK-506}/I_{control}) versus potential (Fig. 4D), the inhibition of KV1.3 by FK-506 increased steeply between −30 and −10 mV, which corresponded with the voltage range for channel activation (F_{2,21} = 11.7, P < 0.01). However, the inhibition in the voltage range between 0 and +40 mV, where the channels are fully activated, did not show any voltage dependence. The linear curve fitting of the data at potentials >0 mV (Fig. 4D) yielded a value approximately equal to zero for the slope of the line: 49.5 ± 4.2% of the control value at 0 mV and 46.8 ± 3.2% of the control at +40 mV (F_{4,35} = 0.09, P > 0.05).

Use-dependent inhibition of KV1.3. Twenty repetitive 200-ms depolarizing pulses of +40 mV from a holding potential of −80 mV were applied at two different frequencies, 1 and 2 Hz (Fig. 5). Under control conditions, the peak amplitude of KV1.3 decayed progressively, eventually reaching a steady state, and was decreased by 55.3 ± 3.6% and by 71.0 ± 3.9%
After the application of a train of depolarizing pulses at 1 and 2 Hz. In the presence of 5 M FK-506, the peak amplitude of Kv1.3 progressively decreased, reaching a steady-state inhibition after approximately six depolarizing pulses. The extent of the steady-state inhibition was 88.0 ± 2.9% and 94.7 ± 1.6% (n = 6) at 1 and 2 Hz, respectively. Thus FK-506 exhibited a strong use-dependent inhibition of Kv1.3.

Effects of FK-506 on steady-state inactivation of Kv1.3. To characterize the inhibitory effects of FK-506 on Kv1.3, we examined the voltage dependence of the steady-state inactivation curve of Kv1.3 (Fig. 6A). Under control conditions, the potential corresponding to the half-inactivation point (V1/2) was −42.8 ± 0.7 mV and slope factor k was 2.84 ± 0.07 mV (n = 8). FK-506 shifted the steady-state inactivation curves (V1/2) to a hyperpolarized potential in a concentration-dependent manner (−52.3 ± 0.5 mV at 5 μM, −56.9 ± 1.1 mV at 30 μM FK-506; F2,21 = 74.8, P < 0.001). In the presence of FK-506, however, the slope factor k was not significantly changed (2.81 ± 0.06 mV at 5 μM, 2.85 ± 0.11 mV at 30 μM FK-506; F2,21 = 0.05). The inhibition, measured as the reduction in the steady-state current in the presence of FK-506 (the IC50 value obtained from concentration-response data as shown in Fig. 1), does not represent the true affinity of the drug for the inactivated states of Kv1.3. Thus the apparent Kᵢ for the inhibition of Kv1.3 by FK-506 in the inactivated state was estimated from the concentration-dependent shift in the steady-state inactivation curve (2). The theoretical value of Kᵢ was calculated to be 0.37 ± 0.08 μM (n = 8; Fig. 6B). Thus the effect of FK-506 on channel inactivation is 15 times more potent than on the steady-state current.

Effects of FK-506 on recovery from inactivation of Kv1.3. Recovery from FK-506-induced inhibition was measured with a double-pulse protocol. Figure 7 shows the peak current amplitudes elicited by the test pulses as a function of the time interval. Under control conditions, the recovery from inactivation could be described by a biexponential function to a fast time constant of 0.65 ± 0.34 ms and a slow time constant of 7.01 ± 0.55 ms (n = 5). In the presence of 5 μM FK-506, the recovery process was also best fitted with a biexponential function (fast time constant of 0.62 ± 0.19 ms, slow time constant of 18.17 ± 3.69 ms; n = 5). The fast time constant obtained in the presence of FK-506 remained unchanged, but the slow time constant was statistically different from that for the current recovery under control conditions (F1,8 = 8.97, P < 0.05).
Effects of FK-506 on Kv1.3 in inside-out patches. Figure 8 shows the effects of FK-506 on Kv1.3 currents recorded from inside-out patches. In the presence of 5 μM FK-506, inhibition of the Kv1.3 currents was characterized by an acceleration in the apparent rate of current decay with little effect on peak amplitude, as was found in whole cell recordings (Fig. 1). FK-506 (5 μM) inhibited the steady-state current of Kv1.3 at the end of a depolarizing pulse of +40 mV by 54.9 ± 2.7% of the control value (n = 7), similar to 58.2 ± 6.7% (n = 9) of the control obtained for whole cell patches.

Concentration-dependent inhibition of Kv1.5 and Kv4.3 by FK-506. Figure 9A shows superimposed Kv1.5 current traces produced by 250-ms depolarizing pulses to +40 mV under control conditions and in the presence of various concentrations of FK-506. Similar to Kv1.3, FK-506 also induced a reduction in the steady-state current of Kv1.5 during the depolarizing pulse, with little effect on peak amplitude. The current amplitude, measured at the end of the 250-ms depolarizing pulse, was also used as an index of the inhibition. A nonlinear least-squares fit of the individual data points to the Hill equation yielded an IC_{50} value of 4.6 ± 0.1 μM and n_H of 1.2 ± 0.1 (n = 5). We further characterized the effect of FK-506 on Kv4.3, a rapidly inactivating A-type current. As shown in Fig. 9B, FK-506 decreased the peak amplitude of Kv4.3 in a concentration-dependent manner. However, the most obvious effect of FK-506 was an acceleration in the rate of the current decay of Kv4.3. To quantify the effect of FK-506 on Kv4.3, therefore, we measured the integral of the total current over the duration of a depolarizing pulse to +40 mV and obtained an IC_{50} value of 53.9 ± 4.6 μM and n_H of 0.9 ± 0.1 (n = 8).

DISCUSSION

The primary focus of this study was to determine the direct effect of FK-506 on the cloned voltage-gated K^+ channels Kv1.3, Kv1.5, and Kv4.3. The findings indicate that FK-506 caused a reversible time-, concentration-, and use-dependent inhibition of Kv1.3 channels. These results suggest that FK-506 inhibits Kv1.3 by mechanisms that do not involve the suppression of the protein phosphatase activity of calcineurin but by a distinct mechanism involving a direct interaction with calcineurin but by a distinct mechanism involving a direct interaction with Kv1.3 channels. This is the first report of such a novel pharmacological property of FK-506 on Kv1.3.

FK-506 is used as an immunosuppressant in organ transplantation and in the treatment of autoimmune diseases (13, 26, 38). The mechanism of its immunosuppressive action is thought to be mediated by a calcineurin-dependent mechanism (23, 36, 45). The findings here, however, indicate that FK-506 directly inhibits Kv1.3 currents by a mechanism that is independent of calcineurin activity, based on the following results. First, rapamycin is another macrolide immunosuppressant drug, and its mechanism of action is different from that of FK-506. Rapamycin has no effect on calcineurin but suppresses later events in signal transduction pathways that are responsible for IL-dependent T lymphocyte activation (1, 36). From an analysis of the concentration-response curve, however, the potencies of rapamycin and FK-506 in inhibiting
Kv1.3 were very similar. The fact that rapamycin and FK-506 have similar structures suggests that this effect might be due to structural similarities rather than the modification of the signal transduction pathways required for T lymphocyte activation, namely, the inhibition of calcineurin activity. Thus we suggest that FK-506 and rapamycin share a common mechanism of action at the molecular level: direct interaction with the Kv1.3 channel. Second, in our experiments, the presence of cypermethrin in the bath solution or calcineurin autoinhibitory peptide in the patch pipette failed to prevent the inhibition of Kv1.3 by FK-506. Because the concentrations of calcineurin inhibitors used in this experiment were sufficiently high to completely inhibit calcineurin activity (12, 15), these results are incompatible with phosphorylation events due to the inhibition of the phosphatase activity of calcineurin by FK-506. Third, the catalytic activity of calcineurin requires a significant increase in the concentration of intracellular free Ca\(^{2+}\) (39).

Our experiments were performed in whole cell recordings in which free Ca\(^{2+}\) was buffered by EGTA (10 mM) to resting concentrations (<10 nM), and low levels of calcineurin activity would be expected in CHO cells. Thus our experimental conditions support a scenario involving the calcineurin-independent inhibition of Kv1.3 by FK-506. However, we cannot

Fig. 5. Use-dependent inhibition of Kv1.3 currents by FK-506. Top: 20 repetitive 200-ms depolarizing pulses of +40 mV from a holding potential of −80 mV were applied at two different frequencies, 1 and 2 Hz, under control conditions and in the presence of FK-506. Bottom: peak amplitudes of the current at each pulse were normalized by the peak amplitudes of current obtained at the 1st pulse and then plotted vs. pulse number (n = 6). Data are expressed as means ± SE.

Fig. 6. Effect of FK-506 on the voltage dependence of steady-state inactivation of Kv1.3. A, top: currents were elicited by 200-ms depolarizing pulses to +40 mV while 30-s preconditioning pulses were varied from −80 to 0 mV under control conditions and after exposure to FK-506. Bottom: steady-state inactivation curves through the data points were drawn according to the Boltzmann equation. B: plot of \(\exp(\Delta V/k)\) against FK-506 concentration. Potential corresponding to the half-inactivation point (\(V_{1/2}\)) and slope factor (k) values were obtained from the steady-state inactivation curves. The concentration-dependent shift in the midpoint (\(\Delta V\)) was determined as the difference between \(V_{1/2}\) values in control conditions and at 5 and 30 \(\mu\)M FK-506 (n = 8). Solid line represents the linear fit to the data \([\exp(\Delta V/k) = 0.66 + 2.70[FK-506]]\), where [FK-506] represents concentration of FK-506. \(K_s\), the reciprocal of the slope, was calculated from this fit. Data are expressed as means ± SE.
completely rule out the possibility that calcineurin-dependent effects may still exist. Finally, in an excised inside-out patch devoid of diffusible cytosolic molecules required for phosphorylation and dephosphorylation, the rapid and reversible effect of FK-506 was also consistent with a direct interaction with the KV1.3 channel rather than through a phosphorylation-dependent mechanism. Furthermore, the rapid onset and reversibility of the inhibition of KV1.5 in whole cell recordings indirectly suggest that the inhibition is not related to phosphorylation.

KV1.3 is a delayed-rectifier K⁺/H11001 channel and inactivates over hundreds of milliseconds during prolonged depolarization. Moreover, the recovery from inactivation in response to a train of repetitive depolarizing pulses (25). Consequently, the presence of a cumulative inactivation in KV1.3 channels can make a significant contribution to controlling membrane potentials. Our data suggest that FK-506 not only accelerates the time course for the intrinsic inactivation of KV1.3 currents but also shifts the voltage dependence of steady-state inactivation in the hyperpolarizing direction. In addition, the inhibition of KV1.3 by FK-506 was voltage dependent, increasing in the voltage range for channel activation. This reflects the voltage dependence of channel inactivation but is not due to a direct voltage dependence of binding to KV1.3 channels. Consistent with this, the inhibitory effect of FK-506 was use dependent, with the effects enhanced at higher rates of channel activation. A possible mechanism of action is that FK-506 is highly lipophilic (36) and is readily partitioned into the plasma membrane, thereby modulating the inactivation kinetics of KV1.3 by an allosteric mechanism. FK-506 binding to inactivated KV1.3 channels stabilizes the inactivated state, and thus decreases the current after steady-state inactivation at any given potential. Under these conditions, the availability of KV1.3 channels is decreased when sustained depolarization by FK-506 occurs in the case of nonexcitable cells, such as T lymphocytes, K⁺/H11001 is the predominant voltage-gated K⁺ channel. This channel plays an important role in the physiology of T lymphocytes, e.g., it limits activating Ca²⁺ signaling by regulating their membrane potential (39). The selective blockade of KV1.3 channels (13) by FK-506 not only decreases the intracellular Ca²⁺ concentration by inhibiting the activation of nonexcitable cells, such as T lymphocytes, but also decreases the availability of KV1.3 channels, consistent with the inhibition of T lymphocyte activation by FK-506.

In human T lymphocytes, KV1.3 is the predominant voltage-gated K⁺ channel. This channel plays an important role in the physiology of T lymphocytes, e.g., it limits activating Ca²⁺ signaling by regulating their membrane potential (39). Therefore, selective blockers of KV1.3 have consistently been of interest and are considered to be an important therapeutic target for immunosuppression (14). In the present study, FK-506 inhibited KV1.3 in a concentration-dependent manner. The depolarization of T lymphocytes was recorded from inside-out patches, and the effects of FK-506 were investigated in the absence and presence of FK-506.

**Fig. 7. Effects of FK-506 on recovery from inactivation of KV1.3.**

- **Top:** A double-pulse protocol was used to characterize the recovery of KV1.3 from inactivation in the absence and presence of FK-506. A 1st prepulse of 200 ms depolarizing pulse of 40 mV from a holding potential of 80 mV was followed by a 2nd identical pulse after increasing the interpulse intervals between 10 ms and 30 s at 80 mV. Pulses were applied every 30 s.
- **Bottom:** Solid lines represent the biexponential fit of the peak amplitude of KV1.3 currents as a function of the interpulse interval (n = 5). Data are expressed as means ± SE.
cytes by FK-506 was also observed by electrophysiology in membrane potential measurements (31). Therefore, this inhibitory effect of FK-506 on KV1.3 channels, which results in the membrane depolarization of T lymphocytes, could be another underlying mechanism by which FK-506 exerts its immunosuppressive effect.

Although FK-506 is used extensively as an immunosuppressive agent in human organ transplantation, it causes numerous side effects including nephrotoxicity, hypertension, and neurotoxicity (13, 38). Moreover, secondary toxic effects have been reported in cardiac tissue, such as arrhythmia and heart failure. KV1.5 has been postulated to be the predominant delayed-rectifier K\(^+\) current responsible for human atrial repolarization (43). KV4.3, a rapidly inactivating A-type current, is expressed at high levels in the heart and is responsible for the early repolarization of the cardiac action potential (28). Theoretically, the inhibition of KV1.5 and/or KV4.3 delays the repolarization of cardiac action potentials. Several studies have reported ventricular tachycardia in patients receiving FK-506, and its putative mechanism involves retarding ventricular repolarization (18, 19). Therefore, it would be expected that, in patients treated with FK-506, arrhythmia may result, at least partly, from the direct interaction of FK-506 with KV1.5 and/or KV4.3 of cardiomyocytes.

The therapeutic plasma concentration of FK-506 is in the range of 0.5–1.5 nM in transplant patients (20). We estimated the \(K_i\) for binding to the inactivated state of KV1.3 to be 0.37 \(\mu\)M, which is well above the therapeutic dose, and this effect appears to have no clinical relevance. However, the concentration of FK-506 in blood is up to 100-fold higher than that in plasma (21). In addition, the concentration of FK-506 in the blood does not necessarily reflect its tissue concentration, and the concentrations in target tissues themselves may be more important in terms of assessing the pharmacological action or side effects of a drug. FK-506 that is highly lipophilic and cell membrane permeant can progressively accumulate in higher concentrations in tissues including T lymphocytes (42). Moreover, the acute cardiovascular effect of FK-506 on electrophysiological and mechanical properties in the isolated guinea pig heart was reported at a micromolar concentration (30). Therefore, under this assumption, our results shed some light on the molecular mechanisms that underlie some of the cardiac toxicity observed in patients who are being administered FK-506.

In this study, we report on a novel calcineurin-independent pharmacological property of FK-506, the direct inhibitory effects of FK-506 on cloned KV1.3 channels. Our findings reveal another mechanism of action of FK-506 for immune suppression.

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