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The immunophilin FKBP52 inhibits the activity of the epithelial Ca\textsuperscript{2+} channel TRPV5

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Gkika, Dimitra, Catalin N. Topala, Joost G. J. Hoenderop, and René J. M. Bindels. The immunophilin FKBP52 inhibits the activity of the epithelial Ca\textsuperscript{2+} channel TRPV5. Am J Physiol Renal Physiol 290: F1253–F1259, 2006. First published December 13, 2005; doi:10.1152/ajprenal.00298.2005.—In the kidney, the epithelial Ca\textsuperscript{2+} channel TRPV5 constitutes the apical entry pathway in the process of active Ca\textsuperscript{2+} reabsorption. The regulation of Ca\textsuperscript{2+} influx through TRPV5 is of crucial importance, because it determines the final amount of Ca\textsuperscript{2+} excreted in the urine. The present study identifies FKBP52 as an auxiliary protein of TRPV5, inhibiting channel activity. FKBP52 shows specific interaction with TRPV5, and both proteins colocalize in the distal part of the nephron. On the functional level, FKBP52 decreases Ca\textsuperscript{2+} influx through TRPV5 as demonstrated in radioactive \(45\text{Ca}\) uptake measurements and electrophysiological studies in TRPV5-overexpressing human embryonic kidney 293 cells. On the other hand, gene silencing of FKBP52 or administration of the FKBP52 blocker FK-506 enhances Ca\textsuperscript{2+} influx through TRPV5. The inhibitory action of FKBP52 on TRPV5 activity is blunted by mutation of its peptidyl-prolyl cis-trans isomerase domain, showing that the FKBP52 catalytic property is critical for channel activity. In conclusion, these results suggest that FKBP52 plays an important role in the regulation of TRPV5 and thus in the process of Ca\textsuperscript{2+} reabsorption.

FKBP52 (also designed as FKBP59, p59, or Hsp56) is a widely expressed cytosolic enzyme that belongs to the FK506-binding proteins (FKBPs) subfamily of immunophilin proteins (11, 18, 24). FKBPs are characterized by their ability to catalyze the cis-trans isomerization of cis-peptidyl-propyl bonds, as well as by their strong affinity to the immunosuppressive drug FK-506 (18). The peptidyl-prolyl cis-trans isomerase (PPIase) activity of FKBP52 plays an important role in diverse cellular processes and, in particular, in the translocation of steroid receptors to the nucleus through dynein association (7, 27). Besides its function as a chaperone, FKBP52 is involved in ion regulation such as Cu\textsuperscript{2+} efflux (25), whereas its Drosophila homolog inhibits Ca\textsuperscript{2+} influx through association with the transient receptor potential (TRP) channel TRPL (10, 28). Concerning the mammalian TRP channels, it was recently demonstrated that FKBP52 associates with TRPC1, -C4, and -C5 orthologs (10, 28), whereas there are no functional data addressing the effect of FKBP52 on these channels.

In the kidney, the TRP channel TRPV5 mediates Ca\textsuperscript{2+} reabsorption in the distal part of the nephron and determines the final amount of Ca\textsuperscript{2+} excreted in the urine (15, 17). Therefore, tight control of TRPV5 expression, trafficking to the plasma membrane, and/or gating of the channel is important for the amount of reabsorbed Ca\textsuperscript{2+} in the kidney (8). There is growing interest in the identification of accessory proteins regulating TRPV5 activity. The Sl100A10-annexin 2 complex modulates TRPV5 trafficking toward the plasma membrane by direct association to the COOH-terminal tail of the channel (30). On the other hand, the 80K-H protein binds to the channel and acts as a Ca\textsuperscript{2+} sensor that facilitates Ca\textsuperscript{2+} influx through the TRPV5 channel (9).

The aim of the present study was to assess whether FKBP52 is a regulator of TRPV5. To this end, we demonstrated by immunohistochemical, biochemical, and functional analysis that FKBP52 inhibits TRPV5-mediated Ca\textsuperscript{2+} influx via its PPIase activity.

MATERIALS AND METHODS

DNA constructs and cRNA synthesis. TRPV5 constructs were generated as described previously (30). FKBP52 was cloned in a pcMV-Sport6 vector (clone IRAkP961125Q2) by the Deutsches Ressourcenzentrum fuer Genomforschung (Berlin, Germany) and then subcloned into pClNeo/ires-green fluorescent protein (GFP) (29), pGEX6p-2 (Amersham Pharmacia Biotech, Uppsala, Sweden), and pT7Ts (19) vectors. The FD67DV mutant of FKBP52 was generated by in vitro mutagenesis (QuickChange site-directed Mutagenesis kit, Stratagene, La Jolla, CA) with the primer set 5'-CTAGATGGCACACAAAGATGTCCATCTGGACCGC-3', 5'-GGGCTTCAAGACTGAGACCACATCTTTGTTGCCATCTAG-3' (the underlined sequence shows the mutated codons). All constructs were verified by sequence analysis, pT7Ts constructs were linearized, and cRNA was synthesized in vitro using T7 RNA polymerase as described previously (16).

Experimental animals. Young adult male Wistar rats were randomly assigned to either control group animals receiving vehicle only (n = 5) or FK-506 (n = 5) (21). TRPV5 knockout (KO) mice were generated as described previously (17). The kidney cortex was sampled and immediately frozen in liquid nitrogen. Subsequently, samples were stored at −80°C until further processing. The animals were kept in a light-temperature-controlled room with ad libitum access to a standard pelleted diet and water. The animal ethics board of the Radboud University Nijmegen approved all experimental procedures.

Real-time quantitative PCR. The FKBP52 mRNA expression level was quantified by real-time quantitative PCR in a mouse cDNA panel including kidney, brain, heart, muscle, duodenum, jejunum, ileum, cecum, colon, bone, and in renal tissue from control and FK-506-treated rats. Total RNA was isolated using TRIzol (GIBCO BRL, Life Technologies, Breda, The Netherlands) from which 2 \(\mu\)g were subjected to reverse transcription using Moloney murine leukemia virus reverse transcriptase. The following primers were used: forward

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TACCCTCTCTCTTG-3
TRPV5 NH2- and COOH-terminal tail fusion proteins immobilized on (Promega) and added to GST, GST-fused FKBP52, or GST-fused TRPV5 NH2- and COOH-terminal tail fusion proteins immobilized on glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) in PBS containing 0.3% vol/vol Triton X-100. After a 2-h incubation at room temperature, the beads were washed extensively and bound proteins were eluted with SDS-PAGE loading buffer, separated on 10% wt/vol SDS-PAGE gels, and visualized by autoradiography.

siRNA. The mammalian expression vector pSUPER (5) was used for expression of siRNA in HEK293 cells. The gene-specific insert specifies a 19-nucleotide sequence, 5'-GAGAGGGCACAGG-TACA-3', corresponding to nucleotides 114–133 downstream of the transcription start site of human FKBP52, which was separated by a 9-nucleotide noncomplementary spacer (5'-TTCAAAAGA-3') from the reverse complement of the 19-nucleotide sequence. HEK293 cells were cotransfected with pSUPER-FKBP52 and pCINeo/IRES GFP-TRPV5 or empty pCINEO/IRES-GFP, whereas the pSUPER-luciferase construct was used as a control. The gene-specific insert for luciferase specifies the 19-nucleotide sequence 5'-CTTACGGTGA-TACCTCGA-3'. Three days after transfection, cells were sorted for GFP expression by flow cytometry (fluorescence-activated cell sorting) using an Epic's Elite flow cytometer (Coulter, Miami, FL). The GFP-positive cells were used for the functional analysis. One day later, they were measured for their functional activity using the 45Ca2+ uptake assay. FKBP52 protein expression was detected by immunoblotting using mouse anti-FKBP52 (Stressgen). 45Ca2+ uptake was determined 2 days after transfection, whereas cotransfected cells were first sorted for GFP by fluorescence-activated cell sorting. For the assays where FK-506 was used, TRPV5-transfected cells were incubated overnight with 10, 100, and 1,000 nM FK-506 (Fujisawa Pharmaceutical, Osaka, Japan). Ca2+ uptake was determined by incubation in uptake medium (110 mM NaCl, 5 mM KCl, 1.2 mM MgCl2, 0.1 mM CaCl2, 10 mM Na-acetate, 2 mM NaH2PO4, 20 mM HEPES/Tris, pH 7.4, supplemented with 10 mM felodipine, 10 μM methoxy-verapamil, and 1 mM BaCl2) for 10 min at room temperature. Radioactive Ca2+ (1 μCi/ml 45CaCl2) was applied to the cells. Each well was washed extensively in stop buffer (110 mM NaCl, 5 mM KCl, 1.2 mM MgCl2, 0.5 mM CaCl2, 1.5 mM LaCl3, 10 mM Na-acetate, 20 mM HEPES/Tris, pH 7.4) at 4°C. Incubated with 0.05% wt/vol SDS, and counted for radioactivity using liquid scintillation.

Electrophysiology and solutions. Patch-clamp experiments were performed in the tight-seal, whole cell configuration at room temperature (20–25°C) using an EPC-9 patch-clamp amplifier controlled by Pulse software (HEKA Electronics). Patch pipettes had resistances between 3 and 5 MΩ after being filled with the standard intracellular solution. Cells were held at 20 mV, and voltage ramps of 450-ms duration, ranging from −100 to +100 mV, were applied every 5 s. Cell capacitance and access resistance were monitored continuously using the automatic capacitance compensation of the Pulse software. Current densities were obtained by dividing the current amplitude measured at −80 mV by the cell capacitance. Ca2+-dependent inactivation was studied using a 3-s voltage step to −100 mV from a holding potential of +70 mV. The inactivation rate was assessed by the time for 10% decay of the current. The standard extracellular solution (Krebs) contained 150 mM NaCl, 6 mM CsCl, 1 mM MgCl2, 10 mM HEPES/NaOH, pH 7.4, and 10 mM glucose. The concentration of Ca2+ ranged between 1 and 10 mM. Divalent-free solutions did not contain added divalent cations, whereas trace amounts of divalent cations were removed with 100 μM EDTA. The standard internal (pipette) solution contained 20 mM CsCl, 100 mM Cs-aspartate, 1 mM MgCl2, 4 mM NaATP, 10 mM BAPTA, and 10 mM HEPES/CSOH, pH 7.2. Cells were kept in a nominal Ca2+-free medium to prevent Ca2+ overload and exposed for a maximum of 5 min to a Krebs solution containing 1.5 mM Ca2+ before sealing of the patch pipette to the cell.

Transcellular Ca2+ transport in primary culture of rabbit kidney connecting tubule and cortical collecting duct. Rabbit kidney connecting tubules (CNT) and cortical collecting ducts (CCD) were
immunodissected from the kidney cortex of New Zealand White rabbits (−0.5 kg) using the antibody R2G9 and then placed in primary culture on permeable filters (0.33 cm²; Costar, Cambridge, MA) as described previously in detail (4). At confluence, monolayers of rabbit CNT/CCD cells growing on permeable filters were incubated overnight with 10, 100, and 1,000 nM FK-506 at the apical and the basolateral side. The next day, filters were washed twice and preincubated in PSS buffer containing 140 mM NaCl, 2 mM KCl, 1 mM K₂HPO₄, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM glucose, 5 mM t-alanine, 5 μM indomethacin, and 10 mM HEPES/Tris, pH 7.4, for 15 min at 37°C. Subsequently, the filters were incubated in PSS (100 μl to the apical and 600 μl to basolateral compartment). After 90 min, transepithelial Ca²⁺ transport was measured by using a colorimetric assay kit as described (4). Transepithelial potential difference and resistance were checked before and after transport measurement to confirm cell confluence and integrity of the monolayer.

Statistical analysis. Values are expressed as means ± SE. Overall statistical significance was determined by ANOVA. In case of significance, differences between the means of two groups were analyzed by an unpaired t-test, whereas multiple comparisons between groups were performed by Bonferroni post hoc tests. P values <0.05 were considered significant. The statistical analyses were performed using SPSS software (SPSS, Chicago, IL).

RESULTS

FKBP52 tissue distribution and localization. FKBP52 expression was investigated at the mRNA level by quantitative real-time PCR analysis, showing a higher abundance in kidney and heart compared with other tissues (Fig. 1A). Subsequently, the localization of FKBP52 was analyzed in the kidney. Immunohistochemical staining of rabbit kidney sections showed partial colocalization of FKBP52 with TRPV5 in DCT and CNT, nephron segments known to be responsible for active transepithelial Ca²⁺ transport (Fig. 1B; see also Supplementary Fig. I. All supplementary materials are online at http://ajprenal.physiology.org/cgi/content/full/00298.2005/DC1.).

Interaction of FKBP52 with TRPV5. To assess the FKBP52 interaction with TRPV5, HEK293 cells were transfected with TRPV5 or the empty vector and cell lysates were subsequently subjected to immunoprecipitation using the TRPV5 antibody. Endogenous FKBP52 was coimmunoprecipitated with TRPV5 from these cells lysates, as represented by the immunopositive band of ∼59 kDa (Fig. 2A). The expression of both proteins in the cell lysates was also verified, as shown in the top and middle.

The interaction between FKBP52 and TRPV5 was further substantiated using GST pull-down assays. FKBP52 was expressed as a GST fusion protein and analyzed for its interaction with in vitro translated full-length [³⁵S]methionine-labeled TRPV5 in the presence (1 mM CaCl₂) or absence of Ca²⁺ (10 mM EGTA) and with different concentrations of FK-506. FKBP52 bound to TRPV5 in a Ca²⁺ (Fig. 2B)- and FK-506 (Fig. 2C)-independent manner. No interaction was observed with GST alone, indicating the specificity of the interaction. To determine the role of TRPV5 tails in the binding with FKBP52, the GST-fused NH₂- and COOH-terminal tails of TRPV5 were analyzed for their interaction with in vitro translated FKBP52. Both tails did not interact with FKBP52 (Fig. 2D; see also Supplementary Fig. II).

Effect of FKBP52 knock-down on Ca²⁺ influx. The role of FKBP52 in Ca²⁺ influx through TRPV5 was investigated using the pSUPER siRNA system. HEK293 cells were cotransfected with pSUPER-FKBP52 (siFKBP52) and TRPV5. The pSUPER-luciferase (siLuc) construct was used as a negative control for the suppression of FKBP52, and the empty vector (mock) as a control for TRPV5 transfection. Application of FKBP52 siRNA significantly reduced FKBP52 protein expression, whereas no effect was observed in the siLuc-transfected cells, demonstrating the specificity of the FKBP52 gene-silencing (Fig. 3A). A ⁴⁵Ca²⁺ uptake assay was performed to deter-

![Fig. 1. Expression profile and localization of FKBP52. A: RNA was extracted from mouse tissues, and after reverse transcription relative FKBP52 expression was determined by real-time PCR analysis. FKBP52 mRNA expression was normalized for the respective hypoxanthine-guanine phosphoribosyl transferase (HPRT) values. B: Immunohistochemical localization of FKBP52 (green) and TRPV5 (red) in rabbit kidney sections. In the merged picture, the partial colocalization of the 2 proteins is depicted in yellow.]
FKBP52 IN TRPV5 REGULATION

Fig. 2. FKBP52 binds the TRPV5 channel. A: interaction of FKBP52 with TRPV5 as demonstrated by coimmunoprecipitation in HEK293 cells transfected with TRPV5 or mock. Equal amounts of cell lysates were subjected to immunoprecipitation using the TRPV5 antibody, and coimmunoprecipitation of the endogenous FKBP52 was detected by immunoblot analysis using the FKBP52 antibody (IP). Total lysates were analyzed for TRPV5 and FKBP52 protein expression (lysates). B: direct interaction of FKBP52 and TRPV5 was confirmed by glutathione S-transferase (GST) pull-down assay. In vitro translated [35S]methionine-labeled TRPV5 associated with the GST-fused FKBP52 in the presence (1 mM CaCl₂) and absence (10 mM EGTA/no CaCl₂ added) of Ca²⁺. C: binding of GST-fused FKBP52 was not altered by the addition of FK-506. D: GST-fused TRPV5 NH₂- and COOH-terminal tails were incubated with in vitro translated [35S]methionine-FKBP52, demonstrating no binding of FKBP52.

To estimate the putative effect of FK-506 on FKBP52 transcription, renal mRNA expression was determined by quantitative real-time PCR in rats treated with FK-506 (1 mg/day) or vehicle for 7 days. The results demonstrated that FK-506 has no significant effect on FKBP52 mRNA expression compared with control (0.32 ±

Role of FKBP52 PPlase activity in TRPV5-mediated Ca²⁺ influx. The enzymatic activity of FKBP52 was abolished by double mutation of two highly conserved amino acid residues (FD67DV mutant) as described previously (22). The FD67DV mutant and wild-type FKBP52 were cotransfected in HEK293 cells with TRPV5 and functionally analyzed using a ⁴⁶Ca²⁺ uptake assay. The FD67DV mutant reversed the inhibitory effect of FKBP52 on TRPV5-mediated Ca²⁺ influx (Fig. 5A). Immunoblot analysis of the cell lysates demonstrated that both wild-type FKBP52 and mutant FD67DV are equally expressed and do not interfere with TRPV5 expression (Fig. 5B; see also Supplementary Fig. IV).

Effect of FK-506 on TRPV5 activity. The immunosuppressant FK-506, known to bind and inhibit FKBP52 enzymatic activity, was assessed for its effect on TRPV5-mediated Ca²⁺ influx. First, the dose-dependent relationship of FK-506 was determined in HEK293 cells, demonstrating that overnight incubation with high concentrations (in the range of 10 nM-100 μM) diminishes cell viability (Fig. 6A). Then, HEK293 cells transiently transfected with TRPV5 were incubated overnight with 10 nM FK-506 and tested for their ⁴⁶Ca²⁺ uptake. Incubation with FK-506 resulted in significantly increased ⁴⁶Ca²⁺ influx in TRPV5-transfected cells (Fig. 6B). To further substantiate this effect, FK-506 action was evaluated in kidney primary cultures. The primary cultures of rabbit CNT/CCD cells form confluent monolayers that exhibit many characteristics of the original polarized epithelia, including parathyroid hormone (PTH)- and vitamin D-stimulated transepithelial Ca²⁺ transport (4). Overnight incubation of 10 nM FK-506 significantly stimulated trancellular Ca²⁺ transport in these primary kidney cells (Fig. 6C). Measurements of transepithelial resistance across the cell monolayer showed that higher concentrations (1 and 100 μM) of FK-506 disturbed the integrity of the confluent cell monolayer.

Regulation of FKBP52 in the kidney. To estimate the putative effect of FK-506 on FKBP52 transcription, renal mRNA expression was determined by quantitative real-time PCR in rats treated with FK-506 (1 mg/day) or vehicle for 7 days. The results demonstrated that FK-506 has no significant effect on FKBP52 mRNA expression compared with control (0.32 ±
Indeed, knock-down of FKBP52 protein expression by siRNA gene silencing, as well as inhibition of FKBP52 enzymatic activity by binding to FK-506, increased Ca\(^{2+}\) influx through TRPV5. In addition, expression of the FKBP52 protein reduced TRPV5-mediated Ca\(^{2+}\) and Na\(^{+}\) currents, suggesting that FKBP52 decreases the number of functional channels at the plasma membrane. In this regulatory process, it seems that the enzymatic activity of FKBP52 plays a critical role, because inactivation of FKBP52 PPlase activity abolished its effect on TRPV5 activity. The ability of FKBP52 to modify its substrates by catalyzing the cis-trans isomerization of proline residues is often a rate-limiting step in protein folding (26). Moreover, it is possible that FKBP52 operates through multiplex targets to regulate the function of the channel, considering that it contains putative ATP, GTP, and calmodulin binding sites (6, 20).

Consistent with our findings on the inhibitory role of FKBP52 in TRPV5 Ca\(^{2+}\) influx is a previous study that

Fig. 3. Enhancement of TRPV5 activity by small-interference (si) RNA-mediated gene silencing of FKBP52. HEK293 cells were cotransfected with the pSUPER-FKBP52 (siFKBP52) construct together with TRPV5 or the empty vector (mock). The pSUPER-Luciferase (siLuc) construct was used as a control for the specificity of FKBP52 gene silencing. A: immunoblot analysis of FKBP52 in lysates from cotransfected HEK293 cells demonstrated suppression of FKBP52 protein. TRPV5 expression was not altered by the FKBP52 protein suppression. B: \(^{45}\)Ca\(^{2+}\) uptake was measured in HEK293 cells, as shown in A. Cells cotransfected with the siFKBP52 and TRPV5 showed an increased \(^{45}\)Ca\(^{2+}\) influx compared with siLuc/TRPV5-transfected cells. Filled bars, cells transfected with siFKBP52; open bars, siRNA control siLuc. Values are means ± SE; n = 4, *P < 0.01 significantly different from the respectively mock-transfected cells. #P < 0.02 significantly different from siLuc TRPV5-transfected cells.

DISCUSSION

The present study demonstrated that FKBP52 binds and colocalizes with the epithelial Ca\(^{2+}\) channel TRPV5 in the distal part of the nephron. FKBP52 reduced TRPV5 activity, as indicated by the functional analysis using FKBP52 siRNA and coexpression studies in HEK293 cells. This inhibitory action was reversed by the abrogation of PPlase activity in FKBP52, as well as by administration of the FKBP52 blocker FK-506. Our findings suggest that the immunophilin FKBP52 is a TRPV5 auxiliary protein regulating channel activity.

FKBP52 transcription was strongly stimulated by two major regulators of Ca\(^{2+}\) homeostasis: vitamin D\(_3\) and dietary Ca\(^{2+}\) (12), in line with the regulation observed for TRPV5 (13). We showed here that renal FKBP52 expression is diminished in TRPV5 KO mice, further supporting functional coupling between both proteins.
identified the FKBP52 Drosophila homolog FKBP59 as a physiological regulator of TRPL channel activity (10). FKBP59 interacts directly with the highly conserved TRPL sequence 701LPPPFNVLP709 and inhibits Ca\textsuperscript{2+}/H\textsuperscript{1001} influx. In Drosophila (10) as well as in mammals (28), the proline residue of the first LP-motif, located next to mouth of the channel pore, plays a crucial role in this association. Conspicuously, TRPV5 contains such an LP motif next to the pore region at the amino acid position 551–552, where the FKBP52 binding site is located. FK-506 has been shown to mimic the LP-dipeptide (23) and to disrupt the FKBP52 interaction with TRPCs (28). However, FK-506 did not interrupt the FKBP52-TRPV5 association, suggesting an alternative mechanism. This would mean that FK-506 binds TRPV5 and modulates its activity without displacing the association of FKBP52, similar to the mechanism previously described for the regulation of the Drosophila homologs FKBP59 and TRPL (10). The physiological relevance of the latter hypothesis needs further confirmation because it is based on in vitro experiments.

FKBP52 is expressed along the apical region of DCT and CNT tubules together with TRPV5. In addition, FKBP52 was also localized in the cytosol. This finding together with the stimulatory effect of FK-506 on Ca\textsuperscript{2+} influx observed in primary CNT/CCD renal cells further substantiated the physiological relevance of FKBP52 in active transepithelial Ca\textsuperscript{2+} transport. Our results imply that although high concentrations of FK-506 are toxic for the cells, submicromolar concentra-

Fig. 5. FKBP52 inhibitory effect on \textsuperscript{45}Ca\textsuperscript{2+} influx through TRPV5 depends on the peptidyl-propyl cis-trans isomerase (PPIase) activity of FKBP52. A: HEK293 cells were cotransfected with TRPV5 and the empty vector (TRPV5), the FKBP52 construct (FKBP52/ TRPV5), or the catalytic inactive FKBP52 mutant (FD67DV/ TRPV5). The inhibitory effect of FKBP52 on TRPV5-mediated \textsuperscript{45}Ca\textsuperscript{2+} influx was abolished by FD67DV. Values are means ± SE; n = 5. *P < 0.01 significantly different from mock-transfected cells. #P < 0.02 significantly different from TRPV5-transfected cells. B: immunoblot analysis for FKBP52 was performed on the lysates of HEK293 cells cotransfected with TRPV5 and the empty vector (TRPV5), the FKBP52 construct (FKBP52/ TRPV5), or the catalytic inactive FKBP52 mutant (FD67DV/ TRPV5) that were applied in the functional assay in A. The results demonstrated equal expression of wild-type and mutated FKBP52, and immunostaining for TRPV5 showed equal expression of the channel.

Fig. 6. FK506-stimulatory effect on Ca\textsuperscript{2+} influx of TRPV5-expressing HEK293 and primary connecting tubule (CNT)/cortical collecting duct (CCD) cells. A: HEK293 cells were incubated overnight with FK-506 at a concentration between 0 and 100 μM. The effect of FK-506 on cell viability was counted with the Trypan blue exclusion assay. B: TRPV5-transfected HEK293 cells were incubated overnight with 0 or 10 nM FK-506. \textsuperscript{45}Ca\textsuperscript{2+} uptake measurements showed a stimulatory effect of FK-506 on \textsuperscript{45}Ca\textsuperscript{2+} influx. C: TRPV5-expressing monolayers of rabbit CNT/CCD primary cell cultures were incubated overnight with FK-506 at a concentration of 0–100 μM. On FK-506 treatment, cells treated with 10 nM FK-506 showed increased Ca\textsuperscript{2+} transport, whereas higher concentrations disturbed the cell monolayer, resulting in reduced Ca\textsuperscript{2+} transport. Values are means ± SE; n = 4. *P < 0.01 vs. control cells.

Fig. 7. Regulation of FKBP52 expression. A: immunoblotting of FKBP52 in total kidney homogenates of TRPV5 knockout (KO) and wild-type littermates. Ten micrograms of total protein were loaded per lane, and each lane represents the renal homogenate from an individual mouse. B: intensity of the immunopositive bands was quantified by densitometry. FKBP52 expression is depicted as a percent ratio to wild-type mice. Values are means ± SE. *P < 0.05 vs. control mice.
tions (10 nM) stimulate transcellular Ca\(^{2+}\) transport. This dose-dependent effect could explain the nephrotoxicity observed in patients treated with the immunosuppressive drug FK-506 (2, 3). On the other hand, it has been previously demonstrated that the hypercalciuric effect of FK-506 is mediated through the decreased expression of calbindin-D_{28K} (1) and TRPV5 (21). Contrary to the reduction in TRPV5 expression on FK-506 administration, we showed that FKBP52 expression is not altered under the same conditions. In this regard, the reduction of the TRPV5/FKBP52 ratio is reminiscent of the transient expression in HEK293 cells, where an excess of FKBP52 inhibits TRPV5-mediated Ca\(^{2+}\) influx. Certainly, given the complexity of the in vivo situation, FK-506 dose-dependent effect could explain the nephrotoxicity observed in patients treated with the immunosuppressive drug FK506.

In conclusion, FKBP52 participates in active transepithelial Ca\(^{2+}\) transport along the distal part of the nephron by reducing TRPV5 activity. This inhibitory effect on TRPV5 is mediated by the enzymatic PPlase activity of FKBP52 and is reversed by FK-506 administration.

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