Published in final edited form as:
Pediatr Cardiol. 2012 August ; 33(6): 988–994. doi:10.1007/s00246-012-0298-4.

The Role of FK506-Binding Proteins 12 and 12.6 in Regulating Cardiac Function

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Abstract

Specifically, FK506-binding proteins 12 (FKBP12) and 12.6 (FKBP12.6) are cis–trans peptidyl prolyl isomerases that are expressed in the heart. Both FKBP12 and FKBP12.6 were previously known to interact with ryanodine receptors in striated muscles. Although FKBP12 is abundantly

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present in the heart, its function in the heart is largely uncertain. Recently, by generating FKBP12 transgenic overexpression and cardiac-restricted knockout mice, we showed that FKBP12 is critically important in regulating trans-sarcolemmal ionic currents, predominately the voltage-gated Na^+ current, I_{Na}, but it appears to be less important for regulating cardiac ryanodine receptor function. Similar genetic approaches also confirm the role of FKBP12.6 in regulating cardiac ryanodine receptors. The current study demonstrated that FKBP12 and FKBP12.6 have very different physiologic functions in the heart.

Keywords

FK506-binding protein; Cardiac function; Arrhythmia; Voltage-gated sodium channel; Ryanodine receptor; Calcium release

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The FK506-binding proteins (FKBP) are immunophilins that interact with immunosuppressants FK506 and rapamycin [23]. At least 10 mammalian FKBPs have been described to date [23]. They commonly contain conserved cis–trans peptidyl prolyl isomerase domains [23]. Both FKBP12 and FKBP12.6, encoded by *Fkbp1a* and *Fkbp1b*, respectively, are small (~12 kDa) cytoplasmic proteins that share 85 % amino acid homology and a near identical tertiary structure [12, 13, 23]. By binding to the immunosuppressive drugs FK506 and rapamycin, FKBP12–FK506 and FKBP12–rapamycin complexes inhibit calcineurin and mTOR (mammalian target of rapamycin), respectively, and subsequently suppress T-cell activation [3, 32].

Both FKBP12 and FKBP12.6 are ubiquitously expressed, including the cardiac and skeletal muscles [9]. A recent report has estimated that the protein concentrations of FKBP12 and FKBP12.6 in cardiomyocytes are, respectively, 1 μmol/l and about 150 nmol/l [6].

Both FKBP12 and FKBP12.6 were found to be associated with calcium release channel ryanodine receptors (RyR) [9]. The stoichiometry of binding is 4 FKBP molecules for every RyR tetramer (i.e., 1 FKBP to 1 RyR monomer). Tightly binding to the skeletal muscle ryanodine receptor (RYR1), FKBP12 can be co-purified through sucrose density gradient centrifugation [7].

Detailed biochemical and physiologic analyses further supported the role of FKBP12 as a channel modulator for RyR1 [9]. The FKBP12 binding site was mapped to a region between amino acids 2,458 and 2,468 of rabbit RyR1, and Val2461–Pro2462 residues were shown to be critical for FKBP12 binding. Each mutation of Val2461-Glu–RyR1, Val2461-Gly–RyR1, and Val2461-Ile–RyR1 abolished RyR1–FKBP12 interaction, and the mutant channels had altered gating frequencies [2, 5]. Additionally, FKBP12.6 can bind to normal RyR1, but with lower affinity [25]. Intriguingly, the normal channel function of Val2461-Ile–RyR1 can be restored by FKBP12.6 [5], suggesting a different binding property and specificity between FKBP12 and FKBP12.6.

Given the extensive studies and a series of pharmacophysiologic analyses, it is widely believed that FKBP12 is important for regulating skeletal muscle excitation–contraction coupling (E–C coupling) [9]. However, the role of FKBP12 in regulating cardiac ryanodine receptors (RyR2) has been highly debatable. Apparently, RyR2 has a significantly higher binding affinity to FKBP12.6 [25]. A recent work using a fluorescence-labeling approach to directly measure in situ binding of FKBP12 and FKBP12.6 in permeabilized cardiomyocytes confirmed a high FKBP12.6–RyR2 affinity (K_d = 0.7 ± 0.1 nmol/l) and a lower FKBP12–RyR2 affinity (K_d = 206 ± 70 nmol/l) despite the fact that both FKBP12.6 and FKBP12 were localized at Z-lines [6].

This study also demonstrated that FKBP12.6, but not FKBP12, inhibits resting RyR2 activity [6]. Studies also suggest that the interaction site of FKBP12.6–RyR2 is very similar to that of FKBP12–RyR1 [21, 26, 27], and Ile2427–Pro2428 residues are essential for the FKBP12.6–RyR2 interaction [15]. However, another group demonstrated that a region between amino acids 1,815 and 1,855 near divergent region 3 is critical for FKBP12.6 binding [16, 31]. In addition, C-terminal region of RyR2 also was suggested as interacting with FKBP12.6 [33]. Apparently, despite a similar expression pattern and protein structure, FKBP12 and FKBP12.6 have distinctly different physiologic functions.

Mice deficient in FKBP12 die in utero due to severe ventricular defects including hypertrabeculation, ventricular noncompaction, and ventricular septal defects (VSD) [22], suggesting its essential role in cardiac development. In contrast, FKBP12.6-deficient mice display normal cardiac development and are viable. However, once mature, adult FKBP12.6-deficient mice display abnormal cardiac physiology including either enlarged hearts [30] or exercise-induced cardiac arrhythmias and sudden death [28]. It has been a highly debated question whether FKBP12, FKBP12.6, or both are key players in regulating cardiac function and various cardiac pathogenetic pathways. More recently, a series using mouse transgenic approaches to determine the biologic function of FKBP12 and FKBP12.6 led to several unexpected results. This brief review aims to summarize and update our recent findings.

Cardiomyocyte-Restricted FKBP12 Knockout Mice

Germline FKBP12 knockout mice ($FKBP12^{-/-}$) demonstrated severe cardiac developmental defects, notably including ventricular hypertrabeculation, noncompaction, and VSD [22]. The majority of $FKBP12^{-/-}$ mice died between E14.5 and birth. A few $FKBP12^{-/-}$ survivors had severe compromised cardiac function and died before reaching to 2 months of age [22].

Our first thought was that the cardiac developmental defects seen in $FKBP12^{-/-}$ mice were due to its essential role in regulating calcium release channel RyR2. To test the functional properties of RyR1 and RyR2 in the absence of FKBP12, skeletal muscle and cardiac muscle membranes from wild-type and FKBP12-deficient mice were prepared and reconstituted into planar lipid bilayers. Both RyR1 and RyR2 from FKBP12-deficient mice demonstrated an increased probability of opening and stayed mostly in subconductance states compared with RyR1 and RyR2 from wild-type mice [22]. Thus, we concluded that FKBP12 was a key modulator for RyR2 [22]. However, the question of how altered RyR2 function led to these specific cardiac developmental defects in $FKBP12^{-/-}$ mice was very puzzling.

Given that FKBP12 is ubiquitously expressed, we generated FKBP12 conditional knockout mice to test a cardiomyocyte autonomous contribution to the cardiac developmental defects. A first report from Tang et al. [24] had demonstrated that ablation of FKBP12 in skeletal muscle using MCK-cre mice led to altered skeletal muscle E–C coupling, confirming the role of FKBP12 in regulating skeletal muscle function and RyR1. Surprisingly, this work also noted that the cardiac function was largely normal in the mutant mice, although FKBP12 expression also was ablated in cardiomyocytes [24], suggesting that FKBP12 was not critical for RyR2 function. These findings were later confirmed by using a cardiomyocyte-specific cre line, MHC-cre mice, further suggesting that the cardiac defects in FKBP12-deficient mice were not primarily derived from FKBP12 deficiency in cardiomyocytes [14]. Thus, the altered RyR2 function seen in FKBP12-deficient mice was not the mechanism underlying the cardiac developmental defects. We currently are in the process of determining whether RyR2 function is altered in the FKBP12 conditional knockout hearts.

Cardiomyocyte-Restricted FKBP12 Overexpression Transgenic Mice

Overexpression of FKBP12 in cultured rabbit ventricular cardiomyocytes via adenovirus gene delivery apparently led to a slight increase in sarcoplasmic reticulum calcium load, which likely was due to the reduced calcium leak, as evinced by a reduced Ca^{2+} spark frequency at rest in the FKBP12 overexpressing cardiomyocytes [20]. To determine the biologic impact of FKBP12 upregulation on cardiac function, we generated cardiomyocyte-restricted FKBP12 transgenic mice using a-myosin heavy chain promoter (MHC-FKBP12). Three independent transgenic lines were generated. Western blot analyses demonstrated that all three transgenic lines exhibited about a ninefold increase in FKBP12 protein levels in the hearts compared with their nontransgenic control mice. All the transgenic mice showed normal development and growth. However, about 38 % of the MHC-FKBP12 transgenic mice died suddenly at 4–6 weeks of age [14].

Surface electrocardiograms (ECGs) recorded from adolescent MHC-FKBP12 transgenic mice showed significant prolongations of the PP interval, P-wave duration, PQ interval, and QRS duration compared with nontransgenic mice [14]. Almost all the MHC-FKBP12 mice presented with various degrees of higher-degree atrioventricular (AV) conduction block. Ambulatory ECG recordings also demonstrated intermittent complete AV block in MHC-FKBP12 mice [14], which was further confirmed by electrophysiologic studies using Langendorff-perfused hearts. The conduction velocity in left ventricular epicardium also was assessed using the optical mapping technique. The conduction velocity in MHC-FKBP12 transgenic hearts was significantly slower than in nontransgenic hearts [14]. Finally, microelectrode measurements of transmembrane action potentials (AP) from the left ventricular epicardium of isolated hearts showed deceleration of the maximal phase 0 upstroke velocity $(dV/dt)_{max}$ and a marked AP prolongation in transgenic hearts (Fig. 1a). These findings suggested that chronic FKBP12 overexpression was associated with abnormal cardiac conduction and repolarization [14].

A major determinant of electrical conduction is the magnitude of Na^+ influx through voltage-gated Na^+ channels during the initial fast membrane depolarization [8]. Accordingly, using the whole-cell voltage-clamp technique, we compared the density and properties of the macroscopic voltage-gated Na^+ current (I_{Na}) in MHC-FKPB12 and wild-type cardiomyocytes. The mean peak I_{Na} density was dramatically reduced (~80 %) in MHC-FKPB12 cardiomyocytes compared with their nontransgenic counterparts, whereas mean whole-cell capacitance was not significantly altered (Fig. 1b). Overexpression of FKBP12 shifted the peak I_{Na}–V curve to more positive potentials, indicating that the number of Na^+ channels activated at a given membrane voltage was reduced.

Furthermore, FKBP12 overexpression significantly slowed both the early and late component of I_{Na} inactivation compared with those of wild-type cardiomyocytes and enhanced a persistent and tetrodotoxin-resistant (3 μmol/l) late I_{Na} component (Fig. 1c) [14]. Interestingly, left ventricular AP recordings from Langendorff-perfused myocardial-specific FKBP12 conditional knockout hearts ($FKBP12^{f/f}$/MHC-Cre) demonstrated a significant acceleration of the maximal phase 0 upstroke velocity compared with the control condition (Fig. 2a), suggesting an increase in I_{Na} density. Whole-cell voltage-clamp experiments confirmed that the peak I_{Na} density in FKBP12-deficient ventricular cardiomyocytes was more than twice as large as that in control cells (Fig. 2b). Thus, FKBP12 ablation profoundly affected peak I_{Na} density in ventricular cardiomyocytes and altered I_{Na} inactivation gating [14].

These findings suggested that FKBP12 is an important regulator of I_{Na}. In contrast to the dramatic alteration of I_{Na}, the voltage dependence of the inwardly rectifying K^+ current

(I_{K1}), the transient outward K^+ current (I_{to}), the sustained K^+ current (I_{Ksus}), the L-type Ca^{2+} current ($I_{Ca,L}$), and the $(Ca^{2+})_i$ transients was either unchanged or only slightly affected [14], suggesting that these channels were not likely to underlie the delay in ventricular repolarization in the transgenic cardiomyocytes. Our current focus was to determine the underlying biochemical mechanism by which FKBP12 regulates voltage-gated sodium channels.

FKBP12.6 Knockout Mice

Two independent mouse strains deficient in FKBP12.6 had been reported. However, intriguingly, they demonstrated two distinctively different cardiac phenotypes. The first strain, generated via the targeting of 129SvEv mouse embryonic stem (ES) cells, displayed gender-specific adult cardiac hypertrophy [10, 30]. The second strain via DBA/lacJ ES cells displayed stress/exercise-induced cardiac sudden death [28]. Interestingly, intracellular calcium release appeared to be altered in both FKBP12.6-deficient mouse models, suggesting that secondary genetic factors may contribute to the final pathogenetic outcome.

To determine whether the cardiac enlargement in FKBP12.6-deficient males was associated with abnormal cardiac gene expression, we analyzed the expression levels of several cardiac markers such as atrial natriuretic peptide (ANP), αMHC, βMHC, skeletal α-actin (acta1), sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a), and phospholamban (pln) [1, 4, 19]. Acta1, ANF, and βMHC, present only in embryonic hearts, are reactivated to persistently higher expression levels in hypertrophic hearts. Typically, SERCA2a and pln, found to be downregulated in hypertrophic and failing hearts, are involved in regulating intracellular Ca^{2+} homeostasis and contractile function of cardiomyocytes [1, 19]. Intriguingly, although cardiac hypertrophy was seen in FKBP12.6-deficient adult males, the mRNA levels of these cardiac hypertrophy markers were not elevated [10]. This observation suggests that FKBP12.6-mediated signaling is likely more relevant to physiologic than to pathologic hypertrophy [11, 17], consistent with the fact that FKBP12.6-deficient mice never display heart failure, a common end stage for the pathologic hypertrophic heart.

To determine whether FKBP12.6-deficient mice in a 129SvEv/C57 background also can experience exercise-induced cardiac arrhythmia and sudden death in addition to hypertrophy, we compared conscious ECG parameters in these mutant mice and male littermates (age, 5–6 months) using a protocol similarly applied in characterizing another FKBP12.6 knockout strain [28]. Continuous recordings of ECG were collected from each mouse. No significant differences were observed for RR intervals, PR intervals, QRS duration, rate-corrected QT intervals, or resting heart rate. Neither FKBP12.6-deficient nor wild-type mice displayed arrhythmia or syncope during ECG probe implantation or under sedentary conscious conditions [10].

Furthermore, we subjected the mutant mice and the wild-type mice to a strenuous exercise protocol followed by intraperitoneal injection of epinephrine as previously described [18, 28]. Although both groups of mice had elevated heart rates after exercise, no syncope or polymorphic ventricular arrhythmia was observed in either group. After intraperitoneal injection of epinephrine, we did not observe differences between the two groups of mice. Again, both groups had elevations of heart rate, but neither FKBP12.6-deficient mice nor their wild-type littermates displayed syncope or polymorphic ventricular arrhythmia. These observations indicated that the 129SvEv/C57B6 strain of FKBP12.6-deficient mouse does not experience exercise-induced arrhythmia and sudden death.

Cardiomyocyte-Restricted FKBP12.6 Overexpression Transgenic Mice

Similarly, we generated MHC-FKBP12.6 transgenic mice [10]. These transgenic mice developed normally and had a normal life span. Histologic and functional characterization of these transgenic mice indicated that overexpression of FKBP12.6 did not significantly alter cardiac structure or function [10]. Expression of FKBP12.6 is ubiquitous. One important question concerned whether the abnormal cardiac hypertrophy seen with FKBP12.6-deficient mice in a 129SvEv/C57B6 background was directly caused by the loss of FKBP12.6 expression in cardiomyocytes. To test this, we generated MHC-FKBP12.6/FKBP12.6$^{-/-}$ (129SvEv/C57B6) compound mice in which FKBP12.6 overexpression was restricted to cardiomyocytes. Heart size and cardiac morphology were compared between littermate adult males (5-months-old) with different genotypes, namely, MHC-FKBP12.6/FKBP12.6$^{-/-}$, FKBP12.6$^{-/-}$, and FKBP12.6$^{+/-}$, and wild type. Our data demonstrated that cardiomyocyte-specific expression of FKBP12.6 prevented cardiac enlargement in FKBP12.6-deficient adult males. This observation strongly suggested a cardiomyocyte autonomous mechanism underlying the development of cardiac hypertrophy in FKBP12.6-deficient males.

Previously, it had been shown that the properties of Ca^{2+} sparks and intracellular calcium transients were altered in FKBP12.6-deficient cardiomyocytes [30]. These findings were consistent with an increase in open probability of RyR2 in the absence of FKBP12.6 and pharmacologic dissociation of FKBP12.6 from RyR2-channel complex [28, 29].

To evaluate Ca^{2+} release in cardiomyocytes isolated from MHC-FKBP12.6 transgenic mice, we measured Ca^{2+} sparks in the cardiomyocytes derived from MHC-FKBP12.6 transgenic hearts. We observed normal Ca^{2+} release in MHC-FKBP12.6 cardiomyocytes compared with wild-type control mice. To determine whether the altered Ca^{2+} release in FKBP12.6-deficient cardiomyocytes was a direct consequence of FKBP12.6 ablation in cardiomyocytes or not, we compared the characteristics of calcium sparks in cardiomyocytes isolated from MHC-FKBP12.6/FKBP12.6$^{-/-}$ (male) with those of Ca^{2+} sparks in cardiomyocytes isolated from sex-matched littermate FKBP12.6$^{-/-}$, MHC-FKBP12.6, and wild-type control mice [10]. These parameters included the frequency and amplitude of the Ca^{2+} spark, full width at half maximum (FWHM) spark size, spark rising time, and half decay time. For all the parameters measured, MHC-FKBP12.6/FKBP12.6$^{-/-}$ cardiomyocytes had normal calcium sparks [10]. These data indicated that cardiomyocyte-specific expression of FKBP12.6 was able to rescue abnormal calcium release in FKBP12.6-deficient cardiomyocytes, which further demonstrated a direct association of FKBP12.6 in regulating calcium release channel RyR2 in cardiomyocytes. Taken together, our findings provided a confirmation of the role played by FKBP12.6 in regulating cardiomyocyte calcium release and cardiac function as well as additional insight into that role.

Conclusion

The role of FKBP12 and FKBP12.6 in regulating cardiac function has been an intriguing question over the past decade. A number of FKBP12 and FKBP12.6 transgenic and knockout mice were generated and analyzed. The findings from these mice provided compelling new data supporting the concept that FKBP12 and FKBP12.6 have very different biologic functions in the heart. Whereas FKBP12 has an important physiologic role in regulating cardiac voltage-gated sodium channels, FKBP12.6 is more relevant to RyR2 function.

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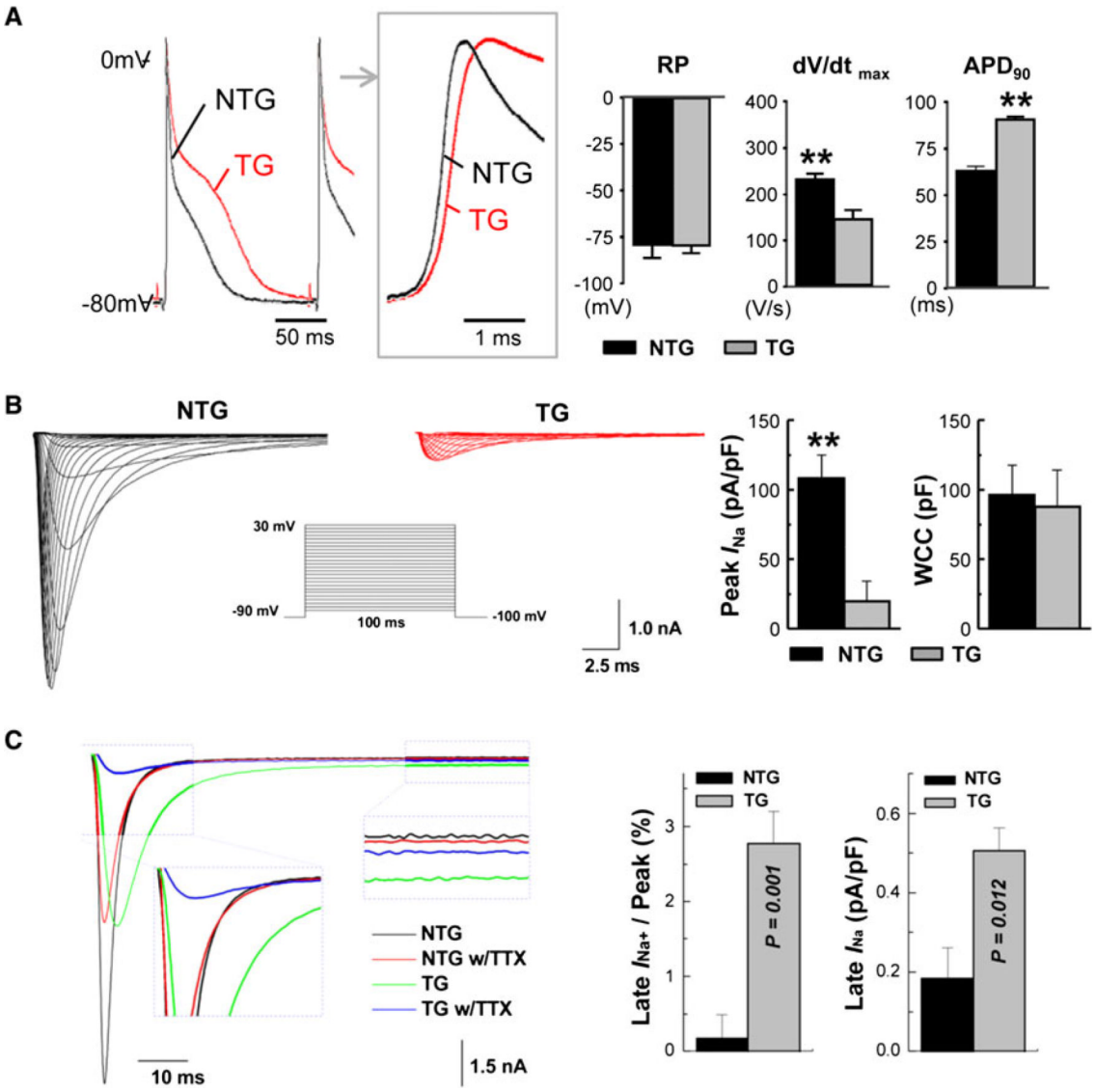

Fig. 1.

Cellular electrophysiology of MHC-FKBP12 ventricular cardiomyocytes. **a** Representative ventricular transmembrane action potentials (APs) recorded from isolated transgenic (TG) and non-transgenic (NTG) hearts. The *box* shows the initial portions of the respective APs in an expanded time scale. On the average, the maximum upstroke velocity of phase 0 of the AP $(dV/dt)_{max}$ was decreased in TG hearts compared with NTG hearts despite similar mean resting membrane potentials (RP). Significantly longer action potential duration (APD) at 90 % repolarization (APD_{90}) was noted in TG hearts. **b** Voltage-clamp analysis of macroscopic I_{Na} in ventricular cardiomyocytes isolated from MHC-FKBP12 and wild-type hearts. Overexpression of FKBP12 decreases the peak Na^+ current. Representative I_{Na} traces are elicited by 120 ms depolarizing pulses to potentials of –90 to +30 mV from a holding potential of –100 mV in 5 mV increments (interpulse interval, 1 s). The insert shows a schematic of the voltage-clamp protocol. The *bar graphs* in (**b**) show Means ± Standard Deviations of maximal peak I_{Na} densities measured at –5 and –25 mV in MHC-FKBP12 and wild-type myocytes, respectively, and whole-cell capacitance (WCC), $^{**}p < 0.01$. **c** FKBP12 overexpression enhances a persistent Na^+ current. Original traces of I_{Na} elicited –

100 to +30 mV for 400 ms before and after exposure to 3 μmol/l tetrodotoxin (TTX) in the external solution. TTX-sensitive components were averaged over the last 100 ms of the depolarizing pulse and normalized to the cell capacitance. Overexpression of FKBP12 increased mean late I_{Na} density ($p < 0.05$)

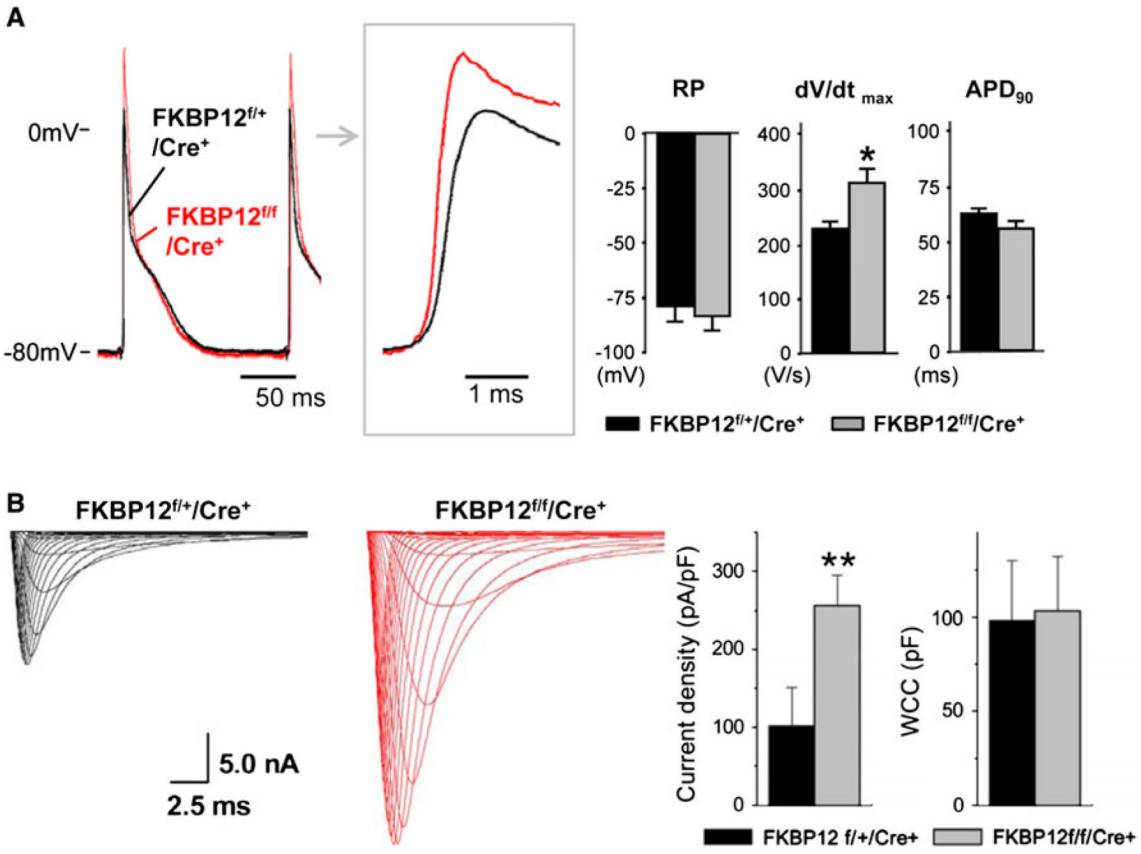

Fig. 2.

Cellular electrophysiologic analyses of FKPB12$^{f/f}$/MHC-Cre^{+} and FKBP12$^{f/+}$/MHC-Cre^{+} hearts. **a** Representative ventricular transmembrane APs recorded from an FKBP12$^{f/f}$/MHC-Cre^{+} and an FKBP12$^{f/+}$/MHC-Cre^{+} heart. The maximum upstroke velocity of phase 0 of the AP $(dV/dt)_{max}$ and the peak action potential amplitude were increased in FKBP12$^{f/f}$/MHC-Cre^{+} hearts compared with control hearts. The means of RP and APD_{90} were similar between FKBP12$^{f/f}$/Cre^{+} and FKBP12$^{f/+}$/Cre^{+} hearts, $*p < 0.05$. **b** I_{Na} traces recorded from isolated FKBP12$^{f/f}$/MHC-Cre^{+} and control FKBP12$^{f/+}$/MHC-Cre^{+} ventricular cardiomyocytes. The maximal peak I_{Na} in FKBP12$^{f/f}$/MHC-Cre^{+} myocytes was increased more than 2.5-fold compared with FKBP12$^{f/+}$/MHC-Cre^{+} cells (15 cells/6 hearts), $**p < 0.01$