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In Vivo Effect of a Dominant Negative Kv4.2 Loss-of-Function Mutation Eliminating $I_{to,f}$ on Atrial Refractoriness and Atrial Fibrillation in Mice

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Background: Gain-of-function K^+ channel mutations cause familial atrial fibrillation (AF) by shortening of the atrial action potential duration (APD). APD-prolonging K^+ channel blockers are an effective therapeutic option in AF. In vitro, the dominant negative Kv4.2W362F mutation (Kv4DN) eliminates $I_{to,f}$ in murine atrial myocytes and markedly prolongs the APD, so whether this loss-of-function of $I_{to,f}$ alters the atrial effective refractory period (AERP) in vivo and/or affects AF-inducibility was investigated in the present study.

Methods and Results: Transvenous electrophysiological studies were performed in vivo in Kv4DN and wild-type littermate control (LMC) mice. Intriguingly, no difference was found between Kv4DN and LMC for the AERP in vivo either at baseline or after carbachol. Consequently, AF-inducibility at baseline (Kv4DN: 10/16 vs LMC: 7/13) and after carbachol (Kv4DN: 9/16 vs LMC: 6/13) did not differ between groups. However, AF-inducibility was associated with a significantly shorter AERP (inducible 51.1±1.4 vs non-inducible 58.4±1.6; $P<0.01$) irrespective of genotype.

Conclusions: The loss-of-function of $I_{to,f}$ prolongs the APD in mouse atrial myocytes in vitro, but this effect on single cells does not translate into measurable AERP prolongation in vivo and hence does not exert an anti-arrhythmic effect. However, the susceptibility of mice to AF in vivo is determined by the individual AERP, irrespective of genotype. (*Circ J* 2009; **73:** 461–467)

Key Words: Atrial fibrillation; Atrial refractoriness; In vivo electrophysiological study; $I_{to,f}$ loss-of-function mutation; Kv4DN transgenic mice

The most common arrhythmia in humans is atrial fibrillation (AF), with increasing prevalence and incidence with aging[1] Electrical remodeling resulting in a shortening of the atrial action potential duration (APD) and of the atrial effective refractory period (AERP) is known to promote AF[2], whereas drug-induced prolongation of APD and the AERP reduces its occurrence[3–5] Recent evidence suggests that mutations in the genes responsible for voltage-gated K^+-channels play an important role in the subset of human patients with familial AF[6–8] These mutations lead to a shortening of the APD and AERP by a gain of function in I_{Ks} or I_{K1} and thereby facilitate AF.

In line with these findings in humans with familial AF, transgenic mice overexpressing Kir2.1, which leads to augmentation of the I_{K1} current, show a shortening of APD and AERP, as well as an increase in the occurrence of spontaneous AF[9] Spontaneous AF was also demonstrated in transgenic mouse models with atrial fibrosis because of either the expression of a constitutively-active form of transforming growth factor (TGF)-β1[10] or an overexpression of tumor necrosis factor (TNF)-α;[11] even in the absence of changes in the APD![10] Similarly, targeted deletion of connexin40 ($Cx40^{-/-}$) slowed atrial and atrioventricular (AV) conduction, and increased vulnerability to AF[12] after transesophageal stimulation.

Interestingly, in the TNF-α heart failure mouse model, the incidence of spontaneous and inducible AF increases with age, predominantly in males;[11] indicating a similar influence of age and gender as seen in human patients.

In experimental studies, the feasibility of inducing AF using in vivo programmed stimulation has been demonstrated in mice; in wild-type (WT) animals, transvenous electrophysiological (EP) studies showed that after administration of the parasympathetic drug carbachol, which is known to facilitate AF and hence is used as standard model for AF induction, the majority (>70%) of animals had AF induced![13] Several mutations protect against pacing-induced AF: in an I_{KACh} knock-out mouse model, AF was inducible after carbachol only in WT animals, but not in knock-out mice![14] In another mouse model, the overexpression of HERG markedly reduced AF inducibility after carbachol administration, despite shortening of the APD, predominantly at slow heart rates, presumably because of a prolonged post-repolarization refractoriness, an increased repolarization reserve and the lack of electrical alternans![15]

Genetic alterations of the atria that either shorten the APD and AERP (eg, by augmenting potassium currents such as I_{Ks} or I_{K1}) or manipulations that slow atrial conduction ($Cx40^{-/-}$ or TGF-β1/TNF-α overexpression) lead to increased vulnerability for AF in mice, and both the elimi-

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Table 1. Electrophysiological Features in Kv4DN and LMC Mice Before and After Carbachol

	SNRT 100 ms			SNRT 200 ms			AV			AERP 100 ms			AERP 200 ms		
	Pre-CC	Post-CC	P value	Pre-CC	Post-CC	P value	Pre-CC	Post-CC	P value	Pre-CC	Post-CC	P value	Pre-CC	Post-CC	P value
Kv4DN	107.5±9.2	116.7±13.2	NS	88.9±10	84.8±13.0	NS	46.97±1.1	47.4±1.4	NS	50.6±1.3	53.2±1.3	NS	50.6±2.1	51.9±2.0	NS
LMC	106.5±20.7	128.7±15.9	NS	146.8±29	122.3±18.2	NS	51.96±2.3	54.6±2.2	NS	54.7±3.4	56.0±2.8	NS	51.7±3.0	54.3±3.9	NS

Electrophysiological features were analyzed in Kv4DN transgenic mice (n=16) and LMCs (n=13) at baseline (PreCC) and after carbachol administration (Post-CC).
LMC, littermate control; SNRT, sinus node recovery time; AV, atrioventricular; AERP, atrial effective refractory period; NS, not significant.

nation of I_{KACh} and the overexpression of HERG seem to be protective against induced AF. Recently, a carbachol-induced heterogeneous shortening of the atrial APD was demonstrated in mice![6] The inducibility of AF in mice by programmed stimulation after carbachol administration, and the fact that atrial APD is shortened after carbachol, is in line with the conceptual model that reduced electrical refractoriness plays an important role in the initiation and perpetuation of AF[2]. Therefore, we tested the hypothesis that genetic manipulation that leads to the prolongation of atrial APD and atrial refractoriness could exert a protective anti-arrhythmic effect against AF.

In mouse myocytes, the transient outward K^+-current, $I_{to,f}$, and the rapidly-activating sustained K^+-current, $I_{K,slow}$, are the major determinants of the peak outward current of the repolarization phase of the atria and ventricles![7] Selective elimination of $I_{to,f}$ in transgenic mice expressing a dominant-negative mutant Kv4.2 α-subunit, Kv4.2W362F, results in a 4-fold prolongation of the atrial APD_{90} in vitro without apparent changes in other ion currents![7] Similar prolongation of the atrial APD has not been demonstrated in other transgenic or knock-out models. In vivo studies focusing on the ventricular EP features in Kv4DN mice have shown a marked prolongation of ventricular APD and a significant QT-prolongation, but despite QT-prolongation this model demonstrates no increase in ventricular arrhythmias![8] Based on those in vitro and in vivo observations, we generated a Kv4DN mouse model to determine the effect of the selective loss of $I_{to,f}$ in atrial myocytes on AERP and AF-inducibility in the intact animal in the absence of any additional electrical and structural remodeling![7]

Methods

This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the German Law on the Protection of Animals.

As previously described, mice were generated using a dominant negative mutation in Kv4.2 (Kv4.2W362F) leading to a loss of function in the K^+-channel responsible for $I_{to,f}$![7–9] Transgenic mice expressing the Kv4.2W362F mutation (Kv4DN) were crossbred with C57/BL6 WT mice and the offspring were screened for the presence of the Kv4.2W362F transgene by PCR![9] The selective expression of the mutant Kv4.2-W362F α-subunits in the atria and ventricles of transgenic Kv4DN mice (positively screened for the Kv4.2W362F transgene) has previously been shown with the use of rtPCR![7] Transgenic Kv4DN and their non-transgenic WT littermate controls (LMC) were subjected to in vivo EP studies.

EP Studies

All EP studies and their offline analysis were performed in a blinded fashion with unblinding only after the analysis of the last animal. Animals were sedated with intraperitoneal ketamine/xylazine (each 50 mg/kg), and a transvenous EP studies was performed at 2 different cycle lengths (CL, 100 and 200 ms) at baseline, after intraperitoneal carbachol administration (CC; 0.05 mg/kg) and after intraperitoneal atropine (40 μg) using an octapolar 2F catheter in spontaneously breathing elderly (age 7–11 months) Kv4DN and LMCs.

Surface ECGs (leads I–III) were obtained from the anesthetized mice by placing subcutaneous 27-gauge electrodes in each limb. After a skin incision of approx 1.4 cm, the

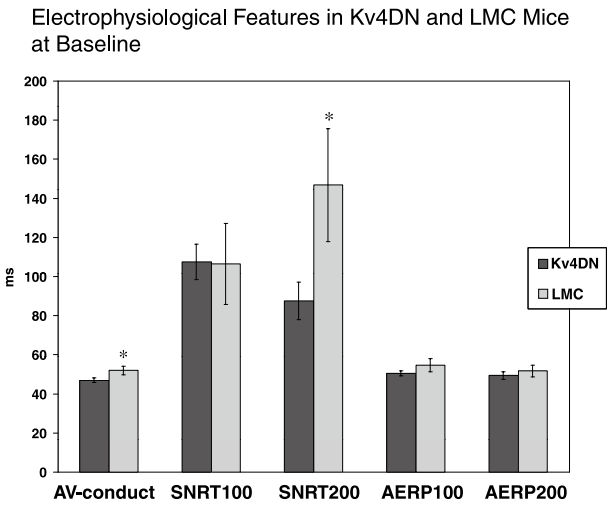

Figure 1. Electrophysiological features in Kv4DN (n=16) and littermate control (LMC; n=13) mice at baseline. Results are presented as mean±SD. *P<0.05 Kv4DN vs LMC. AV, atrioventricular conduction; SNRT100, heart rate-corrected sinus-node recovery time at 100-ms cycle length stimulation; SNRT200, heart rate-corrected sinus-node recovery time at 200-ms cycle length stimulation; AERP100, atrial effective refractory period at 100-ms cycle length; AERP200, atrial effective refractory period at 200-ms cycle length.

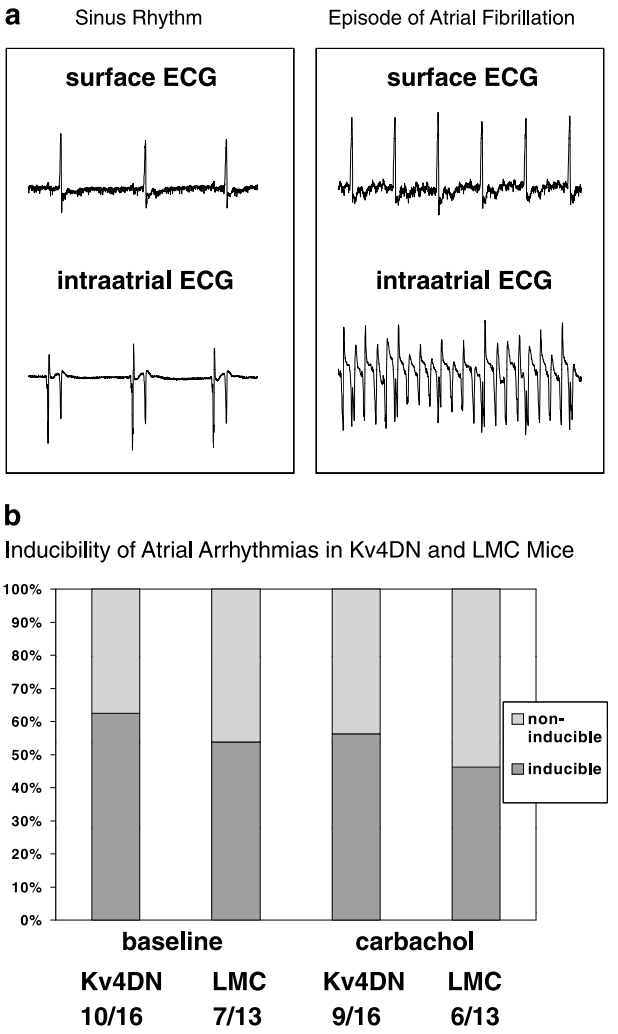

Figure 2. (**a**) Episodes of sinus rhythm and atrial fibrillation (AF) in mouse #114 (Kv4DN) in surface ECG lead I and simultaneous ECG recording of the intra-atrial electrode. (**b**) Inducibility of AF in Kv4DN (n=16) and littermate control (LMC) mice (n=13) at baseline and after carbachol administration. Inducibility rates are presented as percentage of all mice.

right internal jugular vein was exposed and the octapolar mouse 2F electrophysiology catheter (NuMed, NY, USA; 1-mm interelectrode distance) was advanced into the right ventricle, with the proximal electrodes located in the right atrium. Catheter placement was guided by intracardiac signals and pacing thresholds. ECGs (leads I–III and 2 intra-atrial leads) were amplified, filtered (0.1–10,000 Hz), digitized (2 kHz) and stored for blinded offline analysis using a custom-made electrophysiology system based on LabView (Version 6.1, National Instruments) and then analyzed offline by custom-made analysis software (IED, Hamburg, Germany). Only animals that survived at least 24 h after programmed stimulation were included in the study.

We used a modified mouse EP study protocol, which has been described previously in detail.[20] In brief, programmed electrical stimulation was performed at baseline and 3 min after intraperitoneal CC (0.05 mg/kg) at 2 different CL (100 ms and 200 ms). Pacing thresholds were determined, and stimulation was performed for 1.0 ms pulses at twice the threshold amplitude. Standard pacing and programmed electrical stimulation protocols were used to determine sinus node, atrial and AV node (AVN) EP parameters. The sinus node function was evaluated by measuring sinus node recovery time (SNRT) after 20-beat trains and heart-rate corrected SNRT (cSNRT = SNRT – the sinus CL)[20] was calculated. The AERP was analyzed by progressively shortening the S2-interval in 2-ms steps after 20-beat trains. To induce atrial arrhythmias, right atrial burst pacing at rates of 5×80 ms and 5×30 ms and programmed right atrial stimulation with a 200-ms or 100-ms basic CL and up to 3 coupled extrastimuli was performed as described![3]

AF was defined as irregular atrial rhythm on the intracardiac atrial lead lasting more than 1,000 ms and atrial tachycardia (AT) was defined as regular atrial rhythm with changed P-wave morphology (≤10% difference in CL in consecutive beats of the arrhythmia).

Using the surface ECG, the QT-index (QTi) was established as described previously.[21] Using a linear QT-regression graph from pairs of WT animals' QT and RR, the QT expected was calculated: QT expected = 46.49 + 0.1337*RR. The QTi is defined as % of observed vs expected QT.

Statistical Analysis

For normally distributed values, we used Student's t-test (paired and unpaired) to compare the means of 2 groups, and the Mann-Whitney test to compare not normally distributed values. Fisher's exact test was used for categorical variables. Analysis was performed with Prism 4 for Windows (Graphpad; San Diego, CA, USA). All data are presented as mean±SD and a P-value ≤0.05 was considered significant.

Results

In this study, 29 mice (Kv4DN n=16; LMC n=13) were analyzed. Mean age was 339.7±144.5 days (Kv4DN: 323.1±142.4; LMC: 357.6±150.4; NS), 53% were male.

No difference was seen in spontaneous heart rate at base-

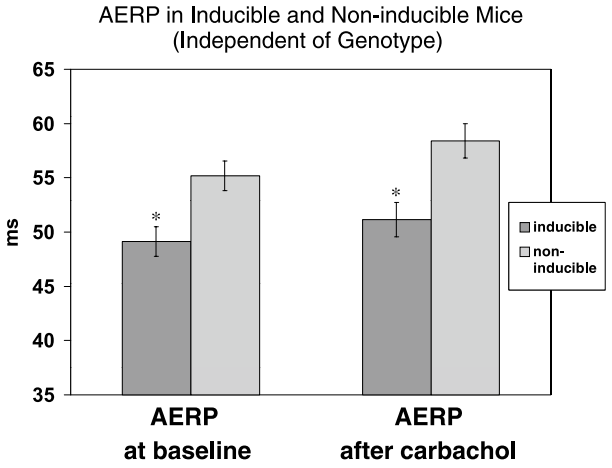

Figure 3. Atrial effective refractory period (AERP) in inducible (n=17) and non-inducible (n=12) mice at baseline and after carbachol-administration. *P<0.05 inducible vs non-inducible mice.

line before CC administration (RR CL: Kv4DN=252.7±14.5 ms vs LMC=289.7±20.5 ms; P=0.148), but a non-significant trend towards shorter CL (faster heart rate) in Kv4DN compared with LMC mice was observed after CC administration (Kv4DN: 307.3±21.7 ms vs LMC 390.8±38.5 ms; P=0.062).

Baseline

Intriguingly, despite the significant APD_{90} prolongation in Kv4DN mice in vitro, the AERP at 100- and 200-ms CL stimulation did not differ significantly between the 2 genotypes (**Figure 1**). In line with this lack of AERP-prolongation in the Kv4DN group, we observed no difference in the inducibility of AT/AF (Kv4DN: 10/16 vs LMC: 7/13) (**Figure 2b**). Importantly, the overall AF/AT inducibility rate in LMC mice was high, which provides an ideal model system for the assumed anti-arrhythmic effect of the Kv4DN mutation.

The duration of AT/AF episodes ranged from 1.4 to 85 s (median 4.2 s), with no difference between the 2 genotypes. The median duration of AT was 4.2 s (from 1.3 to 13 s), and AF episodes lasted 44.2 s (episodes from 3.4 to 85 s). At baseline, episodes of AT (mean CL 156.6±29.6 ms) were inducible in a total of 13 animals; episodes of AF were inducible in 4 animals. All episodes were self-terminating.

Genotype differences were seen at baseline in the heart-rate corrected SNRT at 200-ms CL (**Figure 1**; P=0.03) and in AV conduction time (**Figure 1**; P=0.04), both of which were shorter in Kv4DN than in LMC mice, although the cSNRT at 100-ms CL did not differ (**Figure 1**).

Effect of Carbachol Administration

The administration of CC led to marked hypersalivation in all animals. The mean heart rate decreased (increasing RR interval from 268 to 345 ms, P<0.002), but no difference was observed in refractory period or conduction velocity in either genotype before and after CC administration (ie, no significant change in AERP, cSNRT or AV conduction; **Table 1**).

Similar to baseline conditions, no differences were observed between LMC and transgenic Kv4DN mice for the AERP during CC stimulation (**Table 1**). Consistently, the rate of inducibility of atrial tachyarrhythmias did not differ between genotypes (Kv4DN 9/16 vs LMC 6/13) (**Figure 2b**). Because the AF/AT inducibility rate was already relatively high at baseline, we did not observe an increase in overall AF/AT inducibility after CC administration. However, we observed a trend towards a longer duration of the AF episodes: the duration of AT/AF episodes ranged from 2 to 383 s; in 1 animal the AF episode was not self-terminating and had to be terminated by atrial burst overstimulation. Median duration of AT was 3 s (2–6 s), and the AF episodes lasted from 2 to 383 s. After CC administration, AT episodes (CL: 159.4±35.9 ms) were inducible in 8 animals, and episodes of AF were inducible in 7 animals.

Of note, after atropine administration, no episode of AF was inducible in any animal.

AV conduction remained significantly faster in Kv4DN as compared with LMC mice during CC (**Table 1**, P<0.01).

Influence of Atrial Refractoriness on Inducibility of AF

To further investigate the substrate predisposing mice to AF inducibility, we compared inducible and non-inducible

Table 2. Effect of Gender on AF Inducibility and Electrophysiological Features

	AF inducibility	SNRT 200 ms	AV	AERP 100 ms
Female	10/13	98.40±24.30	49.23±2.82	52.82±3.22
Male	8/16	109.7±12.19	52.81±1.73	54.40±1.73
P value	NS	NS	NS	NS

Electrophysiological features were analyzed in female (n=13) and male (n=16) mice at baseline.
AF, atrial fibrillation. Other abbreviations see in Table 1.

Table 3. ECG Parameters

	P-wave	Intracardiac P-wave	PQ	QRS	QT-index
Kv4DN	14.50±0.65	21.17±0.58	47.82±1.56	13.39±0.35	121.4±4.43
LMC	15.29±0.53	19.47±0.53	52.92±2.33	13.88±0.75	99.93±3.19
P value	NS	NS	0.068	NS	0.001

Surface ECG parameters and intracardiac P-wave duration were analyzed in Kv4DN transgenic mice (n=16) and LMC (n=13) at baseline. Using a linear QT-regression graph from pairs of wild-type animals' QT and RR, the expected QT was calculated: QT expected=46.49+0.1337*RR. The QT-index is defined as % of observed vs expected QT.
Abbreviations see in Table 1.

animals (independent of genotype) and found the AERP to be significantly shorter in inducible than in non-inducible animals irrespective of genotype both before and after CC administration (inducible 51.14±1.35 ms vs non-inducible 58.40±1.58 ms; P=0.002) (**Figure 3**). Importantly, a short AERP were not associated with age or gender in our study.

All other parameters, including SNRT at 100- and 200-ms CL and AV conduction, did not differ between inducible or non-inducible animals.

Gender

Although in humans the prevalence of AF is known to be higher in men than in women, in this experimental setting gender had no significant effect on in vivo AF inducibility in mice (female 10/13 vs male 8/16; P=0.137). No gender-specific difference was seen in any of the EP parameters tested (**Table 2**).

Surface ECG Parameters

We analyzed the surface ECG parameters in both genotypes. No differences were observed in P-wave duration (**Table 3**) or QRS duration. PQ duration did not differ significantly, but we saw a trend towards shorter PQ intervals in the Kv4DN, in line with the EP findings of a faster AV conduction velocity in these animals. As expected from previous observations,[18] the heart-rate corrected QT-index as a marker of ventricular repolarization and refractoriness was significantly prolonged in Kv4.2-W362F transgene positive mice (Kv4DN) as compared with the LMCs (**Table 3**), indicating that the transgenic Kv4DN mice express a biologically functional dominant negative Kv4DN transgene that eliminates $I_{to,f}$.

We further examined genotype differences in intracardiac ECG P-wave duration as a crude marker of intra-atrial conduction velocity. Neither genotype difference nor gender- or age-dependent differences were found.

Discussion

Several mechanisms are known to promote the occurrence and inducibility of AF. AF itself causes a complex cascade of atrial remodeling resulting in shortening of both the APD and AERP,[22] thereby facilitating re-initiation of atrial arrhythmias.[2,23] This has led to the paradigm that "AF begets AF".[24] Parasympathetic stimulation leads to a shortening of APD duration and AERP,[13] thereby promoting re-entry. Recently, several gain-of-function mutations of voltage-gated potassium channels (I_{Ks} and I_{K1}) causing a shortening of AERP have been described as facilitating the arrhythmia in familial AF.[6–8,25] Models of enhanced propensity to AF because of reduced refractoriness provide a therapeutic rationale for prolonging atrial refractoriness. In clinical use, anti-arrhythmic drugs prolonging the APD have proven to be effective in many subjects with AF,[26] with potassium-channel-blocking class III drugs being the most effective (as well as classes IA and C) in conversion to or maintenance of sinus rhythm,[27,28] and even in improving the electrical defibrillation efficacy in patients with persistent AF.[26]

Because APD-shortening has been identified as a pro-arrhythmic factor, we hypothesized that genetic manipulation leading to the prolongation of atrial APD (and thus prolonging the AERP) should have a protective effect against atrial arrhythmias. Loss-of-function mutations in the potassium channels that significantly contribute to atrial repolarization might therefore protect against AF.

Based on the observation that the selective elimination of $I_{to,f}$ in transgenic mice expressing a dominant-negative mutant Kv4.2 α subunit, Kv4.2W362F, results in a 4-fold prolongation of atrial APD_{90} in vitro,[17] we generated a Kv4DN mouse model to determine whether the selective loss of atrial $I_{to,f}$, a major repolarizing current in murine atria, causes prolongation of the AERP in vivo and hence might exert a protective anti-arrhythmic effect against AF-induction in vivo.

Perhaps the most intriguing finding from our study is the lack of prolongation of the AERP in vivo, as opposed to the striking ~4-fold APD prolongation in vitro in isolated atrial myocytes derived from Kv4DN mice (prolongation of APD_{90} 27±8 ms in LMC to 132±34 ms in Kv4DN).[17] Moreover, because a less pronounced (≈2.5-fold) prolongation of the ventricular APD_{90} correlates with prolongation of the ventricular effective refractory period (VERP)[18] and the QT-duration in Kv4DN mice in a previous study,[18] as well as in our Kv4DN mice (**Table 3**), proving that the present Kv4DN mice indeed express a biologically functional Kv4DN transgene eliminating $I_{to,f}$. Electrical remodeling with compensatory upregulation of other ion currents is a known adaptive mechanism, which could theoretically be responsible for the lack of an in vivo effect of elimination of $I_{to,f}$. However, in atrial myocytes derived from Kv4DN mice compensatory upregulation of any other current has not been detected.[17] Previously, compensatory upregulation of $I_{to,s}$ (Kv1.4) was observed in ventricular myocytes of these mice.[19] Despite this compensatory upregulation in ventricular cells, we have found the prolongation of the ventricular APD_{90} to cause a significant prolongation of the VERP in vivo.[18] The APD_{90} was determined in single myocytes derived from Kv4DN mice. In contrast to "whole organ" studies such as our in vivo EP studies, the single cells used for patch clamp studies are extracted from the tissue and hence lack the effect of electrical coupling within the myocardium, which might influence its electrical properties. Furthermore, the relatively rapid inactivation of the $I_{to,f}$ current at the beginning of the repolarization might reduce the current's impact on the AERP (as a reflection of the whole atrial refractoriness) more than on the single-cell APD. Consequently, other ion currents, such as $I_{K,slow}$, might be more important in determining the AERP duration in vivo. This important difference between the in vivo and in vitro findings emphasizes the value of studies in intact hearts and/or intact animals to determine the functional consequences of changes in ion channel expression.

In line with the observation that Kv4DN mice did not show any alteration of the AERP, we did not see any differences in AF inducibility between Kv4DN and LMC. Importantly, however, AERPs were significantly shorter in animals that were inducible for atrial arrhythmias, independent of genotype, gender or age, which is in line with previous observations in other species.[2] However, this also implies that other mouse models with potassium channel loss-of-function mutations leading to significant alterations of the AERP may be needed to test the hypothesis of an anti-arrhythmic effect of a genetic prolongation of the AERP.

Previously, it was shown that AF can be induced in mice in vivo by transvenous stimulation[13,14] during parasympathetic stimulation by the administration of CC. Parasympathetic stimulation is known to facilitate AF by increasing the frequency of re-entry circuits by shortening the wavelength[29] and thereby permitting more re-entry circles to coexist. However, similar to previous studies showing the

general inducibility of atrial tachyarrhythmias with burst pacing, even without vagal stimulation,[30] we were able to induce AF before and after CC administration in a significant proportion of both WT and transgenic Kv4DN mice. In the present data, inducibility was already high at baseline (10/16 Kv4DN, 7/13 LMC), which might account for a lack of a further increase in AF/AT inducibility rates under CC administration. In various studies, the baseline AF inducibility rates have differed remarkably in mice, ranging from 0%[13] up to 50%[14,31] and even 90%.[30] Interestingly, in younger (2–3 months) mice, lower baseline AF inducibility rates were found than in studies focusing on older mice. Similar to our findings (in 7–11 month-old mice) a high inducibility rate was found in 5–7 month-old mice, with a 100% inducibility in the male subgroup.[30] In transgenic mice overexpressing TNF-α, the AF inducibility rate also increased with age![1] Consistent with those observations, in studies of Langendorff-perfused atria of young (2–3 months) and old (22–24 months) male rats,[32] atrial tachyarrhythmias were inducible by in vitro burst stimulation only in the atria of old rats. Therefore, it might be suitable to use older mice with a higher baseline AF inducibility in order to determine a possible protective effect of genetic manipulations presumed to cause a decreased inducibility rate. Because of the fact that a heterogeneously-slowed conduction velocity has been observed in the atria of the older animals,[30] and that atrial electrical remodeling is described as associated with age,[33] parasympathetic stimulation as a trigger for facilitating AF might not be necessarily needed to induce AF in older animals. However, additional parasympathetic stimulation seems to influence the stability of the induced AF episodes, because in the present study the duration of the AF episodes was longer after parasympathetic stimulation compared with baseline.

Interestingly, however, none of the animals of either genotype was inducible for AF after atropine was given to completely block the parasympathetic influence, suggesting that at least some intrinsic parasympathetic activity (which is always present even at baseline) might be mandatory for AF induction.

Of note, we found significantly faster AV conduction in the $I_{to,f}$ loss-of-function transgenic mice as compared with their LMC. Although Na^+ and Ca^{++} currents are the most important ion currents in the AVN, the repolarizing current I_{to} (Kv4) has also been detected in AVN cells of mice.[34] This faster AV conduction in the Kv4DN animals suggests that $I_{to,f}$ might play a role in opposing I_{Na}. Additionally, compensatory electrical remodeling of other ionic currents not affected by the mutation, which is a known adaptive mechanism in transgenic mouse models,[35,36] might also impact on this shortening of AV conduction. However, until now, no analysis of isolated AVN cells has been performed in Kv4DN mice.

Limitation of the Mouse as a Model for Human Arrhythmias

Many differences exist between humans and mice regarding the repolarizing ion currents and the relative contribution of these currents to cardiac repolarization. This leads to significant differences between species in the shape of the AP. Therefore, the relevance of data obtained in a mouse model to understanding human diseases has recently been discussed. In addition, some observations describe opposite effects of certain mutations in humans and mice, such as in the study by Royer et al,[15] which shows a protective effect of HERG-overexpression in mice contrasting with an increased occurrence of AF and ventricular arrhythmias in humans.[25] Nevertheless, significant insights into the mechanisms of arrhythmias have been obtained and certainly will be in the future with the use of genetically altered mouse models.

Because the atrial APD is extremely short in mice, prolongation or shortening of the single-cell APD might not necessarily translate into changes in the AERP. Hence, other animal models with more similarity to humans might be more appropriate for studying these effects. Our data also show that results from studies in single cells in vitro do not necessarily translate into an effect in the intact animal and therefore underline the fact that in vivo studies complement the in vitro experiments. Moreover, because the phenotypic consequences of using a dominant negative strategy, as in the present study, to produce "functional knock-out" of an ion channel could be different from those resulting from targeted gene deletion (ie, "true knock-out"), it might be advisable to use both types of animal models for in vivo studies, if available.

In contrast to human patients, animals require anesthesia in order to perform in vivo EP studies. Several anesthetic drugs have been reported to prolong the QT-interval as a surrogate of cardiac refractoriness in humans or animals; for example, isoflurane,[37] thiopental[38] or propofol.[38] In contrast, ketamine/xylazine does not affect cardiac refractoriness.[39] To avoid anesthesia-induced prolongation of the cardiac refractoriness, which could obscure genotype differences in the AERP, we chose ketamine/xylazine anesthesia to perform the in vivo EP studies. We are aware, however, that ketamine exerts an anticholinergic effect,[40] which might also affect AF inducibility. Ideally, but not applicable, we would perform in vivo EP studies evaluating pro- or anti-arrhythmic effects without anesthesia or would use telemetric ECG monitoring to evaluate potential differences in spontaneous arrhythmias. However, because WT mice do not show spontaneous AF episodes,[11,18] no differences in the spontaneous AF rate would be expected when comparing them with Kv4DN mice. Indeed, we previously reported that no episodes of spontaneous AF were detectable in free-moving, awake Kv4DN mice or LMC![18] A model animal with high rates of spontaneous AF episodes would be necessary to test for the anti-arrhythmic effect of the Kv4DN mutation with the use of telemetric ECG monitoring.

Conclusion

Based on the marked prolongation of the atrial APD in vitro in Kv4DN mice, we aimed to determine the effect of a selective loss of atrial $I_{to,f}$ (in the absence of additional electrical remodeling) on AERP and AF inducibility in vivo. Despite the pronounced APD prolongation in vitro, the Kv4DN transgenic mice in this study failed to show a prolongation of AERP in vivo, which emphasizes the importance of in vivo studies to supplement in vitro studies when determining the functional effect of genetic manipulation of ion channels. Other models with K^+-channel loss-of-function mutations leading to significant alterations of AERP will be needed to evaluate whether a genetic prolongation of AERP will indeed exert an anti-arrhythmic effect.

In summary, in this in vivo model of elderly mice, no differences in AF inducibility before and after parasympathetic stimulation were seen between transgenic Kv4DN and LMC mice, corresponding to the lack of genotype difference in AERP despite a pronounced APD_{90} prolongation

in Kv4DN. Most importantly, however, we found that AF inducibility correlated with a significantly shorter AERP, independent of genotype, gender or age.

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