Spontaneous ventricular tachyarrhythmias in \( \beta_2 \)-adrenoceptor transgenic mice in relation to cardiac interstitial fibrosis

My-Nhan Nguyen,1,2 Helen Kiriazis,1 Diego Ruggiero,1,3 Xiao-Ming Gao,1,2 Yidan Su,1 Anne Jian,1 Li-Ping Han,1,4 Julie R. McMullen,1,2,5 and Xiao-Jun Du1,2

1Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; 2Central Clinical School, Monash University, Melbourne, Victoria, Australia; 3University of Milan, Milan, Italy; 4Wenzhou Medical University, Wenzhou, China; and 5Department of Physiology, Monash University, Melbourne, Australia

Submitted 29 May 2015; accepted in final form 26 June 2015

NEW & NOTEWORTHY

We demonstrated in a mouse model of cardiomyopathy that the severity of myocardial fibrosis correlated significantly with telemetry-quantified frequency of ventricular tachyarrhythmias. Furthermore, severe fibrosis is the prerequisite for the proarrhythmic effect of \( \beta_2 \)-adrenergic activation in this model. Our findings improve current understanding on fibrosis as an arrhythmia substrate.

VENTRICULAR TACHYARRHYTHMIAS (VTAs) incorporate a spectrum of abnormal ventricular rhythm, including ventricular ectopic beat (VEB), ventricular tachycardia (VT), and fibrillation. VTAs are commonly seen in patients with structural heart diseases and represent the most common cause for sudden cardiac death (SCD) in these patients (11, 23, 37, 40). VTA also form an independent risk for heart failure (HF) and mortality in HF patients (1, 43), and, conversely, catheter-based ablation of VTA is associated with functional improvement (34). Despite significant progress in our understanding of the mechanisms and device- or drug-based treatment, the efficacy of current therapies in controlling arrhythmias and related complications is unsatisfactory (37).

Cardiac MRI-based late gadolinium enhancement (LGE) has been increasingly used to detect fibrotic scars or patch fibrosis. Using this technique, clinical studies have established links between the risk of spontaneous or induced VTAs and cardiac histopathologies, including the size of fibrotic scars or patch fibrosis (8, 10, 22, 28, 31). In patients with dilated or hypertrophic cardiomyopathy, LGE-based detection of myocardial fibrosis is associated with increased risk of all-cause mortality and SCD (8, 22, 35). In rats with chronic myocardial infarction, the severity of VTA induced by sympathetic nerve stimulation correlated with the size of scar of the left ventricle (LV) (13). However, there has been no clinical or experimental evidence in vivo to show a quantitative relationship between interstitial fibrosis and VTAs. We previously demonstrated that a strain of transgenic (TG) mice with cardiomyocyte-restricted overexpression of \( \beta_2 \)-adrenoceptors (\( \beta_2 \)-AR mice) and non-TG (NTG) littermates were studied at 4–12 mo of age. VTA was quantified by ECG telemetry. The effect of pharmacological blockade of \( \beta_2 \)-ARs on VTA was examined. Myocardial collagen content was determined by hydroxyproline assay. NTG and TG mice displayed circadian variation in heart rate, which was higher in TG mice than in NTG mice (P <0.05). Frequent spontaneous ventricular ectopic beats (VEBs) and ventricular tachycardia (VT) were prominent in TG mice but not present in NTG mice. The frequency of VEB and VT episodes in TG mice increased with age (P < 0.01). Ventricular collagen content was greater in TG mice than in NTG mice (P <0.001) and correlated with age (r = 0.71, P < 0.01). The number of VEBs or VT episodes correlated with age (r = 0.83 and r = 0.73) and the content of total or cross-linked collagen (r = 0.62–0.66, all P <0.01). While having no effect in younger \( \beta_2 \)-TG mice, \( \beta_2 \)-AR blockade reduced the frequency of VTA in old \( \beta_2 \)-TG mice with more severe fibrosis. In conclusion, \( \beta_2 \)-TG mice exhibit interstitial fibrosis and spontaneous onset of VTA, becoming more severe with aging. The extent of cardiac fibrosis is a major determinant for both the frequency of VTA and proarrhythmic action of \( \beta_2 \)-AR activation.
overdose [ketamine (200 mg/kg), xylazine (40 mg/kg)] and followed by heart removal. The experimental procedures described were approved by the local animal ethics committee and conformed with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition).

**ECG telemetry.** Under isoflurane anesthesia, Data Science (St. Paul, MN) ambulatory telemeters (TA11ETA-F10 and TA10EA-F20) were implanted in mice according to the manufacturer’s instructions and as previously described (36). In brief, an ECG telemetry was implanted into the abdominal cavity with the negative and positive leads subcutaneously positioned at the right foreleg and left side of the chest, respectively, approximating a lead II ECG configuration. After implantation, mice were given a recovery period of 14 days. The ECG was recorded in conscious mice while in their home cage at 1 kHz for 48 h using the Dataquest ART Acquisition System (Data Science).

**Effects of receptor antagonists in vivo.** To determine the influences of autonomic nervous activity on the circadian variation of heart rate (HR) and frequency of arrhythmias, β2-TG and NTG mice were treated with selective antagonists to block β2-ARs (ICI-118,551 at 1 mg/kg, Sigma, St. Louis, MO), β1-ARs (atenolol at 2 mg/kg, Sigma), and muscarinic receptors (atropine at 1.2 mg/kg, Pfizer). All drugs were given subcutaneously as a single bolus. Doses of drugs were selected based on our previous studies (14, 16) showing effective blockade of the respective receptors.

**Recording and analysis of body surface ECG.** Frequent onset of VEB and VT in β2-TG mice by telemetry monitoring implies the possibility that a brief period of ECG recording is able to provide quantitative estimation of the severity of arrhythmias. To test this possibility, we studied separate batches of NTG and β2-TG mice at 3–11 mo of age. Under isoflurane anesthesia (2.0%), chest lead and lead II ECG were simultaneously recorded for 10 min at 2 kHz on Chart 5 Pro (AD Instruments, Bella Vista, NSW, Australia) using the PowerLab 4/30 data-acquisition system and biopotential amplifiers (AD Instruments). Body temperature was monitored using a rectal probe and maintained at 36–37°C during the recording. VEBs were quantified by manual screening and scored based on an arrhythmia score system.

**Echocardiography.** Animals were maintained under light anesthesia by inhalation of 1.8% isoflurane. LV size and function were determined by echocardiography using an iE33 ultrasound machine and a 15-MHz linear transducer (Philips, San Jose, CA). Short-axis two-dimensional image-guided M-mode images crossing the LV at the level of papillary muscles were acquired. LV dimensions at diastole and systole were measured. Fractional shortening was calculated as previously described (19).

**Collagen assay.** At the end of the study, hearts were harvested, immersed in saline at 4°C, dissected into separate parts, and weighed. The LV was frozen in liquid nitrogen and stored in −80°C until use. As previously described (15, 19), total collagen content of the apical portion of the LV (~20 mg) was determined using a hydroxyproline assay. The concentration of soluble and insoluble (cross-linked) collagen was determined by digesting tissue in pepsin with acetic acid for 24 h. This was followed by centrifugation to separate the supernatant (pepsin-soluble collagen) and pellet (insoluble collagen) fractions as previously described (17). Concentrations of soluble and insoluble fractions were then determined by the hydroxyproline assay. Data are expressed as collagen concentration per dry weight.

**Gene expression analysis.** RNA was extracted from LV tissues using TRIzol reagent (Sigma) according to the manufacturer’s instructions. DNase-treated RNA (2 μg) was reverse transcribed into cDNA using a Moloney Murine Leukemia Virus Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Using SYBR green reactions (Roche, Mannheim, Germany) and target primers with the ABI 7500 Fast RT-PCR system, we determined the expression of the following genes related to fibrosis, inflammation, oxidative stress, or electrophysiology: α-smooth muscle actin (α-SMA), transforming growth factor (TGF)-β1, connective tissue growth factor, procollagen types I and III, fibronectin, matrix powerlab 4/30 data-acquisition system and biopotential amplifiers chart 5 pro (AD Instruments, Bella Vista, NSW, Australia) using the
Fig. 2. Telemetry ECG and HR traces showing spontaneous onset of ventricular tachyarrhythmias in β2-TG mice. A and B: ECGs of NTG and β2-TG mice in sinus rhythm. In β2-TG mice, ventricular ectopic beats (VEBs; * in C–E) present as either monomorphic or polymorphic (C) or in the form of bigeminy (D). Episodes of ventricular tachycardia (VT; E) were recorded in β2-TG mice. Time scale = 500 ms.
metalloproteinase-2, IL-6, monocyte chemoattractant protein 1, iso-
forms of NADPH oxidase (NOX2 and NOX4), connexin (Cx)43 and
Cx45, hyperpolarization-activated cyclic nucleotide-gated K+ chan-
nel (HCN2 and HCN4), Na+/Ca2+ exchanger (NCX)1, and sarco(endo-
plasmic reticulum Ca2+-ATPase (SERCA)2a. PCR primers spe-
cific to the respective genes were designed using Primer3, maintained
by the National Center for Biotechnology Information (Bethesda,
MD), and ordered from Sigma. Once received, all primers were
verified against their reference sequence for their specificity before
MD), and ordered from Sigma. Once received, all primers were
verified against their reference sequence for their specificity before
Gene expression was normalized by the expression level of 18S
and GAPDH, and the fold change was calculated according to the
use. Gene expression was normalized by the expression level of 18S
verified against their reference sequence for their specificity before

**Analysis of telemetry ECG and detection of arrhythmias.** Chart 5
Pro (AD Instruments) was used to analyze telemetric ECG recordings.
Basic parameters, including HR, P-wave duration, P-R interval, R-R
interval, and QRS interval, were measured from ECG segments free
of arrhythmias using the ECG analysis module. Episodes of VT were
defined as greater than or equal to four consecutive VEBs, as recom-
meded by the Lambeth Conventions (9). A programmed detection
method was developed to quantify VEBs and episodes of VT from all
48 h of ECG recordings. This method took advantage of increased
amplitudes of R and/or S waves of VEBs by setting positive and
negative voltage thresholds beyond the normal sinus rhythm ampli-
tudes. In doing so, VEBs that exhibit morphologically greater positive
or negative amplitudes were detected. Episodes of VT were detected
using integrals of cumulative slopes derived from consecutive VEBs.
To validate the telemetry results obtained by programmed detection,
1-h ECG recordings were chosen for manual screening, and the results
were compared with those obtained by the programmed detection.
Reproducibility of telemetry data was also examined by comparing
two consecutive 24-h period of recording (i.e., days 1 and 2).

**Statistics.** Analyses of data were performed using either Prism 6
(GraphPad Software, La Jolla, CA) or SPSS Statistics 19 (IBM,
Armonk, NY). Both the absolute number of VEB and VT episodes
failed in passing the Shapiro-Wilk’s test for normal distribution
(\( P < 0.05 \)) and the Levene’s test for equal variance
(\( P < 0.05 \)). Thus, a log(y + 1) transformation was applied, and the transformed data
passed both normality (\( P > 0.05 \)) and equal variance (\( P > 0.05 \)) tests
(9). Transformed data were then compared using one- or two-way
ANOVA. The relationship between the severity of VTA and aging or
extent of cardiac fibrosis was assessed using Pearson correlation
coefficient analysis. Incidence data were compared using a \( \chi^2 \)-test or
Fisher’s exact test. Two-way ANOVA was used to compare arrhyth-
mia results or scores between groups. A Bonferroni post hoc test was
performed if ANOVA indicated a statistically significant difference.
As there were no age-related differences in NTG mice in all param-
ters studied, NTG mice were combined to form a single group for
 clarity, unless otherwise indicated. Parametric results are expressed as
means ± SE. \( P \) values of <0.05 were considered statistically signif-

**RESULTS**

**Circadian changes in HR and physical activity in conscious mice.** Male NTG and \( \beta_3 \)-TG mice of all age groups demon-
strated circadian differences in HR expressed either as hourly
averages over a 48-h period or averaged level of the light
versus dark phase (Fig. 1A). Compared with NTG mice, \( \beta_3 \)-TG
mice at all ages had higher HR levels across 48 h (\( P < 0.05 \); Fig. 1A). HR levels of \( \beta_3 \)-TG mice during the light and dark
phase were similar between different age groups. HR was also
found to be reproducible between the two 24-h periods (data
not shown). NTG and \( \beta_3 \)-TG mice exhibited similar circadian
patterns in physical activity across 48 h, with the value signifi-
cantly greater during the dark phase in all groups studied (\( P <
0.01 \); Fig. 1B).

**Arrhythmic phenotype in \( \beta_3 \)-TG mice.** Typical ECG con-
figurations of NTG and \( \beta_3 \)-TG mice are shown in Fig. 2, A and B.
NTG mice rarely displayed VEBs and never showed VT episodes.
Frequent onset of VEB and VT was noticed in \( \beta_3 \)-TG mice by ECG telemetry (Fig. 2, C–E). VEBs were presented as
polymorphic singles or bigeminy (Fig. 2, C and D). Episodes
of VT were typically brief in duration (Fig. 2E). In addition to

![Fig. 3. Features of ventricular tachyarrhythmias in \( \beta_3 \)-TG mice by telemetry ECG across 48 h.](http://ajpheart.physiology.org/)

**Fig. 3.** Features of ventricular tachyarrhythmias in \( \beta_3 \)-TG mice by telemetry ECG across 48 h. A and B: frequency of VEBs (A) and number of VT episodes (B) in NTG and \( \beta_3 \)-TG mice across three age groups (\( n \) = 15–17 mice/group). Results are either hourly averages (line graphs) or averages of 12-h light or dark phases (bar graphs) and are presented as means ± SE. Results from NTG mice of different ages were combined (\( n = 17 \)). Differences between the light and dark phases were tested by two-way ANOVA for repeated measures with a Bonferroni post hoc analysis. *P < 0.05 vs. the
respective **TG4-6** group; #P < 0.05 vs. the respective
**TG7-8** group. The difference between NTG and **TG**
groups was highly significant (not shown).
VEBs and VTs as major types of arrhythmias, manual validation of telemetry ECG revealed the presence of other types of arrhythmias in some β2-TG mice, including atrial-ventricular (AV) dissociation with well-maintained ventricular rhythm and atrial flutter (data not shown). We validated the accuracy of programmed detection of VEB and VT by manually screening 1-h ECG traces and conducted paired statistical analysis with automatically detected results of the same period. There was no significant difference between the two sets of independently derived arrhythmia data (data not shown). Our data also suggested similarity in frequencies of VEB and VT on day 1 versus day 2 (data not shown).

Circadian differences were evident in the frequency of VEBs and VT episodes across 48 h in β2-TG mice (Fig. 3, A and B). In β2-TG mice, age was associated with increased frequency and severity of spontaneous VEB and VT (both \( P < 0.05 \) by two-way repeated-measures ANOVA; Fig. 3, A and B). For instance, the number of VEBs was ~400 VEBs/h in 4- to 6-mo-old β2-TG (TG4–6) mice but was 10 times greater in 9- to 11-mo-old β2-TG (TG9–11) mice. The number of VT episodes increased from 3 VEBs/h in TG4–6 mice to 54 VEBs/h in TG9–11 mice.

Under isoflurane anesthesia, 10-min ECG recording also detected a high frequency of VTAs in β2-TG mice. The incidence of spontaneous onset of VEBs was detected in >95% of β2-TG mice (\( P < 0.05 \) vs. 6% in NTG mice; Fig. 4A). VT was only observed in β2-TG mice, with its incidence increasing with age (Fig. 4B). An age-dependent increase in the severity of arrhythmias was indicted by the absolute number of VEBs or arrhythmia score (Fig. 4, C and D).

Effects of β1-AR, β2-AR, and muscarinic antagonists on HR and arrhythmias. Next, we studied the effects of selective antagonists with continuous ECG telemetry recording. The timing of drug administration was determined by pilot experiments to ascertain the duration of the drug action and by taking into consideration the HR circadian pattern. Under conscious conditions, the duration of the drug effect on HR was found to be ~7 h for ICI-118,551 and ~2 h for both atenolol and atropine. Assuming that the sharp HR increment around commencement of the dark phase was indicative of sympathetic activation, atenolol was administered 5 min before the dark phase, whereas ICI-118,551 was given 2 h before the commencement of the dark phase. Blockade of β1-AR by atenolol significantly reduced HR in NTG mice (\( P < 0.001 \); Fig. 5A). Conversely, the selective β2-AR antagonist ICI-118,551 had no effect on HR in NTG mice but profoundly lowered HR in β2-TG mice (\( P < 0.001 \); Fig. 6A). Atropine was administered during the middle of the light phase, when HR was at the lowest level. Blockade of muscarinic receptors significantly increased HR, an effect that was similar in NTG and β2-TG mice and lasted for 2 h (\( P < 0.01 \) by repeated-measures ANOVA; Fig. 5B).

Changes in HR confirmed the expected receptor-blocking actions of the drugs. We then examined changes in VTA after drug intervention by comparing the frequency of VEB and VT in the same animals recorded at exactly the same duration of the preceding day. The frequency of VEB and VT was unaffected by atenolol or atropine (Fig. 5C, D). The frequency of VEB in TG4–6 and 7- to 8-mo-old β2-TG (TG7–8) groups was unaffected by treatment with ICI-118,551 but was reduced by ~40% in the TG9–11 group (\( P < 0.05 \); Fig. 6B).

### Relationship of cardiac fibrosis, age, and severity of VTA.

An age-dependent increase in the severity of interstitial fibrosis in β2-TG mice was clearly indicated by LV sections with collagen stained by picrosirus red (Fig. 7A). To illustrate the relationship between the severity of VTA, age, and cardiac fibrosis, hearts were harvested from all NTG and β2-TG mice at the end of the telemetry experiment for the hydroxyproline assay. We determined both total collagen content and the
percentage of insoluble collagen in the LV. Collagen content was similar in NTG mice aged from 4 to 12 mo (Fig. 7B). Compared with NTG mice, both total collagen content and insoluble collagen were significantly higher in β2-TG mice and correlated significantly with age (Fig. 7, A and B). The total number of VEB and VT episodes by 24-h telemetry were positively correlated with age in β2-TG mice (r = 0.83 and r = 0.73, respectively; Fig. 8, A and B). Notably, total collagen content or the percentage of insoluble collagen of the LV was positively correlated with the 24-h counts of VEB (r = 0.63 and r = 0.66, respectively, P < 0.01; Fig. 8, C and D) and VT episodes (r = 0.62 and r = 0.66, respectively, P < 0.01; Fig. 8, E and F).

Age-dependent development of cardiomyopathy. Echocardiography was performed, and organ weights were measured in some of NTG and β2-TG mice. A: changes in conscious HR in NTG and β2-TG mice after administration of atenolol (ATEN; 2 mg/kg sc, n = 5 mice in the NTG group and 16 mice in the TG group; A) or atropine (AT; 1.2 mg/kg sc, n = 5 mice in the NTG group and 14 mice in the TG group; B). C and D: effect of test drugs on the number of VEBs compared with those recorded 24 h before from the same animals. Results were log transformed and are presented as means ± SE. *P < 0.05 vs. pretreatment results of the same animals by two-way repeated-measures ANOVA. Arrows indicate the time of drug administration.

Fig. 5. Effect of antagonists against β2-ARs or muscarinic receptors on conscious HR and frequency of ventricular tachyarrhythmias in β2-TG and NTG mice. A: changes in conscious HR in NTG and β2-TG mice after administration of atenolol (ATEN; 2 mg/kg sc, n = 5 mice in the NTG group and 14 mice in the TG group; A) or atropine (AT; 1.2 mg/kg sc, n = 5 mice in the NTG group and 14 mice in the TG group; B). C and D: effect of test drugs on the number of VEBs compared with those recorded 24 h before from the same animals. Results were log transformed and are presented as means ± SE. *P < 0.05 vs. pretreatment results of the same animals by two-way repeated-measures ANOVA. Arrows indicate the time of drug administration.
selected genes were determined using LVs of NTG and TG mice. These findings indicate the presence of HF in TG mice. TG mice also showed a significant increase in the QRS duration, indicating slow intraventricular conduction (Table 2).

**DISCUSSION**

We made three key findings in the present study: first, we demonstrated a previously unexplored phenotype in the β2-TG strain of mice, i.e., spontaneous and frequent onset of VTA that becomes increasingly severe with aging. Second, the frequency of VEB and VT quantified by telemetry correlated positively with both age and the severity of myocardial fibrosis. Finally, pharmacological blockade of β2-ARs lowered the frequency of VTA in old β2-TG mice with severe fibrosis.

Although the genetic targeting is restricted to cardiomyocytes, one of the early signs of cardiomyopathy in this β2-TG model is interstitial fibrosis, which becomes evident as early as 3–4 mo of age based on our previous study (3) and the present study. This implies that cardiomyocyte β2-ARs act in a paracrine fashion leading to extracellular matrix remodeling with excessive collagen deposition. We previously showed that the molecular mechanism involves enhanced oxidative stress due to the activation of NOX with the subsequent activation of inflammatory, fibrogenic, and apoptotic signaling (46). In β2-TG mice, a comparable functionality of transgene-driven β2-ARs is indicated by the similar increment of HR across all age groups. We previously observed in β2-TG mice an age-dependent progression of cardiomyopathy with premature death occurring from 8 mo of age (15, 19, 46). A similar cardiomyopathy phenotype was observed in the present study on β2-TG mice (C57BL/6 background) that exhibited LV dilatation and a decline in fractional shortening from 9 mo of age. This is in keeping with the 44% downregulation of SERCA2a in the hearts of β2-TG mice at 9–11 mo of age.

The β2-TG model is characterized by spontaneous and frequent onset of VTA, VEBs were observed in all β2-TG mice with polymorphic ECG configurations, suggesting multiple ectopic origins. ECG telemetry also revealed frequent episodes of VT, in keeping with operation of the reentry mechanism in the setting of interstitial fibrosis (32). The frequency of VTA is so high that even a brief ECG recording in mice under isoflurane anesthesia is able to detect VTA in a quantitative...
manner. ECG telemetry did not capture lethal VTA, such as sustained VT or ventricular fibrillation. However, in keeping with previous studies (15, 19), in the present study, four β₂-TG mice died, most likely due to lethal arrhythmias or worsening HF due to frequent VTA. In addition to VEB and VT as the major arrhythmia forms, we also observed in β₂-TG mice proximal atrial flutter and complete AV dissociation. For the latter, the rates of ventricular rhythm and sinus rhythm were very similar, likely attributable to an enhanced automaticity of the AV node driven by an enhanced β₂-adrenergic signaling.

Interstitial fibrosis is generally regarded as a pivotal arrhythmic substrate (32). Thus, the severity of fibrosis would be expected to correlate with the frequency of VTA. However, few clinical or in vivo experimental studies exist that demonstrate such a quantitative relationship. We have provided, for the first time, experimental evidence for a significant and positive correlation between the severity of interstitial fibrosis and frequency of spontaneous VTA. Our finding strongly supports recent clinical reports showing that the presence of fibrotic tissues, measured by LGE, predicts the risk of SCD in patients with cardiomyopathy of diverse etiologies (8, 27, 31, 35). In addition to the severity of interstitial fibrosis, we observed an age-dependent development of LV dilatation and hypertrophy in β₂-TG mice at 9–11 mo. We previously observed in the β₂-TG model histopathology, such as cardiomyocyte apoptosis and hypertrophy (15, 19, 46), but the interstitial fibrosis, estimated by the expression of fibrotic genes, chemical assays, and quantitative histology, is an early sign of cardiomyopathy (3, 46). In the β₂-TG model, both the severity of VTA and interstitial fibrosis are clearly age dependent. Our data imply that the correlation between age and the frequency of VTA is largely attributable to an age-dependent worsening of cardiac fibrosis. Considering the adverse impact by fibrosis on cardiac remodeling and dysfunction and the pivotal role of myofibroblasts in inflammatory, hypertrophic, and apoptotic signaling (18, 44, 45), it is likely that fibrosis is a key player in the age-dependent progression of LV remodeling and dysfunction. The significance of global as well as histological factors...
Fig. 9. Features of age-dependent progression of the cardiomyopathy phenotype in β2-TG mice. A: serial echocardiographic measures of LV dimension at diastole (LVDd) and fractional shortening (FS) in NTG (n = 6 mice/group) and β2-TG mice (n = 8–14 mice/group). *P < 0.05 vs. age-matched NTG mice. B: wet weights of the LV, right ventricle (RV), atria, and lungs of NTG and β2-TG mice (n = 7–11 mice/group) at the end of telemetry experiments. Organ weights were normalized to tibial length (TL). *P < 0.05 vs. NTG mice and †P < 0.05 vs. TG4-6 by two-way ANOVA.

contribution to VTA in this model warrant further investigation. It is also critical to address the question of whether the severity of arrhythmias can be ameliorated by antifibrotic interventions.

The presence of patchy or interstitial fibrosis interferes with electrical coupling of adjacent cardiomyocytes, leading to slow conduction, formation of reentry, and afterdepolarization (32, 37, 45). A recent in vitro study (38) has also revealed direct electrical coupling via the formation of gap junctions between myofibroblasts and cardiomyocytes as an additional proarrhythmic mechanism. In this context, it is important to note the two- to threefold upregulation in the β2-TG heart of both the myofibroblast marker α-SMA and Cx43 as a pivotal gap junction protein (2, 33, 38). Altered intracellular Ca2+ handling, due either to attenuated uptake via SERCA2a or elevated extrusion by NCX1, is known to be arrhythmogenic (21). Graf et al. (20) showed in 3- to 5-mo-old β2-TG mice a significant increase in the amplitude of the HCN channel (i.e., I_{h} channel) together with upregulated expression of HCN4 but not HCN2 (20). Here, we observed HCN4 upregulation but HCN2 downregulation only in TG9–11 mice, when they exhibited signs of cardiac decompensation. HCN4 is known as the main molecule for the HCN channel, and numerous studies have documented the association of HCN4 upregulation and arrhythmias in heart diseases (39). Interestingly, upregulated expression of NCX1 and fibrotic and inflammatory genes was similar across the age groups studied, whereas changes in the expression of SERCA2a, HCN2, and HCN4 were evident only in TG9–11 mice. This implies that HF-associated remodeling of ion channels may contribute additionally to the onset of VTA.

Numerous clinical and experimental studies have demonstrated the role of the autonomic nervous system in the regulation of cardiac electrophysiology and risk of VTA (6, 41). VTAs can be induced by stimulation of sympathetic nerves or administration of β-agonists (12, 13, 26, 41). Conversely, activation of parasympathetic tone generally improves electrophysiological stability (41). After the demonstration of circadian variation of spontaneous VTA, likely driven by circadian changes in autonomic nervous activity in β2-TG mice, we tested if blockade of cardiac receptors of autonomic neurotransmitters, particularly β2-ARs, might reduce the severity of VTA. HR was similarly increased in NTG and β2-TG mice in response to atropine administered during the light phase, suggesting similar parasympathetic tone. The lack of influence by atropine on VTA indicated limited impact of the parasympathetic nervous activity in the β2-TG model. Our previous study (5) showed that ICI-118,551 was effective in blocking β2-ARs in the 2-TG model. We found that pharmacological blockade of β1-ARs or muscarinic receptors failed to alter the frequency of VTA in the β2-TG model. Our previous study (5) showed that ICI-118,551 was effective in blocking β2-ARs in the 2-TG model. This is consistent with the present study on conscious mice, β2-TG mice showed a clear surge of HR immediately before the commencement of the dark cycle. Unlike NTG mice in which the β1-AR blocker atenolol induced bradycardia, ICI-118,551 induced a profound HR reduction in

Table 1. Basic ECG parameters of NTG and β2-TG mice

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>NTG mice</th>
<th>β2-TG mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTG</td>
<td>β2-TG</td>
<td>NTG</td>
</tr>
<tr>
<td>Number of mice/group</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>P duration, ms</td>
<td>8.63 ± 1.11</td>
<td>9.12 ± 0.53</td>
</tr>
<tr>
<td>P-R interval, ms</td>
<td>36.0 ± 0.7</td>
<td>35.7 ± 0.9</td>
</tr>
<tr>
<td>R-R interval, ms</td>
<td>112 ± 3.3</td>
<td>97.3 ± 1.4*</td>
</tr>
<tr>
<td>QRS interval, ms</td>
<td>9.9 ± 0.3</td>
<td>10.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. NTG mice, nontransgenic mice; β2-TG mice, transgenic mice with cardiac overexpression of β2-adrenoceptors. *P < 0.05 vs. age-matched NTG mice.
β2-TG mice, suggesting that the transgene-directed β2-AR becomes predominant over the β1-AR in the heart and that they are able to be activated by endogenous ligands. Here, we have shown that treatment with ICI-118,551 significantly reduced the frequency of VTA in conscious TG9–11 mice, whereas no such effect was observed in TG4–6 and TG7–8 mice. TG9–11 mice exhibited more severe cardiomyopathy, compared with younger β2-TG animals. Thus, while supporting the findings from rabbit or human failing hearts (12, 26), our observation from the β2-TG model with the use of ICI-118,551 further indicates that the proarrhythmic nature of β2-AR activation is dependent on the severity of myocardial histopathology, particularly interstitial fibrosis.

There have been very limited models that exhibit spontaneous VTA, myocardial fibrosis, and ventricular remodeling that better simulate clinical entities. Animal models of nonscience VTA are commonly induced by either chemicals or electrical stimulation in vivo and in vitro (29). These models usually lack cardiac histopathologies that constitute arrhythmic substrates. VTA or SCD have been reported in some strains of mice with mutations of proteins that regulate sympathetic neurotransmission (25), ionic channels (channelopathies), and intracellular Ca2+ transient or electromechanical coupling (4, 42). Again, very few models exhibit significant cardiac pathology. Our study in the β2-TG mouse model documented a positive correlation between the severity of interstitial fibrosis and VTA. As far as we are aware, there have been no similar clinical or experimental reports on such a correlation, albeit the correlation exists between the risk of arrhythmias and SCD and presence of myocardial fibrotic scars due to myocardial infarction (7, 13) or cardiomyopathy (8, 22, 28, 31, 35). Further clinical studies are warranted to quantitatively examine such association between interstitial fibrosis by cardiac MRI (24) and the severity of VTA in patients with defined heart diseases.

In conclusion, our study demonstrates that β2-TG mice develop spontaneous and frequent onset of VTA that are correlated significantly with the extent of interstitial fibrosis, implying a potential mechanistic link. Activation of β2-adrenergic signaling is arrhythmogenic in the setting of severe ventricular remodeling and fibrosis. Future research is warranted to illustrate factors that influence the severity of VTA and to perform therapeutic testing, particularly antifibrotic interventions, of potential antiarrhythmic efficacy.

ACKNOWLEDGMENTS

The authors thank Aya Matsumoto and Geeta Sapra (Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia) for technical support with the ECG telemetry experiments.

GRANTS

This work was supported by the National Health and Medical Research Council (NHMRC) of Australia Grants ID1081710, ID1032687, and...
REFERENCES


