Electrical remodeling in a transgenic mouse model of α1B-adrenergic receptor overexpression

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Rivard K, Trépanier-Boulay V, Rindt H, Fiset C. Electrical remodeling in a transgenic mouse model of α1B-adrenergic receptor overexpression. Am J Physiol Heart Circ Physiol 296: H704–H718, 2009. First published December 26, 2008; doi:10.1152/ajpheart.00337.2008. — Cardiac-specific overexpression of wild-type α1B-adrenergic receptors (α1B-AR) in mice predisposes to dilated cardiomyopathy and sudden death. Although α-adrenergic stimulation is thought to contribute to induction of arrhythmias in heart failure, the electrophysiological consequences of chronic α1-adrenergic activation have not been clearly defined. Thus we characterized ventricular repolarization and monitored incidence of spontaneous arrhythmias in end-stage heart failure α1B-AR mice (9–12 mo) and younger α1B-AR mice (2–3 mo) that do not present signs of heart failure. Compared with aged-matched controls, the corrected QT interval was 34% longer in the 9- to 12-mo α1B-AR mice, and the action potential durations were also significantly prolonged in these mice. These changes were associated with a decrease in the density of the outward K+ currents, Ca2+-independent transient, ultrarapid delayed rectifier, and steady state (at +30 mV), reduction of 68, 64, and 41%, respectively, and underlying K+ channel expression. Electrocardiogram (ECG) recordings revealed that older α1B-AR mice exhibited spontaneous ventricular arrhythmias. The alterations in repolarization can contribute to these rhythm abnormalities and are likely caused by chronic α1B-AR activity. Additional data obtained in 2- to 3-mo α1B-AR mice clearly showed that electrical remodeling was already observed in younger transgenic animals. However, it appeared to be slightly less pronounced than in older mice. These results suggest that there are two waves of remodeling: one due to chronic α1B-AR activity, and a second due to heart failure. Taken together, these data provide strong evidence for a pathological role of chronic α1B-AR activity in the development of repolarization defects and ventricular arrhythmias.

Consistent with an important role in cardiac pathology, Lemire et al. (28) showed that cardiac-specific overexpression of α1B-ARs in transgenic mice leads to heart failure, and that these mice develop a slowly progressing cardiac chamber dilatation followed by premature death with clinical signs of heart failure at around 9–12 mo of age. This phenotype mimics that of idiopathic dilated cardiomyopathy in humans, including differential regulation of hypertrophy/heart failure genes, functional downregulation of the β-adrenergic receptor signaling cascade, progressive loss of left ventricular function, and increased prevalence of arrhythmias (28).

It is well recognized that elevated levels of norepinephrine and increased α1-adrenergic receptor stimulation are implicated in the pathogenesis of heart failure. Increased α1-adrenergic receptor stimulation is also thought to contribute to the higher prevalence of arrhythmias (39). One of the consistent features of heart disease, in both human and experimental model systems, is prolongation of action potential duration (APD), which increases the propensity to develop cardiac arrhythmias (4, 24, 46). Thus it is possible that increased α1-adrenergic stimulation would lead to delayed repolarization, which could increase electrical instability and contribute to arrhythmias in heart failure patients.

A number of studies have shown that acute stimulation with α1-agonists can result in a decreased density of several K+ currents and APD prolongation (2, 12, 13, 15, 19, 20, 33, 36, 52), and α1-adrenergic stimulation can contribute to arrhythmias in heart failure. However, the electrophysiological consequences of chronic α1-adrenergic activity specifically in cardiomyocytes have not been defined. Accordingly, in the present study, we examined the influence of chronic α1B-adrenergic stimulation on ventricular repolarization and on the incidence of spontaneous arrhythmias using a transgenic mouse overexpressing the wild-type α1B-AR (α1B-AR mice) specifically in cardiomyocytes. The cardiac phenotype of this transgenic mouse model has been described in detail elsewhere (17, 28). Results presented here demonstrate that chronic activity of α1B-ARs results in profound alteration in ventricular repolarization, which could contribute to the development of cardiac arrhythmias and sudden cardiac death.

MATERIALS AND METHODS

Animals

The generation of the mouse line with transgenic overexpression of the wild-type form of α1B-AR has been described previously (1). Wild-type α1B-AR was expressed under the control of the murine α-myosin heavy chain promoter, which is highly specific and directs

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transgene expression to the myocardium. The use of this promoter rules out the involvement of peripheral effects in the cardiac phenotype studied. The level of α1B-AR overexpression (43-fold) has been determined previously (29). SJL heterozygous transgenic male mice (α1B-AR) at two different ages, 9–12 mo (presenting dilated cardio-myopathy and heart failure) and 2–3 mo (without cardiac remodeling), and nontransgenic age- and sex-matched littermates (non-Tg) were used throughout this study. All experiments were conducted in accordance to the guidelines published by the Canadian Council of Animal Care and conform to the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Experiments also were approved by the Montreal Heart Institute Animal Care Committee.

**Ventricular Myocytes Isolation**

Non-Tg and α1B-AR mice were heparinized (1 U/kg ip) 20 min before death, anesthetized by inhalation of isoflurane, and then killed by cervical dislocation. The ventricular myocyte isolation protocol has been previously described (47). Briefly, the hearts were rapidly removed and retrogradely perfused (2 ml/min) through the aorta on a modified Langendorff apparatus with the following solutions: 1) 5 min with HEPES-buffered Tyrode solution containing (in mM) 130 NaCl, 5.4 KCl, 1 CaCl2, 1 MgCl2, 0.3 Na2HPO4, 10 HEPES, and 5.5 glucose (pH adjusted to 7.4 with NaOH); 2) 10 min with Tyrode solution without Ca2+ ("Ca2+-free"); 3) 20 min with Ca2+-free Tyrode solution containing 73.7 U/ml collagenase type 2 (Worthington, Freehold, NJ), 0.1% bovine serum albumin (BSA; Fraction V, Sigma Chemicals, St. Louis, MO), 20 mM taurine, and 30 μM CaCl2; and 4) 5 min 30 s with a Kraftbrühe (KB) solution (23) containing (in mM) 100 K+ -glutamate, 10 K+-aspartate, 25 KCl, 10 KH2PO4, 2 MgSO4, 20 taurine, 5 creatine base, 0.5 EGTA, 5 HEPES, 0.1% BSA, and 20 glucose (pH adjusted to 7.2 with KOH). The temperature of the perfusion was maintained at 37 ± 1°C. At the end of the perfusion, the right ventricular free wall was dissected from the heart and placed in KB solution. The tissue was then minced and triturated for 10 min to free individual ventricular myocytes. Myocytes were stored in KB solution at 4°C until used.

**Electrophysiology**

**Cellular electrophysiology.** The whole cell voltage and current recording methods, data acquisition, voltage-clamp protocols, and analysis methods have been described previously (5). Whole cell voltage and current-clamp recordings were performed on the myocytes using the ruptured patch-clamp technique with a patch-clamp amplifier, Axopatch 200 B (Axon Instruments, Foster City, CA). The data were recorded and analyzed with pClamp 8.0 software (Axon Instruments). Series resistance was maintained between 4 and 8 MΩ, and compensation was applied to reduce series resistance by 80–90%. Voltage-clamp currents were low-pass filtered at 1 kHz with a four-pole Bessel analog filter and digitized at 4–10 kHz. To account for differences in cell size, current amplitudes were normalized to the cell capacitance and expressed as densities (pA/pF). Capacitative transients elicited by a 10-mV depolarizing step from a holding potential of −80 mV were recorded at 25 kHz (filtered at 10 kHz). Cell capacitance was measured by integrating the surface area of these capacitative transient. All patch-clamp experiments were carried out at room temperature (RT; 20–22°C).

To record action potential and K+ currents, pipettes were filled with the following solution (mM): 110 K+-aspartate, 20 KCl, 8 NaCl, 1 MgCl2, 1 CaCl2, 10 VA TP, 4 K2ATP, and 10 HEPES (pH 7.2 with KOH), which gave resistances in the range of 1.5–4 MΩ to the pipettes. Cells were continuously perfused with oxygenated Tyrode solution. To compensate for the patch pipette-bath liquid junction potential (K+-aspartate), recorded membrane potentials were corrected by −10 mV.

**Action potential recordings.** Action potentials were recorded at frequency rates of 0.1–4 Hz with the whole cell current-clamp protocol by injection of brief (1–3 ms) stimulus currents (0.4–0.7 nA). The APDs of non-Tg and α1B-AR mouse ventricular myocytes were measured at 20, 50, and 90% of repolarization (APD20, APD50, APD90, respectively). K+ current recordings. Current-voltage (I-V) relationship for the total K+ current (Ipeak) was constructed from the current elicited by each series of 500-ms test potentials varying from −110 to +50 mV in 10-mV increments, from a holding potential of −80 mV, at a frequency rate of 0.1 Hz. The current density of the inward rectifier K+ current (IK1) was determined at the end of the 500-ms voltage steps, ranging from −110 to −40 mV. We then examined the contribution of individual outward K+ currents. First, we eliminated the transient portion [the Ca2+-independent transient outward K+ current (Ito)] by applying an inactivating prepulse (100 ms, −40 mV) preceding the main activation steps. The current remaining after inactivation of Ito is denoted Ikslow and is composed of Icur [the ultrarapid delayed rectifier K+ current or the 4-aminopyridine (4-AP)-sensitive component] and Ias (the steady-state outward K+ current or the 4-AP-resistant component). We then compared the density of Ito obtained by subtracting the current traces measured with and without the inactivating prepulse. We applied 100 μM 4-AP (which blocks Icur) (5) in combination with an inactivating prepulse (which blocks Ito) to record the 4-AP-resistant K+ current, or Ias. We then measured Icur by subtracting currents recorded in the absence or presence of 4-AP (Ikslow − Ias). The current densities of each of the three components of the outward K+ current were determined at the peak current. The description and validation of these pharmacological and biophysical methods have been previously published (5).

**Surface ECG.** Mice were anesthetized with isoflurane. Mouse body temperature was maintained at 37°C using a heating pad. Platinum electrodes were placed subcutaneously, and surface ECG were acquired in lead I configuration, as described previously (6, 7). Acquisition was performed using the Biopac System MP100 at a rate of 2 kHz. The signal was amplified and filtered at 100 Hz (low pass) and 60 kHz (notch filter). Data were analyzed using ECG auto 1.7 (AcqKnowledge 3.7). The QT intervals were calculated manually by a blinded observer from signal-averaged ECG recordings. The rate-corrected QT (QTc) interval was calculated using a modified Bazett’s correction formula, QTc = QT/(RR/100)1/2 (32). Spontaneous ventricular arrhythmias were recorded using surface ECG recordings.

**Telemetry.** In addition, telemetry was also used to record and monitor spontaneous ventricular arrhythmias in conscious, free-moving, unanesthetized mice. Data were acquired with an implantable OpenHeart radiofrequency transmitter (Data Sciences International, Arden Hills, MN). The leads for the transmitters were threaded subcutaneously to the appropriate position and sutured in place to prevent movement. The positive transmitter lead was located on the left anterior chest wall above the apex of the heart, and the negative lead was located on the right shoulder. ECG lead placement represents the conventional lead II position. Recordings were analyzed with ECG Auto (version 1.7, EMKA technologies, Paris, France).

**Western Blot Analysis**

Proteins used for isolation of sarcolemmal-enriched protein and Western blot analysis were identical to those previously reported (7, 30, 47). Proteins were prepared from ventricles of non-Tg and α1B-AR mice (1 heart/sample) homogenized in Tris-EDTA buffer (pH 7.4) containing protease inhibitors (leupeptin, aprotinin, benzamidine, PMSF, and NaN3VO4). The homogenate was centrifuged at 10,000 g for 30 min. The pellet was resuspended in TE buffer containing the protease inhibitors and 0.6 M KCl to dissolve contractile proteins. Proteins (100 μg) were separated on a 10% SDS-PAGE, transferred on nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Bio tech), and blocked in 0.1% Tris-buffered saline-Tween 20 (TBST)
supplemented with 5% nonfat dried milk for 2 h at RT. Membranes were then incubated overnight at 4°C with primary antibodies directed against voltage-gated K⁺ (Kᵥ) 1.5 (Upstate Biotechnology, Lake Placid, NY), Kᵥ1.4, Kᵥ2.1, Kᵥ4.2, Kᵥ4.3, inwardly rectifying K⁺ (Kir) 2.1, TASK-1 (Alomone Labs, Israel), and Kᵥ channel interacting protein type 2 (KChIP2) antibody (Santa Cruz Biotech, CA). After washing three times for 10 min with TBST 0.1%/5% nonfat dried milk, membranes were incubated with secondary antibody (anti-rabbit-horseradish peroxidase or anti-goat-horseradish peroxidase conjugated IgG) for 3 h at RT. Membranes were then washed three times for 10 min with 0.1% TBST before being treated with chemiluminescent substrate and exposed to autoradiography film. Ponceau S was used to confirm uniformity of the protein loading and transfer. Bands were quantified by densitometry using the Quantity One (Bio-Rad).

**Real-time RT-PCR**

Real-time RT-PCR (qPCR) was performed using the technique published previously (16). Ventricular total RNA was isolated using RNeasy Fibrous Tissue kit (Qiagen) and treated with DNase I to prevent genomic DNA contamination. cDNA fragments were synthesized by RT-PCR using the cloned avian myeloblastosis virus reverse transcriptase (Invitrogen). Primers specific to K⁺ channel/accessory protein (Kᵥ1.5, Kᵥ2.1, Kᵥ4.2, and KChIP2) were designed and tested to ensure an amplification of a unique cDNA product. Primer sequences were Kᵥ1.5 forward: GGTGTTCCGAATCTTTCAAGC, reverse: GTCTGCTCTGCGAAGTAG; Kᵥ2.1 forward: CACGTCGTGTGCGAAAGAG; reverse: GCAATGGTGGAGAGGACAATG; Kᵥ4.2 forward: GTGATGCAGACAAATGAGGATG; reverse: GTGACCTTGGTGTGCAAG; and KChIP2 forward: GTCCTCTTTCATGTCTGCCGTCGT, reverse: GTGGCAGACCGTGGAAGTTC. Sequence analysis was performed on all PCR-generated cDNA fragments to ascertain the specificity of the products. The real-time PCR reaction was carried out with Platinum SYBR Green qPCR Supermix (Invitrogen) using a real-time PCR system (MX3005P qPCR system, Stratagene). The PCR reactions were cycled 40 times using a three-step cycle procedure (denaturation at 95°C for 30 s, annealing at 50°C for 45 s, elongation at 72°C for 45 s) after an initial stage at 95°C for 10 min. mRNA expression was quantified relative to murine cyclophilin. To ensure the validity of the results, the linearity and the efficiency criteria were thoroughly respected.
**Statistical Analysis**

Results are expressed as means ± SE. An unpaired Student t-test was used to compare mean data. The results were considered statistically significant when P values were <0.05.

**RESULTS**

**Prolongation of QTc Interval in 9- to 12-mo α1B-AR Mice**

Surface ECG parameters obtained on anesthetized mice were compared between non-Tg and α1B-AR mice. Figure 1A presents typical examples of lead I surface ECG recorded in non-Tg (left) and α1B-AR (right) mice. These examples show that the QT interval was much longer in α1B-AR mice compared with non-Tg mice. Figure 1B presents mean data for heart rate (HR), QT, and QTc intervals. These data indicate that, although the HR was significantly slower in the α1B-AR group, both the QT and QTc intervals from α1B-AR mice were markedly prolonged compared with those from non-Tg animals. The prolonged QTc intervals clearly indicate that the slower HR of the transgenic animals is not sufficient to explain the longer QT intervals.

**Longer APD in 9- to 12-mo α1B-AR Mice**

Thus, to establish whether the increase in the QTc interval reflected a lengthening of APD, current-clamp recordings

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*Fig. 3. Comparison of the individual K⁺ currents in ventricular myocytes isolated from 9- to 12-mo non-Tg and α1B-AR mice. A: Ca²⁺-independent transient outward K⁺ current (Ito) in non-Tg and α1B-AR myocytes. Left: superimposed current records corresponding to Ito. Right: corresponding mean I-V relationships for Ito (non-Tg n = 14; Tg n = 19). B: ultrarapid delayed rectifier K⁺ current (IKur) in non-Tg and α1B-AR myocytes. Left: family of membrane currents corresponding to IKur in non-Tg and α1B-AR myocytes. Right: corresponding mean I-V curves for IKur (non-Tg n = 9; Tg n = 14). C: steady-state outward K⁺ current (IKs) and inward rectifier K⁺ current (IK1) in non-Tg and α1B-AR myocytes. Left: representative examples of IS and IK1 in non-Tg and α1B-AR myocytes. Right: corresponding mean I-V curves for IS and IK1. Current density of IK1, which was activated by voltage steps ranging from -110 to -40 mV, was similar in non-Tg and Tg animals. However, IS was significantly smaller in α1B-AR mouse myocytes (non-Tg n = 9; Tg n = 14). *P < 0.05. (For the description of the current separation, see MATERIAL AND METHODS.)

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were obtained on enzymatically isolated ventricular myocytes from non-Tg and \(\alpha_{1B}\)-AR mice. There was a marked increase in the APD in \(\alpha_{1B}\)-AR myocytes compared with their littermate controls, as illustrated by the data presented in Fig. 1, C and D. Typical examples of action potentials recorded at 4 Hz in non-Tg and \(\alpha_{1B}\)-AR ventricular myocytes are shown in Fig. 1C. Figure 1D summarizes the mean APD data obtained in both groups. These bar graphs compare the APD recorded in non-Tg and \(\alpha_{1B}\)-AR myocytes measured at APD\(_{20}\), APD\(_{50}\), and APD\(_{90}\). APD\(_{20}\) (non-Tg: 2.9 ± 0.1 ms vs. \(\alpha_{1B}\)-AR: 6.1 ± 0.7 ms), APD\(_{50}\) (non-Tg: 4.7 ± 0.2 ms vs \(\alpha_{1B}\)-AR: 20.1 ± 4.0 ms), and APD\(_{90}\) (non-Tg: 18.9 ± 1.0 ms vs. \(\alpha_{1B}\)-AR: 52.4 ± 5.8 ms) were all significantly longer in the \(\alpha_{1B}\)-AR group compared with the non-Tg mice (\(P < 0.05\)). To determine whether the prolongation of the APD was rate dependent, we also recorded APDs at different frequency rates (0.1, 1, 2, 3, and 4 Hz). At lower frequencies, APDs were also significantly longer in \(\alpha_{1B}\)-AR compared with non-Tg mice.

### Alterations in \(K^+\) Currents in 9- to 12-mo \(\alpha_{1B}\)-AR Mouse Ventricular Myocytes

Since APD is mainly determined by repolarizing \(K^+\) currents, these conductances were recorded from freshly isolated ventricular myocytes using the whole cell patch-clamp technique in the voltage-clamp configuration. Figure 2A illustrates typical examples of a family of \(I_{\text{peak}}\) recorded from non-Tg and \(\alpha_{1B}\)-AR ventricular myocytes. Figure 2B is a plot of the mean \(I\)-\(V\) relationships for the peak inward or outward current obtained from 14 non-Tg and 20 \(\alpha_{1B}\)-AR cells. These superimposed current records demonstrate that the total outward \(K^+\) current in myocytes from \(\alpha_{1B}\)-AR was significantly decreased (Fig. 2B). For instance, the mean current densities of the total outward current (\(I_{\text{peak}}\)) measured at +30 mV were 71.1 ± 4.7 pA/pF (n = 14) in non-Tg and 26.4 ± 2.0 pA/pF (n = 20) in \(\alpha_{1B}\)-AR myocytes (\(P = 3.7 \times 10^{-6}\)). Since \(I_{\text{peak}}\) is composed of \(I_{\text{to}}\), \(I_{\text{ss}}\), and \(I_{\text{Kur}}\), we examined these individual components of the outward \(K^+\) currents to evaluate the contribution

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**Fig. 4.** Steady-state inactivation of \(I_{\text{to}}\) and \(I_{\text{Kur}}\) in 9- to 12-mo non-Tg and \(\alpha_{1B}\)-AR mouse ventricular myocytes. **A:** voltage dependence of steady-state inactivation of \(I_{\text{to}}\). Left: example of a family of currents produced by a two-pulse voltage-clamp protocol. The 100-ms inactivating prepulse potential was varied from −110 to −10 mV; the 1-s test pulse potential was +30 mV (protocol shown in inset). Right: plot of voltage dependence of steady-state inactivation of \(I_{\text{to}}\). The amplitude of \(I_{\text{to}}\) for each prepulse membrane potential was determined by subtracting each test pulse current with that obtained with the −40-mV prepulse. The \(I_{\text{to}}\) test pulse amplitude was normalized to the amplitude at the most negative prepulse potential. Data were pooled from 10 non-Tg (N = 3) and 9 \(\alpha_{1B}\)-AR (N = 3) cells. **B:** voltage dependence of steady-state inactivation of \(I_{\text{Kur}}\). Left: example of a family of currents produced by a two-pulse voltage-clamp protocol showing the voltage dependence of the steady-state inactivation of \(I_{\text{Kur}}\). Inactivating pulse (5-s duration) was varied from −110 to −10 mV; the test pulse potential (2.5-s duration) was +30 mV. Right: plot of voltage dependence of steady-state inactivation of \(I_{\text{Kur}}\). The \(I_{\text{Kur}}\) test pulse amplitude was normalized to the test amplitude for the most negative prepulse potential. \(I_{\text{Kur}}\) was obtained by subtraction of the current at the end of the test pulse from the peak test pulse current. Data were pooled from 10 non-Tg (N = 3) and 8 \(\alpha_{1B}\)-AR (N = 3) cells. The smooth curves are the best fit Boltzmann functions.
of each of these currents on the delayed repolarization observed in α1B-AR mice.

$I_{ss}$. The superimposed current records in Fig. 3A demonstrate that $I_{ss}$ in myocytes from α1B-AR mice was significantly decreased compared with non-Tg. The mean $I$-$V$ plots on the right show that the density of $I_{ss}$ in α1B-AR myocytes (at +30 mV: 12.9 ± 1.3 pA/pF, n = 19) was substantially reduced compared with that of non-Tg myocytes (at +30 mV: 40.5 ± 3.0 pA/pF, n = 14) ($P = 6.7 \times 10^{-8}$).

$I_{Kur}$. The family of superimposed current records presented in Fig. 3B correspond to the 4-AP-sensitive current, $I_{Kur}$, obtained in ventricular myocytes from both groups. As illustrated in these examples, $I_{Kur}$ from α1B-AR mice was smaller compared with that from littermate control animals. The mean $I$-$V$ curves (right) show that $I_{Kur}$ in cells from α1B-AR animals was significantly smaller (at +30 mV, 9.4 ± 1.0 pA/pF, n = 14) than that in non-Tg myocytes (at +30 mV, 26.0 ± 3.6 pA/pF, n = 9) ($P = 0.001$).

$I_{K1}$. Combining the inactivating protocol with the use of 4-AP that selectively blocks $I_{Kur}$, we compared the magnitude of $I_{K1}$ and $I_{ss}$ between control and α1B-AR myocytes (Fig. 3C). As illustrated by the representative examples presented on the left and the mean $I$-$V$ plots for $I_{K1}$ (right), the density of $I_{K1}$ was significantly decreased in α1B-AR myocytes (at +30 mV: 5.2 ± 0.3 pA/pF, n = 14) compared with the non-Tg (8.7 ± 0.7 pA/pF, n = 9) ($P = 0.0004$).

$I_{K1}$. In contrast, $I_{K1}$, the repolarizing current that also maintains resting membrane potential, was not changed when non-Tg and α1B-AR myocytes were compared. Indeed, the $I$-$V$ plots in Figs. 2B and 3C illustrate that the density of $I_{K1}$ between non-Tg and α1B-AR were not different (at −110 mV, non-Tg: −17.5 ± 0.9 pA/pF, n = 14; α1B-AR: −15.8 ± 0.8 pA/pF, n = 20; $P = 0.157$). Along with these data, there was no significant difference in the resting membrane potential between the two groups (non-Tg: −71.8 ± 0.5 mV, n = 19; α1B-AR: −73.5 ± 0.8 mV, n = 22).

Taken together, these results suggest that a reduction in the density of the three components of the outward K$^+$ current ($I_{ss}$, $I_{Kur}$, $I_{K1}$) is responsible for the action potential prolongation seen in α1B-AR mice.

Fig. 5. Reactivation of $I_{ss}$ and $I_{Kur}$ in 9- to 12-mo non-Tg and α1B-AR mouse ventricular myocytes. A: recovery from inactivation of $I_{ss}$. Left: example of a family of membrane currents produced by a two-pulse voltage-clamp protocol, showing the time course of recovery of $I_{ss}$ from inactivation. A 500-ms inactivating pulse (+50 mV) was followed at intervals of 10, 20, 30, 40, 50, 60, 80, 100, 200, 400, and 600 ms by an identical 500-ms test pulse. Right: membrane potential dependence of recovery from inactivation of $I_{ss}$. Data were pooled from 11 non-Tg (N = 3) and 8 α1B-AR (N = 3) cells. $P_2/P_1$ is the ratio of test pulse current/prepulse current amplitudes. $I_{ss}$ amplitude was measured as the difference between peak outward current and the current 150 ms after the peak. The holding and interpulse potentials were −80 mV. The smooth lines are best fit single-exponential functions. B: recovery from inactivation of $I_{Kur}$. Left: example of a family of membrane currents produced by a two-pulse voltage-clamp protocol, showing the time course of recovery from inactivation of $I_{Kur}$. A 5-s inactivating pulse was followed at intervals between 50 ms and 3 s by a 2.5-s test pulse. Both pulses were preceded by a brief (100 ms at −40 mV) pulse to inactivate $I_{ss}$. The holding and interpulse potentials were −80 mV. Right: membrane potential dependence of recovery from inactivation of $I_{Kur}$. Data were pooled from 11 non-Tg (N = 3) and 8 α1B-AR (N = 3) cells. $I_{Kur}$ amplitude was measured as the difference between peak test pulse current and the current at the end of the inactivating pulse. The smooth lines are best fit single-exponential functions.
Voltage dependence of steady-state inactivation of $I_{to}$ and $I_{Kur}$. Figure 4A compares the voltage dependence of steady-state inactivation of $I_{to}$ in non-Tg and $\alpha_{1B}$-AR ventricular myocytes, measured using a two-step voltage-clamp protocol. The midpoint potential ($V_{1/2}$) from the Boltzmann equation fit to mean control data was $V_{1/2} = -54.3 \pm 1$ mV, and the slope factor ($k$) was 6.2 $\pm$ 0.9 mV. Measurements of steady-state inactivation of $I_{to}$ in $\alpha_{1B}$-AR mouse ventricular cells yielded a $V_{1/2}$ of $-55.9 \pm 1.6$ mV, and a $k$ value of 5.2 $\pm$ 1.1 mV. Thus the voltage dependence of steady-state inactivation of $I_{to}$ in $\alpha_{1B}$-AR mouse ventricular myocytes was comparable to that in control myocytes.

The steady-state inactivation of $I_{Kur}$ was measured with a two-pulse protocol similar to that used for $I_{to}$, except that pulse durations were longer due to the much slower inactivation kinetics of $I_{Kur}$ (see Fig. 4, insets). In addition, a 100-ms pulse at $-40$ mV was interposed between the inactivating and test pulses to inactivate $I_{to}$. Figure 4B compares typical examples of steady-state inactivation of $I_{Kur}$ measured in control and $\alpha_{1B}$-AR ventricular myocytes. The Boltzmann equations fitted to these data are presented on the right. In non-Tg cells, $V_{1/2}$ was $-48.3 \pm 3.5$ mV compared with $-47.7 \pm 1.9$ mV for the $\alpha_{1B}$-AR group. The $k$ was 8.0 $\pm$ 1.1 mV for the non-Tg and 8.5 $\pm$ 1.2 mV for the $\alpha_{1B}$-AR group. Thus no significant difference was found in the steady-state inactivation of $I_{Kur}$ between the two groups.

Recovery from inactivation of $I_{to}$ and $I_{Kur}$. Figure 5A summarizes the data for the recovery from inactivation of $I_{to}$ in non-Tg and $\alpha_{1B}$-AR mouse ventricular myocytes. The time course of recovery of $I_{to}$ was best fitted by single exponential equations with time constants of 57 $\pm$ 15 ms for control myocytes and 530 $\pm$ 185 ms for the $\alpha_{1B}$-AR myocytes at $-80$ mV ($P = 0.005$). These data indicate that, in $\alpha_{1B}$-AR ventricular myocytes, $I_{to}$ recovered from inactivation much more slowly than in myocytes obtained from non-Tg animals. In contrast, $I_{Kur}$ recovered from inactivation at a similar rate in both groups with time constants of 447 $\pm$ 62 ms in the controls compared with 354 $\pm$ 77 ms ($P = 0.33$) in the $\alpha_{1B}$-AR group, as shown in Fig. 5B.

Reduction in $K^+$ Channels Expression in 9- to 12-mo $\alpha_{1B}$-AR Mouse Ventricle

Western blot experiments were undertaken to compare the protein expression level of $K_{1.5}$ (encoding $I_{K1}$) (31), $K_{4.2}$, and $K_{4.3}$ (underlying $I_{Ku}$) (3, 49), $K_{2.1}$ (responsible for $I_{Ku}$) (50), and Kir2.1 (underlying $I_{K1}$) (51) in non-Tg and $\alpha_{1B}$-AR ventricle. The marked change in recovery from inactivation of $K_{1.5}$, $K_{4.2}$, $K_{4.3}$, $K_{9.2}$, and $K_{9.3}$ due to $\alpha_{1B}$-AR expression in the heart is shown in Fig. 6A. These data are consistent with previous reports that $\alpha_{1B}$-AR expression leads to a decrease in $K^+$ conductance in ventricular myocytes. The time course of recovery of $I_{Kur}$ was best fitted by single exponential equations with time constants of 447 $\pm$ 62 ms in the controls compared with 354 $\pm$ 77 ms ($P = 0.33$) in the $\alpha_{1B}$-AR group, as shown in Fig. 5B.
suggests that Kv1.4 and/or KChIP2 also may be involved. Indeed, an upregulation of Kv1.4 expression could, in part, contribute to the slower $I_{\text{to}}$ reactivation kinetics (18, 21). Similarly, since it has been reported that the accessory subunit KChIP2 can associate with Kv4.2 and Kv4.3 to increase the density of $I_{\text{to}}$ and accelerate its reactivation (26, 34, 42), we also included KChIP2 in these Western blot analysis. These experiments were carried out on sarcolemmal enriched fractions, except for KChIP2, which was examined using cytosolic proteins (30). In the $\alpha_{1B}$-AR mice, there was a marked reduction in the protein expression of Kv1.5, Kv2.1, Kv4.2, and KChIP2. In contrast, Kv4.3 and Kir2.1 were similar between non-Tg and $\alpha_{1B}$-AR ventricles (Fig. 6A). The protein expression of Kv1.4 was increased in $\alpha_{1B}$-AR mice compared with controls.

To determine whether the reduction in the protein expression of Kv1.5, Kv4.2, KChIP2, and Kv2.1 was associated with a parallel reduction of the mRNA levels, real-time PCR experiments were carried out on these subunits. Consistent with the protein expression studies of the underlying K$^+$ channels, the real-time PCR experiments revealed a significant reduction of the transcripts for Kv1.5, Kv4.2, KChIP2, and Kv2.1 in the ventricles of $\alpha_{1B}$-AR mice compared with the non-Tg mice. (Fig. 6B).

**Alterations in Cardiac Rhythm in 9- to 12-mo $\alpha_{1B}$-AR Mice**

Figure 7 depicts ECG traces recorded in 9- to 12-mo $\alpha_{1B}$-AR and littermate control mice. While none of the 24 control mice studied had rhythm abnormalities (Fig. 7A), a variety of spontaneous ventricular arrhythmias were observed in 9- to 12-mo-old $\alpha_{1B}$-AR mice using both surface ECG recordings and telemetry monitoring. Figures 7, B–E show different irregular rhythms present in $\alpha_{1B}$-AR mice; episodes of arrhythmias were reported on 24 of 31 (77%) $\alpha_{1B}$-AR mice. Of these, 19 of 26 (73%) mice experienced spontaneous arrhythmia during a 2-min surface ECG recording, and all 5 $\alpha_{1B}$-AR mice subjected to a 24-h telemetry monitoring exhibited irregular rhythms. These spontaneous arrhythmias were recorded throughout the 24-h recording period, and each episode lasted between 100 ms and 2.5 min.

![Fig. 7. Spontaneous arrhythmias in 9- to 12-mo $\alpha_{1B}$-AR mice. A: representative surface electrocardiogram (ECG) recordings obtained in a non-Tg mouse showing a normal sinus rhythm. B–E: examples of different types of arrhythmias observed in $\alpha_{1B}$-AR mice using surface ECG recordings or telemetry monitoring. B: an irregular rhythm with normal QRS complex. C: a premature ventricular complex, including premature beat followed by a pause. Note that the basal rhythm is delayed after the arrhythmia. D: a nonsustained monophasic ventricular tachycardia, with a sudden increase in ventricular rate and wider QRS complexes. E: a sustained polymorphic ventricular tachycardia. Recordings obtained in B, C, and D were obtained in anesthetized mice using surface ECG, while data presented in E were recorded with telemetry device on freely moving mice. Note the different time scales between the panels.](http://ajpheart.physiology.org/)

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**Characterization of Younger α1B-AR Mice**

As specified earlier, the mice used in the experiments described above were 9–12 mo old and showed clear signs of heart failure (1, 28). A heart failure phenotype could potentially affect ventricular repolarization and QT interval. This might contribute, at least in part, to the observed electrophysiological alterations in α1B-AR myocytes. Using echocardiographic analyses on α1B-AR mice at 3, 6, and 9 mo of age, Lemire et al. (28) previously reported that younger α1B-AR mice (3 mo) do not present signs of heart failure, as opposed to the 9 mo animals. Accordingly, we carried out electrophysiological and expression studies using 2- to 3-mo-old α1B-AR mice to ascertain that the arrhythmogenic changes described in the older transgenic mice were not the result of alterations associated with end-stage heart failure, but rather due to chronic α1-AR activity in the transgenic hearts.

**Younger α1B-AR Present Cardiac Arrhythmia and Delayed Ventricular Repolarization**

Results presented in Figs. 8 and 9 indicated that 2- to 3-mo α1B-AR mice also present cardiac arrhythmias and delayed repolarization. Figure 8 illustrates ECG recordings obtained from 2- to 3-mo control and α1B-AR mice. None of the seven control mice tested had rhythm disturbances (Fig. 8A). In contrast, 5 of 10 (50%) α1B-AR mice subjected to 2-min surface ECG recording exhibited spontaneous arrhythmia. In addition, telemetry monitoring of 2- to 3-mo α1B-AR mice revealed spontaneous arrhythmias in two of two young transgenic mice tested. These arrhythmias were similar to those observed in the older α1B-AR animals. However, these events were seen more frequently and were more sustained in the older transgenic mice compared with the younger α1B-AR animals. For instance, in the 2- to 3-mo α1B-AR mice, the longest arrhythmia lasted 15 s, as opposed to 2.5 min in the older transgenic animals.

Compared with their age-matched littermate controls, the 2- to 3-mo α1B-AR mice had longer QT interval (non-Tg, 49.3 ± 2.5 ms; α1B-AR, 80.3 ± 5.9 ms, P < 0.0002), QTc interval (non-Tg, 42.3 ± 1.6 ms; α1B-AR, 58.1 ± 4.2 ms, P < 0.002), and APD (in ms) (APD20: non-Tg, 2.7 ± 0.1; α1B-AR, 3.8 ± 0.2, P = 0.0001; APD50: non-Tg, 5.5 ± 0.5; α1B-AR, 10.3 ± 0.8, P < 0.00001; APD90: non-Tg, 19.2 ± 1.0; α1B-AR, 34.1 ± 1.8, P < 0.00001) (Fig. 9, A and B).

![Fig. 8. Spontaneous arrhythmias in 2- to 3-mo α1B-AR mice. Representative ECG recordings obtained in non-Tg and α1B-AR mice are shown. A: surface ECG recording showing a normal sinus rhythm in a littermate control mouse. B–E: examples illustrate irregular rhythms observed in 2- to 3-mo α1B-AR mice. B: an irregular rhythm with normal QRS complex obtained with surface ECG recordings. C: a premature ventricular complex followed by a pause (surface ECG). D: an example of premature ventricular complex recorded using telemetry. E: a nonsustained polymorphic ventricular tachycardia with a faster basal rhythm after the ventricular tachycardia recorded with telemetry device.](H712 REPOLARIZATION DELAY IN MUTANT α1B-AR MICE AJP-Heart Circ Physiol • VOL 296 • MARCH 2009 • www.ajpheart.org by 10.220.33.2 on October 30, 2017 http://ajpheart.physiology.org/ Downloaded from)
Younger $\alpha_{1B}$-AR Mice Also Exhibit Reduced Outward $K^+$ Currents

Furthermore, similar to what was seen in the older mice, the 2- to 3-mo $\alpha_{1B}$-AR mice exhibited a decrease in density of the outward component of the $I_{\text{peak}}$ (at $+30 \, \text{mV}$; non-Tg: $54.9 \pm 4.3 \, \text{pA/pF}$ and $\alpha_{1B}$-AR: $30.9 \pm 1.4 \, \text{pA/pF}$, $P = 0.0001$) (Fig. 9C, right). Separation of the individual components of $I_{\text{peak}}$ reveals that the three outward $K^+$ currents were reduced in the young $\alpha_{1B}$-AR mice compared with their age-matched littermates (at $+30 \, \text{mV}$, $I_{\text{to}}$: non-Tg, $28.7 \pm 2.5 \, \text{pA/pF}$, $\alpha_{1B}$-AR, $13.6 \pm 1.2 \, \text{pA/pF}$, $P = 0.0001$; $I_{\text{Kur}}$: non-Tg, $21.8 \pm 3.2 \, \text{pA/pF}$, $\alpha_{1B}$-AR, $11.1 \pm 0.9 \, \text{pA/pF}$, $P = 0.009$; $I_{\text{ss}}$: non-Tg, $11.0 \pm 0.5 \, \text{pA/pF}$, $\alpha_{1B}$-AR, $6.7 \pm 0.6 \, \text{pA/pF}$, $P = 0.00003$) (Fig. 10, A, B, and C, respectively). As for the older mice, $I_{\text{K1}}$ was also comparable between young $\alpha_{1B}$-AR and non-Tg mice (at $-110 \, \text{mV}$, non-Tg: $-18.3 \pm 1.4 \, \text{pA/pF}$, $\alpha_{1B}$-AR: $-16.6 \pm 0.9 \, \text{pA/pF}$, $P = 0.3$) (Fig. 9C and 10C). In line with this finding, resting membrane potentials were also similar between the two groups (non-Tg: $-73.0 \pm 0.5 \, \text{mV}$, $n = 22$; $\alpha_{1B}$-AR: $-75.5 \pm 0.5 \, \text{mV}$, $n = 21$).

Reduced $K^+$ Channel Expression in Young $\alpha_{1B}$-AR Mouse Ventricle

Using the younger $\alpha_{1B}$-AR mice, we also examined the expression of the different $K^+$ channel and accessory subunits that were changed in older $\alpha_{1B}$-AR ventricles (Fig. 7). At 2–3 mo of age, protein and mRNA expression of $K_v1.5$ and KCnIP2 were decreased in transgenic ventricles compared with non-Tg tissues (Fig. 11). As for $K_v4.2$, the expression level of this channel was reduced only at the protein level (Fig. 11) in the younger animals. Similar to what was seen in the older group, $K_v1.4$ protein expression was increased in young $\alpha_{1B}$-AR mice compared with the non-Tg controls. However, as opposed to the results obtained for $K_v2.1$ in the older $\alpha_{1B}$-AR
mice, in the younger animals the protein and mRNA expression of Kv2.1 was similar between control and transgenic animals. To address the possibility that the decrease in \( I_{\text{ss}} \) seen in the young \( \alpha_{1B}\)-AR mice could be due to decreased expression of other channels than Kv2.1, we performed additional experiments to address this possible alternative. Since recently published studies suggested that part of \( I_{\text{ss}} \) could be generated by TASK-1 channel (9, 35), we verified if the protein expression of this channel was altered in the \( \alpha_{1B}\)-AR mice (Fig. 12). These Western blot analyses clearly showed that the protein expression of TASK-1 is similar between control and \( \alpha_{1B}\)-AR mice of both age groups. These new data strongly suggest that TASK-1 does not contribute to \( I_{\text{ss}} \) in mouse ventricular myocytes.

These findings clearly show that electrical remodeling is already present in younger transgenic animals. However, it appears to be somewhat less pronounced than in older mice. These data support an important role for the overexpression of \( \alpha_{1B}\)-AR as a major cause of the repolarization defects and increased incidence of arrhythmias in the Tg mice. In addition, the further reduction of the transcript levels of the \( K_{\text{v1.1}} \) channels and the worsening of the electrical remodeling that occurs in the older animals might be attributed to intracellular signaling linked to cardiac remodeling or failure.

**DISCUSSION**

**Summary of Main Findings**

This study is the first to characterize the electrophysiological consequences of overexpressing the wild-type \( \alpha_{1B}\)-AR in the myocardium of the mouse. Results obtained in 9- to 12-mo \( \alpha_{1B}\)-AR mice revealed that chronic \( \alpha_{1B}\)-AR activity remodels
cardiac expression of K⁺ channel subunits at the mRNA and protein level. These changes are associated with delayed repolarization and spontaneous ventricular arrhythmias. These findings support the hypothesis that chronic α₁B-adrenergic activation is a potentially important control mechanism for cardiac arrhythmias. Since the presence of heart failure in aged α₁B-AR mice could also be associated with delayed repolarization, it was important to determine whether this phenotype is directly related to the transgene and not to the heart failure. The electrical remodeling observed in the younger (2–3 mo) α₁B-AR mice that do not yet have characteristics of heart failure provides further evidence in support of an important role for α₁B-ARs in regulating the expression of cardiac potassium channels and arrhythmogenesis. However, since the alterations in the young mice appear to be slightly less important than in the older transgenic animals, one cannot exclude that part of the electrical remodeling in the older mice could be a consequence of an important role for cardiac arrhythmias. Since the presence of heart failure in aged α₁B-AR mice (2–3 mo) non-Tg, 91 ± 4 pF, n = 17; α₁B-AR, 93 ± 4 pF, n = 21; 9–12 mo: non-Tg, 214 ± 8 pF, n = 20; α₁B-AR, 186 ± 15 pF, n = 20). These observations provide further evidence that the changes observed in the transgenic myocytes do not result from ventricular hypertrophy and remodeling. In support of this, our laboratory recently demonstrated that cardiac-specific overexpression of angiotensin type 1 receptor in a transgenic mouse model leads to delayed repolarization phenotype that did not occur as a consequence of hypertrophy (37). Besides, there is growing evidence suggesting that the reduction of K⁺ currents and subsequent prolongation of APD have been proposed as the first steps in the cascade of events leading to ventricular remodeling and heart failure (25, 27, 41, 44, 52).

Potential Mechanisms Underlying the Reduction of Outward K⁺ Currents

The lower density of Iₜₒ was associated with a reduction of K₄.2 and KChIP2 expression, as well as a slower reactivation time of the current. In support of these findings, previous studies have reported that currents generated by Kᵥ channels that are affected by neurohormonal factors (e.g., α₁-adrenergic agonists and angiotensin II) showed alteration in the expression levels and kinetics of the currents (22, 33). KChIP2 has been proposed to interact with the Kᵥ₄.2 subunit to facilitate its trafficking from the endoplasmic reticulum to the surface membrane (26, 34, 42) and accelerate recovery from inactivation of Iₜₒ (26). Thus the lower expression of KChIP2 in old and young transgenic animals could contribute to explain the reduced density of Iₜₒ and its slower reactivation time. Our laboratory previously reported (16) that the pattern of KChIP2 mRNA expression was similar to that observed for Kᵥ₄.2 during postnatal development in mouse ventricle, suggesting that the increases in KChIP2 mRNA between day 1 and adulthood may be responsible, in part, for the increase in Iₜₒ density observed during this time period. In addition to the changes in KChIP2, the increased expression of Kᵥ₁.4 could also contribute to the slower reactivation kinetics of Iₜₒ in the transgenic animals.

Although both Kᵥ₄.2 and Kᵥ₄.3 K⁺ channel isoforms contribute to Iₜₒ in mouse ventricles (3, 5, 14, 21, 43, 49), we show here that a reduction restricted only to Kᵥ₄.2 α-subunit resulted in a lower Iₜₒ density. It is interesting to note that, under...
different physiological or pathological conditions, changes in $I_{ss}$ could be associated with changes in $K_v4.2$ or $K_v4.3$. For example, Eghbali et al. (11) have shown that, in pregnant mice, a specific reduction of $K_v4.3$ was responsible for the lower $I_{ss}$ in old mice compared to young mice. The reduction in $I_{ss}$ is most likely explained by a combination of posttranscriptional ($K_v4.2$) and transcriptional (KChIP2) regulation. Our results indicate that $I_{ss}$ is decreased in old and young $\alpha_{1B}$-AR mice. The reduction in the older animals is associated with a reduction in $K_v2.1$ protein and mRNA expression. Surprisingly, in the young $\alpha_{1B}$-AR mice, the decrease in the in-cell current density of $I_{ss}$ is not paralleled with a reduction of $K_v2.1$ expression. It is possible that the reduction of $I_{ss}$ would be due to decreased expression of other channels than $K_v2.1$. However, our Western data showed that TASK-1 protein expression was similar between controls and $\alpha_{1B}$-AR mice of both age groups and ruled out a possible involvement of TASK-1 in the generation of $I_{ss}$ in mouse ventricle. Alternatively, the decreased expression of channels other than $K_v2.1$ and TASK-1 remains a possible explanation.

$I_{K1}$ Was Not Affected in $\alpha_{1B}$-AR Mice

Interestingly, the only $K^+$ current that was not different between the $\alpha_{1B}$-AR and the non-Tg mice was $I_{K1}$. Consistent with that, the resting membrane potential and the protein level of $K_{ir}2.1$ were also similar between the two groups. The fact that $I_{K1}$ is not affected by $\alpha_{1}$-adrenergic stimulation supports the hypothesis that chronic activation of $\alpha_{1}$-ARs causes the reduction of $K^+$ currents that activate early in the repolarization process, prolonging the APD at the plateau level and increasing intracellular $Ca^{2+}$ current. $I_{K1}$ is not involved in that process, since its activation produces a prolongation of the APD only at the late phase of the action potential. Moreover, it has been documented in the literature that, in cardiomyocytes isolated from rats, rabbits, and humans (where $I_{K1}$ is prominent), $\alpha_{1}$-adrenergic activation causes prolongation of the APD (13), but does not have a similar effect in guinea pig (10, 40), a species in which $I_{K1}$ is absent. These findings are also in support of the effects of $\alpha_{1}$-adrenergic stimulation on $K^+$ currents involved early in the repolarization process. In contrast to our $I_{K1}$ results, it has been previously reported that the inward rectifier current could be decreased by acute exposure to $\alpha_{1}$-adrenergic agonists in rabbit ventricular myocytes (12). Potential limitations with the pharmacological approach could contribute to explain this discrepancy (e.g., specificity of the agonists/antagonists). However, other factors, such as differences in the underlying $K^+$ channels or species differences, could also explain these differences.

**Limitations**

The data obtained in the younger animals indicate that most of the arrhythmgic changes described here take place before the occurrence of heart failure, strongly supporting the conclusion that these alterations are largely due to chronic $\alpha_{1B}$-AR activity. However, since the young animals also present a less severe phenotype, one cannot exclude that part of the electrical remodeling of the older mice is a consequence of
heart failure. In addition, other remodelings may also contribute to explain the occurrence of arrhythmias. Indeed, in addition to alterations in the repolarization process, the α₁B-AR transgenic mice have a significantly slower HR (Figs. 1 and 9), suggesting that the function and/or regulation of the sinoatrial node may also be affected by chronic α₁B-AR activation. Other studies have also reported that chronic α₁B-AR stimulation causes decreased HR (38). The slower HR observed in the α₁B-AR transgenic mice could be due to sinus bradycardia or junctional rhythm. This might also contribute to the phenotype observed in the transgenic animals. This question will require additional study.

Conclusion

The present study provides strong evidence suggesting that, under pathological conditions, α₁B-AR stimulation is associated with delayed repolarization and cardiac arrhythmias. Our voltage-clamp experiments, along with expression studies, revealed that a number of K⁺ currents/channels are downregulated in α₁B-AR mice, suggesting a causal link to the observed phenotype of rhythm disturbances also reported here. This work improves our understanding of the mechanisms by which α-adrenergic stimulation affects cardiac excitability and cardiac repolarization and our awareness of basic mechanisms of action of autonomic control of cardiac arrhythmia.

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