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Changes underlying arrhythmia in the transgenic heart overexpressing Refsum disease gene-associated protein

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Abstract

Previously, we identified a novel neuron-specific protein (PAHX-AP1) that binds to Refsum disease gene product (PAHX), and we developed transgenic (TG) mice that overexpress heart-targeted PAHX-AP1. These mice have atrial tachycardia and increased susceptibility to aconitine-induced arrhythmia. This study was undertaken to elucidate the possible changes in ion channels underlying the susceptibility to arrhythmia in these mice. RT-PCR analyses revealed that the cardiac expression of adrenergic β_1-receptor (*ADRB1*) was markedly lower, whereas voltage-gated potassium channel expression (*Kv2.1*) was higher in PAHX-AP1 TG mice compared with non-TG mice. However, the expression of voltage-sensitive sodium and calcium channels, and muscarinic receptor was not significantly different. Propranolol pretreatment, a non-specific β-adrenoceptor antagonist, blocked aconitine-induced arrhythmia in non-TG mice, but not in PAHX-AP1 TG mice. Our results indicate that, in the PAHX-AP1 TG heart, the modulation of voltage-gated potassium channel and ADRB1 expression seem to be important in the electrophysiological changes associated with altered ion channel functions, but ADRB1 is not involved in the greater susceptibility to aconitine-induced arrhythmia.

Keywords: Transgenic mouse; Tachycardia; Arrhythmia; Refsum disease; PAHX-AP1; Aconitine; Propranolol

Recently, we identified and characterized a novel neuron-specific protein (PAHX-AP1) that binds to the Refsum disease gene product (phytanoyl-CoA α-hydroxylase [PAHX]) [1]. To test whether the PAHX-AP1 protein is involved in the cardiac symptoms of Refsum's disease, such as tachycardia and gallop rhythm, we created heart-targeted PAHX-AP1 overexpressing transgenic (TG) mice in which PAHX-AP1 was overexpressed under the control of the α-myosin heavy chain promoter [2]. In the PAHX-AP1 TG mice, PAHX-AP1 was expressed within the sinoatrial node of atrium, and these mice have tachycardia and greater susceptibility to arrhythmia induced by drugs, especially aconitine. The duration of the action potential in the left atrial muscle fiber of PAHX-AP1 TG mice shortens with changes in the stimulation frequency much more than the wild-type, and these changes may be caused by tachycardia-induced remodeling of ion channels and receptors in the heart [2].

Aconitine, which is derived from the plant *Aconitum napellus*, binds with high affinity to the open state of sodium channel at epitope 2, thus causing a persistent activation of the sodium channel by blocking its inactivation, and finally leading to inexcitability [3,4]. It was reported that activation of sodium channels and muscarinic receptors by aconitine plays an important role in the aconitine-induced tachyarrhythmia in murine atria; activation of β_1-adrenoceptors by higenamine and dobutamine augmented aconitine-induced tachyarrhythmia [5,6]. These reports and our previous result have strongly suggested that development of susceptibility to

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aconitine-induced tachyarrhythmia in PAHX-AP1 TG mice might be related to changes in expression of these receptors and ion channels.

The present study was undertaken to examine whether the expression levels of receptors and ion channels are changed by tachycardia-induced remodeling in the PAHX-AP1 TG heart, and whether these changes contribute to its susceptibility to aconitine-induced arrhythmia. We found that the altered expression of voltage-gated K^+ channel and adrenergic β_1-receptor (ADRB1) seems to be a major part in the electrophysiological changes of the PAHX-AP1 TG heart associated with modulated ion channel functions by tachycardia-induced remodeling. However, pretreatment of β-adrenoceptor antagonist did not block the aconitine-induced arrhythmia in the TG mice. These findings suggest that voltage-gated K^+ channel rather than ADRB1 may participate in the susceptibility to aconitine-induced arrhythmia in the PAHX-AP1 TG heart. This TG mouse model may be useful for the study of hypersusceptibility to drug-induced tachyarrhythmia.

Materials and methods

All experiments were performed according to the NIH guidelines for care and use of laboratory animals. The experimental protocol was approved by the Chonnam National University Medical School Research Institutional Animal Care and Use Committee. We used FVB PAHX-AP1 TG mice, either sex, weighing 20–25 g, previously developed by Koh et al. [2] and age-matched wild-type littermates.

Cardiovascular responses to aconitine and propranolol. Two-month-old mice were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally [i.p.]), which has a slight effect on the cardiovascular system. Tracheotomy was performed to support spontaneous respiration. The common carotid artery was cannulated for monitoring arterial blood pressure (BP) and heart rate (HR) through a pressure transducer and tachometer connected to a Polygraph (Grass 7E, USA). After the BP and HR were stabilized, aconitine or propranolol was given i.p., and then BP and HR were monitored. For the evaluation of aconitine responses in the presence of propranolol, 4.5 mg/kg of propranolol was injected 10 min prior to aconitine administration.

Experimental procedures for isolated atrial beating. Two-month-old mice were sacrificed by decapitation and exsanguination, and the heart was quickly removed and rinsed in Tyrode's solution. Both atria were separated from the ventricle and mounted in a 10-ml organ bath with one end fixed and the other end connected to a force displacement transducer (FT03, Grass, USA). Atrial beating was measured on a separate channel by a frequency converter amplifier (13-6615-60, Gould). The experiments were performed at 37 °C in organ bath solution (113 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 4.8 mM KCl, and 11.0 mM dextrose) bubbled with a 95% O_2/5% CO_2 mixture. After pre-stretching the atrial strip with a baseline tension of 0.2 g, the strip was equilibrated for 60 min in the aerated solution before the experimental protocols were initiated [7]. Aconitine (10^{-8} M) was given to the organ bath, and atrial contractions were monitored.

Action potential recordings. Two-month-old mice were sacrificed by decapitation and exsanguination. The heart was immediately excised, and the left atrium was dissected in cold Tyrode's solution, which was being continually gassed with a mixture of 95% O_2 and 5% CO_2. Atrial strips were taken from the atria and their action potentials were recorded as previously described [2]. In brief, the atrial preparations were impaled with glass capillary microelectrodes that were filled with 3 M KCl and had tip resistances of 10–30 MΩ. The strips were stimulated with rectangular pulses through a bipolar silver electrode with a Grass stimulator S88 coupled to a Grass stimulus isolation unit. The transmembrane potentials from the microelectrode were amplified (S7071A electrometer, WPI), displayed on a Tektronix oscilloscope, and recorded with a Polaroid camera (C-51, Tektronix). Action potential duration was measured after 90% repolarization (APD_{90}, ms).

RNA isolation and RT-PCR analysis. To isolate total RNA, mouse heart was homogenized with a polytron homogenizer in 4.0 M guanidine thiocyanate, 1% β-mercaptoethanol, and RNA was purified by centrifugation through 5.7 M CsCl as described [1]. RNA samples were quantitated by spectrophotometry at 260 nm. Reverse transcription was performed at 42 °C for 60 min with 200 ng of total RNA, 100 pmol of random primers, and reverse transcriptase (Invitrogen). PCR was performed on first strand cDNAs to the exponential phase, which was determined to be 30–35 cycles. All reactions involved an initial denaturation at 94 °C for 5 min followed by 30 or 35 cycles of the following: 94 °C for 1 min, the appropriate annealing temperature for 1 min, and elongation at 72 °C for 2 min (Mastercycler Personal PCR system, Eppendorf, Germany). To allow quantitative comparisons among the first strand cDNAs, control PCR was performed for 25–27 cycles with primers for the housekeeping gene, GAPDH. After confirming that the quantity of GAPDH among them was the same by analyzing the samples on agarose gels, PCRs for the desired genes were performed using specific primers. The annealing temperatures were 60 °C for mouse adrenergic β_1-receptor (ADRB1), voltage-gated K^+ channel (Kcnab2), Kv2.1, Kv1.5, and GAPDH; and 58 °C for acetylcholine muscarinic M2 (AchM2) receptor, voltage-sensitive Ca^{2+} channel (Cacn), and Na^+ channel (Scn). The amplification products were analyzed on 1.2% agarose gels and visualized by ethidium bromide staining.

The specific mouse PCR primers were as follows: Scn sense, 5′-TCCTGTGCATCTCCTGTAAGCG-3′ and antisense, 5′-TACACC ATCTCTGCCACAAGCC-3′; ADRB1 sense, 5′-ATCGTTCTGC TCATCGTGGTGG-3′ and antisense, 5′- TGACGAAATCGCAGCA CTTGG-3′; Kcnab2 sense, 5′- TGACCTTGGCCTACGATAATGG-3′ and antisense, 5′-TTCCGCTTGCTCACAGATGG-3′; Kvβ2.1 sense, 5′-CGGCAGTTCAACCTGATCCC-3′ and antisense, 5′-TTTATTG CCCAGAATGCTGTCG-3′; Kv1.5 sense, 5′-GGTCGCACTTCTCC AGTATCCC-3′ and antisense, 5′-AGGTCCACGTTGCTCTTGGC-3′; AchM2 receptor sense, 5′-GCGTGGGTTCTTTCCTTCATC-3′ and antisense, 5′-ACAGACGTGGAGTCATTGGAGC-3′; Cacn sense, 5′- GACGCTATGGGCTATGAGTTGC-3′ and antisense, 5′- GAAC ACCAGGAAGATCACGAGC-3′; and GAPDH sense, 5′-TATGAC AACTCCCTCAAGAT-3′ and antisense, 5-AGATCCACAACGGAT ACATT-3′.

Drugs. Aconitine and propranolol (Sigma) were dissolved in saline immediately before administration. Pentobarbital sodium was obtained from TCI (Japan).

Statistics. The values of action potential durations are presented as means ± SEM ($n = 5$), and differences in the values between TG and non-TG mice were evaluated using ANOVA and unpaired Student's t test. Differences were regarded as significant when $p < 0.05$.

Results and discussion

Tachyarrhythmic properties of the transgenic mice

To confirm whether the heart of PAHX-AP1 TG mice exhibited tachyarrhythmic properties in response to aconitine, the drug was injected i.p. into PAHX-AP1 TG and non-TG mice. When 1.5 mg/kg of aconitine was

injected into the mice, the mean blood pressures (BP) of TG and non-TG mice were 87.0 ± 4.0 mmHg ($n = 5$) and 89.8 ± 5.7 mmHg ($n = 5$), respectively, in sex- and age-matched littermates (Fig. 1A, upper panel). The heart rate (HR) of non-TG mice was 358 ± 12 beats/min with the normal rhythm, but the HR of TG mice was 412 ± 13 beats/min and showed an irregular pattern (Fig. 1 A, lower panel). In non-TG mice, 0.5 and 1.5 mg/kg of aconitine did not cause cardiac arrhythmia (Fig. 1A, Table 1), but 4.5 mg/kg of aconitine-induced arrhythmia in 67% of non-TG mice (Table 1). In TG mice, however, 0.5, 1.5, and 4.5 mg/kg of aconitine produced arrhythmia in 50, 75, and 100% of mice, respectively, (Table 1).

Aconitine was also added to isolated atrial strips in an organ bath. Control beats of non-TG and TG mice were 318.5 ± 19.5 beats/min ($n = 6$) and 382.7 ± 19.3 beats/min ($n = 7$), respectively, with a regular rhythm. However, 10^{-8} M of aconitine produced irregular beats with increased HR in PAHX-AP1 TG hearts, but not in non-TG hearts (Fig. 1B). The action of aconitine is generally dependent on basal heart rate or stimulation frequency when it is used as an arrhythmogenic agent [8]. Because PAHX-AP1 TG mice have higher heart rate than the non-TG mice, this raises the question of whether the TG mice really have increased susceptibility to aconitine-induced arrhythmia, or merely present arrhythmia due to a higher basal heart rate. However, PAHX-AP1 TG mice had the abnormal rhythm with aconitine in a dose-dependent manner (Table 1). Thus, the PAHX-AP1 TG mouse heart really does have an arrhythmogenic potential.

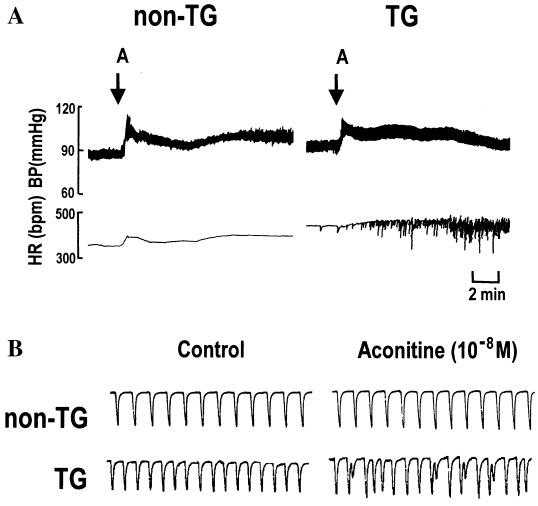

Fig. 1. Effect of aconitine on cardiovascular function in vivo and in vitro. (A) The representative recordings of BP and HR before and after aconitine (1.5 mg/kg) in non-TG and PAHX-AP1 TG mice are shown. Aconitine given i.p. (arrow with A) induced arrhythmia in the PAHX-AP1 TG mice (right panel), but not in the non-TG mice (left panel). There was no significant difference in BP in either type of mice in response to the drug. The irregular feature in HR reflected the development of arrhythmia. The positions of the calibration markers for BP and HR are shown on the left. Timescale bar is 2 min; bpm, beat per min. (B) Representative recordings of beats of isolated non-TG and PAHX-AP1 TG atria in the organ bath are shown. After administration of 10^{-8} M aconitine, the HR of the PAHX-AP1 TG atria increased significantly and irregular atrial beats occurred.

To determine whether there are any changes in the action potential phenotype, we measured the action potential duration (APD_{90}) at the 1, 4, and 7 Hz stimulus frequencies in left atrial fibers. The APD_{90} in the non-TG atrial fibers at 1 Hz stimulus frequency was 89.5 ± 19.7 ms and shortened in a frequency-dependent manner to 63.5 ± 4.7 mmHg at 4 Hz and 58.3 ± 9.3 ms at 7 Hz. APD_{90}s were also shortened in the PAHX-AP1 TG atrial fibers, but the magnitudes of the shortenings at each frequency were significantly greater (Fig. 2A). APD_{90} in the PAHX-AP1 TG atria was shortened to 26.7 ± 4.4 ms at 7 Hz (Figs. 2A and B). These results support our previous reports [2], showing that PAHX-AP1 TG mice have an increased susceptibility to aconitine-induced tachyarrhythmia and an alteration of the action potential duration. The present study also showed that PAHX-AP1 TG mice produced arrhythmia in response to aconitine with more sensitivity than non-TG mice both in vivo and in vitro. The action potential duration following the frequency-dependent stimulation was more shortened in PAHX-AP1 TG mice, indicating that its electrophysiological change was affected by tachycardia-induced atrial remodeling of receptors and ion channels [9–12].

Expression of receptors and ion channels in the transgenic heart

It has been suggested that the increased arrhythmogenic sensitivity to aconitine in PAHX-AP1 TG mice

Table 1
Incidence of aconitine-induced arrhythmia between non-transgenic (non-TG) and PAHX-AP1 transgenic mice (TG) mice, and the effect of propranolol (0.5 mg/kg) pretreatment on aconitine-induced arrhythmia

	Aconitine (mg/kg)						Propranolol + Aconitine (4.5)	
	0.5		1.5		4.5			
	non-TG	TG	non-TG	TG	non-TG	TG	non-TG	TG
Normal rhythm	4	2	4	1	2	0	6	0
Abnormal rhythm	0	2	0	3	4	4	1	6

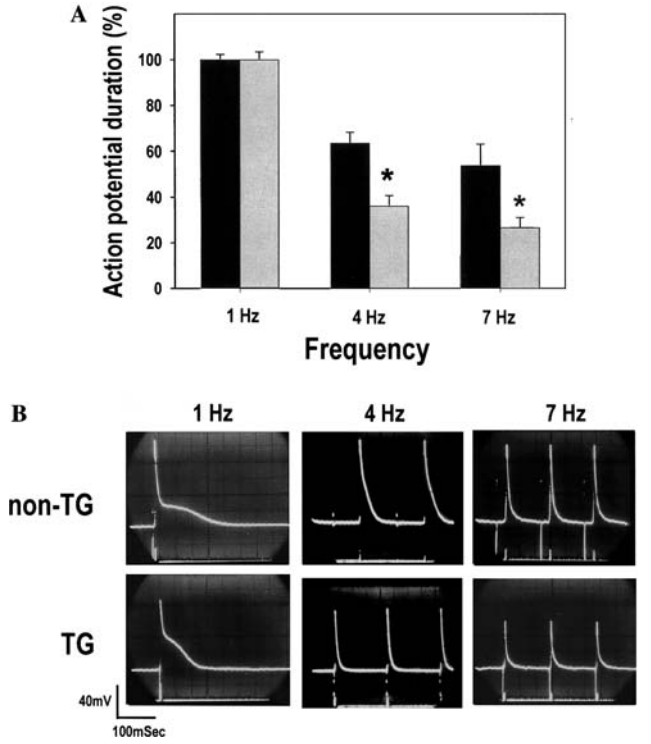

Fig. 2. Action potential duration in the left atrial fibers. (A) Changes in action potential durations (APD_{90}) at 1, 4, and 7 Hz stimulation are shown. The APD_{90} shortened in a stimulus frequency-dependent manner in the non-TG and PAHX-AP1 TG left atrial fibers. The magnitudes of these shortenings in the PAHX-AP1 TG atria at each stimulus frequency were significantly greater than those in the non-TG hearts ($*p < 0.05$). (B) The representative AP recordings from non-TG and TG left atrial fibers at 1, 4, and 7 Hz are shown.

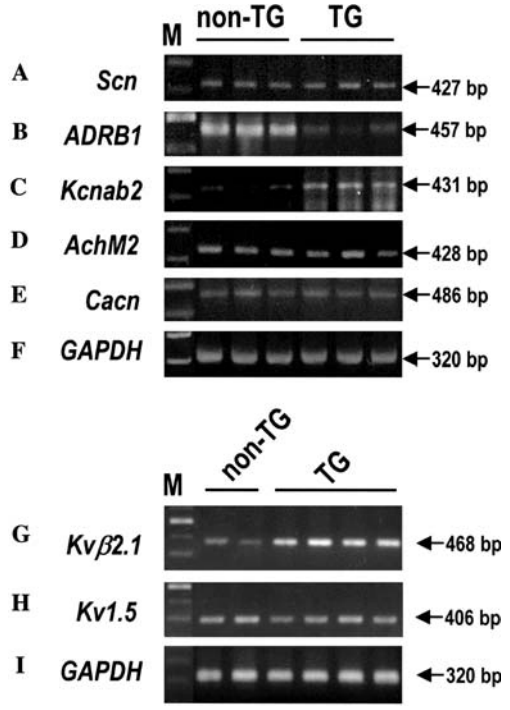

Fig. 3. RT-PCR analysis of ion channels and receptors in the mouse heart. In PAHX-AP1 TG mice, the expression of sodium (*Scn*) channel (A) was not significantly different. The expression of adrenergic β_1 (*ADRB1*) receptor (B) was markedly lower, but voltage-gated K^+ channel (*Kcnab2*) expression (C) was higher compared with non-TG mice. However, the expression of muscarinic receptor *AchM2* (D) and voltage-gated calcium channel *Cacn* (E) showed the similar level in TG and non-TG heart. The expression of *Kvβ2.1* (G) was higher in PAHX-AP1 TG heart than that of non-TG heart. In contrast, the expression of *Kv1.5* showed the similar level as compared to that of non-TG heart (H). *GAPDH* was used to confirm RNA fidelity (F,I). M represents the molecular size marker.

might be due to receptor and ion channel remodeling in the heart [10–12]. To determine whether changes in the expression of receptors and ion channels are present in the PAHX-AP1 TG-mice, we used RT-PCR to analyze the expression of β_1-adrenergic (ADRB1) receptors, muscarinic (AchM2) receptors, voltage-sensitive Na^+ (Scn) channels, K^+ (Kcnab2, β member 2) channels, and Ca^{2+}(Cacn) channels in three TG and three non-TG hearts.

In PAHX-AP1 TG hearts, expression of *Scn* was not significantly different compared with that of non-TG hearts (Fig. 3A). It is very interesting that Na^+ channel expression was not changed, because the PAHX-AP1 TG mice have the susceptibility to aconitine, a Na^+ channel activator. In PAHX-AP1 TG mice, cardiac expression of *ADRB1* was markedly lower (Fig. 3B), whereas *Kcnab2* expression was higher compared with non-TG mice (Fig. 3C). Both *AchM2* (Fig. 3D) and *Cacn* (Fig. 3E) were expressed at similar levels in TG and non-TG hearts.

To identify a candidate subunit for the increased K^+ channel expression in PAHX-AP1 TG heart, we examined *Kvβ2.1* and *Kv1.5* expressions in four PAHX-AP1 TG and two non-TG hearts by RT-PCR. The expression of *Kvβ2.1* was higher in PAHX-AP1 TG hearts than in the non-TG hearts (Fig. 3G), whereas the expression of *Kv1.5* showed a similar level in all hearts (Fig. 3H). This result suggests that the increased K^+ channel message in PAHX-AP1 TG heart may be derived from Kv2.1, which may not be associated with the Kv1.5 channel to modulate the cardiac functions of PAHX-AP1 TG mice.

Thus, our results indicated that the major electrophysiological changes of the PAHX-AP1 TG heart might not be directly related to the Na^+ channel, but related to the ADRB1 and K^+ channels. Other studies also support this finding. Down-regulation of the atrial adrenergic β_1 subunit isoforms has been observed in a chronic atrial fibrillation patient [13]. Reduced responsiveness of ADRB1 to catecholamines has been observed in the supraventricular tachycardia-induced cardiomyopathy of the pig [14].

Effects of propranolol on aconitine-induced arrhythmia

Because PAHX-AP1 TG mice have down-regulated ADRB1 in the heart without changes in Na^+ channel

expression, we examined the effect of propranolol on the development of aconitine-induced arrhythmia. In non-TG mice, 4.5 mg/kg of aconitine-induced arrhythmia in 67% of non-TG mice, whereas the same dose of aconitine produced arrhythmia in 100% of TG mice (Table 1). Thus, 4.5 mg/kg of aconitine was administered to induce arrhythmia in both groups (Fig. 4A). Pretreatment with propranolol (0.5 mg/kg) blocked the development of abnormal rhythm in 86% of non-TG mice, but not in PAHX-AP1 TG mice (Fig. 4B, Table 1). The ability of propranolol to block the arrhythmogenic effect of the aconitine in non-TG mice indicated that the β-adrenergic receptor mediates the development of aconitine-induced cardiac arrhythmia.

Pretreatment with propranolol has been reported to elevate the threshold to aconitine-induced arrhythmia, and withdrawal of propranolol increased vulnerability to aconitine-induced arrhythmia in rats [15]. Aconitine and higenamine are components of aconite root, and both aconitine and higenamine potentiate the action of each other. Tachyarrhythmia induced by aconitine (0.16–0.25 μM) in right atrium can be attenuated by quinidine, atropine, and AF-DX 116, suggesting that aconitine is activating sodium channels and muscarinic receptors [5]. Higenamine and dobutamine did not cause

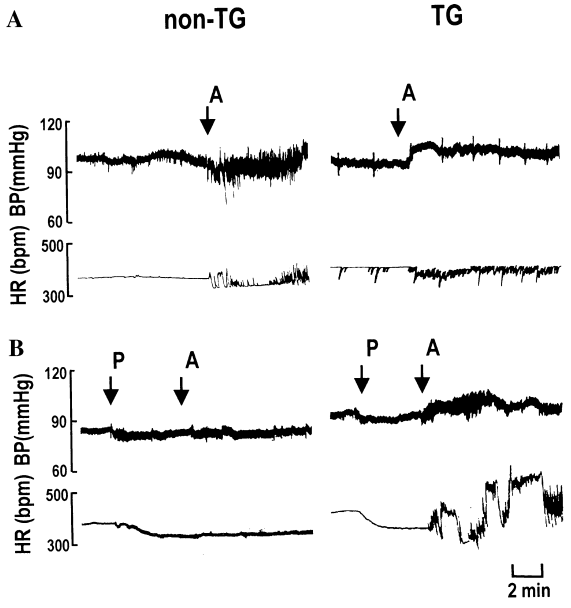

Fig. 4. Effects of the propranolol on aconitine-induced arrhythmia. (A) Representative recordings of BP and HR before and after injection with 4.5 mg/kg of aconitine are shown. The arrhythmia in response to aconitine (arrow with A) was induced in both non-TG and PAHX-AP1 TG mice. (B) The representative recordings of BP and HR after 4.5 mg/kg of aconitine administration under the propranolol (0.5 mg/kg) pretreatment are shown. In non-TG mice, pretreatment with propranolol i.p. (arrow with P) resulted in the suppression of aconitine-induced arrhythmia (left panel), but in the PAHX-AP1 TG mice, the arrhythmia still remained (right panel). The positions of the calibration markers for BP and HR are shown on the left. Timescale bar is 2 min.

chronotropic effects by themselves, but enhance aconitine-induced tachyarrhythmia [5]. These results indicate that higenamine is a β_1-adrenoceptor full agonist in murine atria and that the aconitine-induced tachyarrhythmia is augmented by the β_1-adrenergic action of higenamine. Higenamine exhibited positive chronotropic effects on the isolated right atria of mice and potentiated the aconitine-induced positive chronotropic effect. These positive chronotropic effects were blocked by practolol, a β_1-selective adrenergic antagonist, but not by butoxamine, a β_2-specific adrenergic receptor antagonist [6]. Pretreatment with cholera toxin and forskolin reversed the potentiating interaction between aconitine and higenamine. Thus, it is suggested that the potentiating interaction between aconitine and higenamine involves the β_1-adrenergic signaling pathway and Gi-protein [6]. However, in this study, propranolol did not block the aconitine action in PAHX-AP1 TG heart. Considering the down-regulation of ADRB1 in the PAHX-AP1 TG heart (Fig. 3B), the cardiac adrenergic β-receptor does not seem to have a major role in the hypersusceptibility to aconitine-induced arrhythmia in PAHX-AP1 TG mice.

In the heart of the PAHX-AP1 TG mice, the voltage-gated K^+ channel was up-regulated, like in other reports of atrial tachycardia-induced remodeling [10,11]. However, voltage-sensitive Ca^{2+} channel expression was unchanged. We also observed that Kv2.1 subtype K^+ channel expression was higher in the PAHX-AP1 TG heart. Atrial tachycardia has been observed to result in a decrease or increase of the ion currents caused by changes in the expression of some ion channels. The pattern of atrial tachycardia-induced remodeling is variable, depending on the species, severity and duration of symptoms, drugs used, and genes introduced. For example, in the dog model of atrial tachycardia-related atrial fibrillation (AF), $I_{Ca.L}$, I_{to}, and I_{Na} decreased quantitatively in parallel with changes in mRNA levels for corresponding pore-forming ion channel subunits [16]. In patients with AF, particularly those with long-standing (>6 months) arrhythmia, Ca^{2+} channel 1c subunit mRNA concentration was decreased [17–19]. The substantial decreases in Kv 4.3 mRNA, paralleling changes in I_{to}, have also been reported in patients with AF, along with unchanged expression of Kv1.5 mRNA and the corresponding current I_{sus} [20]. A more recent study of tachycardia-induced atrial remodeling in the rat showed rather rapid increases in Kv1.5 mRNA concentration as early as 30 min after the onset of atrial tachycardia, with slightly slower decreases in Kv4.2 and Kv4.3 expression after 3–4 h. This finding implied that increased Kv1.5 gene expression, having a transient nature, might be responsible for the biochemical electrical remodeling unique to paroxysmal tachycardia [21].

If the K^+ channel activity is elevated as a consequence of mRNA up-regulation, K^+ outward current in

myocardiocytes may be increased, and the membrane will be more electrophysiologically excitable, with shortening of the action potential duration, as we observed in the present study. The up-regulation of K^+ channel and its activation, followed by alteration of K^+ current and action potential, may be the tachyarrhythmogenic mechanism and contribute to the susceptibility to aconitine-induced arrhythmia in the PAHX-AP1 TG heart. In a previous study, we observed various arrhythmogenic effects of K^+ channel blockers (4-AP, glibenclamide, and TEA) [2]. The arrhythmogenic potential of 4-AP was higher in the TG mice than non-TG mice, but there were no differences in the arrhythmogenic potentials of glibenclamide and TEA. The different effects of K^+ channel blockers may be due to their differential effects on the subtypes of the K^+ channel. Further studies are needed to know whether specific K^+ channel blockers suppress the aconitine-induced tachyarrhythmia, and the K^+ channel alterations seen in the PAHX-AP1 TG heart. It might be possible that the density of the distinct potassium channel types in the conduction system and in arrhythmogenic areas of transgenic heart is completely different from normal tissue, possibly increasing the role of potassium channels, such as the Kv1.1 or Kv2.1 channel, which are assumed to not contribute to the potassium currents of the normal cardiomyocyte [22].

K^+ channel mediates the repolarization following an action potential and altered expression of this channel is the possible pathogenic mechanism of a variety of arrhythmias. The effects of commonly used antiarrhythmic drugs on the rapidly activating cardiac voltage-gated potassium channels have been studied in the expression system of the *Xenopus* oocyte, and this study demonstrated marked differences in sensitivity to antiarrhythmic drugs within the group of voltage-operated cardiac potassium channel types [23]. This study also suggests that several antiarrhythmic drugs exert significant effects at rapidly activating cardiac potassium channels. The different effect of the drugs was not related to the fast or slow current inactivation of the potassium channels, but the profiles of the effects of propafenone and flecainide at the different potassium channel types were identical for Kv2.1. Propafenone blocked the Kv2.1 channel in the open state from the intracellular side by entering the inner vestibule of the channel in a time- and voltage-dependent manner [24]. Thus, it seems that PAHX-AP1 TG heart may be the suitable model for testing the activity of new antiarrhythmic drugs on the Kv2.1 channel which is assumed to contribute to the potassium currents of the pathologic cardiomyocyte [22].

Amiodarone, commonly used class III anti-arrhythmic drug for atrial and ventricular arrhythmias, inhibits many membrane currents including Na^+, Ca^{2+}, and K^+ currents. But, its precise mechanism on subtypes of these electrolyte channels is not demonstrated clearly [25]. It was reported that amiodarone suppressed aconitine-induced ventricular tachyarrhythmias by inhibiting Na^+ and Ca^{2+} channels [26]. Thus, the effect of amiodarone on suppression of aconitine-induced ventricular tachyarrhythmias could be explained by its action on inhibiting ion channels. However, in this study, we showed that voltage-gated K^+ channel (Kv2.1) among the ion channels might contribute to the susceptibility to aconitine-induced arrhythmias in heart-targeted PAHX-AP1 transgenic mouse. Basically, the findings of this study are confined to atrial tachyarrhythmia of transgenic heart and further studies are needed to examine the effects of amiodarone on the aconitine-induced arrhythmias in heart-targeted PAHX-AP1 transgenic mouse. It is speculated that the large differences in the effects of different drugs on the different potassium channel types might be a basic principle of antiarrhythmic therapy. Therefore, a disturbed expression pattern of potassium channel types in the arrhythmogenic area of PAHX-AP1 TG heart could be reversed by the suitable antiarrhythmic drug, thus reducing the probability of generating arrhythmic events.

Even with the advancement of molecular biology, the basic mechanism of arrhythmias and the action of antiarrhythmic agents still remained to be revealed. As discussed above, changed potassium channel in PAHX-AP1 transgenic mouse is similar pattern as with many circumstances of arrhythmias in human beings. Three types of atrial tachycardia have been known by automatic, triggered, and reentrant. Especially in children and young adult, triggered activity and enhanced automaticity are important mechanisms for atrial tachycardia [27,28]. Also, aconitine-induced arrhythmia was reported to derive from triggered activity [26]. Thus, this transgenic mouse, having greater susceptibility to aconitine-induced arrhythmia, might be a good model in this situation. And the effectiveness and choice of antiarrhythmic agents in this type of atrial tachycardia could be evaluated in this transgenic mouse. Again, heart-targeted PAHX-AP1 transgenic mouse is a good animal model, especially to study the effects of anti-arrhythmic agents on atrial tachyarrhythmias.

Acknowledgments

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