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Forum Minireview

New Aspects for the Treatment of Cardiac Diseases Based on the Diversity of Functional Controls on Cardiac Muscles: Effects of Targeted Disruption of the Type 5 Adenylyl Cyclase Gene

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Abstract. Cyclic AMP (cAMP) is known to play a major role in regulating cardiac function. Difference in adenylyl cyclase (AC) isoforms is a potential mechanism by which the cAMP signal, a common second messenger signal, can be regulated in a tissue-specific manner. However, the physiological significance of expressing multiple AC isoforms in a tissue and how each specific isoform regulates the cAMP signal remains poorly understood. In a genetically engineered mouse model in which the expression of the type 5 AC is knocked out (AC5KO), we identified the attenuation of autonomic regulation and calcium-mediated inhibition of cardiac function. We also identified that disruption of type 5 AC preserves cardiac function in response to chronic pressure-overload and catecholamine stress, at least in part, through the inhibition of cardiac apoptosis, which plays a major role in the development of heart failure. The protection against both apoptosis and development of cardiac dysfunction induced by left ventricular pressure overload in AC5KO makes this molecule potentially important for developing future pharmacotherapy, where suppressing the activity of type 5 AC, and not the entire β-adrenergic signaling (β-AR) signaling pathway, may have an advantage over the current β-AR–blockade therapy in the treatment of heart failure.

Keywords: cardiac adenylyl cyclase, knockout mouse, autonomic nervous system, heart failure, longevity, cardiac disease

Introduction

Activation of the sympathetic nerves initiates the most potent stimulus for enhancing cardiac function, both acutely and chronically. Norepinephrine is released on the synaptic cleft at sympathetic nerve terminals binds to β-adrenergic receptors (β-AR) on the cardiac sarcolemma and activates the stimulatory guanine nucleotide binding protein Gs by promoting the exchange of GDP for GTP. This reaction catalyzes the dissociation of the GTP-bound Gsα subunit from G$\beta\gamma$. GTP-bound Gsα then binds to and stimulates adenylyl cyclase (AC). AC is a membrane-bound enzyme that catalyzes the conversion of ATP to cyclic AMP (cAMP) (1) (Fig. 1). cAMP, an intracellular second messenger, activates protein kinase A (PKA) by dissociating its regulatory subunit from the catalytic subunit (2). The AC isoform originally isolated from the brain (designated as type 1) is calmodulin-sensitive and expressed only in the brain. Subsequently, several groups, including our own, isolated additional AC isoforms (types 2 through 9). So far, at least nine isoforms are known (3, 4). Types 5 and 6 are both calcium (Ca^{2+})- and Gi-inhibitable and share most, if not all, of their biochemical properties (3, 4). Although these isoforms were cloned from different tissue sources, such as the heart (5, 6), liver (7), and neuronal cells (8), these represent the major AC isoforms in the heart. Although the mRNAs of the ubiquitous isoforms, in particular, types 2, 3, 4, 7, and 9, are detectable in the

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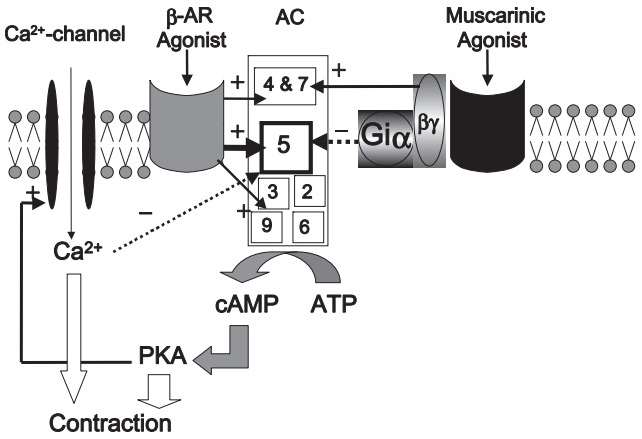

Fig. 1. cAMP signaling in the heart. Cardiac AC in WT consists of mainly type 5 and 2, 3, 4, 6, 7, 9 AC isoforms. All ACs are stimulated by Gsα. Only type 5 and type 6 are strongly inhibited by Giα and Ca^{2+}. G$\beta\gamma$ stimulates type 4 and type 7, but not the other isoforms. PKA increases Ca^{2+} influx through the activation of L-type Ca^{2+} channel, and then the increased intracellular Ca^{2+} inhibits type 5 and type 6 AC (negative feedback system).

heart, the steady state mRNA levels for the type 5 and 6 isoforms are much higher than those of the other isoforms (9). Expression of the type 5 isoform has been most abundant in only two tissues, that is, brain and heart, whereas type 6 is expressed in most tissues at a low level (5, 6). Furthermore, type 5 appears to be an adult isoform, whereas type 6 is more highly expressed in the neonate, at least in rats (10, 11). Expression of the type 6 isoform is most abundant in the fetus, gradually declines with age, and reaches its lowest level in mature adults. The expression of type 5 follows an opposite pattern. The expression is lowest in the fetus, gradually increases with age, and reaches a maximum in the mature adult. Thus, two major isoforms in the heart are developmentally regulated in an opposite manner, a pattern similar to the expression of the α- and β-myosin heavy chains (12). Nevertheless, by mRNA analysis, these two isoforms are the most abundant in the heart at each stage of development. It should be apparent that sympathetic regulation of the heart is importantly determined by the unique AC isoforms expressed in this tissue. Although much data in vitro have been obtained to support this contention, the physiological relevance of this unique pattern of AC isoform expression remains to be determined. The biochemical diversity afforded by the multiplicity of AC isoforms enables a cell, by the nature of the AC isoforms it expresses, to integrate and modulate uniquely its responsiveness to both internal signals (e.g., calcium) and to external stimuli (e.g., hormones and neurotransmitters). The type 5 isoform is the dominant isoform in the adult heart and thereby plays a key role in determining the cardiac response to a variety of stimuli, in particular, stimulation by the sympathetic nerves (Fig. 1).

Changes in the expression level of type 5 AC and those of other isoforms in pathophysiological states may contribute to cardiac dysfunction. Some of the aforementioned hypotheses can be approached using transgenic animal models. Whereas all prior studies have examined these questions in vitro, or in vivo, using pharmacological stimulation, or even by overexpressing isoforms of AC in the heart, we selected the approach of targeted disruption of AC (Fig. 2). However, this experimental design is complicated by the fact that AC consists of nine mammalian transmembrane isoforms. We selected type 5 AC to knock out in the mouse (AC5KO), since this isoform is one of the most prominent in adult cardiac tissues and expressed negligibly in other organs except for the brain. In AC5KO, both basal and isoproterenol (ISO)-stimulated AC activities were decreased by 30% – 40% in cardiac membrane and compensatory increase of the other AC isoforms could not be identified in the heart (Fig. 2D). So far, we have demonstrated a number of interesting pathophysiological roles of type 5 AC using AC5KO (9, 13 – 16), which we will describe below.

Role of type 5 AC in the autonomic and calcium-mediated cardiac regulation

AC assay using crude cardiac membranes showed that increases of AC activities in the presence of ISO were attenuated in AC5KO. Also, decreases of AC activities in the presence of carbachol, a muscarinic agonist, or low concentration of Ca^{2+} (less than 1 μM) was absent in AC5KO. Echocardiography was performed to examine cardiac function. We anticipated that cardiac function [left ventricular ejection fraction, (LVEF)] should be decreased in AC5KO compared to that in wild type (WT). However, to our great surprise, it was not different between AC5KO and WT at baseline, even if the increase of LVEF following ISO and the decrease following acetylcholine on top of ISO was significantly attenuated in AC5KO (Fig. 3: A and B). Conscious AC5KO had a higher heart rate (HR) compared with WT (613 ± 8 vs. 523 ± 11 beats/min, $P<0.01$, $n = 14 – 15$). In addition, baroreflex-mediated bradycardia following phenylephrine was attenuated in AC5KO (Fig. 3C). Electrophysiological studies also demonstrated that Ca^{2+}-channel function was diminished in AC5KO. These finding suggest that disruption of type 5 AC attenuates not only sympathetic responsiveness but also impairs parasympathetic and Ca^{2+}-mediated regulation of the heart (9).

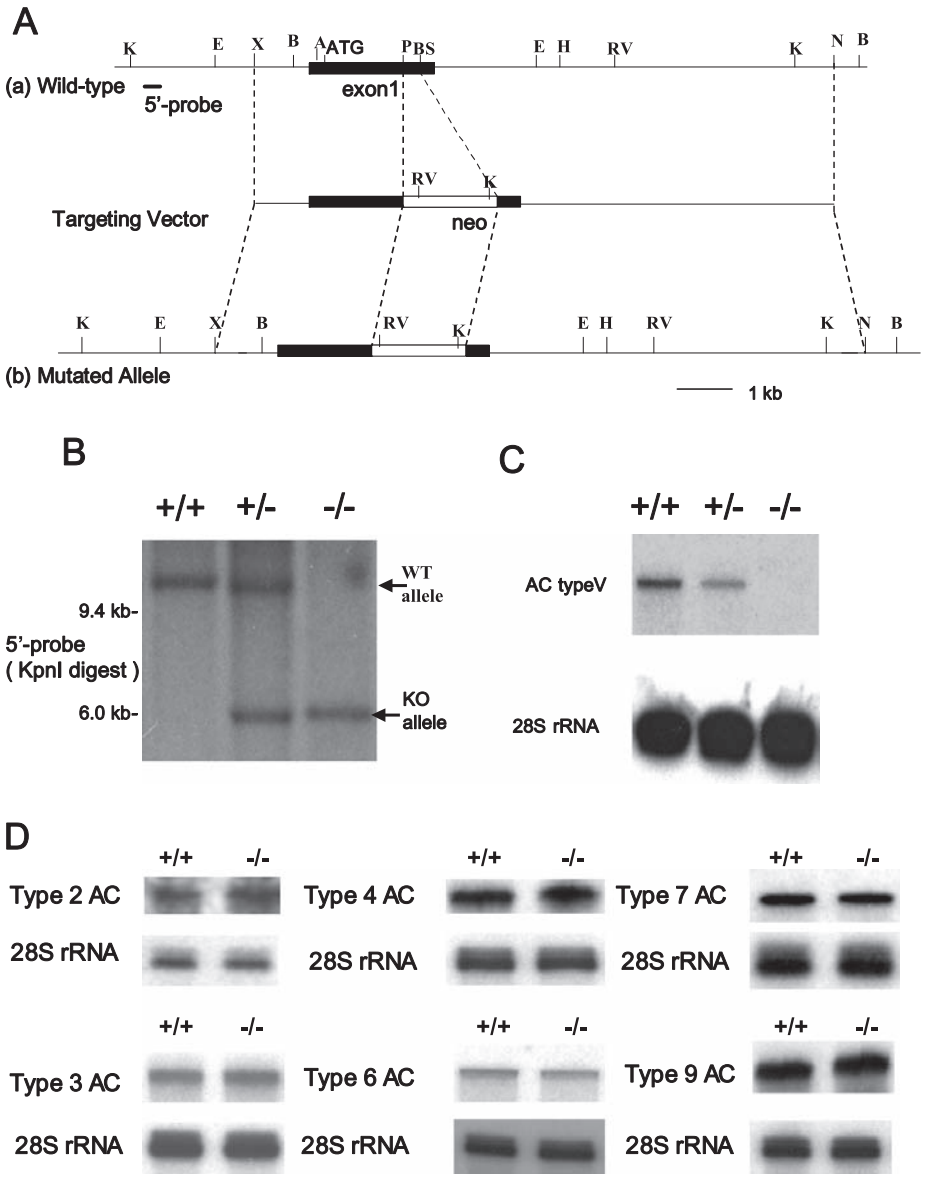

Fig. 2. Generation of AC5KO. A: Targeted disruption of type 5 AC gene in a non-conditional manner. a: Partial structure of the type 5 AC gene, targeting vector construct, and b: resultant mutated allele are shown. The positions of the phosphoglycerate kinase promoter-neo cassette (neo) and 5'-probe (*Eco*RI-*Hind*III, 400-bp) are indicated. K, *Kpn*I; E, *Eco*RI; X, *Xho*I, A, *Apa*I; P, *Pst*I; BS, *Bss*HII, H, *Hind*III, RV, *Eco*RV; N, *Nco*I; B, *Bam*HI. B: Southern blot analysis of genomic DNA from the offspring of F1-heterozygote intercross using 5'-probe. C: RNase protection analysis of type 5 AC and 28S rRNA mRNA in the hearts of WT (WT: +/+), AC5KO heterozygous (Het: +/−), and AC5KO homozygous (KO: −/−) mouse. D: RNase protection assays of types 2, 3, 4, 6, 7, 9 AC and 28S rRNA in the hearts of WT (+/+) and AC5KO (−/−). cRNA of the 28S rRNA was used as an internal control. Types 1 and 8 AC were hardly detectable. Modified from Ref. 9 with permission.

Role of type 5 AC on cardiac function against pressure overload

We next examined the effects of pressure overload, induced by thoracic aortic constriction (TAC), in AC5KO. Cardiac hypertrophy [left ventricular weight / tibial length ratio (LVW/TL)] was not different between WT and AC5KO at baseline and increased progressively and similarly in both groups at 1 and 3 weeks after TAC. However, LVEF was decreased in WT at 3 weeks after TAC with LV dilatation. In contrast, AC5KO did not show a fall in LVEF at 1 and 3 weeks after TAC (Fig. 4). Apoptosis, which has been demonstrated recently to play an important role in the development of heart failure (17 – 19), was examined by TUNEL staining. The number of apoptotic myocytes was similar at baseline, but it increased significantly in WT (4-fold) at both 1 and 3 weeks after TAC, and the number was significantly less in AC5KO (2-fold). More importantly, cardiac apoptosis was induced prior to cardiac dysfunction in WT. Bcl-2, which is known as an anti-apoptotic protein (20), was increased significantly more in AC5KO with pressure overload. These findings suggest that disruption of type 5 AC plays a protective role in response to pressure overload and the development of heart failure, potentially through limiting the induction of cardiac apoptosis.

Role of type 5 AC in the desensitization with chronic catecholamine stress

Desensitization of the β-adrenergic signaling protects

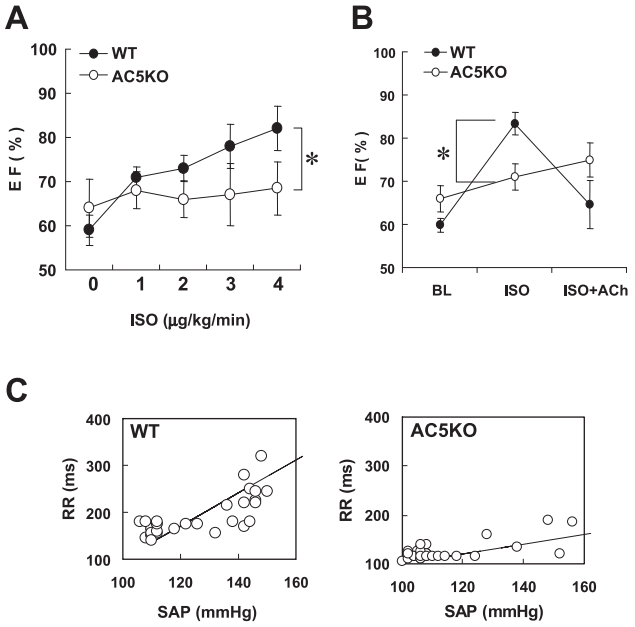

Fig. 3. In vivo cardiac function. A: Baseline (BL) ejection fraction (EF) was similar between WT and AC5KO, but did not increase normally in response to β-AR stimulation with ISO in AC5KO. B: Acetylcholine (ACh; 25 mg/kg, i.p.) superimposed on ISO (0.04 μg/kg/min, i.v.) reduced EF in WT, but not in AC5KO. C: In vivo conscious heart rate (HR). Parasympathetic slowing of HR in response to phenylephrine (0.02 μg/g, i.v.)-induced increase in arterial pressure is shown by the plot of systolic arterial pressure (SAP) vs. R-R interval (ms). The depressed slope indicates that reflex parasympathetic bradycardia was impaired in AC5KO. Modified from Ref. 9 with permission.

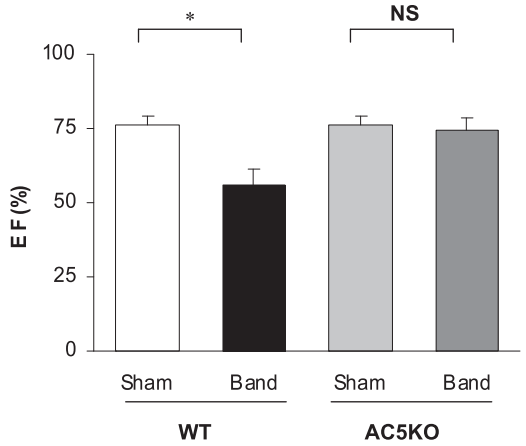

Fig. 4. Echocardiographic measurements of LV ejection fraction (EF) were performed in WT and AC5KO after 3 weeks of banding (Band). The data were compared with those from sham (Sham)-operated controls at 3 weeks. LVEF was significantly decreased after 3 weeks of banding in WT (n = 8) but not in AC5KO (n = 10), while both determinations were unchanged between in sham-operated in WT (n = 6) and AC5KO (n = 6). *P<0.05; NS, not significant. Modified from Ref. 13 with permission (Copyright 2003, National Academy of Sciences, U.S.A.).

the heart against chronic catecholamine stress, thus preventing the development of apoptosis. However, previous studies have been mainly focused at the level of adrenergic receptors, but less at the level of AC. Thus, the effects of chronic ISO infusion (30 – 60 mg/kg per day for 1 – 2 weeks) were examined using AC5KO, and we found that the responses of LVEF to ISO infusion were attenuated in AC5KO compared with WT, that is, physiological desensitization was more effective in AC5KO (Fig. 5A). The mechanism for the less effective desensitization in WT was at least in part through the paradoxical upregulation of type 5 AC protein expression. As in the TAC models, the increase of apoptotic myocytes was smaller and upregulation of Bcl-2 after chronic ISO infusion was greater in AC5KO with the activation of PDK1 (phosphoinositide-dependent protein kinase 1) -Akt signaling, which was recently demonstrated to be one of the strong protective and anti-apoptotic pathways in the heart. These results suggest

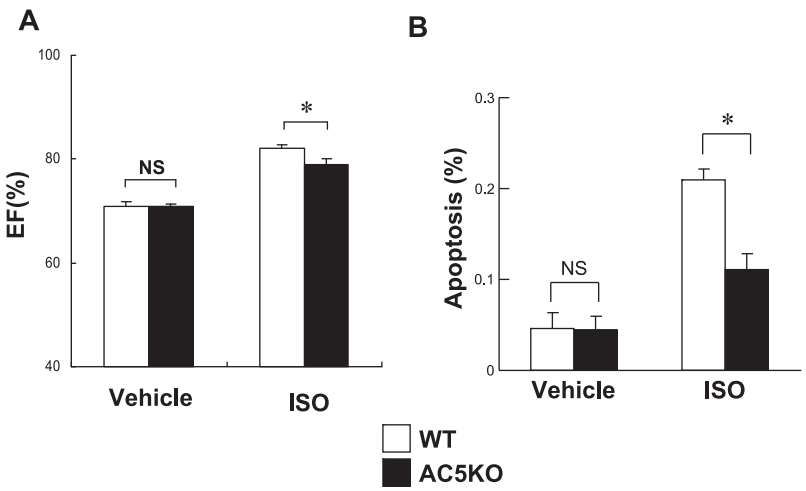

Fig. 5. Cardiac function and apoptosis after catecholamine stress. A: Changes in LV ejection fraction (EF) after chronic ISO-infusion (60 mg/kg /day for 7 days) in WT and AC5KO. LVEF was not different between WT and AC5KO with vehicle, but was greater in WT than AC5KO after chronic ISO-infusion. *P<0.05, n = 9 – 11. B: TUNEL-positive myocytes in LV myocardium were counted in WT and AC5KO and expressed as % of myocytes. The number of TUNEL-positive myocytes was significantly smaller in AC5KO than in WT after chronic ISO. *P<0.05; NS, not significant, n = 7 – 10 each. Modified from Ref. 14 with permission.

that the disruption of type 5 AC results in more effective desensitization after chronic catecholamine stress and protects against the development of myocyte apoptosis and deterioration of cardiac function (Fig. 5B) (14).

Role of type 5 AC in the regulating life span

AC5KO has an increased median lifespan (30%) and are protected from reduced bone density and susceptibility to fractures of aging. Old AC5KO mice are also protected from aging-induced cardiomyopathy, e.g., hypertrophy, apoptosis, fibrosis, and reduced cardiac function with the activation of the Raf/MEK/ERK signaling pathway and upregulation of cell protective molecules, including superoxide dismutase. Fibroblasts isolated from AC5KO mice exhibited ERK-dependent resistance to oxidative stress. These results suggest that type 5 AC plays an important role in regulating lifespan and stress resistance (15).

Role of type 5 AC in stabilizing HR under microgravity

Autonomic nervous activity is known to be altered under microgravity, leading to disturbed regulation of cardiac function, such as heart rate. In order to examine the role of type 5 AC for regulating cardiac function under microgravity, we analyzed HR variability of AC5KO under microgravity induced by parabolic flight. The inverse of HR, that is, the R-R interval was much more variable in AC5KO than that in WT (Fig. 6B). The standard deviation of normal R-R intervals (SDNN), a marker of total autonomic variability, was significantly greater in the microgravity phase in both AC5KO and WT, but the magnitude of increase was much greater in AC5KO (Fig. 6A). These results suggesting that type 5 AC plays a major role in stabilizing HR under microgravity (16).

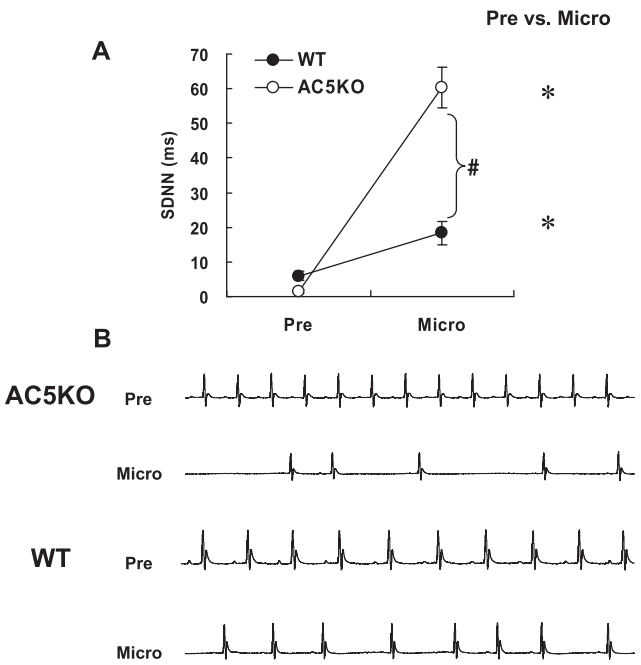

Fig. 6. The effect of parabolic flight on R-R interval in AC5KO. A: The standard deviation of normal R-R intervals (SDNN) between pre-microgravity (Pre) and microgravity (Micro). SDNN was compared between pre-microgravity and microgravity (*P<0.01 vs. pre-microgravity, n = 4 – 8) and between WT and AC5KO at microgravity ($^{\#}P$<0.01, n = 4 – 8). B: Representative ECG under pre-microgravity (Pre) and microgravity (Micro) in each group. Modified from Ref. 16 with permission.

Conclusion

From our intensive experiments using AC5KO, type 5 AC has been demonstrated to play an important role not only in autonomic regulation but also Ca^{2+}-mediated regulation in the heart. Also, disruption of type 5 AC protects the heart against chronic pressure-overload and catecholamine stress, which is known to play a major role in the development of heart failure (21). These findings, taken together, make type 5 AC potentially important for studies designed to develop future β-AR blockade therapy, where suppressing the activity of type 5 AC may have an advantage in the treatment of heart failure (22, 23).

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