Adenylyl cyclase type 5 in cardiac disease, metabolism, and aging

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Adenylyl cyclase (AC) is the enzyme that catalyzes the conversion of ATP to adenosine 3',5'-cyclic monophosphate (cAMP), a key intracellular second messenger, which in the heart mediates inotropy and chronotropy. There are nine isoforms of membrane-bound AC, with type 5 being one of the two major isoforms in the heart. Since the role of AC in the heart in regulating cAMP and acute changes in inotropic and chronotropic state are well known, this review will address our current understanding of the distinct regulatory role of the AC5 isoform in response to chronic stress. Transgenic overexpression of AC5 in cardiomyocytes of the heart (ACS-Tg) improves baseline cardiac function but impairs the ability of the heart to withstand stress. For example, chronic catecholamine stimulation induces cardiomyopathy, which is more severe in ACS-Tg mice, mediated through the AC5/sirtuin 1/forkhead box O3a pathway. Conversely, disrupting AC5, i.e., AC5 knockout, protects the heart from chronic catecholamine cardiomyopathy as well as the cardiomyopathies resulting from chronic pressure overload or aging. Moreover, AC5 knockout results in a 30% increase in a healthy life span, resembling the most widely studied model of longevity, i.e., calorie restriction. These two models of longevity share similar gene regulation in the heart, muscle, liver, and brain in that they are both protected against diabetes, obesity, and diabetic and aging cardiomyopathy. A pharmacological inhibitor of AC5 also provides protection against cardiac stress, diabetes, and obesity. Thus AC5 inhibition has novel, potential therapeutic applicability to several diseases not only in the heart but also in aging, diabetes, and obesity.

Adenylyl cyclase type 5; cardiomyopathy; aging; metabolism; AC5 inhibitor
an intracellular NH2-terminus, two repeats of six transmembrane domains (M1 and M2) and two cytoplasmic catalytic domains of ~40 kDa each (C1 and C2). Crystal structure-coupled with biochemical data indicate that two cytosolic domains form the catalytic core pocket, and ATP binds at one of two pseudosymmetric binding sites at the C1-C2 interface (10, 75). Forskolin binds in two almost equivalent pockets at either end of C1 and C2 domains (87). For the isoforms of AC1, AC2, and AC5, expression of either the α-half (M1/C1) or the β-half (M2/C2) of the molecule alone is insufficient to generate enzymatic activity. The specificity of AC response likely depends on the creation of intracellular microdomains containing signaling molecules. In the submicromolar range of Ca2+, the sensitivity of ACs for Ca2+ is coupled with distinct subcellular localization of Ca2+-sensitive AC isoforms (82, 83), suggesting a temporally and spatially distinct pattern of cAMP signaling, depending on the localization of ACs in Ca2+ microdomains within the plasma membrane or cytoplasm. For instance, studies in overexpression models suggested that AC8 may augment cardiac contractility by preferentially activating Ca2+ loading of sarcoplasmic reticulum through cAMP compartmentation, rather than enhancing Ca2+ influx via L-type Ca2+ channels (21). Dyachok et al. (12) suggested that oscillations of cAMP lead to selective target activation by restricting the spatial redistribution of PKA (12). β-Adrenergic receptors (β-AR) are selectively located in plasma membrane lipid raft microdomains, resulting in more efficient coupling to AC compared with nonlipid raft microdomain receptors, such as the E-prostanoid-2 receptor. Signaling modules that include AC isoforms also contain A kinase anchoring proteins (AKAPs), PKA, and anchored phosphodiesterases to provide microdomains of cAMP production and signaling (2, 34, 82, 86).

Since AC signaling in general and AC5 signaling in particular have been extensively reviewed (31, 56, 67), this review will focus on AC5 and its regulation of responses to chronic stress and disease. We will also provide a brief overview of the potential translational direction of this work, discussing some of our recent findings with a pharmacological AC5 inhibitor.

β-AR-G Protein-AC-cAMP Signaling Pathway

The β-AR-G protein-AC-cAMP signaling pathway is one of the major pathophysiological mechanisms for regulation of cardiac function (31, 45, 47, 78). By targeting Ca2+ handling proteins, it provides strong inotropic and chronotropic response in times of need, such as in fight or flight (22, 48, 70, 72). Throughout much of the 20th century, it was believed that stimulation of this pathway could provide inotropic support and should be used in heart failure therapy. It was shown that transgenic (Tg) mice with up to 60-fold overexpression of β2-AR had enhanced cardiac function without signs of cardiac pathology (46, 51). Furthermore, β2-AR transgene experiments showed improvement in function in failing rabbit hearts (76). More recent work with adenoviral-mediated β2-AR transgene overexpression demonstrated enhanced cardiac function in a rat model of heart failure (65). However, the concept of treating heart failure with chronically enhanced β-AR stimulation became controversial when patients responded positively to acute β-AR inotropic therapy, particularly with dopamine and dobutamine, but had poor outcomes when on prolonged inotropic therapy (14, 44, 55). An experimental study that first highlighted the adverse effects of chronic β-AR signaling was shown in Gsα Tg mice (36). Although these animals had higher responsiveness to isoproterenol (Iso) when young, a picture of cardiomyopathy developed as they aged, including myocardial hypertrophy, fibrosis and necrosis, and depression of cardiac function (1, 36, 37). Later studies using β1-AR (15, 16, 63) and β2-AR (11, 63)-overexpressed models confirmed these findings, i.e., hyperfunction at young age and deterioration of function with aging. These studies (1, 11, 15, 16, 37, 63) in combination with clinical studies showing poor outcomes in patients on β-AR agonists (14, 44, 55) and Bristow’s classical study in The New England Journal of Medicine demonstrating desensitization of the β-AR in patients with heart failure (4) changed the paradigm from treating patients with heart failure with β-AR agonists to antagonists (7, 8, 30, 60, 68). Heart failure still remains as the leading cause of mortality and morbidity in the United States. For this reason, targeting components distal to the β-AR signaling, such as ACs, will be important for the development of future treatment of heart failure.

AC in the Heart

Whereas AC2, -3, -4, -5/6, and -7 are detected in rat cardiac fibroblasts (59), AC5 and AC6 are the two major isoforms expressed in the adult mammalian heart (23, 35). Both AC5 and AC6 regulate heart rate and contractility, but AC6 plays a more significant role at baseline in view of the relatively minor reduction in AC content and corresponding reductions in cardiac contractility observed in AC5 knockout (AC5-KO) hearts (58). However, the role of these two major isoforms in the heart in mediating the response to cardiac stress is controversial. In this article, we first review the studies demonstrating an adverse effect of overexpression of AC5 and beneficial effects of disrupting AC5 on cardiomyopathies induced by chronic Iso stimulation, aging, and pressure overload in either AC5-Tg or AC5-KO mice. This leads to a discussion of other factors involved in AC5 protection against aging, e.g., metabolism and diabetes. Since not all studies are in agreement, we then discuss those with an opposite point of view and reconcile the differences. The controversial studies on AC6 overexpression and disruption are beyond the scope of this review, which focuses on AC5.

Regulation of Cardiomyopathy by AC5

Chronic catecholamine cardiomyopathy. Chronic Iso increased oxidative stress and induced a more severe cardiomyopathy in AC5-Tg compared with wild-type (WT) mice, as reflected by a greater impairment of left ventricular (LV) ejection fraction (EF) along with greater LV dilation and increased fibrosis, apoptosis, and hypertrophy (41) (Fig. 1, A and B). LV EF fell more (P < 0.05) in AC5-Tg than WT mice (~35 ± 2 vs. −18 ± 1%). Oxidative stress induced by chronic Iso was greater in AC5-Tg hearts, whereas protein expression of manganese superoxide dismutase (MnSOD), which protects against oxidative stress, was reduced by 36%, suggesting that the increased severity of the cardiomyopathy in AC5-Tg may have resulted as a consequence of decreased MnSOD expression. This was confirmed by mating AC5-Tg with MnSOD-Tg mice. These bigenic mice no longer responded to chronic Iso with more severe cardiomyopathy than WT mice. In fact, LV EF fell less in AC5-Tg × MnSOD-Tg (~13 ± 1%) versus either AC5-Tg or WT mice. LV EF fell similarly in
MnSOD-Tg alone (−13 ± 2%). Conversely, AC5-KO mice are protected from the cardiomyopathy induced by chronic Iso treatment (58), as reflected by less of a reduction with chronic Iso (\(P < 0.05\)) in AC5-KO than WT (−10 ± 2 vs. −19 ± 2%) mice, and this protection was lost in bigenic AC5-KO mice mated with MnSOD heterozygous KO mice, where LV EF fell by −18 ± 3% (Fig. 1, C and D). The decrease in LV EF with chronic Iso in the bigenic AC5-KO × MnSOD heterozygous mouse was similar to that in the MnSOD heterozygous alone (−18 ± 3 vs. −18 ± 4%). The decreases in LV EF must be interpreted with the histological changes in the heart consistent with chronic cardiomyopathy, e.g., fibrosis and apoptosis. When the data are all taken together, the picture of intensification of cardiomyopathy with AC5-Tg and protection with MnSOD-Tg or AC5-KO becomes even more apparent. We also demonstrated that AC5, but not AC6, regulates MnSOD at the transcriptional level via the sirtuin 1/forkhead box O3a pathway (Fig. 2). Thus the cardiomyopathy induced by chronic catecholamine stress is intensified in AC5-Tg by inhibiting sirtuin 1/forkhead box O3a, which downregulates MnSOD transcription, resulting in oxidative stress intolerance (41).

In addition, it was reported that myocardial AC5 mRNA expression was increased from 5–12 wk in spontaneously hypertensive rats, which was accompanied by development of LV hypertrophy and hypertension (20). Recently, from mi...
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CR on the AC5-KO would combine their potentially different mechanisms mediating longevity resulting in a superlongevity model. This hypothesis was not correct, and superimposing CR on the AC5-KO was uniformly lethal within a month (79, 84). AC5-KO mice on CR developed a syndrome similar to starvation, as evidenced by greater decrease in body weight, blood glucose, fat, and glycogen storage, and greater increase in ketone bodies than either AC5-KO or CR alone. Accordingly, we adopted the converse hypothesis that the longevity mechanisms were similar in the two models. To test this, we recently compared AC5-KO model with CR in terms of physical phenotype as well as metabolic and gene expression profiles (84). Similar to the mice on CR, AC5-KO mice exhibit a lower body weight, reduced fat accumulation (Fig. 4B) and glycogen stores, and lower fasting blood glucose levels. However, in contrast to CR with restricted food intake, AC5-KO mice eat more compared with their WT littermates. Microarray analysis revealed a remarkable similarity of gene profiles between AC5-KO and CR mice in the heart, skeletal muscle, and brain (Fig. 4A). Many tissue-specific pathways in the regulation of metabolism, longevity, and stress resistance overlap in the AC5-KO and CR mouse models, including sensory perception in heart and brain, muscle function in skeletal muscle, and lipid metabolism in liver (Fig. 4C). Importantly, the similarly regulated genes and pathways for AC5-KO and CR will begin to establish a unified theory for longevity, stress resistance, and potentially for diabetes and obesity.

Diabetic cardiomyopathy. A key extrapolation from the above study comparing AC5-KO and CR is that both models of longevity protect against glucose intolerance and insulin resistance (24, 32, 80, 84) and, taken together with AC5-KO’s ability to protect against pressure overload and catecholamine cardiomyopathy, raises the likely probability that AC5-KO also might protect against diabetic cardiomyopathy. Even at

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Aging cardiomyopathy. The genetically engineered mouse model in which type 5 AC was knocked out, i.e., AC5-KO mice, have increased median life span of ~30% (Fig. 3A) and are protected from aging-induced cardiomyopathy (85), including decreased LV hypertrophy, decreased fibrosis, and decreased apoptosis compared with WT as they age (Fig. 3B). Using a proteomic-based approach, we demonstrated a significant activation of the Raf/MEK/ERK signaling pathway, which results in protection from oxidative stress, leading to longevity in AC5-KO mice (Fig. 2). In addition to the prolonged life and protection against aging cardiomyopathy in AC5-KO mice, this model also exhibits protection against the osteoporosis of aging (85). Furthermore, both young and old AC5-KO mice had better exercise endurance than WT mice of the corresponding age. These beneficial effects of AC5 disruption on aging are synergistic in clinical relevance of AC5 inhibition, since elderly patients have an increased prevalence of heart failure (42, 43).

AC5-KO model vs. calorie restriction models of longevity. Calorie restriction (CR) is the most widely studied model of longevity (5, 50, 71). Our hypothesis was that superimposing CR on the AC5-KO would combine their potentially different mechanisms mediating longevity resulting in a superlongevity model. This hypothesis was not correct, and superimposing CR on the AC5-KO was uniformly lethal within a month (79, 84). AC5-KO mice on CR developed a syndrome similar to starvation, as evidenced by greater decrease in body weight, blood glucose, fat and glycogen storage, and greater increase in ketone bodies than either AC5-KO or CR alone. Accordingly, we adopted the converse hypothesis that the longevity mechanisms were similar in the two models. To test this, we recently compared AC5-KO model with CR in terms of physical phenotype as well as metabolic and gene expression profiles (84). Similar to the mice on CR, AC5-KO mice exhibit a lower body weight, reduced fat accumulation (Fig. 4B) and glycogen stores, and lower fasting blood glucose levels. However, in contrast to CR with restricted food intake, AC5-KO mice eat more compared with their WT littermates. Microarray analysis revealed a remarkable similarity of gene profiles between AC5-KO and CR mice in the heart, skeletal muscle, and brain (Fig. 4A). Many tissue-specific pathways in the regulation of metabolism, longevity, and stress resistance overlap in the AC5-KO and CR mouse models, including sensory perception in heart and brain, muscle function in skeletal muscle, and lipid metabolism in liver (Fig. 4C). Importantly, the similarly regulated genes and pathways for AC5-KO and CR will begin to establish a unified theory for longevity, stress resistance, and potentially for diabetes and obesity.

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Fig. 3. Disruption of AC5 protects against cardiac stress. A: effects of AC5-KO on longevity (85). B: effects of AC5-KO on aging cardiomyopathy. LV EF, apoptosis, and fibrosis were significantly different in old AC5-KO vs. old WT. *p < 0.05. Figures used are modified with permission from Yan et al. (85).
In the absence of a high-fat diet, levels of fasting glucose and insulin resistance were lower in AC5-KO (Fig. 4D). Our preliminary results suggest that AC5-KO protects against diabetic cardiomyopathy (32). When the AC5-KO and their WT were placed on a high-fat diet, the WT rapidly developed a reduction in cardiac function with histopathological evidence of cardiomyopathy, as typically reported in the literature (6, 18, 62). However, the AC5-KO was protected against high-fat diet-induced cardiomyopathy (32). These observations underlie several important and clinical relevant questions. For example, is the protection of the cardiomyopathy due to an action of AC5-KO on the heart, i.e., the target organ of the cardiomyopathy, or is it indirectly due to an action on metabolism, i.e., the initiating cause of the cardiomyopathy? These and other related investigations are currently underway.

Controversy in role of AC5 in the heart. Not all studies have found that overexpression of AC5 is deleterious or that its disruption is salutary. For example, when AC5 is overexpressed in the heart, LV function is improved as well as the response to exercise (17). This is not particularly surprising since increasing any component of the β-AR signaling pathway, even at the level of the β-receptor, improves cardiac performance at baseline and in the response to exercise, as we have also observed in our AC5-Tg models. The adverse effects appear much later with chronically enhanced β-AR stimulation. The bottom line is that patients with heart failure respond favorably to β-AR blockade over the long haul but have increased mortality with chronically enhanced β-AR stimulation. A more controversial finding is that AC5-Tg was able to rescue Gq overexpression-induced cardiomyopathy (74) but not cardiomyopathy induced by cardiac overexpression of β-AR (64). Conversely, AC5-KO mice were not able to rescue Gq overexpression-induced cardiomyopathy (77). These seemingly different results from rescue of cardiomyopathy (57,
58, 77) are not likely due to different backgrounds in the KO mice, but rather reconciliation of the differences in these studies is more apparent when understanding the signaling pathways. For example, Tg mice with cardiac-specific overexpression of Gαq showed that the cardiomyopathy was mediated by PKC with a significant reduction in AC5. Therefore, it is logical that replacing AC5 in this situation would be beneficial and that reducing it further, as with the AC5-KO, would not be beneficial. However, β-AR or chronic Iso-stimulated cardiomyopathy is mediated by PKA with increased levels of AC5 (58). These results, taken together, support our hypothesis that chronically elevated levels of AC activation, like β-AR (11, 16, 63) or Gαq (1, 36, 37), are deleterious and facilitate development of cardiomyopathy. In contrast, when a cardiomyopathy develops associated with reduced levels of AC5, restoration of AC5 expression may be beneficial for normal cardiac function under these conditions.

**Clinical Relevance of AC5**

Although hundreds, if not thousands, of novel and exciting discoveries have been made by alterations in genes in genetically engineered mice, relatively few have translated into improving clinical care. One reason for the lack of success is that it is difficult to overexpress or delete a gene in patients. Therefore, the goal becomes to have a pharmacological analag of the altered gene that can be safely delivered to patients. A current goal of our laboratory is to translate the beneficial effects of the AC5-KO model to clinical therapy. In this connection, while screening for a commercially available drug for the AC5 inhibition, adenine-9-β-D-arabinofuranoside (Ara-A; Vidarabine) showed a selective inhibition of AC5. Recent studies in our laboratory demonstrated that Ara-A selectively inhibits AC5 activity in AC5-Tg mice, but not in AC6-Tg mice. In cardiac membrane preparations with Iso stimulation, Ara-A (10 μM) reduced cAMP production much more in AC5-Tg (49%) than in WT and not at all in AC5-KO (38). Ara-A was originally developed as an antiviral drug, which was approved by the United States Food and Drug Administration. It has been clinically used for treatment of herpes virus infection, but it was found to be less efficient for viral therapy than the newer drug, acyclovir. We also found that this pharmacological AC5 inhibitor recapitulates the favorable effects of AC5 disruption and ameliorated the development of cardiomyopathy and heart failure induced by either permanent coronary artery occlusion or chronic catecholamine infusion (38). Ara-A significantly improved the survival rate and LV function compared with vehicle after 3 wk of coronary artery occlusion, and these beneficial effects of Ara-A were abolished by U0126, a MEK inhibitor, suggesting the involvement of the downstream MEK-ERK pathway of AC5 (38). This is significant since the same signaling pathway was found mediating the longevity in AC5-KO (85). In heart failure, Ara-A has also been shown to reduce autophagy by inhibition of AMPK (49). Since toxicology for the drug has found little to be contraindicated in heart failure and since adverse effects were only manifest with very high chronic doses, low dose Ara-A is a strong candidate for a clinical trial for heart failure since it selectively inhibits AC5, has been shown to protect against heart failure without adverse effects, and has been already approved by the United States Food and Drug Administration. One potential limitation to this drug is that only an intravenous formulation is currently available. Accordingly, drug discovery studies will have to be pursued for oral delivery and optimizing the compound for heart failure applications.

**Conclusions**

There are several take-home messages. First, although AC5 and AC6 are the two major isoforms in the heart, they mediate dramatically different functions, particularly in response to stress. Second, although AC5 is one of the major cardiac isoforms of AC, potentially its most important role will be in mediating diabetes, obesity, and longevity, even more so than in cardiac protection. Finally, it may be possible to translate the beneficial effects of the AC5-KO to the bedside, by using a pharmacological analog, which preferentially inhibits AC5.

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**DISCLOSURES**

S.F. Vatner and D.E. Vatner both work for Vasade Biosciences, which also has research interests in AC5 but has no intellectual property and did not provide support for this study. All research was funded by the NIH.

**AUTHOR CONTRIBUTIONS**

L.Y. drafted manuscript; S.F.V., M.P., G.J.A.L., L.L., K.I., Y.I., J.E.P., and D.E.V. edited and revised manuscript; S.F.V. and D.E.V. approved final version of manuscript; M.P. prepared figures.

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