Protein Kinase A Transgenes
The Many Faces of cAMP

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The β-adrenergic receptor/adenyl cyclase/protein kinase A (PKA) axis is the central signaling pathway that serves to stimulate cardiac function. It is classically perceived as a linear signaling cascade (Figure), and cAMP is thought to be the second messenger responsible for the positive inotropic, chronotropic, and lusitropic effects of catecholamines. New data gained from a series of transgenic animals overexpressing various elements of this axis show that this system has a far more complex makeup than previously thought.

It has been known for about two decades that the β-adrenergic receptor system is dysfunctional in heart failure.1 The loss of receptor responsiveness has been attributed to a reduced β-receptor number, particularly of the β1-subtype, plus a functional desensitization of the remaining receptors—presumably mediated via increased activity of G protein–coupled receptor kinases.2 The extent of the receptor downregulation and the functional loss correlate with the severity of the disease.3,4 This phenomenon has long been regarded as detrimental to the compromised function of the failing heart, and it has been proposed that restoration of β-adrenergic receptor responsiveness might be a strategy to improve cardiac function.

However, recent clinical studies and observations on a panel of transgenic animals suggest that the opposite is the case. Sustained stimulation or overexpression of cardiac β1-adrenergic receptors leads to hypertrophy, fibrosis, and heart failure,5–7 and blockade of β-adrenergic receptors with several antagonists—bisoprolol, carvedilol, and metoprolol—improves survival in heart failure patients.8 Thus, the loss of β-adrenergic receptor responsiveness in heart failure is more likely to represent a protective mechanism than a causative factor in heart failure,9 and further protection can be achieved in patients by shutting off this system altogether with β-receptor antagonists.

This concept receives support from transgenic animals overexpressing other components of the β-adrenergic receptor axis (Figure) as well as recent studies on human heart samples. These transgenic animals include mice that overexpress or lack the β1- and/or β2-adrenergic receptors, the G protein–coupled receptor kinase GRK2 or its C-terminal domain, Gaß, adenyl cyclases, and phospholamban. A new study by Antos et al10 in this issue of Circulation Research, on overexpression of the catalytic subunit of PKA can be added to this list. Most of these studies support the view that sustained overactivity of this system is detrimental to the heart, but some models appear to contradict this notion.

Mice lacking β1- and/or β2-adrenergic receptors have essentially normal basal cardiac function11 whereas overexpression of the β1-receptor is detrimental.6,7 Altered Ca2+ handling may be responsible for the latter phenotype.12 Surprisingly, such changes do not occur in ß2-subtype–overexpressing animals unless overexpressed at very high levels.13,14 Overexpression of the α-subunit of Gaß, the next element in the axis, causes a phenotype similar to the β1-receptor transgene and is characterized initially by increased responsiveness to catecholamines but later by myocyte hypertrophy, fibrosis, and heart failure.15

A few of the downstream targets of PKA have also been studied in transgenic or knockout models. Overexpression of phospholamban, the negative regulator of the sarcoplasmic Ca2+-ATPase (SERCA), causes reduced cardiac function16 and its knockout is associated with enhanced myocardial contractility.17 In line with these observations, overexpression of SERCA itself seems to be beneficial.18,19 It is well established that heart failure in patients and in many experimental models is characterized by reduced function and/or expression of SERCA.

While all these models point to a negative role of persistent activity of the β-adrenergic receptor/cAMP axis, overexpression of two types of adenylyl cyclase in the heart has been reported to result in enhancement of adrenergically stimulated cAMP levels,20,21 improved cardiac function, and absence of fibrosis even in older animals.21 These observations raised the question whether the negative consequences of overexpression of the upstream members of the signaling cascade were perhaps not mediated via cAMP and PKA.

To address this controversial issue of the role of cAMP in the development of heart failure, Antos et al10 have overexpressed the catalytic subunit of PKA in the hearts of transgenic mice. PKA is composed of four subunits, two regulatory and two catalytic subunits. Upon binding of cAMP to the regulatory subunits, the catalytic subunits are released and thereby become active. Overexpression of the catalytic subunit alone therefore resulted in constitutive activity of this kinase independent of the upstream elements. Like the β1-receptor and Gaß-overexpressing animals, these animals showed cardiomyocyte hypertrophy, fibrosis, and a progressive decline in cardiac function resulting ultimately in death from cardiac failure; these effects were more prominent at higher levels of PKA overexpression. The observations of the present study fit well into the general picture that sustained...
stimulation of the β-adrenergic receptor/cAMP pathway is harmful for the heart. These observations further seem to suggest that the damage in the β1-adrenergic receptor- and Gαs-overexpressing models is indeed mediated by cAMP and PKA.

Several intriguing questions arise from this important new study. The most puzzling aspect is the comparison with the observation that adenylyl cyclase overexpression permits the rescue of a heart failure model.22 Thus, overexpression of upstream (β1-adrenergic receptor, Gαs) and downstream (PKA) elements of adenylyl cyclase seems to cause cardiac damage, whereas adenylyl cyclase itself is beneficial. A related issue is the question why overexpression of β1- but not β2-adrenergic receptors causes damage, since they both signal via cAMP and PKA. These contrasting observations cannot be explained by the linear signaling cascade that is depicted in the Figure. Various modifications of the classical model of this cascade and explanations of the findings can be envisioned.

A very general explanation is that transgenic overexpression of the various proteins may not be equivalent to stimulation of the endogenous proteins. There may be differences between endogenous and overexpressed proteins with respect to their localization, interaction with other proteins, and specificity of signaling. This should lead to caution in interpreting data from overexpressing animals. A more interesting potential explanation is the activation of alternative signaling pathways. Thus, it has been argued that a key difference between the β1- and the β2-adrenergic receptors is the ability of the latter to couple not only to Gs but also to Gi, and to activate signaling pathways other than cAMP/PKA, for example, the mitogen-activated protein kinase pathways. It is not known whether the overexpression of adenylyl cyclase affected cAMP-independent signals. However, although distinct signaling of β1- versus β2-adrenergic receptors has been shown in short-term experiments, it remains to be proven that the same occurs during long-term stimulation and that it is causally related to the cardiac phenotype.

A third potential explanation is that it matters where and when cAMP is generated. There may be a different temporal activation pattern of the various overexpressed proteins. For example, while there is continuous PKA activity in the PKA transgene, the adenylyl cyclase transgene is under the control of Gαs and thus dependent on β-adrenergic receptor activation. Indeed, compared with the robust increase in basal PKA activity in the PKA transgenes, basal levels of cAMP are low in the adenylyl cyclase transgenes and increase only after agonist stimulation. This dosing of cAMP “as needed” might then avoid the deleterious consequences of continuous stimulation. However, the same argument should apply to the overexpressed β1-receptors, since these have comparably little spontaneous activity23 and would also largely depend on activation by catecholamines to produce a continuous signal and a corresponding phenotype. An alternative explanation for the observed differences would be that it matters where the cAMP is produced in the cardiomyocyte, that is, there might be distinct spatial formation of cAMP. Following this theory of compartmentation, the overall cAMP content of a cell is of limited value to assess its biological impact, but rather the precise localization of the generated cAMP determines its cellular targets and effects.24,25 Such compartmentation of cAMP production has been proposed to account for the remarkable differences between β1- and β2-adrenergic signaling in cardiac myocytes. In support of this proposal, Xiao et al26 have shown that the inotropic response to β-adrenergic stimulation in rat cardiac myocytes is independent of the phosphorylation of classical PKA targets, including phospholamban and troponin I, whereas it has been shown to be dependent on phosphorylation of these proteins in the human heart.27 The concept of distinct spatial types of cAMP generation has gained additional support from studies indicating specific localization of β1- versus β2-adrenergic receptors on cardiac myocytes.28 This concept is further extended by the observation that glucagon-like peptide-1 stimulation raises cAMP in the heart without increasing contractility.29 Taken together, these data suggest that depending on its spatial and perhaps also temporal distribution cAMP may or may not be linked to cardiac contractility and to long-term damage.

Another set of questions concerns the downstream targets responsible for the effects of cAMP. Antos et al30 propose hyperphosphorylation of the cardiac ryanodine receptor as the mechanism responsible for the development of heart failure. This hyperphosphorylation has been recently described to occur in heart failure in patients as well as in animal models.30 Although this results in an increased open probability of the ryanodine receptor, a causal relationship to the development of hypertrophy and finally heart failure remains to be established. It would be interesting to know whether the cytosolic calcium levels during diastole and the sarcoplasmic calcium content are in fact altered in these mice. These experiments might also give further insights as to why the robust increase in phospholamban phosphorylation, which should result in enhanced removal of Ca2+ from the cytosol during diastole, cannot compensate for the postulated enhanced sarcoplasmic Ca2+ release. Finally, the role of other downstream targets of

Transgenic models of the cardiac β-adrenergic receptor/PKA system. The signaling cascade is represented as a simple linear chain. Proteins that are detrimental when overexpressed are shown in black; those causing beneficial effects upon overexpression are depicted in white. PLB indicates phospholamban.
PKA remains to be studied—in this as well as in other transgenic models of this system.

What types of experiments need to be conducted to clarify these issues? First, we will have to understand that cells are more than empty bags where proteins and secondary messengers are free to diffuse and act all over the place. The spatial and temporal organization of signals and signaling molecules will need to be identified, and this will include comparisons between endogenous and transgenic proteins.

Second, it will be important to understand the role and importance of additional pathways in β-adrenergic signaling. The many exciting short-term studies need to be extended to more long-term and more physiological settings.

Third, the mechanisms linking elevated PKA activity to the cardiac phenotype will need to be identified. The study of Antos et al10 suggests that it may be an imbalance between “good” (phospholamban) and “bad” (ryanodine receptor) phosphorylation that determines the ultimate outcome.

Thus, the seemingly well-known cardiac β-adrenergic receptor/PKA system is turning out to be a puzzle far from being resolved. However, the progress made in recent years both in terms of understanding the mechanisms and in treating heart failure patients makes this one of the most fascinating biological systems to be studied.

References


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