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► To cite this version:

Hind Mehel, Aziz Guellich, Rodolphe Fischmeister. Cyclic AMP Synthesis and Hydrolysis in the Normal and Failing Heart. *Pflügers Archiv European Journal of Physiology*, 2014, 466 (6), pp.1163-1175. 10.1007/s00424-014-1515-1 . hal-02610569

HAL Id: hal-02610569

<https://hal.science/hal-02610569>

Submitted on 17 May 2020

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Cyclic AMP Synthesis and Hydrolysis in the Normal and Failing Heart

Aziz GUELLICH, Hind MEHEL and Rodolphe FISCHMEISTER

INSERM UMR-S 769, LabEx LERMIT, Châtenay-Malabry, France

University Paris-Sud, Faculty of Pharmacy, Châtenay-Malabry, France

Correspondence to:

Rodolphe Fischmeister

INSERM UMR-S 769

Université Paris-Sud, Faculté de Pharmacie

5, Rue J.-B. Clément

F-92296 Châtenay-Malabry Cedex

France

Phone: +33-1-46.83.57.71

Fax 33-1-46.83.54.75

E-mail: rodolphe.fischmeister@inserm.fr

Summary

Cyclic AMP regulates a multitude of cellular responses and orchestrates a network of intracellular events. In the heart, cAMP is the main second messenger of the β -adrenergic receptor (β -AR) pathway producing positive chronotropic, inotropic and lusitropic effects during sympathetic stimulation. Whereas short term stimulation of β -AR/cAMP is beneficial for the heart, chronic activation of this pathway triggers pathological cardiac remodelling which may ultimately lead to heart failure (HF). Cyclic AMP is controlled by two families of enzymes with opposite actions: adenylyl cyclases which control cAMP production and phosphodiesterases which control its degradation. The large number of families and isoforms of these enzymes, their different localization within the cell and their organization in macromolecular complexes leads to a high level of compartmentation, both in space and time, of cAMP signaling in cardiac myocytes. Here, we review the expression level, molecular characteristics, functional properties and roles of the different adenylyl cyclase and phosphodiesterase families expressed in heart muscle and the changes that occur in cardiac hypertrophy and failure.

Introduction

Cyclic AMP regulates a multitude of cellular responses and orchestrates a network of intracellular events. In the heart, cAMP is the main second messenger of the β -adrenergic receptor (β -AR) pathway producing positive chronotropic, inotropic and lusitropic effects during sympathetic stimulation. These effects involve mainly the activation of cAMP-dependent protein kinase (PKA) and the phosphorylation of several key proteins involved in the excitation-contraction (EC) coupling, such as the L-type Ca^{2+} channel (LTCC), phospholamban (PLB), ryanodine receptor (RyR2) and troponin I (TnI) (11). The stimulation of β -AR can also act *via* another pathway that implicates the Ca^{2+} /calmodulin kinase (CaMKII). CaMKII activation can result from PKA-dependent increases in Ca^{2+} , or in a PKA-independent manner, can be targeted downstream of a guanine nucleotide exchange protein directly activated by cAMP (Epac) (45). Whereas short term stimulation of β -AR/cAMP is beneficial for the heart, chronic activation of this pathway triggers pathological cardiac remodelling which may ultimately lead to heart failure (HF) (91, 101). All forms of chronic HF in humans are associated with an elevated level of catecholamines that is inversely correlated with survival (71). Although the exact mechanisms are not fully elucidated, chronic activation of β -ARs or downstream cAMP targets, such as PKA and CaMKII, results in altered Ca^{2+} signalling, cardiac hypertrophy and fibrosis, leading to ventricular dysfunction (102) and cardiac arrhythmias (4, 13, 29, 88, 89). In particular, hyperphosphorylation of RyR2 by PKA and/or CaMKII is proposed to increase diastolic Ca^{2+} leak and consequently to favour fatal cardiac arrhythmias in HF (3, 76, 109). This ultimately leads to the development of chamber dilatation and heart failure (90, 91). This is a complex process that eventually causes down regulation of various components in the cAMP signaling pathway.

Cyclic AMP is controlled by two families of enzymes with opposite actions: adenylyl cyclases which control cAMP production and phosphodiesterases which control its degradation. The large number of families and isoforms of these enzymes, their different localization within the cell and their organization in macromolecular complexes around A kinase-anchoring proteins (AKAPs), a family of scaffolding proteins, and PKA substrates (25, 35) leads to a high level of compartmentation, both in space and time, of cAMP signaling in cardiac myocytes (35, 58, 120). Ensuring that the right amount of cAMP is present in the right place, at the right moment, is actually a key feature of the cell to respond to the diversity of external stimuli. However, such organization is fragile and any change in the localization,

translational and post translational modifications of these proteins will affect the cellular response to hormones and neuromediators. Here, we will review the expression level, molecular characteristics, functional properties and roles of the different adenylyl cyclase and phosphodiesterase families expressed in heart muscle and the changes that occur in cardiac hypertrophy and failure.

Adenylyl cyclases: structure and function

Cyclic cAMP is synthesized by adenylyl cyclases (AC) in response to activation of G_s protein-coupled receptors (G_s PCRs). ACs catalyse the conversion of ATP to cAMP and pyrophosphate. The first AC isotype (AC1) was cloned in 1989 (62). To date, 9 transmembrane and one soluble AC isoforms (sAC), coded by separate genes, have been described in mammals. These isoforms are differentially expressed across tissues. They are also characterized by specific regulatory properties and are usually classified based on this regulatory pattern (50).

Each AC has a pseudosymmetric structure consisting of two hydrophobic domains each with six transmembrane spans (M1 and M2) alternating with two cytoplasmic domains loops C1, C2. The latter form the catalytic core of the enzyme. Both N-terminus and C-terminus are intracytoplasmic and are involved in the regulation of the activity of the enzyme by allowing its interaction with different partners. They are highly variables among ACs, in contrast to the catalytic core which is well conserved (123). The tertiary structure of AC has been recently resolved at high resolution (2.3 Å). The crystal structure consists of a molecular complex with a catalytically active part of AC (C1-C2), the stimulatory heterotrimeric G_s protein α subunit ($G_{s\alpha}$) and forskolin. A molecular mechanism is proposed for the activation of AC by $G_{s\alpha}$ (144). Based on these data, different kinetic models of the activation and inhibition of AC in physiological conditions or in response to different compounds were proposed.

In the heart muscle, the main AC isoforms responsible for cAMP synthesis are AC5 and AC6. However, two additional AC isoforms, AC1 and AC8, are expressed in sinoatrial node cells (78, 157). Unlike AC5/6 which are inhibited by Ca^{2+} , AC1 and AC8 are Ca^{2+} -activated and their expression in pacemaker cells allows Ca^{2+} modulation and PKA-dependent phosphorylation of Ca^{2+} cycling proteins that contributes to the generation of rhythmic action potentials (78, 157). Recent studies have also reported a cardiac role of sAC which is

activated by bicarbonate and calcium and insensitive to heterotrimeric G proteins and forskolin. Following ischemia, cAMP generated by this isoform activates PKA leading to the phosphorylation of Bax, a proapoptotic protein which is a member of the Bcl-2 family, and its translocation to the mitochondria leading to activation of caspases and reactive oxygen species production during reperfusion phase. Inhibition of sAC may limit cell apoptosis (17). Recently, sAC was shown to be expressed in the mitochondrial matrix of hepatocytes (2) and cardiac myocytes (22) where it regulates oxidative phosphorylation and ATP synthesis (21).

While AC5 and AC6 share 65% amino acid (AA) homology and many regulatory characteristics (8, 112), accumulating data from the literature suggest that AC5 and AC6 exert opposite effects on cardiac contractile function, cell survival, and Ca^{2+} handling: beneficial for AC6 and deleterious for AC5 (137, 156). Theoretically, these differences could be due to differences in i) their physical localization, ii) specific regulatory properties, and/or iii) intrinsic enzymatic activity. The lack of specific antibodies suitable for immunohistology studies makes it difficult to resolve the question of localization. When overexpressed in mice, AC6 has been detected in caveolae, sarcoplasmic reticulum (SR), mitochondria and nuclear envelope (39, 42). Both AC5 and AC6 isoforms are activated by $G_{s\alpha}$ and inhibited by $G_{i\alpha}$, PKA and by submicromolar (physiological) concentrations of Ca^{2+} (8). The only known difference so far is the effect of PKC-dependent phosphorylation on the activity of these two isoforms: PKC α and ξ stimulate AC5 while PKC δ and ϵ inhibit AC6 (50). Different laboratories have examined the enzymatic activity of AC5 and AC6 leading to different results. For instance, in one study, it was reported that the maximal activity of AC5 is 7 times greater than that of AC6 (16), while another study showed almost a similar activity (44).

The respective role of AC5 and AC6 in the heart has been the subject of intense investigation (123). Using mouse models with AC5 or AC6 gene deletion or cardiac overexpression, these studies were globally marked by the diametrically opposite results between both transgenic (Tg) mice overexpressing or lacking AC5 or AC6 in response to different heart stress conditions. It is important to note that for knockout mice, there is no tissue and/or time controlled deletion and it is always difficult to compare AC activity between strains from different laboratories because of the differences in measurement methods.

Deletion of AC5

Two AC5^{-/-} mouse lines have been generated by two independent groups (56, 140). Even though both exhibit a similar decrease in basal and isoproterenol or forskolin stimulated AC activity *in vitro* and in isolated cells, differences have been reported in left ventricular (LV) contractility. In the model developed by Tang *et al.* (140) basal contractile function was enhanced whereas no change has been observed in Okumura *et al.*'s model (98). Tang *et al.* reported a decreased sensitivity to β -AR stimulation (140) with no change in the maximal contractile response, whereas Okumura *et al.* reported a decreased maximal response (96, 99). Parasympathetic regulation mediated by the G_i protein was almost totally blunted which could explain the “paradoxal” increase in basal heart rates observed in this model (96). In contrast to Tang *et al.* who reported a significant reduction in G_{sα} protein, no differences in G protein or other compounds of the β -AR cascade including AC levels was found in the Okumura line. Curiously, this strain exhibited Parkinsonian-like phenotype with neuromotor dysfunction, abnormal movement coordination and bradykinesia evidenced by specific tests. Some locomotor difficulties were also present (56). Despite these serious abnormalities, the authors reported later that these AC5^{-/-} mice live longer and both young and old mice had better exercise endurance than WT littermates (152). LV function is preserved in aged mice and this was attributed to increased antioxidant capacities (up regulation of MnSOD via MEK/ERK pathway) (156). Disrupting the PKA regulatory subunit-RII β in mouse also increases lifespan and confers resistance to age-related abnormalities such as cardiac dysfunction (30, 113).

Consistent with the theory of a beneficial effect of limiting the overactivation of the neurohormonal system and cAMP in HF, AC5^{-/-} mice have been reported to be protected against catecholamine infusion in terms of LV function and cardiac remodelling (99). The molecular mechanism underlying this protective effect seems to be related to the phosphorylation of GSK3 α and the activation of the forkhead transcription factor (FKHR) (99). In the HF model induced by pressure overload, the decline in LV ejection fraction (LVEF) was attenuated and progression to HF slowed down in AC5^{-/-} compared to WT mice (97). The underlying protective mechanism in AC5^{-/-} mice involved up-regulation of Bcl-2 and reduction of apoptosis (97). However, in the HF model of Tg mice with cardiac-specific overexpression of G_{αq}, AC5 deletion improved neither survival, cardiac function, cardiac dilation nor electrical remodelling (148).

Overexpression of AC5

Three Tg mouse lines with a cardiac-specific overexpression of AC5 have been generated in three independent groups (Hanoune, Liggett and Vatner) (32, 63, 142). In the Hanoune's line, a 2-fold increase in cAMP generation was observed without any impact on cardiac performance, heart rate or mean blood pressure (32). However, these AC5 Tg mice showed enhanced exercise capacity with a significantly longer distance run than WT animals. In the Liggett's line, a similar 2-fold increase in basal AC activity was observed, but in this case this led to a higher heart rate and fractional shortening (echocardiography) compared to WT mice (142). This was associated with increased PKA activity and PLB phosphorylation. Consequently, these AC5 Tg mice showed a reduced inotropic, lusitropic and chronotropic response to a β_1 -AR stimulation with dobutamine (142). In the Vatner's line, basal AC activity was increased 13-fold, and LV function, PKA activity and PLB phosphorylation were all strongly elevated (63).

To our knowledge, there has been no study aimed at exploring cardiac function under stress conditions in the Hanoune AC5 Tg line. It has only been reported that as they age, these mice show myocardial remodelling with cellular degeneration and fibrosis, and compensatory hypertrophy of the remaining cardiomyocytes (50). The Liggett's model was investigated in the setting of G_{α_q} cardiomyopathy. While deletion of AC5 failed to rescue this cardiomyopathy, AC5 overexpression showed a clear improvement in cardiac contractility even if hypertrophy and hypertrophy-associated fetal gene expression were not modified. In contrast, the Liggett AC5 Tg mice were not protected from the cardiomyopathy induced by cardiac β_1 -AR overexpression (111). The Vatner model was recently studied in a model of chronic catecholaminergic infusion. In their model, overexpression of AC5 exacerbates the cardiomyopathy induced by chronic catecholamine stress due to a decrease in MnSOD as a result of downregulation of the SIRT1/FoxO3a pathway, resulting in oxidative stress intolerance (63).

Deletion of AC6

Deletion of AC6 decreases PKA activity without affecting basal AC activity. However it leads to significant decrease in β -AR-stimulated cAMP generation. LV systolic and diastolic dysfunction was observed in these mice with profound alterations in LV Ca^{2+} handling,

including marked reductions in PLB phosphorylation and SERCA2a activity and marked impairment in SR Ca^{2+} storage capacity. AC6 deletion was also associated with diminished Akt phosphorylation and activity (136). The only available study on the effect of AC6 deletion on stressed heart was performed in a model of LV pressure overload. Interestingly, AC6 deletion is associated with attenuation of LV hypertrophy and prevention of LV dilation, and relative preservation of LV function, but surprisingly only in females (139). Reactivation of fetal gene program as well as expression of collagens are reduced in hearts from AC6^{-/-} mice after pressure overload. This is associated with decreased expression of FHL1 and periostin, two mediators known for their role in promoting cardiac hypertrophy and fibrosis, respectively (139).

Overexpression of AC6

Two different groups have studied the effect of cardiac overexpression of AC6 in mice. Several lines with different levels of overexpression of the AC6 transgene were obtained in Hammond's laboratory, including one with temporal control (tet-on/tet-off). Globally, these mice have similar cAMP generation in cardiac myocytes and do not exhibit any change in heart rate or LV contractile function under basal conditions. However, in response to β -AR stimulation, LV contractility increases (38). The line developed by Vatner's lab (46) has normal heart rate in basal conditions with a moderate increase in LVEF. Studies from Hammond's lab have reported no decline in cardiac function or fibrosis in 17-month old mice (37) which is in contrast with what has been reported with AC5 overexpression (50). Different studies from this group have reported a beneficial effect on ischemic cardiomyopathy in terms of mortality, LV contractile function and remodelling and reduced incidence of high-grade AV block (65, 112, 135, 137). This was not related to infarct size which was similar to that observed in WT mice (135). Most of these beneficial effects were due to improvement in Ca^{2+} handling with increased phosphorylation of PLB, increased SR Ca^{2+} uptake and SR Ca^{2+} storage in cardiac myocytes. Detailed molecular studies have shown that increased AC6 expression in cardiac myocytes leads to increased PLB phosphorylation on Ser-16 by activation of Akt, independently of the activation of the β -AR/PKA pathway (40). AC6 overexpression also decreases PLB expression (112) via an increase in ATF3, a transcriptional suppressor shown to bind to the PLB promoter and repress its expression (40). Eventually, this relieves the inhibition of SERCA2a. For note, restoration of SERCA2a was

shown to be beneficial in different models of HF and in humans (47). AC6 overexpression also inhibits PH domain and leucine rich repeat protein phosphatase 2 (PHLPP2) activity, activates the cardioprotective signaling PI3K and Akt, and increases p70S6 kinase phosphorylation and Bcl-2 expression (41, 42). Overexpression of AC6 in rat cardiac fibroblasts inhibits collagen synthesis in response to serum or other activators (133) and facilitates nodal atrioventricular conduction (124) which are other mechanisms of cardioprotection (103, 134).

As reported with AC5, overexpression of AC6 in the context of $G_{\alpha q}$ cardiomyopathy improves survival and increases cardiac function. In contrast to AC5, AC6 overexpression decreases myocardial hypertrophy in this model (122). AC6 overexpression also normalizes the prolonged action potential duration and the increased K^+ I_{to} and I_{K1} current density (145). The tet-on/tet-off model was used to study the effect of AC6 overexpression on pressure overload (131), aging related LV dysfunction (138) and HF induced by chronic occlusion of the left coronary artery (65). With pressure-overload, cardiac AC6 overexpression improves function of the failing heart. This is due to improved Ca^{2+} handling due to increased PLB phosphorylation, reduced expression of the sodium/calcium exchanger protein NCX1 and phosphatase 1 (PP1), and increased SR Ca^{2+} content (131). However, in these experiments the transgene was switched ON ten days prior to transaortic constriction (TAC). In both aging and myocardial infarction induced HF, AC6 overexpression improved LV function and remodelling without reduction in long term mortality in the MI group (65). However, the beneficial effects of AC6 overexpression on pressure overload stressed heart were not found in Vatner's lab (46). Indeed, in this study, AC6 Tg mice behaved worse than WT mice in response to such stress (46).

Overexpression of inactive AC6

In order to explain the paradoxical effect of AC6 overexpression, Hammond's group has generated recently a catalytically inactive AC6 mutant by directed AA substitution targeting the catalytic core (41). Both AC6 and AC6 mutant proteins were localized in plasma membrane (associated with caveolin), nuclear envelope, and sarcoplasmic reticulum. cAMP production in response to isoproterenol was reduced by 74% compared to WT mice. Many of the beneficial biological effects seen with overexpression of the catalytically active normal form of AC6 were reproduced with the mutant inactive AC6 including decreased PLB

expression, enhanced Ca^{2+} handling, and protection against β -AR induced cardiac myocyte hypertrophy and apoptosis (41). The expression of this mutant form was also associated with increased expression of the small Ca^{2+} -binding protein S100A1 in LV. This directly shows that the beneficial effect AC6 overexpression does not require cAMP production. To our knowledge, there has been no study yet aimed at characterizing how these mice respond to stress-induced HF.

Double AC5 and AC6 deletion

Double KO mice have been recently generated by mating AC5 and AC6 KO. These mice show a high mortality starting three weeks after birth. Cardiomyocytes from these mice are unable to increase their LTCC current in response to β -AR stimulation (147). For obvious reason, these mice were not evaluated under HF conditions.

Adenylyl cyclases and heart failure

Although acute stimulation of the cAMP pathway is physiological and has beneficial effects on cardiac function to perform daily living activities and is necessary for the fight-or-flight response, long term sustained activation produces detrimental effects on the myocardium by inducing LV hypertrophy, dysfunction and ultimately HF. This has been shown using different Tg animal models including cardiac overexpression of the catalytic subunit of PKA, β_1 - and β_2 -ARs, G_{sa} protein which all show dilated cardiomyopathy and HF as they age. Inhibition of this pathway seems to be beneficial in HF in both animal models and humans, with β -blocker therapy being today one of the most widely used treatment of HF. Recent studies have led to the conclusion that cardiac ACs actually form an exception to this rule. The beneficial and harmful effects depend on the isoform, raising again the question of the meaning and the role of the coexistence in the cardiomyocyte of AC5 and AC6, two in appearance very similar isoforms.

Due to the lack of specific antibodies, studies on the expression of these two isoforms in normal and pathological conditions were limited to mRNA giving conflicting results. Recently, Hu *et al.* have developed a specific AC5 antibody and measured the level of AC5 in different animal models at different ages (54). Contrary to AC5 mRNA levels which were

found to increase with age (31), these authors surprisingly found a higher level of AC5 protein expression at birth which decreases with age (54). Interestingly, in pressure overload induced LV hypertrophy, AC5 expression was found to increase, as seen for other fetal genes, and AC6 expression to decrease (54). The study however was limited to one time point and did not cover advanced stages of HF.

The cytoarchitecture and function of cardiomyocytes are profoundly altered in HF and this strongly impacts on the subcellular organisation of the cAMP signalling. In a recent study, Nikolaev *et al.* (94) have shown using ventricular cardiomyocytes isolated from Tg mice expressing a fluorescence resonance energy transfer (FRET)–based cAMP sensor that the selective stimulation of β_1 -AR and β_2 -AR leads to distinct physiological and pathophysiological responses with β_1 -AR-cAMP signalling response covering the entire cell, whereas β_2 -AR-cAMP responses were locally delimited (see also (93)). Using ion conductance microscopy (SICM) with measurements of cAMP production by FRET, the authors have reported a redistribution of the β_2 -AR from the T-tubules to the cell crest in failing cardiomyocytes with loss of the confined cAMP response of β_2 -AR stimulation. Timofeyev *et al.* (147) have further shown that AC6 is responsible for the β_1 -AR stimulation of the LTCC current $I_{Ca,L}$ and that AC5 is the major isoform involved in β_2 -AR signaling, both being colocalized by caveolin 3 within the T-tubules (146, 155).

A kinase-anchoring proteins (AKAPs) play an important role in tethering PKA and other upstream and downstream signaling proteins including ACs (126). Several AKAPs have been identified in heart including mAKAP (also known as AKAP6), Yotiao (AKAP9), AKAP15/18 (AKAP7), AKAP-Lbc (AKAP13), AKAP79/150 (AKAP5) and Gravin (AKAP12) (28). AKAP79/150 was shown to bind both AC5 and AC6 in the heart through their N terminal part and this facilitates their phosphorylation by PKA to terminate the signal (20). AKAP79/150 also allows the recruitment of AC5/6 to a membrane-associated complex of signaling molecules which direct the PKA phosphorylation of PLB, RyR2, and a subpopulation of LTCCs (28). mAKAP β was found anchored in nuclear envelope and in the SR of cardiomyocytes where it is associated with AC5 but not AC6 isoforms (59). Disruption of the binding of mAKAP β to AC5 leads to an increase in cAMP and to cellular hypertrophy in the absence of agonist stimulation (59). It is still unclear, however, how these AKAPs change during HF and how this may affect temporal and spatial regulation of cAMP signaling.

Cardiac adenylyl cyclases as therapeutical targets

Due to the different role of AC isoforms and their localization in different subcellular compartments, ACs may turn out to be innovative therapeutic targets provided that isoform-specific modulators become available (108). So far, the only AC activator used in clinic is colforsin (also known as NKH477), a water-soluble forskolin analogue (i.e. non-selective) which is used in Japan in acute therapy (61).

Iwatsubo *et al.* (57) reported a cardiac beneficial effect of adenine 9-D-arabinofuranoside (Ara-A). Ara-A, also known as vidarabine, is an adenosine analog used clinically as an antiviral drug to inhibit DNA synthesis and viral replication. The authors reported that Ara-A is an AC5 selective inhibitor and when infused one week prior to permanent coronary occlusion using an osmotic pump in mice, it significantly reduced mortality, and partially prevented LV dysfunction and fibrosis (57). The same results were found in a model of chronic catecholamine infusion. The authors showed that Ara-A increased mitogen/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) phosphorylation (57). Other studies from the same group have reported beneficial effect of AC5 inhibition on diabetes, obesity, etc., extending the potential application and therapeutic areas for this pharmacological inhibitor. However, the specificity of this compound was recently questioned by two independent groups (14, 15) who showed that this inhibitor was unable to discriminate between AC5 and AC6. In addition, virtual molecular docking studies did not support the AC5 specificity of Ara-A (15).

The concept of AC6 activation is progressively moving towards clinic. In 2004, Lai *et al.* (64) showed the beneficial effect of intracoronary adenovirus encoded AC6 (Ad.AC6) in a model of HF induced by rapid pacing in pig. A gene-transfer phase 1/2 randomized, double blind clinical trial using adeno-associated virus type 5 encoding AC6 (ClinicalTrials.gov NCT00787059) in patients with HF is currently in progress. The study started in July 2010 and should be completed by the end of 2014. The primary outcome combines exercise treadmill time, LV function, +dP/dt and -dP/dt at baseline and during dobutamine infusion.

Cyclic AMP phosphodiesterases

The main physiological process for lowering cAMP (and cGMP) levels in cardiac cells is through catalytic hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). Eleven PDE families including 21 genes and about 100 isoforms have been identified so far and they are classified based on their primary AA sequence as well as kinetic and regulatory properties (7, 18, 36). PDEs share a conserved catalytic domain (C-domain) showing approximately 25–52% AA sequence identity, but differs markedly in their regulatory domain (N-domain). Some PDE families selectively hydrolyze cAMP (PDE4, 7 and 8) or cGMP (PDE5, 6, and 9) while other are promiscuous (PDE1, 2, 3, 10, 11). Out of these 11 PDE families, 6 are expressed in cardiac myocytes: PDE1, PDE2, PDE3, PDE4, PDE5 and PDE8. In this section, we will review the structure, subcellular distribution, regulation and role of the four main cardiac PDEs responsible for cAMP hydrolysis, i.e. PDE1-4. Data on PDE8 is so far limited to one single study in which a gene deletion approach was used to demonstrate that this enzyme controls cardiac RyR2 Ca^{2+} leak and β -AR regulation of LTCC channels and Ca^{2+} transients (106).

Phosphodiesterase type 1 (PDE1)

PDE1 family is encoded by three genes: *PDE1A*, *B* and *C*. They have alternative promoters and give rise to a multitude of proteins by alternative splicing. In humans, PDE1A and 1B preferentially degrade cGMP while PDE1C hydrolyzes cAMP and cGMP with high affinity. PDE1 is activated by Ca^{2+} /calmodulin complex which binds to the two regulatory domains in the N-terminal region. Phosphorylation of the enzyme by PKA or CaMKII near the regulatory domains decreases the affinity of PDE1 for Ca^{2+} /calmodulin, and thus constitutes a negative feedback for PDE1 activity. In the heart, PDE1 was thought to be expressed in nonmyocyte cells (12) until the presence of PDE1C isoform was established in human cardiomyocytes (151). PDE1C activity is essentially detected in the soluble fraction, suggesting that hydrolysis of cAMP and cGMP via PDE1C mainly takes place in the cytosol (151). Because of the lack of a commercially available selective PDE1 inhibitor, little is known on the role of this PDE family in cardiac function.

Phosphodiesterase type 2 (PDE2)

PDE2 family is encoded by a single *PDE2A* gene, which generates three splice variants: PDE2A1-3. These isoforms share the same C-terminal sequence but differ in their N-terminal region which determines their subcellular localization: cytosolic for PDE2A1; particulate for PDE2A2 and 3. PDE2 hydrolyzes both cAMP and cGMP and is stimulated by cGMP binding to a N-terminal allosteric regulatory site known as GAF-B domain (75, 105). This feature makes of PDE2 a crucial negative feedback regulator of cAMP when cGMP rises as during production of nitric oxide (10). PDE2 is therefore able to establish a cross-talk between the cAMP and cGMP signaling pathways (72).

Several studies have reported a PDE2 expression in isolated cardiomyocytes and myocardium. Although PDE2 activity is relatively low compared to other cardiac PDEs, its presence at the plasma membrane contributes to the regulation of LTCC activity when cGMP levels are increased in many species including humans (34). This was first demonstrated in frog ventricular cardiomyocytes dialyzed with cAMP and cGMP, wherein activated PDE2 is able to reduce $I_{Ca,L}$ amplitude (51). The same mechanism accounts for the effects of high concentrations of cGMP or NO donors on $I_{Ca,L}$ in human atrial cells (149). More recently, a study performed on neonatal rat cardiomyocytes using the FRET-based imaging technique showed that by decreasing the level of cAMP, PDE2 significantly attenuates the effects of a β -AR stimulation downstream of β_3 -AR (130).

Phosphodiesterase type 3 (PDE3)

PDE3 is characterized by its high affinity for cAMP and cGMP, but the rate of cGMP hydrolysis is ~10% that of cAMP, thus making of cGMP a competitive inhibitor of cAMP hydrolysis. The PDE3 family is encoded by two different genes, *PDE3A* which encodes three isoforms by alternative splicing (PDE3A1-3) and *PDE3B* which encodes one isoform (154). Both PDE3A and PDE3B are expressed at the cardiac level with PDE3A being the dominant isoform (107). The isoforms differ from each other at the N-terminal region by the presence or absence of NHR1 and NHR2 (N-terminal hydrophobic region), which are important for subcellular localization of the enzymes (154). Thus, PDE3A1 and 3B are localised exclusively in the particulate fraction, PDE3A3 is cytosolic and PDE3A2 is present in both fractions. These isoforms also differ in the presence and/or the number of phosphorylation sites for PKB, PKC and PKA (48, 154), resulting in differential regulation of their catalytic activity and protein interactomes (150).

PDE3 represents the major cardiac PDE activity in higher mammals. PDE3 inhibitors exert a direct positive inotropic effect in the heart of large mammals (73) and are used for acute treatment of acute HF (79). However, their long-term use in congestive HF is associated with increased incidence of arrhythmias and sudden death (104). Evidence from global PDE3A and PDE3B knockout mice has revealed that PDE3A but not PDE3B is responsible for the inotropic and chronotropic effects of PDE3 inhibitors (132). PDE3A regulates cardiac contractility mainly by modulating PLB-SERCA2 activity and subsequent SR Ca^{2+} uptake (9). However, chronic PDE3A downregulation or inhibition also induces myocyte apoptosis *in vitro* (23) while on the contrary myocardial overexpression of the PDE3A1 isozyme protects against ischemia/reperfusion-induced cardiomyocyte apoptosis and cardiac injury (95). Together with two isoforms of the PDE4 family, PDE3B is anchored at the cell membrane by PI3K γ (107), which also anchors PKA (110) allowing a high cAMP hydrolytic activity of the enzyme. Disruption of the PI3K γ /PDE3B/PKA complex leads to abnormal activation of the cAMP signaling cascade causing arrhythmias (43), necrotic cardiac tissue damage and fibrosis (107).

Phosphodiesterase type 4 (PDE4)

PDE4 enzymes hydrolyze cAMP only and represent the largest family of PDEs (7, 36). They are encoded by 4 different genes, *PDE4A-D*, with multiple promoters, giving rise to a multitude of proteins by alternative splicing. Over 25 human isoforms have been identified. In the heart, only PDE4A, PDE4B and PDE4D genes are expressed (117, 119), with a rank order of hydrolytic activity PDE4D>PDE4B>PDE4A in neonatal rat heart (87) and PDE4A>PDE4B>PDE4D in adult rat heart (1). PDE4 is also expressed in human ventricle (119) and atrium (86) with PDE4D being the dominant isoform.

Structurally, the PDE4 family members can be divided into four groups based on the organization of their N-terminal domain: the long isoforms contain two regions UCR (upstream conserved region), UCR1 and UCR2; the short isoforms lack UCR1; the super-short isoforms have an N-terminally truncated UCR2; the dead-short isoforms lack UCR1 and UCR2 and have an inactive catalytic unit that is both N- and C-terminally truncated (53). These two modules are important for the catalytic activity and dimerization of the protein (5, 55, 100). Indeed, under basal conditions, UCR2 interacts with UCR1 and C-domain exerting an auto-inhibition of the catalytic activity. PKA phosphorylation of a serine in UCR1 disrupts

the UCR2 inhibitory influence and activates the enzyme (52). Hence, in cardiomyocytes, PKA inhibition leads to a strong increase in the cytosolic cAMP response to a β -AR stimulation (83, 121). Except for PDE4A, all PDE4 isoforms contain an Erk2 phosphorylation site in the C-terminal portion. Erk2 phosphorylation activates the short forms and inhibits the long forms (52).

The function of PDE4 isoenzymes differs in different places within the cell as it depends on their interaction with other molecular partners in specific signalling complexes. For instance, PDE4D3 is present in the perinuclear region of cardiac myocytes by an association with mAKAP (26). In this compartment, mAKAP also binds Epac1 and Erk5 kinase, so that when Epac1 is activated by cAMP, it recruits Rap1 which allows the suppression of Erk5 activation and relieves the inhibition of PDE4D3 (26). This complex allows a spatial control of PKA activity by its anchoring to mAKAP, and a temporal regulation of cAMP signals by the presence of PDE in the immediate vicinity (27, 141). PDE4D3 is also localized in the sarcomeric region of the myocytes *via* an anchoring to myomegalin (153), at the dyad in association with RyR2 (67), and in the sarcolemma in association with the KCNQ1/KCNE1 potassium channel responsible for I_{Ks} (143). PDE4D5, PDE4D8 and PDE4D9 have also been shown to associate with β -ARs either directly (116, 118) or indirectly by binding to β -arrestin (6, 19). Finally, PDE4D5 has been found to form a complex with Hsp20, a small heat-shock protein with cardioprotective properties when phosphorylated by PKA (33, 74).

In rats and mice, PDE4 represents the major cAMP-PDE (69). Yet, unlike PDE3 inhibitors, PDE4 inhibitors do not induce any positive inotropic effect under basal conditions (81, 127). This is because PDE3 and PDE4 work in a different range of cAMP concentrations. Only when intracellular cAMP level is elevated, e.g. with forskolin, isoprenaline or after PDE3 inhibition, or in cells where basal AC activity is high such as human atrial myocytes, do PDE4 inhibitors produce an additional increase in cAMP, $I_{Ca,L}$ or contraction (69, 81, 86, 120, 121). In adult mouse cardiomyocytes, both PDE4B and PDE4D were found to be part a molecular complex with LTCCs. However, evidence from global PDE4B and PDE4D knockout mice has revealed that only PDE4B regulates LTCC phosphorylation and $I_{Ca,L}$ amplitude during β -AR stimulation (70, 83), while both PDE4B and PDE4D control the phosphorylation of RyR2 (68, 83).

Phosphodiesterases and heart failure

Numerous studies have shown a decrease in expression and/or activity of PDE3 and PDE4 in various models of cardiac hypertrophy and/or HF: cardiomyopathic hamsters (77), failing dog hearts (125, 129), mouse (24) and rat heart (1) subjected to transverse aortic constriction, including human HF (23). On the contrary, hypertrophy and/or HF is usually accompanied by an increase in expression and/or activity of PDE1A (84, 85), PDE2 (80) and PDE5 (92, 114). The question is whether these changes are bystander effects of the cellular pathological remodelling, or whether they correspond to an adaptive or maladaptive process.

Downregulation of PDE3 and PDE4 in hypertrophy, two PDEs that mainly (PDE3) or exclusively (PDE4) hydrolyze cAMP, might be regarded as an initial adaptive process because it partly compensates for the deficit in cAMP synthesis. However, such PDE remodelling may be maladaptive in the long term, because of a loss of cAMP compartmentation and thus the loss of specificity of cAMP action (82). In that respect, PDE3 inhibition, initially described as a therapeutically relevant strategy to treat HF, has been associated with increased mortality in failing patients following chronic use (104). Similarly, PDE4 inhibition or gene deletion causes ventricular arrhythmias in animal models (43, 70) and a PDE4 downregulation is observed in patients with atrial fibrillation (86). However, if inhibiting all cardiac PDE3 or PDE4 enzymes is clearly deleterious in the long run, one must consider the possibility that inhibiting only a subpopulation of PDE3 or PDE4 at the right subcellular localization might actually turn out to be beneficial for the heart. Such a strategy has recently been tested in neonatal cardiomyocytes by inhibiting the activity of PDE4D5 only where it controls the amount of cAMP available to activate PKA phosphorylation of Hsp20 (128). Under resting conditions, PDE4D5 activity prevents Hsp20 from being phosphorylated but the interaction between Hsp20 and PDE4D5 can be disrupted by means of an exogenous peptide. This causes an increase in Hsp20 phosphorylation with the anticipated result of diminishing the hypertrophic response to chronic β -AR stimulation (128).

Upregulation of PDE1A and PDE5 in HF, two PDEs that preferentially (PDE1A) or exclusively (PDE5) hydrolyze cGMP, thus causing a reduction in intracellular cGMP concentration, can clearly be seen as maladaptive. Indeed, cGMP is known to inhibit pathological cardiac remodelling (49) and Tg mice with cardiac-specific overexpression of PDE5 are predisposed to adverse LV remodelling after myocardial infarction (114), while on the contrary pharmacological inhibition of PDE1 (84) or PDE5 reduces hypertrophy and

ameliorates cardiac pressure and volume overload (60, 66, 84). However, it is worth noting that in a recent clinical trial, PDE5 inhibition in HF patients with preserved ejection fraction did not improve significantly exercise capacity or clinical status compared to the placebo group (115).

Finally, upregulation of PDE2 in HF might be regarded as an adaptive process because specific PDE2 inhibition was shown to restore β -AR responsiveness in diseased cardiomyocytes, whereas PDE2 overexpression blunts catecholamine responsiveness and protects against pathological hypertrophy (80). Thus, PDE2 may be considered as a new member of the group of proteins contributing to the well-known phenomenon of β -AR desensitization in chronic HF.

Conclusion

Since the discovery of cAMP by Earl Sutherland and Ted Rall in the late fifties, an amazing number of studies have appeared on how this second messenger is synthesized or degraded, on what makes its level go up or down, on what it does to target effectors by either covalent or non-covalent mechanisms, and on how it affects a countless number of cellular functions. In the heart, the cAMP pathway has been so fully explored over the years that one can wonder what else is there to be found. What this review shows is that if the big picture has not changed that much, *the devil is in the details*. Hopefully, going deeper and deeper into the intricate mechanisms and organisation of the cAMP cascade will not drag us to hell but will on the contrary help us opening interesting avenues for new therapeutic strategies to combat HF.

Acknowledgements

Our own work reviewed here was supported by the Fondation Leducq for the Transatlantic Network of Excellence cycAMP grant 06CVD02 (to RF), the European Union contract LSHM-CT-2005-018833/EUGeneHeart (to RF) and by the Investment for the Future program ANR-11-IDEX-0003-01 within the LABEX ANR-10-LABX-0033 (to Dr. Fischmeister). AG was a recipient of a postdoctoral grant from the CORDDIM program of Région Ile-de-France. HM was a recipient of a doctoral grant from the Fondation pour la Recherche Médicale.

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