TOPICAL REVIEW

Raf kinase inhibitor protein: lessons of a better way for β-adrenergic receptor activation in the heart

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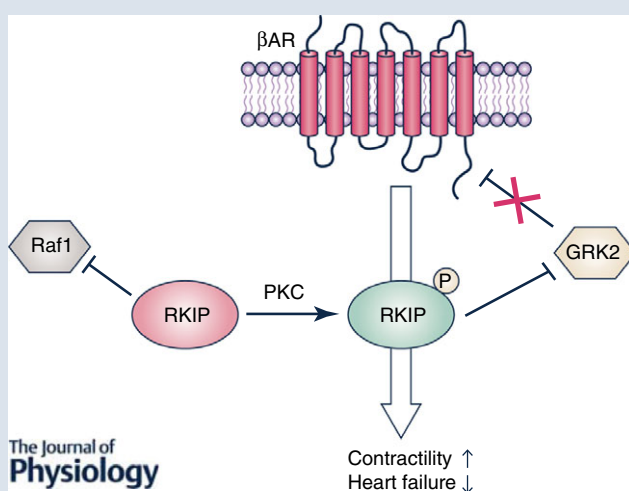
Abstract Stimulation of β-adrenergic receptors (βARs) provides the most efficient physiological mechanism to enhance contraction and relaxation of the heart. Activation of βARs allows rapid enhancement of myocardial function in order to fuel the muscles for running and fighting in a fight-or-flight response. Likewise, βARs become activated during cardiovascular disease in an attempt to counteract the restrictions of cardiac output. However, long-term stimulation of βARs increases the likelihood of cardiac arrhythmias, adverse ventricular remodelling, decline of cardiac performance and premature death, thereby limiting the use of βAR agonists in the treatment of heart failure. Recently the endogenous Raf kinase inhibitor protein (RKIP) was found to activate βAR signalling of the heart without adverse effects. This review will summarize the

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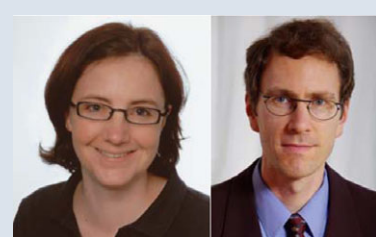
current knowledge on RKIP-driven compared to receptor-mediated signalling in cardiomyocytes. Emphasis is given to the differential effects of RKIP on β_1- and β_2-ARs and their downstream targets, the regulation of myocyte calcium cycling and myofilament activity.

Abstract figure legend Raf kinase inhibitor protein (RKIP) activates β-adrenergic receptors and protects the heart. RKIP inhibits Raf1 and switches to G-protein-coupled receptor kinase 2 (GRK2) upon phosphoryation by protein kinase C. RKIP-mediated GRK2 inhibition prevents receptor desensitization and internalization and, thus, increases β-adrenergic receptor signalling. This leads to increased cardiac contractility and relaxation and protects from the development of heart failure.

Abbreviations AAV, adeno-associated virus; AC6, adenylyl cyclase 6; βAR, β-adrenergic receptor; βARKct, β-adrenergic receptor kinase, C-terminus; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cAMP, cyclic adenosine monophosphate; cMyBPC, cardiac myosin binding protein C; Epac, exchange protein directly activated by cAMP; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GPCR, G-protein-coupled receptor; GRK, G-protein-coupled receptor kinase; G$_i$, inhibitory G-protein; G$_s$, stimulatory G-protein; KO, knock-out; LTCC, L-type Ca^{2+} channel; MEK, mitogen-activated protein kinase kinase; NCX, sodium–calcium exchanger; PDEIII, phosphodiesterase III; PEBP, phosphatidylethanolamine-binding protein; PKA, protein kinase A; PKC, protein kinase C; PLN, phospholamban; PMCA, sarcolemmal Ca^{2+}-ATPase; RKIP, Raf kinase inhibitor protein; RyR2, ryanodine receptor 2; S100A1, S100 calcium binding protein A1; SERCA2a, sarco-/endoplasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; TnI, troponin I.

Introduction

Heart failure occurs if cardiac output is reduced to an extent that it cannot meet the body's needs. It represents one of the leading causes of morbidity and mortality in developed countries and results from the loss and/or dysfunction of cardiomyocytes due to literally any insult to the heart, most frequently chronic arterial hypertension, myocardial infarction, aortic stenosis or infectious diseases. The activation of the sympathetic nervous system via β-adrenergic receptors (βARs) is the most important compensatory mechanism in heart failure, acting to stabilize the hemodynamic situation by accelerating cardiac contraction and relaxation (Ponikowski *et al.* 2016).

Free calcium ions (Ca^{2+}) are the critical intermediary that translates sympathetic activity into myofilament movement. Changes of beat-to-beat myocyte Ca^{2+} cycling are also among the hallmarks of heart failure. Further, local Ca^{2+} release events (Ca^{2+} sparks) and/or altered Ca^{2+} sensitivity of cardiomyocytes contribute to contractile dysfunction and increase the risk of cardiac arrhythmias in failing hearts as well as in diseases like inherited cardiomyopathy or early after myocardial infarction (Cho *et al.* 2016). The profound effects on cardiac function of even small modifications to elements of the βAR signalling cascade, the receptor cascade that controls Ca^{2+} handling to the myofilaments, necessitates precise regulation of the entire system.

In the treatment of heart failure, pharmacological activation of βARs is beneficial in acute situations due to its ability to rapidly increase cardiac output. However, sustained activation of βARs is detrimental to the heart; it promotes cardiomyocyte death and myocardial fibrosis and increases patient mortality (Engelhardt *et al.* 1999; Tacon *et al.* 2012; Ponikowski *et al.* 2016). A strategy that would increase cardiac output, but without the adverse effects of chronic βAR stimulation, is still lacking. This review will discuss novel therapeutic approaches aimed at selective activation of specific components of βAR signalling with a main focus on the Raf kinase inhibitor protein (RKIP). Upon phosphorylation by protein kinase C (PKC), RKIP potentiates βAR signalling through inhibition of receptor desensitization, which has proven beneficial effects on myocyte Ca^{2+} regulation and murine heart failure (Lorenz *et al.* 2003; Schmid *et al.* 2015).

βAR signalling in the heart

Sympathetic activity is transmitted to cardiac muscle via neuronally and circulating catecholamines that predominantly activate βARs on cardiomyocytes as those are the receptor subtypes with the highest density in the ventricular myocardium. Two different βAR subtypes are expressed in the heart: β_1- and β_2-adrenergic receptors, at a ratio of 80:20. Stimulation of cardiac βARs mediates an increase in contractile force (positive inotropy), speed of relaxation (positive lusitropy), atrio-ventricular conduction (positive dromotropy) and heart rate (positive chronotropy) (Bristow *et al.* 1986; Brodde 1991; Jensen *et al.* 2009). β_3ARs are a third subtype of cardiomyocyte βAR. Their role in the heart, however,

is still largely unclear. They induce distinct intracellular signalling pathways and a negative inotropic effect. Since their expression is increased in several subtypes of human cardiomyopathy, they may have a potential role in heart failure. Mice with cardiac β_3AR overexpression showed reduced hypertrophic remodelling through nitric oxide synthase activation (Balligand 2013; Belge *et al.* 2014).

β_1- and β_2ARs couple to stimulatory G-proteins (G_s) that stimulate adenylyl cyclases to produce the second messenger cyclic adenosine monophosphate (cAMP), which in turn activates the cAMP-dependent protein kinase A (PKA). In cardiomyocytes, regulators of beat-to-beat Ca^{2+} cycling and sarcomere proteins represent major substrates of PKA. Activation of PKA causes phosphorylation of L-type Ca^{2+} channels (LTCCs). This increases Ca^{2+} influx; phosphorylation of phospholamban (PLN), which accelerates the reuptake of Ca^{2+} into the sarcoplasmatic reticulum (SR); phosphorylation of ryanodine receptors 2 (RyR2), which increases SR Ca^{2+} release; phosphorylation of troponin I (TnI) and of cardiac myosin binding protein C (cMyBPC),

which decreases myofilament Ca^{2+} sensitivity; and phosphorylation of titin, which reduces the sarcomeric passive stiffness (Lefkowitz *et al.* 2002; Rockman *et al.* 2002; Krüger & Linke, 2006; Baker, 2014; Najafi *et al.* 2016). Taken together, these PKA-mediated phosphorylation events enhance Ca^{2+} cycling and reduce myofilament Ca^{2+} sensitivity in cardiomyocytes, leading to the increases in force and increases in the rates of contraction and of relaxation. Myocyte Ca^{2+} is also important for the formation of the Ca^{2+}–calmodulin complex, which activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a kinase that also impacts on Ca^{2+} homeostasis by phosphorylation of RyR2, PLN or cMyBPC, thereby further potentiating βAR-mediated cardiac contraction and relaxation (Fig. 1; Rockman *et al.* 2002; Maier & Bers, 2007; Lehnart *et al.* 2009; Sadayappan *et al.* 2011; Uchinoumi *et al.* 2016). In addition, βARs activate an exchange protein directly activated by cAMP (Epac). Epac1 seems to contribute to cardiac hypertrophy and is upregulated in heart failure, whereas Epac2 seems to be involved in CaMKII-induced SR Ca^{2+} leak and
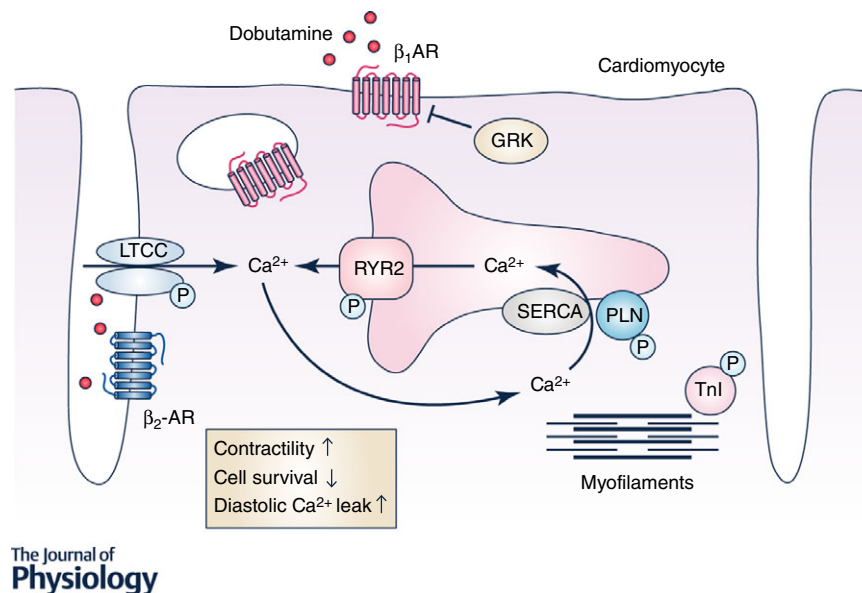
Figure 1. Acute dobutamine application induces positive inotropy; chronic dobutamine application deteriorates cardiac function

Dobutamine activates β_1- and β_2-adrenergic receptors (β_1AR and β_2AR). Activated βARs increase contractility and relaxation of cardiomyocytes via the activation of stimulatory G-proteins (G_s), which in turn activate protein kinase A and Ca^{2+}/calmodulin-dependent protein kinase II. These kinases increase Ca^{2+} cycling: upon phosphorylation, phospholamban (PLN) dissociates from sarco-/endoplasmatic reticulum Ca^{2+}-ATPase (SERCA2a). This leads to increased SERCA2a-mediated Ca^{2+} re-uptake into the sarcoplasmatic reticulum and cardiomyocyte contractility. Phosphorylation of troponin I (TnI) decreases Ca^{2+} sensitivity and thereby increases cardiomyocyte relaxation. However, G-protein-coupled receptor kinase (GRK) phosphorylates activated G-protein-coupled receptors (GPCR) as for example β_1AR and β_2AR, which induces receptor desensitization and internalization. This blunts βAR signalling and the initial increase in cardiomyocyte contractility upon dobutamine application. Further, chronic βAR stimulation induces apoptosis, fibrosis and arrhythmia, in particular via hyperphosphorylation of the ryanodine receptor 2 (RyR2) and L-type Ca^{2+} channels (LTCC), thereby leading to increased diastolic Ca^{2+} leak.

arrhythmia. Despite these detrimental effects, Epac has also been reported to promote cardiomyocyte survival in heart failure (Métrich *et al.* 2008, 2009; Pereira *et al.* 2013).

Thus, βARs, as major drivers of heart rate, contractile force, speed of contraction and relaxation, play an important role in so-called fight-or-flight situations or whenever cardiac output needs to be enhanced. Analogously, βAR agonists such as adrenaline, dobutamine and dopamine are used to stabilize patients in *acute* cardiac failure (Felker, 2001; Tacon *et al.* 2012; Ponikowski *et al.* 2016). However, long-lasting application of these β-AR agonists for several days or even weeks induces structural cardiac damage, including cardiac hypertrophy, cardiomyocyte apoptosis and interstitial fibrosis (Fig. 1; Engelhardt *et al.* 1999; O'Connor, *et al.* 1999; Felker, 2001; Tacon *et al.* 2012; Vidal *et al.* 2012; Ponikowski *et al.* 2016). As a natural defence against this damage, prolonged activation of βAR leads to receptor desensitization via phosphorylation by G-protein-coupled receptor kinases (GRK), thereby protecting the heart from long-term sympathetic overdrive. The predominant GRK subtype in the heart is GRK2. Phosphorylation of βARs by GRK2 increases the affinity of the receptor for β-arrestin, a protein that blocks G-protein coupling upon receptor stimulation and promotes receptor internalization and degradation, rendering myocytes less responsive to agonist binding of βARs (Rockman *et al.* 2002). However, such loss of βAR function also promotes contractile decline of failing hearts. This dilemma between the need for positive inotropy of failing hearts on one side and receptor desensitization to prevent cardiac damage upon sustained βAR activation on the other side pushes for a well-synchronized, well-balanced and fine-tuned way to regulate βAR signalling in the heart.

βAR signalling in heart failure

βAR agonists augment cardiac contraction at the beginning of treatment. In contrast, sustained βAR stimulation is cardiotoxic, consistent with the finding that noradrenaline plasma levels correlate with the degree of cardiac dysfunction and mortality of heart failure patients (Thomas & Marks, 1987; Cohn *et al.* 1993; Zhang *et al.* 2013). Under conditions of increased sympathetic nervous system activation or chronic βAR agonist treatment, both βAR density at the surface of the cell membrane and the responsiveness of the remaining receptors are reduced. These molecular characteristics of failing hearts correlate well with the stage of heart failure independent of the underlying cause of the disease (Ohsuzu *et al.* 1994). The pattern of βAR subtype downregulation, however, seems to depend on the aetiology of heart failure: β_1ARs but not β_2ARs are downregulated in the majority of heart failure cases, but in mitral valve disease and ischaemic cardiomyopathy both βAR subtypes are affected to a similar extent (Brodde *et al.* 1986; Bristow *et al.* 1991; Steinfath *et al.* 1991, 1992).

Toxic effects mediated through βAR activation appear to originate from β_1ARs, because cardiac overexpression of β_1ARs in mice led to cardiac hypertrophy, interstitial fibrosis and cardiac dysfunction (Engelhardt *et al.* 1999; Zhang *et al.* 2013). Furthermore, β_1ARs mediate pro-apoptotic signalling through the kinases PKA and CaMKII. For example, selective inhibition of β_1AR resulted in protection of catecholamine-induced apoptosis in rat ventricular myocytes (Zaugg *et al.* 2000; Shizukuda & Buttrick, 2002).

β_2ARs in contrast, have been described as cardioprotective receptors (Liggett *et al.* 1998; Siedlecka *et al.* 2008). Cardiac overexpression of β_2ARs in mice prevented myocardial remodelling and contractile dysfunction in a genetic model of heart failure generated by $G_{\alpha q}$ overexpression (Dorn *et al.* 1999). However, favourable effects were achieved only at relatively low levels of β_2AR overexpression, whereas higher expression levels turned out deleterious, suggesting that specificity of β_2AR signalling must be preserved to achieve beneficial effects via this activation. In addition, β_2AR overexpression of up to 60-fold was tolerated in ageing mouse hearts without detriment for a period of at least 1 year (Liggett *et al.* 2000). Further, selective β_2AR activation protected from stress-induced apoptosis in isolated cardiomyocytes as well as from myocardial dysfunction and apoptosis in a rat model of heart failure (Paur *et al.* 2012) and mice lacking β_2ARs had a higher mortality than wild-type mice in response to chronic isoproterenol application (Patterson *et al.* 2004). Also, in human heart failure an Ile164 polymorphism in the β_2AR, which reduces its signalling efficiency, was found to worsen patients' prognosis (Liggett *et al.* 1998). Beneficial effects of β_2ARs in the heart are often associated with β_2AR coupling to inhibitory G-proteins (G_i). In line with this, the unfavourable outcome of the Ile164 polymorphism was suggested to result from the loss of β_2ARs coupled to G_i and their protective effects on apoptosis (Chesley *et al.* 2000). On the other hand, enhanced β_2AR–G_i signalling is also reported to contribute to cardiac deterioration in heart failure by further reducing cardiac contractility. Thus, several groups hypothesized that a combination of β_1AR blockade with β_2AR–G_s activation may be ideal for improving cardiac contractility without adverse effects (Ahmet *et al.* 2008; Woo & Xiao, 2012); others, however, suggested β_1AR blockade combined with β_2AR–G_i activation as the preferred strategy for heart failure therapy with particularly striking results in a model of Takotsubo cardiomyopathy (Siedlecka *et al.* 2008, clenbuterol as β_2AR–G_i biased β_2-agonist; Paur *et al.* 2012). Takotsubo cardiomyopathy is characterized by ballooning and contractile dysfunction only of the apical portions of the heart in response to excessive

emotional stress and subsequent exposure to high levels of catecholamines. Prevention of adrenaline-mediated G_i effects increased mortality, thus providing strong evidence for the beneficial effects of G_i coupling in activating β_2ARs. β_2AR–G_i signalling may thus be essential to counteract hyperactivated β_1AR–G_s signalling (Gorelik *et al.* 2013).

Finally, the general view of the β_1AR as the 'bad' and the β_2AR as the 'good' receptor in heart failure also has been challenged by the finding that deletion of β_2AR was cardioprotective in a model of genetic cardiomyopathy. Deletion of β_1AR in this particular mouse mutant was proposed to worsen the phenotype via a PKA-independent pathway employing Epac (Fajardo *et al.* 2013; Zhang *et al.* 2013). In summary, even though chronic β_1AR signalling is generally thought to be cardiotoxic and chronic β_2AR signalling cardioprotective, the outcome of β_1AR *vs.* β_2AR activation depends at least partially on the underlying disease type.

Strategies in heart failure that target *β*AR signalling

While pharmacological stimulation of βARs is commonly used to stabilize a failing heart in an acute situation, blockage of βARs in chronic heart failure turned out to be beneficial due to disruption of the vicious circle between sympathetic overdrive and maladaptive remodelling processes. Unlike initial expectations from negative inotropic drugs, antagonists of βARs (β-blockers) improve patients' symptoms and significantly promote survival when applied carefully at slowly increasing dosages. Multiple studies within the last two decades have shown that β-blockers improve survival for chronic heart failure by up to 30% (Packer *et al.* 1996*a*,*b*; Lechat *et al.* 1998). Low dosages of β-blockers are sufficient to protect from sympathetic overdrive, thereby preventing β_1AR-mediated remodelling processes and restoring β-adrenergic function by re-sensitization and increased expression of βARs (Felker, 2001; Lompré *et al.* 2010; Tacon *et al.* 2012; Ponikowski *et al.* 2016). However, not all patients tolerate β-blockers well and the withdrawal rate is high due to side effects like fatigue, sleep disturbance, depression, weight gain, pulmonary side effects and sexual dysfunction (Packer *et al.* 1996*a*,*b*). In heart failure, depression of cardiac contractility further hampers the use of β-blockers in general or at least at the desired dose. The ideal drug in the treatment of heart failure would increase cardiac output and thereby instantly alleviate symptoms, but without the adverse effects of chronic βAR stimulation.

Several new experimental strategies have been added in recent years to increase cardiac contractility in heart failure by activation of βAR or modulation of βAR downstream signalling, particularly by targeting regulators of myocyte Ca^{2+} cycling. Most attempts to reconstitute *βAR signalling* failed, because they accelerated rather than attenuated deterioration of cardiac morphology and function. These studies evaluated the use of isoproterenol or dobutamine; the inhibition of phosphodiesterase III (PDEIII), an enzyme that degrades cAMP; activation of PKA; inhibition of protein phosphatase 1, an enzyme that reduces PKA-mediated activation of calcium cycling proteins; or activation of CaMKII (El-Armouche *et al.* 2008; Lehnart *et al.* 2009; Lompré *et al.* 2010; Tacon *et al.* 2012; Bers, 2014; Ponikowski *et al.* 2016).

Studies aiming at myocyte *Ca^{2+} cycling* yielded more promising results for the treatment of heart failure. Ca^{2+} coordinates myofilament activity in the contractile apparatus of the cardiac myocyte. Upon electrical stimulation, the concentration of Ca^{2+} in the contractile units increases at least 10-fold, thereby inducing the formation of cross-bridges between myofilaments. The subsequent conformational changes of the myosin head finally lead to myocardial contraction. Therefore, levels of cellular Ca^{2+} directly correlate with the heart's mechanical function and enhancing myocyte Ca^{2+} cycling increases mechanical force of the contractile units and the rate of contraction and relaxation. Potential therapeutic strategies were evaluated in animal models aiming at nodal points of the signalling cascade such as sarco-/endoplasmatic reticulum Ca^{2+}-ATPase (SERCA2a), which plays an important role in diastolic Ca^{2+} removal. SERCA2a activity was modulated via deletion of the SERCA2a inhibitor PLN, overexpression of SERCA2a or overexpression of S100 calcium binding protein A1 (S100A1). Further, the LTCC was targeted using the $G\beta\gamma$ scavenger C-terminus of the β-adrenergic receptor kinase (βARKct), which leads to disinhibition of G-protein ($G\beta\gamma$)-mediated inhibition of the channel (Slack *et al.* 2001; Schmitt *et al.* 2009; Pleger *et al.* 2011; Völkers *et al.* 2011; Kairouz *et al.* 2012). In healthy hearts, none of these strategies led to rapid deterioration of cardiac function and they all successfully rescued animal models of heart failure. The most-progressed target, SERCA2a, was evaluated in patients with moderate to severe heart failure in the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial. In phase 1/2, intracoronary infusion of a recombinant adeno-associated virus (AAV) vector for delivery of SERCA2a DNA appeared promising; however, in a follow-up study that evaluated the effects on hospitalization and mortality, SERCA2a gene transfer turned out to be safe but did not improve the endpoints. Technical issues leading to inefficient cellular uptake of the viral vector are discussed as likely causes for the failure of the phase 2b CUPID trial. Further investigation of this trial is needed to avoid failure of future gene therapy trials (Pleger *et al.* 2014; Greenberg *et al.* 2014, 2016; Greenberg, 2015; Lother & Hein, 2016).

The underlying reasons why reconstitution of βAR signalling is particularly prone to cardiac damage but

reconstitution of Ca^{2+} cycling is rather well-tolerated or even protective are not yet understood. Remarkably, over-expression of adenylyl cyclase 6 (AC6) safely increased left ventricular function beyond standard heart failure therapy in a recently published phase 1/2 trial of AC6 gene transfer in heart failure patients (Pleger *et al.* 2014; Hammond *et al.* 2016). Unlike other AC subtypes, AC6 has no effect on basal cAMP levels and is only responsive to βAR stimulation suggesting that selective and non-constitutive activation of βAR downstream targets may be crucial in distinguishing well-tolerated from detrimental positive inotropy. AC6 is also thought to improve cardiac performance via cAMP-independent mechanisms that still need to be elucidated (Gao *et al.* 2002; Tang *et al.* 2012).

Recently, RKIP was suggested as a promising strategy to stimulate cardiac contractility and to reconstitute βAR signalling of failing hearts by chronic β_1AR activation without triggering adverse effects. Unlike AC6, RKIP enhances adrenergic signalling in cardio-myocytes at a different level. RKIP attenuates GRK2 activity and thereby produces a balanced activation of β_1ARs and β_2ARs. The following discussion will summarize the potential benefits of this differential activation in failing cardiomyocytes and evaluate RKIP as a therapeutic agent against heart failure. We will further discuss the effects of RKIP on key components of downstream βAR signalling, particularly myocyte Ca^{2+} kinetics, diastolic Ca^{2+} leak and myofilament Ca^{2+} sensitivity since they show characteristic alterations in failing hearts that lead to contractile dysfunction and arrhythmia.

RKIP – a governor of intracellular signalling

RKIP belongs to the evolutionarily conserved phosphatidylethanolamine-binding protein (PEBP) family, which has been characterized as a modulator of signal transduction cascades in mammalian cells and has been reviewed in detail by Trakul & Rosner (2005), Granovski & Rosner (2008) and Lorenz *et al.* (2014*a*). PEBP/RKIP proteins possess a central β-sheet surrounded by smaller β-strands and two carboxy-terminal α-helices. These structural elements are connected by loops of variable length. Characteristic for this family is a cavity at the surface that consists of dynamically arranged amino acid residues and displays high affinity for small anionic groups such as phosphates, phospholipids and nucleotides (Hengst, 2000; Granovski & Rosner, 2008; Granovski *et al.* 2009). This cavity is also implicated in the binding of RKIP to the kinase Raf-1 to the extent that reduced flexibility of the cavity favours Raf binding (Granovski *et al.* 2009). Raf-1 is a member of the Raf–mitogen-activated protein kinase kinase (MEK)–extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) cascade that is involved in differentiation,

proliferation, cell survival and hypertrophy. RKIP has been shown to inhibit Raf-1 signalling (Yeung *et al.* 1999), but the mechanism is not yet entirely clarified. While over-expressed RKIP has been postulated to interfere with the interaction of Raf-1 with its substrate, MEK, endogenous RKIP rather interferes with Raf-1 activation (Trakul & Rosner, 2005). Phosphorylation of RKIP by protein PKC at serine 153 mediates the release of Raf-1 from RKIP (Corbit *et al.* 2003; Lorenz *et al.* 2003; Deiss *et al.* 2012). Interestingly, serine 153 phosphorylation triggers an additional mechanistic/structural feature with impact on the control of RKIP interaction partners: it induces RKIP dimerization. A loop structure at the surface of RKIP and in immediate proximity to the PKC phosphorylation site was identified as a part of the dimerization interface (Deiss *et al.* 2012). RKIP dimerization facilitates the release of Raf-1 but also participates in the substrate switch of RKIP from Raf-1 to GRK2 since inhibition of RKIP dimerization prevented RKIP/GRK2 binding and, *vice versa*, a dimeric RKIP mutant was able to bind GRK2 in the absence of RKIP^{Ser153} phosphorylation (Deiss *et al.* 2012). As mentioned above, GRK2 is a kinase that phosphorylates activated G-protein-coupled receptors (GPCRs), thereby initiating their desensitization and internalization and subsequently blunting receptor signalling (Pierce *et al.* 2002). In mammalian cells, GRK2 is a major feedback inhibitor of GPCRs and has been implicated in diseases such as immune diseases or heart failure. RKIP does not inhibit the catalytic activity of GRK2 but interferes with the GRK2–receptor interaction via its binding to the N-terminus of GRK2, a part of GRK2 that is important for GRK–receptor interaction (Lorenz *et al.* 2003). This inhibitory mechanism of RKIP enables a largely specific interference of RKIP with GRK2 towards receptor substrates while cytosolic substrates of GRK2 are not affected (Schmid *et al.* 2015). GRK2–RKIP interaction prevents GPCR internalization leading to enhanced GPCR signalling, which, in the heart, enhances contraction and relaxation.

Even though other kinase signalling cascades such as the nuclear factor κ-light-chain-enhancer of activated B-cells (NFκB) and glycogen synthase kinase-3β are also known to be regulated by RKIP in cultured cells, thus far only Raf-1, MEK1 and ERK2 as well as GRK2 have been identified as direct interaction partners of RKIP of which only Raf-1 and GRK2 have been validated under endogenous conditions (Yeung *et al.* 2001; Lorenz *et al.* 2009, 2014*b*). In line with its influence on several kinase signalling cascades, RKIP impacts on diverse physiological processes including cell transformation, cell cycle, inflammation, metastasis and cardiomyocyte contractility (Granovsky & Rosner, 2008; Lorenz *et al.* 2014*a*; Brietz *et al.* 2016). Deletion or downregulation of RKIP resulted for example in deterioration of metastatic cancer, Alzheimer's disease, pulmonary hypertension and heart failure and increased

replication of the Newcastle disease virus (Lorenz *et al.* 2014*a*; Schmid *et al.* 2015; Yin *et al.* 2016).

RKIP and its function in the heart

Cardiac RKIP expression is up-regulated in heart failure patients and in mice with pressure overload-induced heart failure, which implies that RKIP is part of the physiological response to stress in cardiac diseases. Indeed, mice with cardiac overexpression of RKIP are protected from heart failure induced by chronic pressure overload (induced by transverse aortic constriction) while RKIP deficiency exaggerated heart failure under these conditions. AAV9-mediated gene transfer protected wild-type and RKIP knockout mice from transverse aortic constriction-induced heart failure (Schmid *et al.* 2015). Recent findings strongly suggest that RKIP provides a new and well-tolerated mode of sustained βAR activation in the heart by differential stimulation of protective *vs.* detrimental β-adrenergic signalling (Schmid *et al.* 2015; Fig. 2). The data indicate that the effects of RKIP in the heart are characterized by the following qualities:

(1) RKIP stimulates β_1AR–G_s signalling, which results in enhanced contraction and relaxation via increased PLN and TnI phosphorylation and subsequently increased SERCA2a activity, higher SR Ca^{2+} load and decreased Ca^{2+} sensitivity of myofilaments. Cardiac contractility of RKIP-overexpressing mice was improved compared to control animals up to an age of at least 12–14 months; and lifespan of RKIP-overexpressing mice under these conditions was at least as long as of non-transgenic mice (Schmid *et al.* 2015).

(2) Despite enhanced β_1AR–G_s signalling, RKIP-stimulated hearts are still able to respond adequately to physiological stress situations because the size of the dobutamine response of RKIP-overexpressing and wild-type hearts is similar. This moderate or sub-maximal activation may play an important role for the observed reduction of cardiomyocyte apoptosis, interstitial fibrosis, brain natriuretic peptide and collagen expression in RKIP-overexpressing mice compared to wild-type controls and the overall well-tolerated positive inotropic phenotype of RKIP-overexpressing mice (Schmid *et al.* 2015).

(3) Besides β_1AR–G_s-signalling that stimulates the activity of both PKA and CaMKII, RKIP activates β_2AR–G_i in mouse hearts. The simultaneous
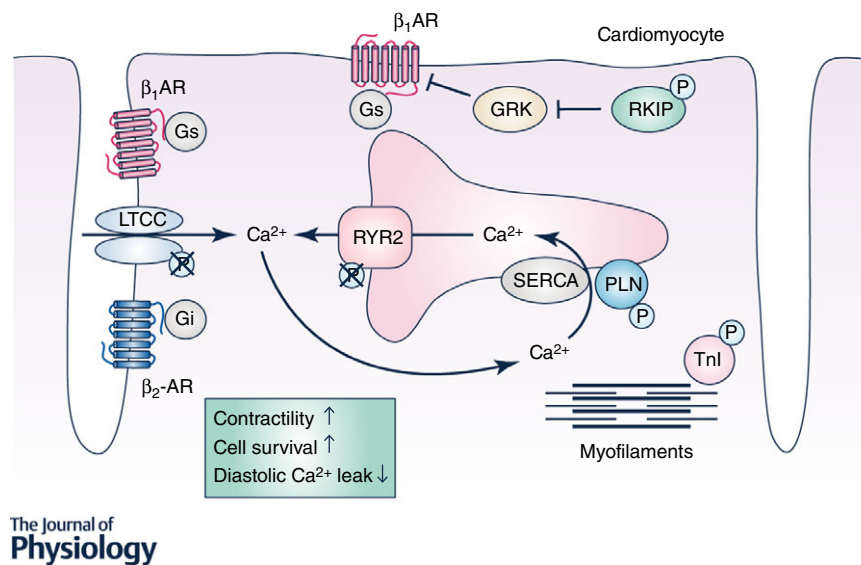
Figure 2. RKIP induces positive inotropy and protects from cell death and diastolic Ca^{2+} leak
The Raf kinase inhibitor protein (RKIP) binds GRK2 and inhibits G-protein-coupled receptor kinase (GRK)-mediated receptor phosphorylation, which prevents receptor desensitization and internalization and, thus, increases β-adrenergic receptor signalling. RKIP increases contractility and relaxation of cardiomyocytes via activated β_1-adrenergic receptor (β_1AR) coupled to stimulatory G-proteins (G_s): phosphorylated phospholamban (PLN) dissociates from sarco-/endoplasmatic reticulum Ca^{2+}-ATPase (SERCA2a) and thereby increases SERCA2a activity, Ca^{2+} loading of the sarcoplasmatic reticulum and cardiomyocyte contractility. Phosphorylation of troponin I (TnI) decreases Ca^{2+} sensivity and thereby increases cardiomyocyte relaxation. RKIP mediates anti-apoptotic, anti-fibrotic and anti-arrhythmic effects via increased β_2-adrenergic receptor (β_2AR) signalling. Continuous signalling of β_2AR coupled to inhibitory G-proteins (G_i) prevents β_1AR-stimulated increases in ryanodine receptor 2 (RyR2) and L-type Ca^{2+} channel (LTCC) phosphorylation and protects from diastolic Ca^{2+} leak.

activation of β_2AR–G_i within the transverse (t)-tubular region prevents the adverse effects of mere β_1AR–G_s, such as diastolic Ca^{2+} leak and cardiac arrhythmia due to hyperphosphorylation and subsequent activation of the RyR2 or hyperphosphorylation of the LTCC. These RKIP effects appear to be mediated by β_2AR–G_i signalling since this protection is absent in RKIP-overexpressing mice lacking β_2ARs and in the presence of the G_i inhibitor pertussis toxin (Communal *et al.* 1999; Xiao *et al.* 1999; Lehnart *et al.* 2009; Eschenhagen, 2010; Nikolaev *et al.* 2010; Bers, 2014; Schmid *et al.* 2015). Of note, these experiments show that RKIP predominantly activates β_2AR coupled to G_i in mouse hearts, even though RKIP in principle is capable of activating β_2AR–G_s as demonstrated in cell cultures (Lorenz *et al.* 2003). Further evidence for a central role of β_2AR on the protective effects of RKIP in the heart is the absence of protection from cardiac remodelling, i.e. apoptosis and interstitial fibrosis, in RKIP transgenic mice lacking the β_2AR as well as a reduced overall survival compared to β_2KO controls – effects that are reported to result from mere β_1AR–G_s signalling (Schmid *et al.* 2015). The switch of β_2AR from G_s to G_i in RKIP transgenic mice seemed to be due to the enduring β_1AR–G_s–PKA activation as indicated by the characteristic phosphorylation patterns of βAR downstream targets (Daaka *et al.* 1997; Xiao *et al.* 1999). Remarkably, overall β_2AR phosphorylation is significantly reduced in RKIP transgenic mice, consistent with GRK2 inhibition and absence of β_2AR desensitization in RKIP transgenic mice (Rockmann *et al.* 2002; Houslay & Baillie, 2005; Schmid *et al.* 2015).

(4) RKIP was found to inhibit βAR downregulation and thereby secures sustained positive inotropy, which is not achieved by direct agonist-mediated βAR stimulation (Lorenz *et al.* 2003; Schmid *et al.* 2015) (Figs 1 and 2).

(5) RKIP promotes cell survival. RKIP overexpression reduced cardiomyocyte apoptosis, whereas deletion of RKIP (RKIP$^{-/-}$) dramatically increased it. This effect is β_2AR dependent since the protection from apoptosis is absent in RKIP-transgenic mice lacking the β_2AR. Interestingly, β_2AR–G_i is known to stimulate the kinase Akt, which in turn mediates anti-apoptotic effects (Chesley *et al.* 2000; Talan *et al.* 2011). In line with β_2AR–G_i activation by RKIP, Akt activation was enhanced in RKIP-overexpressing mice and was dependent on β_2AR and pertussis toxin-sensitive G_i proteins. These findings suggest that RKIP mediates cell survival via Akt.

However, as described above, RKIP not only increases GPCR signalling via GRK inhibition, but also inhibits mitogen-activated protein kinase signalling dependent on its phosphorylation status: RKIP acts as a GRK inhibitor in its PKC phosphorylated form (pRKIP^{Ser153}), but in the absence of Ser153 phosphorylation it acts as a Raf-1 inhibitor. In the heart, Raf–MEK–ERK1/2 signalling promotes cell survival (Punn *et al.* 2000; Harris *et al.* 2004; Heineke & Molkentin, 2006; Purcell *et al.* 2007; Sheikh *et al.* 2008; Cheng *et al.* 2011; Van Berlo *et al.* 2011). Thus, in its unphosphorylated form, RKIP could potentially increase cardiomyocyte death. However, RKIP in the heart mainly exists in its phosphorylated form, so that the Raf-1 inhibitory effect of RKIP is absent in the heart. Even moderate RKIP overexpression of up to 8-fold revealed no inhibitory effect of RKIP on Raf-1/MEK/ERK1/2. Potential side effects of this new cardioprotective strategy may occur at very high overexpression levels of RKIP that exceed the ability of PKC to fully phosphorylate RKIP and may result in RKIP-mediated Raf/MEK/ERK1/2 inhibition and increased apoptosis associated with signs of heart failure as seen by Fu *et al.* or by cardiac overexpression of a phosphorylation-deficient mutant of RKIP, RKIP^{S153A} (Bueno *et al.* 2000; Lorenz *et al.* 2003; Fu *et al.* 2013; Ruppert *et al.* 2013; Schmid *et al.* 2015).

Compared to other positive inotropic strategies in heart failure therapy, the biochemical and phenotypic effects of RKIP substantiate the hypothesis that a successful positive inotropic strategy should not induce an unselective activation of βAR downstream targets (as for βAR agonists, PDEIII inhibitors or β_1AR-overexpressing mice) but rather circumvent activation of the RyR2 (as in PLN$^{-/-}$ mice, SERCA2a or βARKct overexpression) or even protect from RyR2 sensitization (as in GRK2$^{-/-}$ mice, S100A1 and RKIP transgenic mice) (Kairouz *et al.* 2012; Respress *et al.* 2012; Bers, 2014; Pleger *et al.* 2014; Ritterhoff *et al.* 2015). RKIP achieves this well-tolerated βAR stimulation with positive inotropy and lusitropy in RKIP-overexpressing mice by concomitant activation of β_2ARs (in their G_i-coupled mode) that counteracts several maladaptive β_1AR effects such as RyR2 sensitization, diastolic Ca^{2+} leaks and arrhythmia as well as apoptosis and fibrosis. Indeed, 'sole' β_1AR activation as provided in RKIP transgenic mice lacking the β_2AR (β_2KO) increased cardiac contractility, but also reduced overall survival of ageing RKIP transgenic mice (RKIP/β_2KO) compared to β_2KO controls (Schmid *et al.* 2015). Taken together, the consequences of enhanced βAR signalling in the heart appear to be highly dependent on the type and the extent of activated signalling elements.

In sum, RKIP differentially modulates several molecular events downstream of βAR, which appears promising for heart failure therapy. In the following, we will discuss the differential regulation of βAR receptors by RKIP, its effects on myocyte Ca^{2+} kinetics and distribution and how failing hearts may benefit from these alterations.

Depressed myocyte Ca^{2+} cycling and heart disease

Ca^{2+} enters the cardiomyocyte via the LTCCs, which are predominantly located within the t-tubuli of the sarcolemma in close neighbourhood to the sarcoplasmic Ca^{2+} release channels, RyR2. These functional dyads facilitate the rapid increase of cytosolic Ca^{2+} levels upon depolarization of the cell leading to myofilament contraction. Cardiac relaxation is initiated by Ca^{2+} removal from the cytosol. In human myocytes, 74% of diastolic Ca^{2+} removal is accomplished by the Ca^{2+}-ATPase SERCA2a, 24% by the Na$^+$/Ca^{2+} exchanger (NCX), 1% by the sarcolemmal Ca^{2+}-ATPase (PMCA) and 1% by the mitochondrial Ca^{2+} uniporter) (Bers, 2014). Since SERCA2a eliminates the largest share (even 93% of Ca^{2+} removal in myocytes from mice and rats), the SERCA2a regulator PLN plays a pivotal role in modulating myocyte Ca^{2+} distribution and kinetics. PLN inhibits SERCA2a, thereby attenuating the rate of Ca^{2+} transport to the SR. Phosphorylation of PLN by PKA at serine 16 can almost fully relieve this inhibitory effect leading to a pronounced acceleration of SR Ca^{2+} uptake. As Ca^{2+} is sequestered by the SR, in the sarcomeres TnI inhibition of actin–myosin interactions is re-established and myocytes relax (Rockman *et al.* 2002).

In heart failure, depressed contractility is associated with depressed myocyte Ca^{2+} cycling. Although the causes of heart failure can vary widely, e.g. myocardial infarction, arterial hypertension, infections or genetic defects, the pattern of abnormal Ca^{2+} metabolism is relatively uniform (overview in Lehnart *et al.* 2009). In failing hearts, Ca^{2+} release is typically reduced, consistent with a decrease in contractility and force generation (van der Velden *et al.* 2004; Avner *et al.* 2011; Haghighi *et al.* 2014). Further, SR Ca^{2+} reuptake during diastole is slow and diastolic Ca^{2+} levels are elevated due to a diastolic Ca^{2+} leak (via RyR2), but primarily due to reduced SERCA2a activity. Depression of the Ca^{2+} pump results from reduced SERCA2a expression in failing hearts, whereas PLN levels remain stable leading to a reduced SERCA/PLN ratio (Hasenfuss & Pieske, 2002). SR Ca^{2+} transport is further inhibited by a reduction of PLN phosphorylation, most likely as a result of increased protein phosphatase-1 activity and downregulation of βAR density (Weber *et al.* 2016).

The identification of inherited mutations in Ca^{2+} regulatory proteins that caused alterations of protein function and induced dilated cardiomyopathy and terminal heart failure finally proved the concept that the Ca^{2+} cycling alterations in failing hearts are not secondary events or bystander in the course of the disease, but play a causative role in myocardial remodelling and the deterioration of cardiac function (Haghighi *et al.* 2003; Schmitt *et al.* 2003). Therefore, restoration of SR Ca^{2+} cycling holds promise for the treatment of heart failure.

RKIP seems to provide a promising approach for the restoration of depressed Ca^{2+} cycling. It increases myocyte Ca^{2+} transients at baseline and also upon bolus application of caffeine indicative of increased Ca^{2+} release. Increased Ca^{2+} release during systole in RKIP-overexpressing cardiomyocytes is most likely due to accelerated Ca^{2+} reuptake into the SR during diastole, which is mainly due to enhanced PLN phosphorylation at serine 16 (PKA site) and threonine 17 (CaMKII site) leading to efficient release of PLN from SERCA2a and subsequent activation of SERCA2a. The RKIP-induced increase in Ca^{2+} reuptake during diastole may further be supported by an accelerated Ca^{2+} release from the myofilaments mediated by enhanced TnI phosphorylation at the serine residues 23 and 24. Further, via GRK inhibition, RKIP prevents βAR desensitization and βAR degradation in a heart under chronic sympathetic stress, which subsequently secures efficient and continuous Ca^{2+} cycling. In addition, RKIP overexpression is able to prevent a loss of SERCA2a expression in a failing mouse heart, which in turn also ensures effective Ca^{2+} cycling in cardiomyocytes.

In sum, RKIP improves cardiac performance in healthy hearts and in failing hearts. Since the extent of the contractile response depends on the amount of activating Ca^{2+}, the enhanced Ca^{2+} load would explain the hypercontractile phenotype of RKIP-overexpressing hearts and the improved cardiac function in a mouse model of heart failure due to chronic pressure overload (Schmid *et al.* 2015). RKIP is an elegant example of achieving a stable and physiological (i.e. still regulatable) increase in Ca^{2+} cycling on several molecular levels via restoring expression and function of both βAR and direct regulators of myocyte Ca^{2+} cycling.

Increased Ca^{2+} sensitivity and heart disease

Ca^{2+} sensitivity of myofilaments affects contraction, relaxation and remodelling of the myocardium as well as cardiac rhythm. Increased Ca^{2+} sensitivity was observed in end-stage heart failure and in heart tissue 3–4 days after myocardial infarction (van der Velden *et al.* 2004; Avner *et al.* 2011). Further, in hypertrophic cardiomyopathy, the most frequent cause of sudden cardiac death in the young population, an increase of myofilament Ca^{2+} sensitivity has been proposed as a central disease mechanism (Landstrom & Ackerman 2012; Deftereos *et al.* 2016). Increased Ca^{2+} sensitivity is often associated with high susceptibility for ventricular tachycardia and sudden cardiac death. Desensitization of myofilaments to Ca^{2+} was suggested to reduce the risk of arrhythmias by stabilizing action potential generation and propagation, because high Ca^{2+} sensitivity would prolong Ca^{2+} transients and slow down the propagation of action potentials, thereby fostering the generation of electrical re-entry (Huke & Knollmann, 2010; Tardiff *et al.* 2015). The

causative relation to arrhythmia was underlined by human and animal studies that found increased episodes of ventricular tachycardia after myocardial infarction and in heart failure upon treatment with the Ca^{2+} sensitizer levosimendan (Flevari *et al.* 2006). In contrast, the myosin inhibitor blebbistatin reduced myofilament Ca^{2+} sensitivity and prevented ventricular tachycardia in troponin T mutant mice (Baudenbacher *et al.* 2008). These examples demonstrate the broad therapeutic possibilities of Ca^{2+} desensitizing agents to fight arrhythmias and myocardial remodelling as well as contractile dysfunction.

Schmid *et al.* (2015) found RKIP to increase TnI phosphorylation at S23/S24. This PKA-dependent phosphorylation decreases Ca^{2+} sensitivity of the Tn complex because phosphorylation reduces its Ca^{2+} affinity (Cheng *et al.* 2015). The strong therapeutic potential of decreasing Ca^{2+} sensitivity by TnI phosphorylation was demonstrated by the expression of a pseudo-phosphorylated TnI mutant that rescued the morphological and functional changes of the heart in an animal model of hypertrophic cardiomyopathy caused by an E180G α-Tm mutant with increased myofilament Ca^{2+} sensitivity (Alves *et al.* 2014). RKIP appears as a particularly attractive tool for Ca^{2+} desensitization of myofilaments because it also exhibits positive inotropy via enhanced β_1AR signalling and antiarrhythmic effects via reduced RyR2 and LTCC phosphorylation, and it prevents apoptosis and maladaptive remodelling by Akt stimulation.

Diastolic Ca^{2+} leak and heart disease

Besides desensitization of myofilaments by increasing TnI phosphorylation, Schmid *et al.* (2015) also showed RKIP to reduce the frequency of Ca^{2+} sparks and Ca^{2+} waves. Ca^{2+} sparks occur if a cluster of RyR2 produces a local Ca^{2+} release from the SR. With every heartbeat, the action potential synchronizes the almost simultaneous opening of thousands of RyR2 clusters within a myocyte leading to a Ca^{2+} transient that initiates contraction. In contrast, the local Ca^{2+} increase caused by a spontaneous Ca^{2+} spark can trigger Ca^{2+} release only from neighbouring RyR2 clusters via a Ca^{2+}-induced Ca^{2+} release. The resulting propagating wave may induce Ca^{2+} elimination by the NCX causing an inward current (1 Ca^{2+} out–3 Na^+ in) and both early and delayed afterdepolarizations that trigger aberrant electrical activity and arrhythmias of the heart (reviewed in Bers, 2014). The clinical relevance of this pathomechanism was demonstrated by the finding of disease-causing RyR2 mutations in patients with catecholaminergic polymorphic ventricular tachycardia, because the genetic defects put affected individuals at risk for stress-induced ventricular tachycardia (Priori & Chen, 2011). Not only do Ca^{2+} sparks trigger arrhythmia, but the diastolic loss of Ca^{2+} also reduces SR Ca^{2+} content. As

a consequence of this loss, the systolic Ca^{2+}-induced Ca^{2+} release is smaller leading to reduced contraction of the heart. The Ca^{2+} leak also impairs myocardial relaxation, because it slows down cytosolic Ca^{2+} clearance during diastole of the heart and it may cause diastolic activation of contractile proteins.

RKIP reduces the frequency of Ca^{2+} sparks and Ca^{2+} waves by reducing phosphorylation of RyR2 at SS2808/2814 (Schmid *et al.* 2015). Hyperphosphorylation of RyR2 is known to induce diastolic SR Ca^{2+} leakage, which predisposes for arrhythmias (Bers, 2014). The respective role of S2808 and S2814 phosphorylation by PKA or CaMKII in this scenario, however, is controversial in the field (Eschenhagen, 2010; Bers, 2014). The reduction of RyR2 phosphorylation by RKIP despite enhancing β-adrenergic signalling is explained by β_2AR–G_i-coupled signalling induced by RKIP (Schmid *et al.* 2015). Since β_2ARs are believed to be concentrated within the t-tubular region of the cardiomyocyte (Kuschel *et al.* 1999; Orchard & Brette, 2007; Nikolaev *et al.* 2010), β_2AR–G_i signalling within the t-tubular region seems to protect βAR targets in close proximity to β_2ARs such as the RyR2 and LTCC from hypersensitization. It is noteworthy that the resulting stabilization of RyR2 is strong enough to reduce the incidence of Ca^{2+} sparks despite increased SR Ca^{2+} contents, a condition that can trigger spontaneous Ca^{2+} release.

Pharmacological and gene therapeutic studies are aimed at fixing the Ca^{2+} leak, increasing the Ca^{2+} transient and enhancing cytosolic Ca^{2+} clearance (reviewed in Marks, 2013). RKIP is a positive inotropic strategy that circumvents RyR2 sensitization and seems to fulfil all of these requirements. RKIP (1) reduces the occurrence of Ca^{2+} sparks by β_2AR-mediated stabilization of RyR2, (2) accelerates cytosolic Ca^{2+} elimination by PKA-dependent phosphorylation of PLN and (3) increases SR Ca^{2+} filling and therefore the size of the Ca^{2+} transients. Finally, RKIP overexpression prevented morphological and functional maladaptation of the heart in mice subjected to long-term pressure overload. Future studies will show if these beneficial effects hold true for the rescue of independent models of heart failure.

Conclusion

RKIP is an endogenous protein that exhibits a combination of favourable effects for heart failure patients: (1) the gain/increase of cardiac contractile efficiency by the activation of G_s signalling/β_1AR leading to functional recovery of the heart and (2) the protection of the heart under sympathetic stress from exaggerated β_1AR downstream signalling including protection from apoptosis and pro-arrhythmic adverse effects via β_2AR activation. This approach promises a new therapeutic strategy to achieve well-tolerated long-term increases in cardiac contractility.

RKIP comprises several favourable characteristic effects on calcium cycling, calcium sensitivity, G-protein recruitment to βARs and a physiological extent or range of βAR activation and has proven protective in murine heart failure. Future studies will further unravel the signalling network induced by RKIP that is responsible for the well-tolerated mode of βAR activation and evaluate its therapeutic efficacy in various disease entities.

References

Ahmet I, Krawczyk M, Zhu W, Woo AYH, Morrell C, Poosala S, Xiao RP, Lakatta EG & Talan MI (2008). Cardioprotective and survival benefits of long-term combined therapy with β2 adrenoreceptor (AR) agonist and β1AR blocker in dilated cardiomyopathy postmyocardial infarction. *J Pharmacol Exp Ther* **325**, 491–499.

Alves ML, Dias FA, Gaffin RD, Simon JN, Montminy EM, Biesiadecki BJ, Hinken AC, Warren CM, Utter MS, Davis RT, Sadayappan S, Robbins J, Wieczorek DF, Solaro RJ & Wolska BM. (2014) Desensitization of myofilaments to Ca^{2+} as a therapeutic target for hypertrophic cardiomyopathy with mutations in thin filament proteins. *Circ Cardiovasc Genet* **2**, 123–143.

Avner BS, Shioura KM, Scruggs SB, Grachoff M, Geenen DL, Helseth DL, Farjah M, Goldspink PH & Solaro JR (2011). Myocardial infarction in mice alters sarcomeric function via post-translational protein modification. *Mol Cell Biochem* **363**, 203–215.

Baker AJ (2014). Adrenergic signaling in heart failure: a balance of toxic and protective effects. *Pflugers Arch* **466**, 1139–1150.

Balligand JL (2013). Beta3-adrenoreceptors in cardiovasular diseases: new roles for an "old" receptor. *Curr Drug Deliv* **10**, 64–66.

Baudenbacher F, Schober T, Pinto JR, Sidorov VY, Hilliard F, Solaro RJ, Potter JD & Knollmann BC (2008). Myofilament Ca^{2+} sensitization causes susceptibility to cardiac arrhythmia in mice. *J Clin Invest* **118**, 3893–3903.

Belge C, Hammond J, Dubois-Deruy E, Manoury B, Hamelet J, Beauloye C, Markl A, Pouleur AC, Bertrand L, Esfahani H, Jnaoui K, Götz KR, Nikolaev VO, Vanderper A, Herijgers P, Lobysheva I, Iaccarino G, Hilfiker-Kleiner D, Tavernier G, Langin D, Dessy C & Balligand JL (2014). Enhanced expression of β3-adrenoceptors in cardiac myocytes attenuates neurohormone-induced hypertrophic remodeling through nitric oxide synthase. *Circulation* **129**, 451-462.

Bers DM (2014). Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol* **76**, 107–127.

Brietz A, Schuch KV, Wangorsch G, Lorenz K & Dandekar T (2016). Analyzing ERK 1/2 signalling and targets. *Mol Biosyst* **12**, 2436–2446.

Bristow MR, Anderson FL, Port JD, Skerl L, Hershberger RE, Larrabee P, O'Connell JB, Renlund DG, Volkman K, Murray J & Feldman AM (1991). Differences in beta-adrenergic neuroeffector mechanisms in ischemic versus idiopathic dilated cardiomyopathy. *Circulation* **84**, 1024–1039.

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P & Jamieson S (1986). β_1- and β_2-Adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. *Circ Res* **59**, 297–309.

Brodde OE (1991). β_1- and β_2-Adrenoceptors in the human heart: properties, function, and alterations in chronic heart failure. *Pharmacol Rev* **43**, 203–242.

Brodde OE, Schüler S, Kretsch R, Brinkmann M, Borst HG, Hetzer R, Reidemeister JC, Warnecke H & Zerkowski HR (1986). Regional distribution of beta-adrenoceptors in the human heart: coexistence of functional β_1- and β_2-adrenoceptors in both atria and ventricles in severe congestive cardiomyopathy. *J Cardiovasc Pharmacol* **8**, 1235–1242.

Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R, Hewett TE, Jones SP, Lefer DJ, Peng CF, Kitsis RN & Molkentin JD (2000). The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *EMBO J* **19**, 6341–6350.

Cheng H, Kari G, Dicker AP, Rodeck U, Koch WJ & Force T (2011). A novel preclinical strategy for identifying cardiotoxic kinase inhibitors and mechanisms of cardiotoxicity. *Circ Res* **109**, 1401–1409.

Cheng Y, Rao V, Tu A-Y, Lindert S, Wang D, Oxenford L, McCulloch AD, McCammon JA & Regnier M (2015). Troponin I mutations R146G and R21C alter cardiac troponin function, contractile properties and modulation by PKA-mediated phosphorylation. *J Biol Chem* **68**, 3045–3119.

Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG & Crow MT (2000) The β_2-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G_i-dependent coupling to phosphatidylinositol $3'$-kinase. *Circ Res* **87**, 1172–1179.

Cho GW, Altamirano F & Hill JA (2016). Chronic heart failure: Ca^{2+}, catabolism, and catastrophic cell death. *Biochim Biophys Acta* **1862**, 763–777.

Cohn JN, Johnson GR, Shabetai R, Loeb H, Tristani F, Rector T, Smith R & Fletcher R (1993). Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias, and plasma norepinephrine as determinants of prognosis in heart failure. The V-HeFT VA Cooperative Studies Group. *Circulation* **87**, VI5–VI16.

Communal C, Singh K, Sawyer DB & Colucci WS (1999). Opposing effects of β_1- and β_2-adrenergic receptors on cardiac myocyte apoptosis: role of a pertussis toxin-sensitive G protein. *Circulation* **100**, 2210–2212.

Corbit KC, Trakul N, Eves EM, Diaz B, Marshall M & Rosner MR (2003). Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein. *J Biol Chem* **278**, 13061–13068.

Daaka Y, Luttrell LM & Lefkowitz RJ (1997). Switching of the coupling of the β_2-adrenergic receptor to different G proteins by protein kinase A. *Nature* **390**, 88–91.

Deftereos S, Papoutsidakis N, Giannopoulos G, Angelidis C, Raisakis K, Bouras G, Davlouros P, Panagopoulou V, Goudevenos J, Cleman MW & Lekakis J. (2016) Calcium ions in inherited cardiomyopathies. *J Med Chem* **2**, 139–150.

Deiss K, Kisker C, Lohse MJ & Lorenz K (2012). Raf kinase inhibitor protein (RKIP) dimer formation controls its target switch from Raf1 to G protein-coupled receptor kinase (GRK) 2. *J Biol Chem* **287**, 23407–23417.

Dorn GW, Tepe NM, Lorenz JN, Koch WJ & Liggett SB (1999). Low- and high-level transgenic expression of β_2-adrenergic receptors differentially affect cardiac hypertrophy and function in Galphaq-overexpressing mice. *Proc Natl Acad Sci USA* **96**, 6400–6405.

El-Armouche A, Wittkopper K, Degenhardt F, Weinberger F, Didie M, Melnychenko I, Grimm M, Peeck M, Zimmermann WH, Unsold B, Hasenfuss G, Dobrev D & Eschenhagen T (2008). Phosphatase inhibitor-1-deficient mice are protected from catecholamine-induced arrhythmias and myocardial hypertrophy. *Cardiovasc Res* **80**, 396–406.

Engelhardt S, Hein L, Wiesmann F & Lohse MJ (1999). Progressive hypertrophy and heart failure in β_1-adrenergic receptor transgenic mice. *Proc Natl Acad Sci USA* **96**, 7059–7064.

Eschenhagen T (2010). Is ryanodine receptor phosphorylation key to the fight or flight response and heart failure? *J Clin Invest* **120**, 4197–4203.

Fajardo G, Zhao M, Urashima T, Farahani S, Hu DQ, Reddy S & Bernstein D. (2013) Deletion of the β_2-adrenergic receptor prevents the development of cardiomyopathy in mice. *J Mol Cell Cardiol* **63**, 155–164.

Felker G (2001). Inotropic therapy for heart failure: An evidence-based approach. *Am Heart J* **142**, 393–401.

Flevari P, Parissis JT, Leftheriotis D, Panou F, Kourea K & Kremastinos DT (2006). Effect of levosimendan on ventricular arrhythmias and prognostic autonomic indexes in patients with decompensated advanced heart failure secondary to ischemic or dilated cardiomyopathy. *Am J Cardiol* **98**, 1641–1645.

Francis GS, Cohn JN, Johnson G, Rector TS, Goldman S & Simon A (1993). Plasma norepinephrine, plasma renin activity, and congestive heart failure. Relations to survival and the effects of therapy in V-HeFT II. The V-HeFT VA Cooperative Studies Group. *Circulation* **87**, 40–48.

Fu X, Koller S, Abd Alla J & Quitterer U (2013). Inhibition of G protein-coupled receptor kinase 2 (GRK2) triggers the growth-promoting mitogen-activated protein kinase (MAPK) pathway. *J Biol Chem* **288**, 7738–7755.

Gao MH, Bayat H, Roth DM, Yao Zhou J, Drumm J, Burhan J & Hammond HK (2002). Controlled expression of cardiac-directed adenylylcyclase type VI provides increased contractile function. *Cardiovasc Res* **56**, 197–204.

Gorelik J, Wright PT, Lyon AR & Harding SE (2013). Spatial control of the AR system in heart failure: the transverse tubule and beyond. *Cardiovasc Res* **98**, 216–224.

Granovsky AE & Rosner MR (2008). Raf kinase inhibitory protein: a signal transduction modulator and metastasis suppressor. *Cell Res* **18**, 452–457.

Granovsky AE, Clark MC, McElheny D, Heil G, Hong J, Liu X, Kim Y, Joachimiak G, Joachimiak A, Koide S & Rosner MR (2009). Raf kinase inhibitory protein function is regulated via a flexible pocket and novel phosphorylation-dependent mechanism. *Mol Cell Biol* **29**, 1306–1320.

Greenberg B (2015). Gene therapy for heart failure. *J Cardiol* **66**, 195–200.

Greenberg B, Butler J, Felker GM, Ponikowski P, Voors AA, Pogoda JM, Provost R, Guerrero J, Hajjar RJ, Zsebo KM (2016). Prevalence of AAV1 neutralizing antibodies and consequences for a clinical trial of gene transfer for advanced heart failure. *Gene Ther* **23**, 313–319.

Greenberg B, Yaroshinsky A, Zsebo KM, Butler J, Felker GM, Voors AA, Rudy JJ, Wagner K & Hajjar RJ (2014). Design of a phase 2b trial of intracoronary administration of AAV1/SERCA2a in patients with advanced heart failure: the CUPID 2 trial (calcium up-regulation by percutaneous administration of gene therapy in cardiac disease phase 2b). *JACC Heart Fail* **2**, 84–92.

Haghighi K, Kolokathis F, Pater L, Lynch RA, Asahi M, Gramolini AO, Fan G-C, Tsiapras D, Hahn HS, Adamopoulos S, Liggett SB, Dorn GW, MacLennan DH, Kremastinos DT & Kranias EG (2003). Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. *J Clin Invest* **111**, 869–876.

Haghighi K, Bidwell P & Kranias EG (2014). Phospholamban interactome in cardiac contractility and survival: A new vision of an old friend. *J Mol Cell Cardiol* **77**, 160–167.

Hammond HK, Penny WF, Traverse JH, Henry TD, Watkins MW, Yancy CW, Sweis RN, Adler ED, Patel AN, Murray DR, Ross RS, Bhargava V, Maisel A, Barnard DD, Lai NC, Dalton ND, Lee ML, Narayan SM, Blanchard DG & Gao MH (2016). Intracoronary gene transfer of adenylyl cyclase 6 in patients with heart failure: a randomized clinical trial. *JAMA Cardiol* **1**, 163–171.

Harris IS, Zhang S, Treskov I, Kovacs A, Weinheimer C & Muslin AJ (2004). Raf-1 kinase is required for cardiac hypertrophy and cardiomyocyte survival in response to pressure overload. *Circulation* **110**, 718–723.

Hasenfuss G & Pieske B (2002). Calcium cycling in congestive heart failure. *J Mol Cell Cardiol* **34**, 951–969.

Heineke J & Molkentin JD (2006). Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol* **7**, 589–600.

Hengst U (2000). The phosphatidylethanolamine-binding protein is the prototype of a novel family of serine protease inhibitors. *J Biol Chem* **276**, 535–540.

Houslay MD & Baillie GS (2005). β-Arrestin-recruited phosphodiesterase-4 desensitizes the AKAP79/PKA-mediated switching of β_2 adrenoceptor signalling to activation of ERK. *Biochem Soc Trans* **33**, 1333–1336.

Huke S & Knollmann BC (2010). Increased myofilament Ca^{2+}-sensitivity and arrhythmia susceptibility. *J Mol Cell Cardiol* **48**, 824–833.

Jensen BC, Swigart PM, De Marco T, Hoopes C & Simpson PC (2009). α_1-Adrenergic receptor subtypes in nonfailing and failing human myocardium. *Circ Heart Fail* **2**, 654–663.

Kairouz V, Lipskaia L, Hajjar RJ & Chemaly ER (2012). Molecular targets in heart failure gene therapy: current controversies and translational perspectives. *Ann N Y Acad Sci* **1254**, 42–50.

Krüger M & Linke WA (2006). Protein kinase-A phosphorylates titin in human heart muscle and reduces myofibrillar passive tension. *J Muscle Res Cell Motil* **27**, 435–444.

Kuschel M, Zhou YY, Cheng H, Zhang SJ, Chen Y, Lakatta EG & Xiao RP (1999). G_i protein-mediated functional compartmentalization of cardiac β_2-adrenergic signaling. *J Biol Chem* **274**, 22048–22052.

Landstrom AP, Ackerman MJ. (2012). Beyond the cardiac myofilament: hypertrophic cardiomyopathy-associated mutations in genes that encode calcium-handling proteins. *Curr Mol Med* **5**, 507–518.

Lechat P, Packer M, Chalon S, Cucherat M, Arab T & Boissel JP (1998). Clinical effects of β-adrenergic blockade in chronic heart failure: a meta-analysis of double-blind, placebo-controlled, randomized trials. *Circulation* **98**, 1184–1191.

Lefkowitz RJ, Pierce KL & Luttrell LM (2002). Dancing with different partners: protein kinase a phosphorylation of seven membrane-spanning receptors regulates their G protein-coupling specificity. *Mol Pharmacol* **62**, 971–974.

Lehnart SE, Maier LS & Hasenfuss G (2009). Abnormalities of calcium metabolism and myocardial contractility depression in the failing heart. *Heart Fail Rev* **14**, 213–224.

Liggett SB, Tepe NM, Lorenz JN, Canning AM, Jantz TD, Mitarai S, Yatani A & Dorn GW (2000). Early and delayed consequences of β_2-adrenergic receptor overexpression in mouse hearts: critical role for expression level. *Circulation* **101**, 1707–1714.

Liggett SB, Wagoner LE, Craft LL, Hornung RW, Hoit BD, McIntosh TC & Walsh RA (1998). The Ile164 β_2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. *J Clin Invest* **102**, 1534–1539.

Lompré A-M, Hajjar RJ, Harding SE, Kranias EG, Lohse MJ & Marks AR (2010). Ca^{2+} cycling and new therapeutic approaches for heart failure. *Circulation* **121**, 822–830.

Lorenz K, Lohse MJ & Quitterer U (2003). Protein kinase C switches the Raf kinase inhibitor from Raf-1 to GRK-2. *Nature* **426**, 574–579.

Lorenz K, Schmid E & Deiss K (2014*a*). RKIP: a governor of intracellular signaling. *Crit Rev in Oncog* **19**, 489-496.

Lorenz K, Schmitt JP, Vidal M & Lohse MJ (2009). Cardiac hypertrophy: targeting Raf/MEK/ERK1/2-signaling. *Int J Biochem Cell Biol* **41**, 2351–2355.

Lorenz K, Stathopoulou K, Schmid E, Eder P & Cuello F (2014*b*). Heart failure-specific changes in protein kinase signalling. *Pflugers Arch* **466**, 1151–1162.

Lother A & Hein L (2016). Pharmacology of heart failure: From basic science to novel therapies. *Pharmacol Ther* **166**, 136–149.

Maier LS & Bers DM (2007). Role of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. *Cardiovasc Res* **73**, 631–640.

Marks AR (2013). Calcium cycling proteins and heart failure: mechanisms and therapeutics. *J Clin Invest* **123**, 46–52.

Métrich M, Berthouze M, Morel E, Crozatier B, Gomez AM & Lezoualc'h F (2009). Role of the cAMP-binding protein Epac in cardiovascular physiology and pathophysiology. *Pflugers Arch* **459**, 535–546.

Métrich M, Lucas A, Gastineau M, Samuel J-L, Heymes C, Morel E & Lezoualc'h F (2008). Epac mediates β-adrenergic receptor-induced cardiomyocyte hypertrophy. *Circ Res* **102**, 959–965.

Najafi A, Sequeira V, Kuster DWD & van der Velden J (2016). β-Adrenergic receptor signalling and its functional consequences in the diseased heart. *Eur J Clin Invest* **46**, 362–374.

Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE & Gorelik J (2010). β_2-Adrenergic receptor redistribution in heart failure changes cAMP compartmentation. *Science* **327**, 1653–1657.

O'Connor CM, Gattis WA, Uretsky BF, Adams KF Jr, McNulty SE, Grossman SH, McKenna WJ, Zannad F, Swedberg K, Gheorghiade M & Califf RM (1999). Continuous intravenous dobutamine is associated with an increased risk of death in patients with advanced heart failure: insights from the Flolan International Randomized Survival Trial (FIRST). *Am Heart J* **138**, 78–86.

Ohsuzu F, Katsushika S, Akanuma M, Hakamada N, Hamada H, Maie S, Muneoka K, Okumori M & Nakamura H (1994). Reduction of β-adrenergic receptors in atrial cell membranes of patients following mild to moderate heart failure. *Jpn Heart J* **35**, 27–34.

Orchard C & Brette F (2007). t-Tubules and sarcoplasmic reticulum function in cardiac ventricular myocytes. *Cardiovasc Res* **77**, 237–244.

Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM & Shusterman NH (1996*a*). The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. carvedilol heart failure study group. *N Engl J Med* **334**, 1349–1355.

Packer M, Colucci WS, Sackner-Bernstein JD, Liang CS, Goldscher DA, Freeman I, Kukin ML, Kinhal V, Udelson JE, Klapholz M, Gottlieb SS, Pearle D, Cody RJ, Gregory JJ, Kantrowitz NE, LeJemtel TH, Young ST, Lukas MA & Shusterman NH (1996*b*). Double-blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure. THE PRECISE trial. Prospective randomized evaluation of carvedilol on symptoms and exercise. *Circulation* **94**, 2793–2799.

Patterson AJ, Zhu W, Chow A, Agrawal R, Xiao RP & Kobilka B (2004). Protecting the myocardium: a role for the beta2 adrenergic receptor in the heart. *Crit Care Med* **32**, 1041–1048.

Paur H, Wright PT, Sikkel MB, Tranter MH, Mansfield C, O'Gara P, Stuckey DJ, Nikolaev VO, Diakonov I, Pannell L, Gong H, Sun H, Peters NS, Petrou M, Zheng Z, Gorelik J, Lyon AR & Harding SE (2012). High levels of circulating epinephrine trigger apical cardiodepression in a β_2-adrenergic receptor/Gi-dependent manner: a new model of Takotsubo cardiomyopathy. *Circulation* **126**, 697–706.

Pereira L, Cheng H, Lao D-H, Na L, van Oort RJ, Brown JH, Wehrens XHT, Chen J & Bers DM (2013). Epac2 mediates cardiac β1-adrenergic-dependent sarcoplasmic reticulum Ca^{2+} leak and arrhythmia. *Circulation* **127**, 913–922.

Pierce KL, Premont RT & Lefkowitz RJ (2002). Signalling: Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* **3**, 639–650.

Pleger ST, Raake P, Katus HA & Most P (2014). Cardiac calcium handling on trial: targeting the failing cardiomyocyte signalosome. *Circ Res* **114**, 12–14.

Pleger ST, Shan C, Ksienzyk J, Bekeredjian R, Boekstegers P, Hinkel R, Schinkel S, Leuchs B, Ludwig J, Qiu G, Weber C, Raake P, Koch WJ, Katus HA, Muller OJ & Most P (2011). Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. *Sci Transl Med* **3**, 92ra64.

Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH & van der Meer P; Authors/Task Force Members; Document Reviewers (2016). 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur J Heart Fail* **18**, 891–975.

Priori SG & Chen SR (2011). Inherited dysfunction of sarcoplasmic reticulum Ca^{2+} handling and arrhythmogenesis. *Circ Res* **7**, 871–883.

Punn A, Mockridge JW, Farooqui S, Marber MS & Heads RJ (2000). Sustained activation of p42/p44 mitogen-activated protein kinase during recovery from simulated ischaemia mediates adaptive cytoprotection in cardiomyocytes. *Biochem J* **350**, 891–899.

Purcell NH, Wilkins BJ, York A, Saba-El-Leil MK, Meloche S, Robbins J & Molkentin JD (2007). Genetic inhibition of cardiac ERK1/2 promotes stress-induced apoptosis and heart failure but has no effect on hypertrophy in vivo. *Proc Natl Acad Sci USA* **104**, 14074–14079.

Respress JL, van Oort RJ, Li N, Rolim N, Dixit SS, deAlmeida A, Voigt N, Lawrence WS, Skapura DG, Skardal K, Wisloff U, Wieland T, Ai X, Pogwizd SM, Dobrev D & Wehrens XHT (2012). Role of RyR2 phosphorylation at S2814 during heart failure progression. *Circ Res* **110**, 1474–1483.

Ritterhoff J, Völkers M, Seitz A, Spaich K, Gao E, Peppel K, Pleger ST, Zimmermann WH, Friedrich O, Fink RHA, Koch WJ, Katus HA & Most P (2015). S100A1 DNA-based inotropic therapy protects against proarrhythmogenic ryanodine receptor 2 dysfunction. *Mol Ther* **23**, 1320–1330.

Rockman HA, Koch WJ & Lefkowitz RJ (2002). Seven-transmembrane-spanning receptors and heart function. *Nature* **415**, 206–212.

Ruppert C, Deiss K, Herrmann S, Vidal M, Oezkur M, Gorski A, Weidemann F, Lohse MJ & Lorenz K (2013). Interference with ERK(Thr188) phosphorylation impairs pathological but not physiological cardiac hypertrophy. *Proc Natl Acad of Sci USA* **110**, 7440–7445.

Sadayappan S, Gulick J, Osinska H, Barefield D, Cuello F, Avkiran M, Lasko VM, Lorenz JN, Maillet M, Martin JL, Brown JH, Bers DM, Molkentin JD, James J & Robbins J (2011). A critical function for Ser-282 in cardiac Myosin binding protein-C phosphorylation and cardiac function. *Circ Res* **109**, 141–150.

Schmid E, Neef S, Berlin C, Tomasovic A, Kahlert K, Nordbeck P, Deiss K, Denzinger S, Herrmann S, Wettwer E, Weidendorfer M, Becker D, Schäfer F, Wagner N, Ergün S, Schmitt JP, Katus HA, Weidemann F, Ravens U, Maack C, Hein L, Ertl G, Müller OJ, Maier LS, Lohse MJ & Lorenz K (2015). Cardiac RKIP induces a beneficial beta-adrenoceptor-dependent positive inotropy. *Nat Med* **21**, 1298–1306.

Schmitt JP, Ahmad F, Lorenz K, Hein L, Schulz S, Asahi M, MacLennan DH, Seidman CE, Seidman JG & Lohse MJ (2009). Alterations of phospholamban function can exhibit cardiotoxic effects independent of excessive sarcoplasmic reticulum Ca^{2+}-ATPase inhibition. *Circulation* **119**, 436–444.

Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, Kranias EG, MacLennan DH, Seidman JG & Seidman CE (2003). Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science* **299**, 1410–1413.

Sheikh F, Raskin A, Chu P-H, Lange S, Domenighetti AA, Zheng M, Liang X, Zhang T, Yajima T, Gu Y, Dalton ND, Mahata SK, Dorn GW II, Heller Brown J, Peterson KL, Omens JH, McCulloch AD & Chen J (2008). An FHL1-containing complex within the cardiomyocyte sarcomere mediates hypertrophic biomechanical stress responses in mice. *J Clin Invest* **118**, 3870–3880.

Shizukuda Y & Buttrick PM (2002). Subtype specific roles of β-adrenergic receptors in apoptosis of adult rat ventricular myocytes. *J Mol Cell Cardiol.* **34**, 823–831.

Siedlecka U, Arora M, Kolettis T, Soppa GKR, Lee J, Stagg MA, Harding SE, Yacoub MH & Terracciano CMN (2008). Effects of clenbuterol on contractility and Ca^{2+} homeostasis of isolated rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* **295**, H1917–H1926.

Slack J, Grupp IL, Dash R, Holder D, Schmidt A, Gerst MJ, Tamura T, Tilgmann C, James PF, Johnson R, Gerdes AM & Kranias EG (2001). The enhanced contractility of the phospholamban-deficient mouse heart persists with aging. *J Mol Cell Cardiol* **33**, 1031–1040.

Steinfath M, Geertz B, Schmitz W, Scholz H, Haverich A, Breil I, Hanrath P, Reupcke C, Sigmund M & Lo HB (1991). Distinct down-regulation of cardiac β_1- and β_2-adrenoceptors in different human heart diseases. *Naunyn Schmiedebergs Arch Pharmacol* **343**, 217–220.

Steinfath M, Lavicky J, Schmitz W, Scholz H, Döring V & Kalmár P (1992). Regional distribution of β_1- and β_2-adrenoceptors in the failing and nonfailing human heart. *Eur J Clin Pharmacol* **42**, 607–611.

Tacon CL, McCaffrey J & Delaney A (2012). Dobutamine for patients with severe heart failure: a systematic review and meta-analysis of randomised controlled trials. *Intensive Care Med* **38**, 359–367.

Talan MI, Ahmet I, Xiao R-P & Lakatta EG (2011). $\beta2$ AR agonists in treatment of chronic heart failure: long path to translation. *J Mol Cell Cardiol* **51**, 529–533.

Tang T, Gao MH & Hammond HK (2012). Prospects for gene transfer for clinical heart failure. *Gene Ther* **19**, 606–612.

Tardiff JC, Carrier L, Bers DM, Poggesi C, Ferrantini C, Coppini R, Maier LS, Ashrafian H, Huke S & van der Velden J (2015). Targets for therapy in sarcomeric cardiomyopathies. *Cardiovasc Res* **105**, 457–470.

Thomas JA & Marks BH (1987). Plasma norepinephrine in congestive heart failure. *Am J Cardiol* **41**, 233–243.

Trakul N & Rosner MR (2005). Modulation of the MAP kinase signaling cascade by Raf kinase inhibitory protein. *Cell Res* **15**, 19–23.

Uchinoumi H, Yang Y, Oda T, Li N, Alsina KM, Puglisi JL, Chen-Izu Y, Cornea RL, Wehrens XHT & Bers DM (2016). CaMKII-dependent phosphorylation of RyR2 promotes targetable pathological RyR2 conformational shift. *J Mol Cell Cardiol* **98**, 62–72.

Van Berlo JH, Elrod JW, Aronow BJ, Pu WT & Molkentin JD (2011). Serine 105 phosphorylation of transcription factor GATA4 is necessary for stress-induced cardiac hypertrophy in vivo. *Proc Natl Acad Sci USA* **108**, 12331–12336.

van der Velden J, Merkus D, Klarenbeek BR, James AT, Boontje NM, Dekkers DH, Stienen GJ, Lamers JM & Duncker DJ (2004). Alterations in myofilament function contribute to left ventricular dysfunction in pigs early after myocardial infarction. *Circ Res* **11**, 85–95.

Vidal M, Wieland T, Lohse MJ & Lorenz K (2012). β-Adrenergic receptor stimulation causes cardiac hypertrophy via a Gβγ/Erk-dependent pathway. *Cardiovasc Res* **96**, 255–264.

Völkers M, Weidenhammer C, Herzog N, Qiu G, Spaich K, Wegner von F, Peppel K, Muller OJ, Schinkel S, Rabinowitz JE, Hippe HJ, Brinks H, Katus HA, Koch WJ, Eckhart AD, Friedrich O & Most P (2011). The inotropic peptide ARKct improves AR responsiveness in normal and failing cardiomyocytes through Gβγ-Mediated L-Type calcium current disinhibition. *Circ Res* **108**, 27–39.

Weber S, Meyer-Roxlau S, El-Armouche A (2016). Role of protein phosphatase inhibitor-1 in cardiac beta adrenergic pathway. *J Mol Cell Cardiol* **101**, 116–126.

Woo AYH & Xiao R-P (2012). β-Adrenergic receptor subtype signaling in heart: From bench to bedside. *Acta Pharmacol Sin* **33**, 335–341.

Xiao RP, Avdonin P, Zhou YY, Cheng H, Akhter SA, Eschenhagen T, Lefkowitz RJ, Koch WJ & Lakatta EG (1999). Coupling of β_2-adrenoceptor to G_i proteins and its physiological relevance in murine cardiac myocytes. *Circ Res* **84**, 43–52.

Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, Fee F, Katsanakis KD, Rose DW, Mischak H, Sedivy JM & Kolch W (1999). Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. *Nature* **401**, 173–177.

Yeung KC, Rose DW, Dhillon AS, Yaros D, Gustafsson M, Chatterjee D, McFerran B, Wyche J, Kolch W & Sedivy JM (2001). Raf kinase inhibitor protein interacts with NF-κB-inducing kinase and TAK1 and inhibits NF-kB activation. *Mol Cell Biol* **21**, 7207–7217.

Yin R, Liu X, Zhang P, Chen Y, Xie G, Ai L, Xue C, Qian J, Bi Y, Chen J, Sun Y, Stoeger T & Ding Z (2016). A Raf kinase inhibitor demonstrates antiviral activities both *in vitro* and *in vivo* against different genotypes of virulent Newcastle disease virus. *Antiviral Res* **133**, 140–144.

Zaugg M, Xu W, Lucchinetti E, Shafiq SA, Jamali NZ & Siddiqui MA (2000). β-Adrenergic receptor subtypes differentially affect apoptosis in adult rat ventricular myocytes. *Circulation* **102**, 344–350.

Zhang X, Szeto C, Gao E, Tang M, Jin J, Fu Q, Makarewich C, Ai X, Li Y, Tang A, Wang J, Gao H, Wang F, Ge XJ, Kunapuli SP, Zhou L, Zeng C, Xiang KY & Chen X (2013). Cardiotoxic and cardioprotective features of chronic β-adrenergic signaling. *Circ Res* **112**, 498–509.

Additional information

Competing interests

The authors have no competing interests.

Author contributions

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