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Sarcolemmal α2-adrenoceptors in feedback control of myocardial response to sympathetic challenge

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ABSTRACT

α2-adrenoceptor (α2-AR) isoforms, abundant in sympathetic synapses and noradrenergic neurons of the central nervous system, are integral in the presynaptic feed-back loop mechanism that moderates norepinephrine surges. We recently identified that postsynaptic α2-ARs, found in the myocellular sarcolemma, also contribute to a muscle-delimited feedback control capable of attenuating mobilization of intracellular Ca^{2+} and myocardial contractility. This previously unrecognized α2-AR-dependent rheostat is able to counteract competing adrenergic receptor actions in cardiac muscle. Specifically, in ventricular myocytes, nitric oxide (NO) and cGMP are the intracellular messengers of α2-AR signal transduction pathways that gauge the kinase-phosphatase balance and manage cellular Ca^{2+} handling preventing catecholamine-induced Ca^{2+} overload. Moreover, α2-AR signaling counterbalances phospholipase C – PKC-dependent mechanisms underscoring a broader cardioprotective potential under sympathoadrenergic and angiotensinergic challenge. Recruitment of such tissue-specific features of α2-AR under sustained sympathoadrenergic drive may, in principle, be harnessed to mitigate or prevent cardiac malfunction. However, cardiovascular disease may compromise peripheral α2-AR signaling limiting pharmacological targeting of these receptors. Prospective cardiac-specific gene or cell-based therapeutic approaches aimed at repairing or improving stress-protective α2-AR signaling may offer an alternative towards enhanced preservation of cardiac muscle structure and function.

Contents

Abbreviations: AC, Adenylyl cyclase; Akt, Protein kinase B (PKB); AR, Adrenergic receptor; cAMP, Cyclic adenosine monophosphate; cGMP, Cyclic guanosine monophosphate; I_{CaL}, Voltage-gated L-type Ca^{2+} current; NO, Nitric oxide; NOS, Nitric oxide synthase; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; PKA, Protein kinase A; PKC, Protein kinase C; PKG, Protein kinase G; PP1, Protein phosphatase 1; SERCA, Sarco/endoplasmic reticulum Ca^{2+}-ATPase.

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1. Introduction

Physiological systems aimed at augmenting bodily performance, for instance in the case of the "fight or flight" response, are equipped with compensatory feedback mechanisms to prevent stress-induced cellular energy overuse and sustain a metabolic level necessary for confrontation or evasion of threats (Goldstein, 2013; Selye, 1950; Swaab, Bao, & Lucassen, 2005; Zingman, Hodgson, Alekseev, & Terzic, 2003). Stress responses in peripheral organs mediated by the sympathoadrenal system act through release of catecholamines (epinephrine, norepinephrine) from neuronal synapses and via catecholamine secretion to the blood stream from the adrenal medulla. In addition, adrenergic cells (Huang et al., 1996) intrinsic to the heart can also contribute to local cathecholamine levels and participate both in the stress response and in basal cardiac regulation and cardiogenesis (Donald & Shepherd, 1963; Lehmann et al., 2013; Thomas, Matsumoto, & Palmiter, 1995). Aberrant feedback control of the sympathetic response results in a spectrum of pathologies (Goldstein, 2013; Maletic, Eramo, Gwin, Offord, & Duffy, 2017; Mittal et al., 2017; Zingman et al., 2003).

Catecholamines target G protein-coupled adrenergic receptors (ARs) originally grouped as alpha (α-) and beta (β-) receptors (Ahlquist, 1980; Bylund, 2007). Based on both pharmacological and molecular properties, ARs were further subdivided into three types – α1-AR, α2-AR and β-AR – each represented by three (or more) receptor subtypes (Bylund et al., 1994). While α1-ARs primarily trigger dissociation of $G_{\alpha q}/G_{\beta\gamma}$-type complexes, β-ARs and α2-ARs mainly act via "$G_{\alpha s}$ - stimulatory" (G_s) and "$G_{\alpha i}$ - inhibitory" (G_i) proteins, respectively (Dzimiri, 1999; Gilsbach & Hein, 2008; Salazar, Chen, & Rockman, 2007). Diverged cellular responses induced by catecholamines are defined via respective adrenoceptor coupling with different heterogeneous $G_\alpha/G_{\beta\gamma}$-protein complexes (Gilman, 1987; Gudermann, Kalkbrenner, & Schultz, 1996; Simon, Strathmann, & Gautam, 1991). At least 20 G_α, 6 G_β and 12 G_γ proteins have been identified to date, which provide almost 1500 combinatorial signal transduction options; those are further multiplied by a variety of effector isoforms (Clapham & Neer, 1997; Gudermann, Kalkbrenner, Dippel, Laugwitz, & Schultz, 1997; Offermanns & Schultz, 1994). Possible regulation of effectors either by G_α or $G_{\beta\gamma}$ subunits, by their bipartite or synergistic effects are also known to occur (Clapham & Neer, 1997).

In contrast to presynaptic α1- and β-ARs that drive neuronal activity and release of neurotransmitters, α2-ARs have a suppressing role. Indeed, $G_{\alpha q}$-coupled α1-ARs, abundantly present in the brain, activate phospholipase C (PLC), which via hydrolysis of phosphatidylinositol 4,5 biphosphate (PIP2) leads to the stimulation of protein kinase C (PKC) isoforms following the ensuing increase of intracellular Ca^{2+} (Birnbaum, 2004; Hubbard & Hepler, 2006). Activation of β-ARs followed by the dissociation of G_s subunit stimulates adenylyl cyclase increasing production of the second messenger 3′-5′-cyclic adenosine monophosphate (cAMP), which activates protein kinase A (PKA) (Gilsbach & Hein, 2008). The catalytic subunit of PKA phosphorylates and activates voltage-gated Ca^{2+} channels enhancing transmitter secretion in neurons (Dzimiri, 1999). In contrast, activation of the presynaptic α2-ARs leading to the dissociation of G_i protein results in suppression of cAMP levels, opening of inwardly-rectifying K^+ channels and inhibition of voltage-gated Ca^{2+} channels directly affecting the exocytotic machinery (Miller, 1998; Wu & Saggau, 1997). Thus, α2-ARs have been recognized as short-loop feedback suppressors of sympathetic and adrenal catecholamine release and thereby generally have an inhibitory influence on sympathoadrenergic drive (Altman et al., 1999; Brede et al., 2003; Hein, Altman, & Kobilka, 1999; Trendelenburg, Klebroff, Hein, & Starke, 2001). In this way, α2-ARs are mainly involved in the antinociceptive, sedative, central hypotensive, hypothermic, and behavioral actions of specific agonists (Hunter et al., 1997; Lähdesmäki et al., 2002; Lakhlani et al., 1997; MacMillan, Hein, Smith, Piascik, & Limbird, 1996) (Table 1).

Table 1
Pharmacological use of α2-AR agonists.

Agonists	Clinical treatments	Adverse effects
Clonidine (guanabenz, guanfacine)	Hypertension	After abrupt cessation: rebound hypertension, tachycardia (Campbell & Reid, 1985; Mehta & Lopez, 1987) and associated symptoms, such as headache, anxiety, tremor, sweating, nausea, vomiting (Karachalios et al., 2005).
	Post-, intraoperative and chronic pain	At intrathecal infusion: severe hypotension, low systemic vascular resistance index (Puskas, Camporesi, O'Leary, Hauser, & Nasrallah, 2003), impotence (Koman, Alfieri, Rachinger, Strauss, & Scheller, 2012).
	Anxiety, attention deficit/hyperactivity disorder	Somnolence, fatigue, dry mouth, bradycardia, irritability, sore throat, insomnia, constipation, increased body temperature, ear pain, nausea, hypotension, headache (Connor, Fletcher, & Swanson, 1999; Sharma & Couture, 2014).
	Postoperative shivering	Sedation (Panneer, Murugaiyan, & Rao, 2017)
	Abstinence symptoms	Significant hypotension (Gold, Pottash, Sweeney, & Kleber, 1980)
Tizanidine	Spasticity, myofascial pain, muscle spasm and cramps	asthenia, dry mouth, dizziness, somnolence, gastrointestinal disorders (Henney III & Chez, 2009; Nance et al., 1997; Wagstaff & Bryson, 1997)
Dexmedetomidine	Spinal anesthesia shivering, intensive care unit sedation	hypotension, bradycardia, sinus arrest, nausea, vomiting, fever, hypoxia, tachycardia, anemia, tachyphylaxis (Giovannitti, Thoms, & Crawford, 2015; Pediatric Advisory Committee, 2016)

To date, four different α2-AR subtypes have been identified, termed α2A, α2B and α2C encoded by the intronless genes *adra2A*, *adra2B* and *adra2C*, respectively (Bylund et al., 1992; Lomasney et al., 1990), as well as α2D having two duplicates encoded by the genes *adra2Da* and *adra2Db* (Ruuskanen et al., 2005, 2004). While mammalian species express only the first three adrenoceptors, all other vertebrates (except crocodiles) possess the whole repertoire of four *adra2* genes (Céspedes, Zavala, Vandewege, & Opazo, 2017). In mammals, primarily the α2A- and α2C- receptor subtypes are present in the central neural system whereas all three receptor isoforms are broadly distributed in peripheral organs. Animal models with genetic ablation of either α2A or α2C subtypes demonstrate that while deletion of the α2A-AR led to increased plasma levels of norepinephrine, α2C-AR predominantly inhibited secretion of catecholamines from chromaffin cells in a Ca^{2+}-dependent manner similarly to the feedback mechanism observed in nerve terminals (Brede et al., 2003; Moura, Afonso, Hein, & Vieira-Coelho, 2006). The potency of norepinephrine-induced inhibition of transmitter release, measured in these animal models, suggested ~10 fold higher affinity of norepinephrine for the α2C-AR (K_i inhibitory constant values = 650 nM) compared to the α2A-AR (K_i = 5.8 μM) (Hein et al., 1999). The distinct responsiveness of these receptor isoforms to neuronal stimulation frequencies indicated that the α2C-AR inhibits transmitter release at low levels of sympathetic nerve activity, whereas α2A receptors regulate release at higher frequencies (Hein et al., 1999; Philipp, Brede, & Hein, 2002; Uys, Shahid, & Harvey, 2017).

As sustained cardiac sympathetic stimulation has been implicated in maladaptive cardiac remodeling as well as in development and progression of heart failure, a defective function of α2-AR has been considered as a risk for cardiovascular disorders. Indeed, excess mortality in mice lacking α2A and α2C isoforms was attributed to elevated circulating catecholamines, aggravated left ventricular hypertrophy, fibrosis and

development of heart failure (Brede et al., 2002). However, targeted rescue of these receptor isoforms specifically in sympathetic neurons provided only partial restoration of the left ventricular response to intravenous infusion of α2-AR agonists (Gilsbach et al., 2010). Concomitantly, pre-conditioning using the α2-AR agonist dexmedetomidine has been found to promote phosphorylation of Erk1/2, Akt and eNOS in left ventricular myocytes and to improve cardiac recovery after ischemia/reperfusion (Ibacache et al., 2012; Yoshikawa, Hirata, Kawaguchi, Tokinaga, & Yamakage, 2018). Such observations may point towards a functional role of α2-ARs in cardiac tissue, where they can participate in regulation of the peripheral adrenergic response. While, such contribution of postsynaptic α2-ARs remains insufficiently understood, a number of recent findings warrant reevaluation of the functional role of these receptors in myocardial cells.

2. α2-Adrenoceptors in ventricular myocytes

While β-AR and α1-AR have been directly implicated in myocardial contractility (Dzimiri, 1999; Myagmar et al., 2017; Terzic, Pucéat, Clément, Scamps, & Vassort, 1992), α2-ARs were traditionally considered to have a limited role in the heart. α2-AR agonists were primarily found to act in coronary arteries and prejunctional cardiac nerves but not in the myocardium itself (Bloor et al., 1992; Dukes & Vaughan Williams, 1984; Hayashi & Maze, 1993; Hongo et al., 2016). Specifically, α2-AR agonists (clonidine, dexmedetomidine) induce α2A-mediated antihypertensive and bradycardic effects confirmed by genetic deletion of gene encoding this specific receptor subtype or its subtle mutation (D79N) (Altman et al., 1999; MacMillan et al., 1996). Several functional studies reported no direct inotropic outcomes of the selective α2-AR agonist dexmedetomidine neither in isolated whole hearts nor in isolated cardiac papillary muscles (Flacke, Flacke, Blow, Mclntee, & Bloor, 1992; Housmans, 1990). In fact, poly(A)$^+$RNA expression of α2A, α2B and α2C in rat hearts was found negligible compared to levels of these receptors in neuronal, kidney, liver or aortic tissues (Lorenz et al., 1990). RNAase protection analysis with isoform-specific cDNAs has revealed a weak hybridization only of the α2B probe in rat cardiac tissue (Handy, Flordellis, Bogdanova, Bresnahan, & Gavras, 1993). However, more recent studies using Western blots have identified protein expression of all three α2 receptors in whole rat hearts, but only α2A- and α2C- receptor subtypes were found in ventricular myocytes (Ibacache et al., 2012). In humans, distribution of α2-AR mRNA was found to be generally different from rats, and ventricular tissue has been characterized by the predominant presence of α2C and by the absence of α2A mRNA (i.e. by the presence of $\alpha2_{C4}$ but not $\alpha2_{C10}$ subtypes, respectively, according to the isoform nomenclature based on chromosome localization) (Berkowitz, Price, Bello, Page, & Schwinn, 1994). The identified expression of α2-AR isoforms in myocardial cells is intriguing and would rather point towards the potential contribution of these receptors to myocardial regulation.

Contrary to the paradigm that α2-ARs have minimal direct effects in cardiomyocytes, our recent studies with isolated rat ventricular myocytes have detected not only the presence of all three α2A, α2B and α2C subtypes, both at mRNA and protein expression levels, but also revealed their previously unrecognized cardioprotective potential (Kokoz et al., 2016; Maltsev et al., 2014). Furthermore, we identified that α2-AR are expressed through embryonic development of the heart into adulthood in mice, as well as in cardiomyocytes differentiated in vitro from embryonic stem cells (Martinez-Fernandez et al., 2014; Martinez-Fernandez, Li, Hartjes, Terzic, & Nelson, 2013)(Fig. 1). This indicates that α2-AR isoforms can be involved in cardiogenic mechanisms and may serve as targets for manipulation in potential cell based therapies.

Using L-arginine, the substrate for intracellular nitric oxide (NO) synthase, the presence of which was a prerequisite during cell isolation and subsequent experimental procedures, we found that NO and cGMP were central intracellular messengers mediating

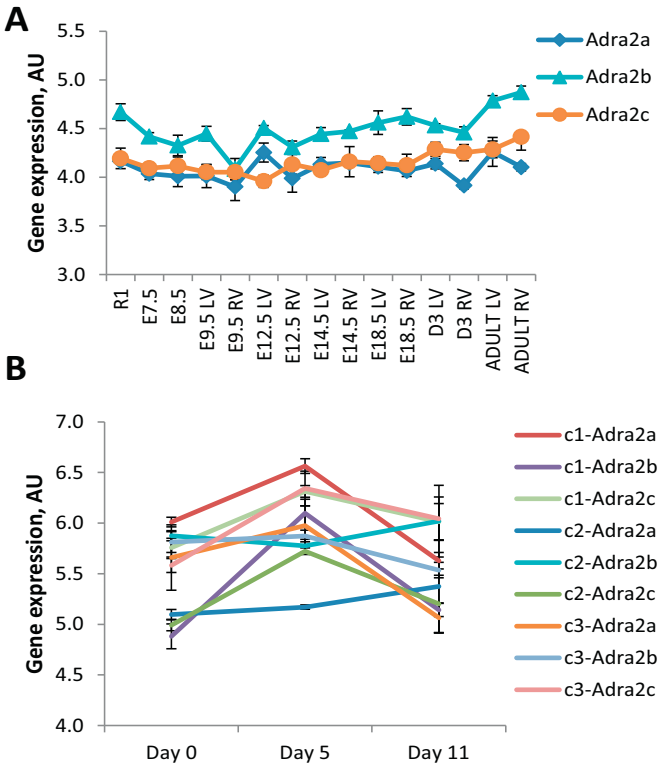

Fig. 1. *Adra2*-encoded α2-adrenoceptors are expressed throughout natural cardiac development (A) and in vitro cardiac differentiation (B). A: gene expression profile of the three different *adra2* isoforms in embryonic stem cells (R1), at different stages of embryonic cardiac development (embryo days 7.5 thru 18.5), three days after birth (D3) and at three months (adult) in left and right ventricles (LV & RV) where appropriate. B: gene expression profiles of the same *adra2* isoforms during in vitro cardiac differentiation of three different clones (c1–3) of pluripotent stem cells (Reyes, Martinez-Fernandez, Li, & Nelson, unpublished observation).

α2-AR-signaling in ventricular myocytes (Kokoz et al., 2016; Maltsev et al., 2014). The conducted inhibitory analysis demonstrated that targeting α2-AR by the prototypic agonist guanabenz stimulates the endothelial type NO synthase (eNOS), rather than the neuronal (nNOS) isoform, via the α2-AR – PI3K – Akt(PKB) pathway (Maltsev et al., 2014) (Fig. 2).

It has been established that activation of Akt/PKB and eNOS leads to phosphorylation and direct S-nitrosylation of phospholamban (Brittsan & Kranias, 2000; Catalucci et al., 2009; Filice et al., 2011; Garofalo, Parisella, Amelio, Tota, & Imbrogno, 2009) and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) (Adachi et al., 2004; Murphy, Kohr, Sun, Nguyen, & Steenbergen, 2012; Tong, Evangelista, & Cohen, 2010) (Fig. 2), which in isolated cardiac myocytes was manifested by suppression of spontaneous Ca^{2+} waves (Bai et al., 2013; Hüser, Bers, & Blatter, 1998; Maltsev et al., 2014). Under ongoing excitation-contraction coupling, this pathway would promote Ca^{2+} re-uptake into the sarcoplasmic reticulum (SR) and would potentiate Ca^{2+} release (Braz et al., 2004). Indeed, since inhibition of eNOS is capable of reducing cardiac output, left ventricular maximal developed pressure and dP/dt_{max}, α2-AR activation in cardiomyocytes by promoting SERCA activity could facilitate cardiac performance (Casadei & Sears, 2003; Prendergast, Sagach, & Shah, 1997).

Furthermore, activation of the α2-AR – PI3K – Akt(PKB) – NO – sGC – cGMP – PKG pathway in cardiomyocytes suppressed L-type Ca^{2+} current (I_{CaL}) (Kokoz et al., 2016) (Fig. 2). The identified α2-AR-mediated inhibition of I_{CaL} may imply several plausible mechanisms. The first is a direct function-suppression phosphorylation of the channel protein by PKG (Jiang et al., 2000; Schröder et al., 2003). The second is a cGMP – PKG-dependent stimulation of protein phosphatases 1 (PP1) and/or

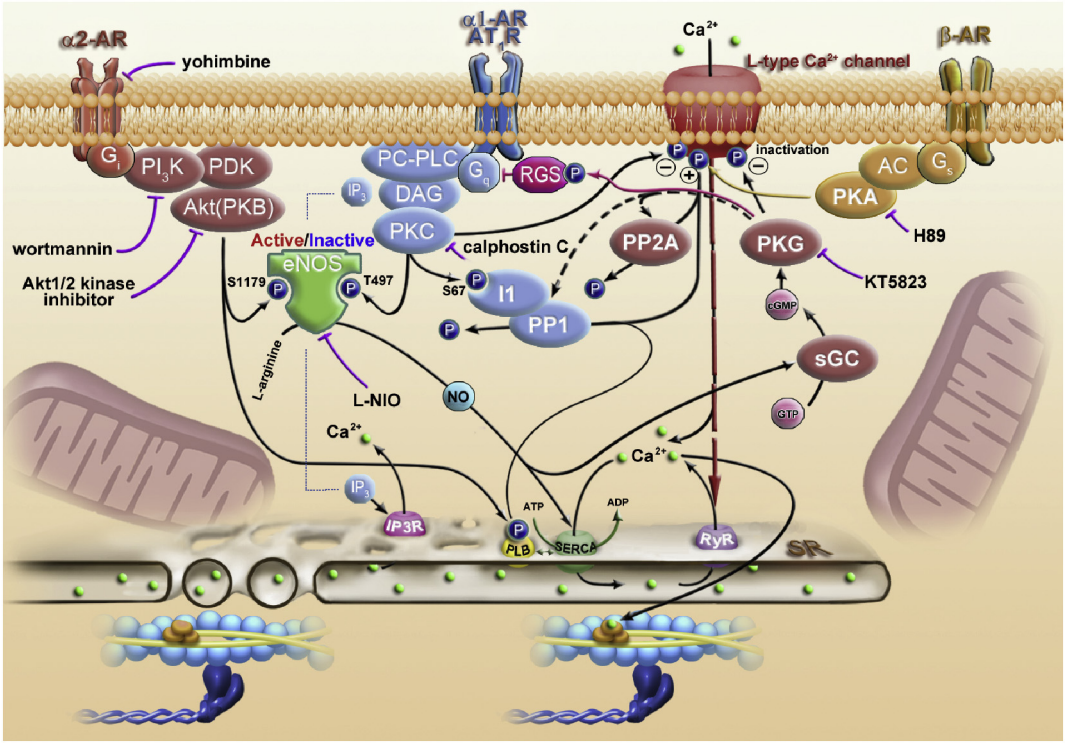

Fig. 2. Suggested interference of the main G_i-coupled α2-AR signaling cascade with the selective signaling pathways of G_s and G_q protein-coupled receptors targeting intracellular Ca^{2+} handling in cardiomyocytes, based on performed inhibitory analysis. For clarity, not all signaling pathways affecting intracellular Ca^{2+} handling in cardiomyocytes are illustrated. Designations: PI_3K, phosphatidylinositol-3-kinase; PDK, phosphatidylinositol-dependent kinase; Akt(PKB), Akt-kinase (protein kinase B); eNOS, endothelial NO-synthase; SERCA, sarcoplasmic Ca^{2+}-ATPase; RyR, ryanodine receptor; PLB, phospholamban; sGC, soluble guanylate cyclase; PKG, cGMP-dependent protein kinase; RGS, regulator of G-protein signaling that following interaction with and phosphorylation by PKG turns off G_q-coupled signaling; PP1 and PP2A, protein phosphatases 1 and 2A; AC, adenylyl cyclase; PKA, protein kinase A that denotes β_2-adrenoceptor-mediated phosphorylation activating L-type Ca^{2+} channels that induces Ca^{2+} release from sarcoplasmic reticulum (SR). PC-PLC, phosphatidylcholine-specific phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; PKC-dependent phosphorylation of phosphatase inhibitor I1 enhances PP1 activity; IP_3, inositol trisphosphate that via interaction with IP_3 receptor (IP3R) induces Ca^{2+} release from SR. Intracellular Ca^{2+} defines the contractility of cardiac muscle. Dashed line represent proposed direct impact of cGMP-dependent activation on PP1 and PP2A via PKG reducing a number of phosphorylated L-type Ca^{2+} channels. "Plus" and "minus" denote activating and inhibiting phosphorylation of L-type Ca^{2+} channels, respectively, induced by particular protein kinases. Adrenergic α1, β and angiotensin AT_1 (G_s- and G_q-coupled) receptors mobilize cellular pathways, which can be counteracted by the α2-AR signaling, and can activate adaptive cardiac muscle remodeling (hypertrophy) that following exhaustion of cardiac adaptive resources lead to maladaptation and development of heart failure. Agents used in the conducted inhibitory analysis are also indicated (Kokoz et al., 2016; Maltsev et al., 2014).

2A (PP2A) (Kokoz et al., 2016; Xu, Lee, & Han, 2013) that dephosphorylate L-type Ca^{2+} channels (duBell et al., 2002; duBell & Rogers, 2004; Hescheler, Kameyama, Trautwein, Mieskes, & Söling, 1987). A third option is α2-AR – G_i protein mediated stimulation of phosphodiesterases (PDE) leading to a reduction in cAMP levels (Dong, Guo, Ye, & Hare, 2014; Isidori et al., 2015; Keef, Hume, & Zhong, 2001). Finally, the inhibitory effect of $G_{\beta\gamma}$ subunits on I_{CaL} cannot be excluded (Clapham & Neer, 1997; Stephens & Mochida, 2005). While PP1- and PP2A-catalyzed dephosphorylation can oppose PKA-stimulated I_{CaL}, the counteracting effect of these serine/threonine phosphatases against PKG phosphorylation remains unclear. Nevertheless, we can conclude that, in cardiac myocytes, α2-AR signaling pathways by altering the kinase-phosphatase balance can optimize utilization of intracellular Ca^{2+} to prevent potential catecholamine-induced Ca^{2+} overload known to precipitate cardiac hypertrophy and heart failure (Bers, Eisner, & Valdivia, 2003; Karch & Billingham, 1986; Tham, Bernardo, Ooi, Weeks, & McMullen, 2015; Vassalle & Lin, 2004; Wehrens & Marks, 2004).

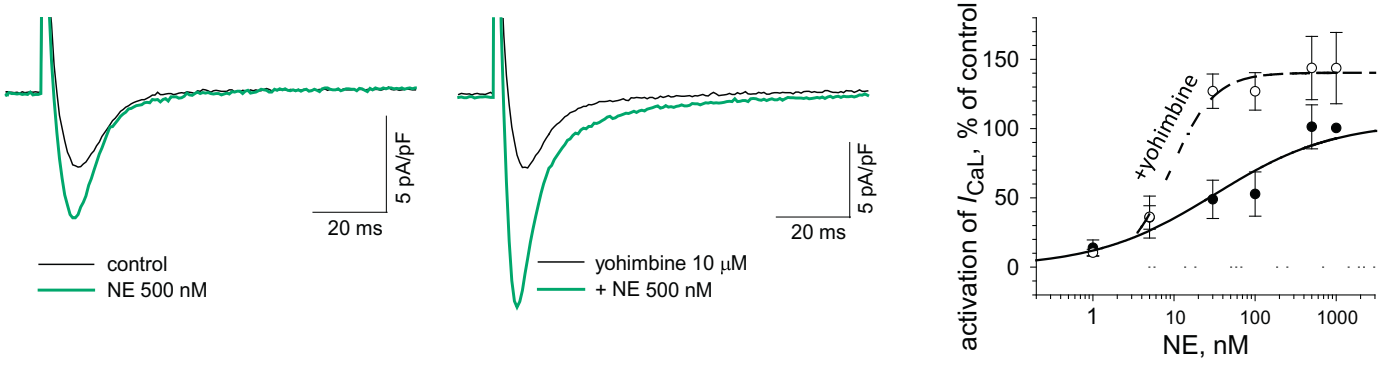

Fig. 3. In isolated rat left ventricle cardiac myocyte norepinephrine (NE) significantly accentuated inward I_{CaL} in the presence of yohimbine, an antagonist of α2-AR. Current recordings were performed using the perforated mode of the whole-cell patch-clamp technique in response to depolarization from −50 to 0 mV of membrane potential. The dose-dependent relationships constructed using peak current values. Reprinted from (Kokoz et al., 2016).

The counterbalancing input that α2-ARs can exert against sympathoadrenal stimulation of isolated cardiomyocytes could be illustrated by the receptor-dependent control of I_{CaL} in response to norepinephrine (Fig. 3). Application of norepinephrine to isolated cardiac myocytes collectively targets all sets of sarcolemmal α- and β-adrenoceptors. Dose-dependence of the norepinephrine effect reached the saturating level of I_{CaL} activation, around 100% above control currents, at ~500 nM. The α2-AR antagonist yohimbine substantially elevated I_{CaL} (~140% above control values) at norepinephrine levels >10 nM indicating the threshold above which α2-ARs start to contribute to the suppression of I_{CaL} (Fig. 3). Furthermore, in accord to the established signaling pathway, antagonism of α2-AR by yohimbine in the presence of norepinephrine magnified intracellular Ca^{2+} transients, induced by electrical stimulation of isolated cardiomyocytes, which, in turn, resulted in a positive inotropic effect, elevating myocardial workload (Kokoz et al., 2016). These data indicate that in addition to the established role of synaptic α2-AR to suppress sympathoadrenergic outflow, postsynaptic α2-AR in cardiomyocytes can oppose α1- and β- stimulation preventing detrimental Ca^{2+} overload and energy overuse in ventricular myocytes during sympathoadrenergic drive. Thus, we have identified that beyond the established α2-AR-mediated feedback suppression of sympathetic and adrenal catecholamine release, α2-ARs in myocellular sarcolemma comprise a sparing muscle-delimited feedback mechanism that attenuates mobilization of intracellular Ca^{2+} and myocardial contractility induced by other adrenergic receptors (Fig. 4).

3. α2-AR signaling interferes with pathological stimuli in ventricular myocytes

Persistent targeting of α1 and β adrenoceptors by catecholamines has been linked to elevation of intracellular Ca^{2+} resulting in Ca^{2+}/calmodulin complex formation and activation of calcineurin- and PKC-dependent transcription factors (Terzic, Pucéat, Vassort, & Vogel, 1993). Both enzymes respond to dysregulated calcium signaling, with increase in their expression and activity associated with cardiac maladaptive remodeling (Lymperopoulos, Rengo, & Koch, 2013; Mudd & Kass, 2008; O'Connell, Jensen, Baker, & Simpson, 2014; Tham et al., 2015). However, a substantial number of studies demonstrate that

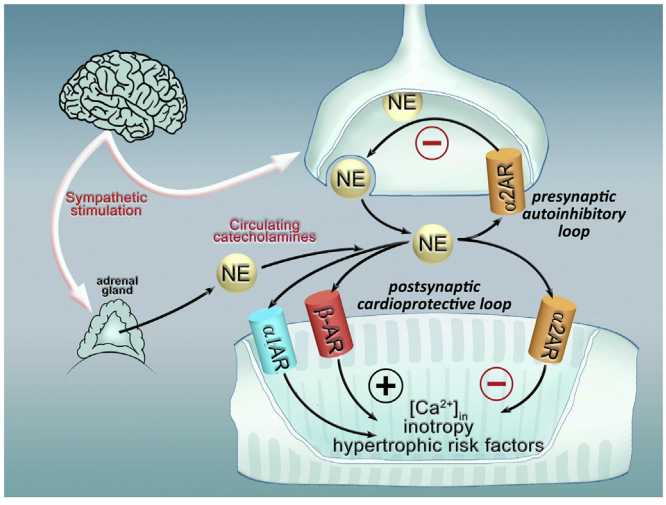

Fig. 4. α2-AR feedback loops controlling sympathetic signaling. α2-AR located in sympathetic synapses and in noradrenergic neurons of central nervous system when stimulated by norepinephrine activate a feedback loop decreasing the release of norepinephrine. Sarcolemmal α2-ARs in cardiomyocytes when stimulated by synaptic and circulating norepinephrine released from adrenal medulla activate an effector stress-reactive response that attenuates mobilization of intracellular Ca^{2+} and myocardial contractility induced by activation of other adrenergic receptors in cardiac myocytes. Reprinted from (Kokoz et al., 2016).

long-term activation of G_q-coupled α1-ARs can, at least partially, negate overstimulation of β-ARs in heart failure (O'Connell et al., 2014). Activation of other G_q-coupled receptors, such as angiotensin receptors type 1, AT_1R, and type2, AT_2R, the main effectors of the renin-angiotensin system (RAS), or endothelin receptors (Fig. 2) induce a program of hypertrophy (Capote, Mendez Perez, & Lymperopoulos, 2015; Schlüter & Wenzel, 2008), contributing to the pathogenesis of heart malfunction. The consensus is that hypertrophic stimuli of the G_q- or the functionally similar G_{11}- coupled receptors activate phospholipase C at the plasma membrane (Berridge, 2016; Hubbard & Hepler, 2006) leading to stimulation of Ca^{2+}-dependent (PKCα, PKCβ) and Ca^{2+} independent (PKCδ, PKCε) PKC isoforms and increase of Ca^{2+} release from the sarcoplasmic reticulum (Dorn & Force, 2005; Eskildsen-Helmond, Bezstarosti, & Dekkers, 1997; Goutsouliak & Rabkin, 1998; Mudd & Kass, 2008). In isolated cardiomyocytes, activation of the PLC – PKC – eNOS pathway suppressed NO production and reduced SERCA activity (Maltsev et al., 2014), an effect counterbalanced by α2-AR activation (Table 2). Of note, the propagation of spontaneous Ca^{2+} waves and elevation of free intracellular Ca^{2+} were similarly detected in the presence of phenylephrine, an α1-AR agonist, or angiotensin II (Ang II) that also increases Ca^{2+} wave frequencies via a PKC-dependent mechanism (Bkaily et al., 2005; Zeng et al., 2014).

Furthermore, it has been reported that G_q-signaling can directly interfere with PI3K and Akt activities (Ballou, Lin, Fan, Jiang, & Lin, 2003; Howes et al., 2003), downstream effectors of the α2-AR cascade in cardiomyocytes (Kokoz et al., 2016; Maltsev et al., 2014) (Fig. 2). Intriguingly, the α2-AR pathway could affect the regulator of G-protein signaling (RGS), which has emerged from studies of vascular smooth muscle cells (M. Tang et al., 2003) and can specifically counter-regulate the hypertrophic effects linked to activation of G_q-protein coupled receptors (Klaiber et al., 2010; Shimizu & Minamino, 2016) (Fig. 2). Activation of cGMP-dependent PKG facilitates its binding to and phosphorylation of RGS, which in turn accelerates GTPase activity of G_α subunits converting G protein to an inactive state resulting in termination of G-protein-mediated signal transduction (Xie & Palmer, 2007). In many aspects, the signaling pathways activated by α2-AR are similar to β3-AR signaling, which in the ventricular myocardium is also mainly coupled with G_i proteins (Gauthier et al., 1998). In fact, targeting of β3-ARs has been proposed to benefit the failing heart through a NO/cGMP-dependent signaling pathway (Balligand, 2016; Cannavo & Koch, 2017). Hence, a unique aspect of the activation of α2-AR in cardiomyocytes is an ability to counteract the G_q – PLC – PKC-dependent mechanisms, which underscores the prospective implementation of the cardioprotective features of α2-AR under sympathoadrenergic and angiotensinergic cardiac loads. Since intracellular signal cascades mediated by different PKC isoforms share in common the production of hypertrophic markers (e.g. TGFβ, p38, NFκB etc.) (Braz et al., 2004; Palaniyandi, Sun, Ferreira, & Mochly-Rosen, 2008; Ruf, Piper, & Schlüter, 2002; Schlüter & Wenzel, 2008; J. Wang, Liu, Arneja, & Dhalla, 1999), it could be suggested that the activation of α2-AR in cardiac myocytes can affect the expression of these markers and mitigate the development of cellular hypertrophy and associated heart failure (Fig. 4). Indeed, a recent pilot study in transgenic rats harboring the human angiotensinogen gene [TGR(hAGT)L1623], which are hypertensive and display cardiac hypertrophy and mild systolic dysfunction, suggested that activation of α2-ARs by oral administration of guanabenz (in a dose that does not reduce blood pressure) limits the development of cardiac hypertrophy characteristic of this animal model (Reyes, Varagic, VonCannon, Cheng, & Ferrario, 2018).

4. Defective α2-AR signaling in the development of cardiac dysfunction

Several sequence variants of α2-AR subtypes have been identified in the translated region of human α2A-, α2B-, and α2C-adrenoceptor genes with potential consequences on human cardiac disorders.

Table 2
G protein-coupled signaling in cardiac myocytes suggested to interfere with α2A-AR pathways.

G-protein	G_s	G_q	G_i
Receptor	β1, β2	α1A/B/D AT1 ET	α2A/B/C, β3, β2 (at prolonged activation)
Endogenous agonist[a]	Epinephrine (β2), norepinephrine (β1)	Epinephrine, norepinephrine (α1), angiotensin II, endothelin	Epinephrine (α2, β3), norepinephrine (β3)
Primary effector	Adenylyl cyclase	Phospholipase C	eNOS, nNOS
Messenger	cAMP	IP_3, DAG	NO, cGMP
Cell response	↑PKA ↑L-type Ca^{2+} channel	↑PKC, ↑PP1, MAPK	↑PKG, ↓PKA ↓L-type Ca^{2+} channel
Outcomes	Increased cardiac output (chronotropy, dromotropy, inotropy)	Increased contractility, expression of hypertrophic factors, post-ischemic necrosis, neonatal cardiac grows	Improved of Ca^{2+} handling, controlled increase of contractility, counteraction of hypertrophic stimuli (?)

[a] parentheses denote preferential target for the catecholamines (Rang, Ritter, Flower, & Henderson, 2016, p. 179), regardless of the possible difference in the catecholamine affinity among α receptor isoforms.

Primarily, such variants are associated with mutations or amino acid deletions in the third intracellular loop leading to decreased receptor – G-protein coupling (Heinonen et al., 1999; Small, Forbes, Brown, & Liggett, 2000; Small, Forbes, Rahman, Bridges, & Liggett, 2000; Snapir et al., 2001). Specifically, lack of three glutamates in the acidic stretch within the third intracellular loop of the α2B-AR has been recognized as a genetic risk factor for acute coronary events, but not for hypertension (Snapir et al., 2001). The same genetic polymorphism in middle-aged patients has been associated with increased risk of sudden cardiac death and acute myocardial infarction before the age of 55. It has been suggested that patients with the mutated receptor genotype may be prone to spasm of coronary arteries in the vicinity of preexisting stenotic lesions contributing to the severity and ultimate fatality of ongoing coronary events (Snapir et al., 2003).

Another genetic variant is represented by a 4-amino acid deletion in the third intracellular loop of the α2C-AR (α2CDel322–325), which greatly attenuates the ability of these receptors to suppress norepinephrine release (Brede et al., 2002; Small, Forbes, Rahman, et al., 2000). Although individuals carrying this mutation are expected to be susceptible to the development of heart failure, population studies have revealed only a weak correlation between the α2CDel322–325 polymorphism and disease incidence (Small, Wagoner, Levin, Kardia, & Liggett, 2002). Individuals with the Arg389 mutation in the β1-AR (β1Arg389), which resulted in a two-fold increase in agonist-stimulated activity compared to the wild-type β1Gly389 receptor (Mason, Moore, Green, & Liggett, 1999), have also been suggested at high risk for developing heart failure. However, these individuals were also found not susceptible to cardiac dysfunction (Small et al., 2002). Only rare patients carrying both the α2CDel322–325 and β1Arg389 mutations exhibited marked increase in heart failure risk associated with homozygosity (Small et al., 2002). The lack of overt cardiac disease outcome in patients with α2CDel322–325 may be due to the control of catecholamine levels by alternative presynaptic α2-AR isoforms. Furthermore, it also may not be excluded that under deficient α2C receptor function, other α2-AR isoforms in ventricular myocytes could compensate for detrimental stimulation of cardiac adrenoceptors imposed by high levels of catecholamine (Kokoz et al., 2016).

5. α2-AR signaling in cardiac hypertrophy

Similar to humans, the spontaneously hypertensive rat (SHR) is a model of genetic hypertension which develops left ventricle (LV) hypertrophy and heart failure with aging (Bing, Conrad, Boluyt, Robinson, & Brooks, 2002). While remodeling of the extracellular matrix and microtubules along with dysregulation in cell death pathways, intracellular calcium, β-adrenergic stimulation and mitochondrial function are recognized traits in SHR, the mechanisms responsible for tissue dysfunction and transition from hypertrophy to heart failure are only partially understood (Bing et al., 2002; Palmer, Chen, Lachapelle, Hendley, & LeWinter, 2006; Y. Tang, Mi, Liu, Gao, & Long, 2014). Of note, the development of LV hypertrophy and dysfunction in SHR appears to be not solely due to hypertension and may be linked to other traits (Palmer et al., 2006). Indeed, SHR rendered normotensive by means of peripheral sympathectomy still developed LV hypertrophy and heart failure (Cutilletta, Benjamin, Culpepper, & Oparil, 1978; Cutilletta, Erinoff, Heller, Low, & Oparil, 1977). The mechanisms primarily underlying cardiac remodeling of SHR have been suggested to include elevated sympathetic activity, both systemic and localized to cardiac tissue (Palmer et al., 2006). It has been shown that sympathetic activity was higher in the left ventricle of SHR compared to control, and would accentuate hypertension once cardiac and vascular hypertrophy are fully established (Adams, Bobik, & Korner, 1989). Finally, characteristics of systolic and diastolic Ca^{2+} dynamics demonstrated impaired regulation of intracellular Ca^{2+} in SHR cardiac myocytes compared to control animals (Palmer et al., 2006).

While we found that α2A-ARs were overexpressed in SHR cardiomyocytes, the regulation of I_{CaL} and Ca^{2+}-dependent NO synthesis by the receptor agonist guanabenz was reduced compared to cardiomyocytes isolated from controls (Kokoz et al., 2016). Contributing to such dysregulation could be genetic variants of the α2-AR, yet the involvement of α2-AR common polymorphisms into SHR ontogeny is not evident (Kobayashi et al., 1994; Sun, Chun, McArdle, Kimberling, & Pettinger, 1993). Remarkably, in SHR cardiomyocytes the blunted efficacy of α2-AR signaling was accompanied by increased transcript levels of mRNAs corresponding to all α2-AR isoforms (Kokoz et al., 2016). The impaired α2-AR signaling detected in SHR cardiomyocytes could be a consequence of the receptor desensitization induced by increased expression of β-arrestin under mechanical cardiac overload (Lymperopoulos & Bathgate, 2013; You et al., 2017). β-Arrestins, acting in concert with cofactors, G-protein coupled receptor kinases (GRKs), uncouple the receptor from its cognate G proteins resulting in the receptor functional desensitization and internalization (Ferguson, 2001; Lymperopoulos & Bathgate, 2013; Reiter & Lefkowitz, 2006). In fact, little is known about the possible mechanisms of desensitization/internalization of α2-AR particularly in cardiomyocytes, yet in other tissues agonist-promoted desensitization of certain α2-AR isoforms has been shown to occur via GRK-induced receptor phosphorylation (Lembo, Ghahremani, & Albert, 1999; Liggett et al., 1992). Comparison of the amino acid sequences in the third intracellular loop of α2-AR isoforms, the region responsible for receptor-arrestin interaction following phosphorylation of multiple serine or threonine residues, revealed little sequence homology suggesting the subtype selective desensitization mechanisms (Eason & Liggett, 1992). In this way, only the α2A and α2B isoforms are substrates for GRK2-catalyzed phosphorylation and β-arrestin binding leading to receptor desensitization during short-term epinephrine activation (Eason & Liggett, 1992; Wang et al.,

2004). During long-term agonist exposure, the three α2-AR subtypes reveal the following extent of desensitization: α2A = α2B > α2C. While during prolonged activation α2A and α2B displayed internalization and receptor down-regulation, the desensitization of α2C isoform relies solely on a decrease in G_i protein levels (Eason & Liggett, 1992; Liggett et al., 1992). Since desensitization of α2-ARs under upregulation of GRK plays a detrimental role in cardiac pathophysiology, suppression of β-arrestin action, directly or indirectly, for example, by regulation of expression of GRK2 in cardiomyocytes, has been proposed to be pursued for prevention of maladaptive cardiac remodeling and heart failure (Lymperopoulos, 2018; Lymperopoulos & Bathgate, 2013; Wang et al., 2004).

Aberrant α2-AR response in SHR may impair downstream effectors. Indeed, eNOS protein expression was reduced in young SHR, yet doubled in 1-year-old SHR compared to normotensive controls (Piech, Dessy, Havaux, Feron, & Balligand, 2003). Such profile of NOS expression hardly can explain the detected inability of α2-ARs to accelerate the rate of NO synthesis and suppression of I_{CaL}, while cGMP content in SHR animals was found to be well-preserved (Piech et al., 2003). Furthermore, bypass of the eNOS – sGC signaling using an NO donor or cGMP analog revealed that in SHR cardiomyocytes downstream NO- and cGMP-dependent pathways seem to be inoperative (Kokoz et al., 2016). In this setting, the observed α2-AR overexpression may result from an adaptive cellular program aimed at attempting to compensate the sympathoadrenal cardiac overload in this animal model. It seems feasible to test whether the aberrant α2-AR signaling observed in a model of spontaneous hypertension is also characteristic of an alternative model of cardiac pressure overload, such as, for instance, transverse aortic constriction. A similar profile of α2-AR dysregulation would provide convincing evidence that correction of aberrant α2-AR signaling could be a practicable therapeutic approach to prevent the progression of myocardial hypertrophy and its decompensation towards heart failure. Therefore, the link between α2-AR signaling and myocardial remodeling highlights the potential translational value of repairing/correcting the efficacy of sarcolemmal α2-ARs or their downstream effectors in cardiomyocytes. In particular, restoration or increase of the surface density of α2-AR in the plasma membrane, increase in the affinity of particular receptor isoform(s) to ligands and/or repair of defective downstream effectors could be envisioned options.

6. α2-AR in cardiac therapeutic applications

Despite considerable progress in the treatment of chronic heart failure with angiotensin-converting–enzyme inhibitors, aldosterone antagonists, β-receptor blockers, resynchronization therapy and cardiac transplantation, heart failure is associated with high mortality (McMurray & Pfeffer, 2005; Neubauer, 2007; Yancy et al., 2016), which warrants improved therapeutic strategies (Braunwald, 2015; Honig & Terzic, 2017; Kveiborg, Major-Petersen, Christiansen, & Torp-Pedersen, 2007). For instance, β-adrenergic receptor blockade does not abolish the detrimental effects of the sympathetic overflow observed in heart failure. It cannot attenuate the effects of vasoactive sympathetic co-transmitters, such as dopamine and neuropeptide Y (Du, 2001; Maisel et al., 1989; Mittal et al., 2017).

A tempting approach in heart failure management could be to reduce detrimental systemic neurotransmitter spillover via agonism of α2-ARs. Despite the observed dramatic reduction of norepinephrine levels induced by clonidine in control subjects, in patients with heart failure such administration resulted only in a minor reduction of regional norepinephrine spillover due to a significant desensitization and downregulation of α2-AR (Aggarwal, Esler, Socratous, & Kaye, 2001; Lang et al., 1997). Alternatively it has been suggested that the inhibition of whole-body catecholamine release can be achieved by activation of central imidazoline receptors (Guyenet, 1997; Szabo, 2002). Suppression of sympathetic nerve activity by pharmacological targeting of imidazoline I1 receptors by selective agonists, such as moxonidine or rilmenidine, results in reduction of sympathetic outflow (Hausberg, Tokmak, Pavenstädt, Krämer, & Rump, 2010; Head, Burke, & Chan, 1997). However, the Moxonidine Congestive Heart Failure (MOXCON) clinical trial reported more deaths in the moxonidine-treated group, which led to termination of this trial (Coats, 1999; Cohn et al., 2003). Closure of this trial indicates only partial comprehension of imidazoline receptors-associated pathways, mechanisms of its agonist action and the distribution of binding sites among tissues. Local targeting of I1 receptors in cardiac myocytes activated PKC, which via eNOS-dependent pathway inhibited SERCA lowering SR Ca^{2+} stores (Maltsev et al., 2014) and via NO-independent mechanism stimulated PP1 phosphatase activity (Braz et al., 2004). Of note, deletion of the gene encoding PKCα rescued cardiomyopathy associated with overexpression of PP1 (Braz et al., 2004). These results are consistent with the paradigm that loss of cardiac function is associated with a general increase in total cardiac protein phosphatase activity and an increase in PKC protein content and activity (Bayer et al., 2003; Bowling et al., 1999; Carr et al., 2002; Neumann, 2002). It is plausible that beyond systemic targeting of I1 receptors, local activation of these receptors in cardiomyocytes could provoke increased mortality in patients with cardiac muscle dysfunction.

Thus, instead of inhibition of sympathetic outflow by activation of centrally located α2-ARs, which induces a number of adverse effects (Table 1), cardiomyocyte-specific targeting of α2-AR would provide cardiac muscle-delimited stress control and enhance the efficacy of the current standard regimen of RAS- and β-blockade in the treatment of heart failure. From this standpoint, cardiovascular gene- or cell-based regenerative therapies should be considered in order to potentially repair and/or enhance the efficacy of α2-AR as an alternative or an adjuvant approach in the treatment of heart failure (Fig. 5). Delivery of cells into diseased hearts or gene therapy has been associated with a safe profile and signs of efficacy (Behfar et al., 2008; Singh et al., 2018; Tani et al., 2018; Terzic & Behfar, 2016). However, the development of such advanced therapeutic approaches would require to resolve at the very least the following practical questions: 1) are α2-AR isoforms interdependent or linked to different signaling pathways encompassing cardioprotective mechanisms?; 2) which α2-AR isoforms in cardiomyocytes control Ca^{2+} homeostasis and myocardial contractility?; 3) how does activation of specific α2-AR isoforms affect the expression profile of intracellular hypertrophic and heart failure molecular markers?; 4) which mechanisms underline aberrant α2-AR signaling in heart failure?

Hence, tissue-specific targeting of α2-AR in cardiomyocytes using gene or cell-based therapies could provide prospects in the prevention and treatment of cardiac hypertrophy and heart failure. In principle, enhancement of α2-AR signaling in cardiomyocytes could invigorate a cardiac stress-resistance phenotype without central suppression of the general adaptation syndrome critical to overcome life-threatening conditions.

7. Concluding remarks

In cardiac myocytes, in response to catecholamines, G-protein–coupled α1- and β-adrenoceptors mediate activation of cellular processes leading to increase of intracellular Ca^{2+} or Ca^{2+} responsiveness thereby promoting cardiac contractile force aimed at augmenting cardiac output under stress. Recent findings indicate that α2-AR isoforms (α2A-, α2B- and α2C) are also expressed in cardiac myocytes with the potential to safeguard cardiac muscle under adrenergic surge by governing intracellular Ca^{2+} handling and contractility. By adjusting the balance between protein kinase and phosphatase activities, sarcolemmal α2-ARs are capable of counterbalancing signaling cascades that provoke hypertrophic cardiac remodeling under chronic activation of adrenergic and angiotensinergic signaling. In this regard, the reprograming gene or cell based therapies aimed at cardiac specific restoration or enhancement of α2-AR signaling may represent future therapeutic directions for prevention or treatment of heart failure. This is in

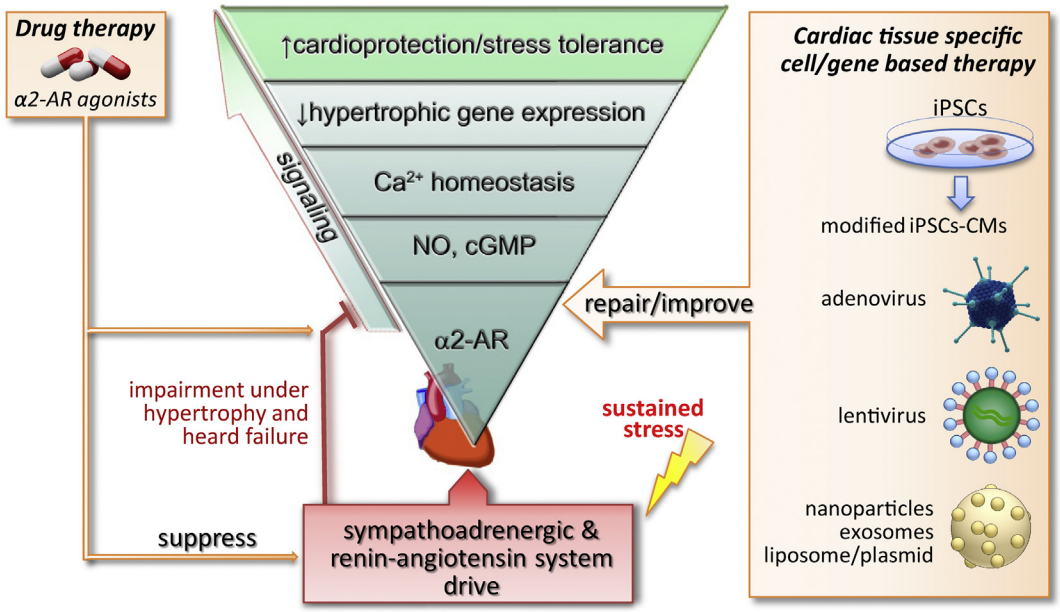

Fig. 5. Under disease-induced desensitization and downregulation of α2-AR signaling, tissue specific repair or improvement of the α2-AR sparing feedback machinery in cardiomyocytes using reprograming gene- or cell-based therapies may offer an alternative towards enhanced preservation of cardiac muscle structure and function.

accord with the modern opinion that refined heart failure therapies would require a paradigm shift from treating its secondary effects (neurohormonal activation, abnormal hemodynamics, arrhythmia, renal dysfunction etc.) to direct heart targeting with the goal of improving cardiac structure and function (Gheorghiade et al., 2016).

Conflict of interest statement

All authors declare that they have no conflict of interest.

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