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Review

Cardiac Adrenoceptors: Physiological and Pathophysiological Relevance

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Abstract. At present, nine adrenoceptor (AR) subtypes have been identified: α_{1A}-, α_{1B}-, α_{1D}-, α_{2A}-, α_{2B}-, α_{2C}-, β_1-, β_2-, and β_3AR. In the human heart, β_1- and β_2AR are the most powerful physiologic mechanism to acutely increase cardiac performance. Changes in βAR play an important role in chronic heart failure (CHF). Thus, due to increased sympathetic activity in CHF, βAR are chronically (over)stimulated, and that results in β_1AR desensitization and alterations of down-stream mechanisms. However, several questions remain open: What is the role of β_2AR in CHF? What is the role of increases in cardiac G_i-protein in CHF? Do increases in G-protein-coupled receptor kinase (GRK)s play a role in CHF? Does βAR-blocker treatment cause its beneficial effects in CHF, at least partly, by reducing GRK-activity? In this review these aspects of cardiac AR pharmacology in CHF are discussed. In addition, new insights into the functional importance of β_1- and β_2AR gene polymorphisms are discussed. At present it seems that for cardiovascular diseases, βAR polymorphisms do not play a role as disease-causing genes; however, they might be risk factors, might modify disease, and/or might influence progression of disease. Furthermore, βAR polymorphisms might influence drug responses. Thus, evidence has accumulated that a β_1AR polymorphism (the Arg389Gly β_1AR) may affect the response to βAR-blocker treatment.

Keywords: heart failure, cardiac β_1- and β_2-adrenoceptors, β-adrenoceptor polymorphism, cardiac G-protein, G-protein-coupled receptor kinase

1. Introduction

There are several cardiac receptor systems that are involved in regulation of contractility and/or heart rate. Among these, there are receptors coupled to the G_s-protein-adenylyl cyclase pathway (β-adrenoceptors (AR), histamine-receptors, serotonin-receptors), receptors coupled to the G_i-protein-adenylyl cyclase pathway (muscarinic-receptors, adenosine-receptors), and receptors that couple to the $G_{q/11}$-protein-phospholipase C-protein kinase C pathway (α_1AR, endothelin-receptors, angiotensin II-receptors). A vast body of evidence has accumulated that in heart failure, α- and βAR changes play an important role. The aim of this review was to critically discuss recent findings in the field and their physiological and pathophysiological relevance.

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Invited article

2. α- and β-Adrenoceptors in the human heart

2.1 β-Adrenoceptors

At present, three βAR subtypes have been identified in mammals, β_1-, β_2-, and β_3AR (for a review see ref. 1). In the human heart, β_1- and β_2AR coexist, whereby β_1AR predominate; in general, the ratio β_1-:β_2AR is about 70%:30% in the atria and 80%:20% in the ventricles. On the other hand, total βAR number appears to be equally distributed over atria and ventricles (2).

Both βAR subtypes couple to the G_s-protein, thereby elevating the intracellular level of cyclic AMP and cause positive inotropic and chronotropic effects, in vitro as well as in vivo. In atria, stimulation of both β_1- and β_2AR can evoke maximal increases in force of contraction (in vitro on isolated tissues) and heart rate (in vivo in healthy subjects), whereas in ventricles, only stimulation of β_1AR causes maximal increases in force of contrac-

tion, while stimulation of β_2AR causes only submaximal increases (2).

However, accumulated evidence indicates that at least in the rat and murine heart, stimulation of β_1AR causes not only positive inotropic and chronotropic effects, but can also promote apoptosis of cardiomyocytes (3 – 7). Moreover, β_2AR in rat and murine heart have been shown to couple not only to the G_s-protein but also to the G_i-protein (8 – 10), thereby inducing antiapoptosis (3 – 7). Interestingly, very recent data indicate that in rat heart (isolated ventricular cardiomyocytes), β_2AR-agonists appear to be able to activate either the G_s-protein pathway or the G_s- *and* G_i-protein pathway in an agonist-specific fashion. Thus, in isolated ventricular cardiomyocytes from spontaneously hypertensive rats (SHR) with heart failure, the contractile response to several β_2AR-agonists, including terbutaline, salbutamol, zinterol, and procaterol, could be markedly enhanced when the cardiomyocytes were pretreated with pertussis toxin (PTX), thereby inactivating the G_i-protein. Interestingly, however, the contractile response to another β_2AR-agonist, fenoterol, was not affected by PTX-treatment (11). Similarly, in rat ventricular cardiomyocytes, fenoterol fully inhibited phenylephrine-evoked hypertrophic response [evoked by α_{1A}AR stimulation (12)], while terbutaline and salbutamol evoked only partial inhibition. PTX-treatment of the cardiomyocytes did not affect fenoterol-effects, but converted terbutaline- and salbutamol-evoked partial inhibition into complete inhibition (Fig. 1) (13). Taken together, it appears that at least in the rat cardiomyocyte, fenoterol activates only the β_2AR-G_s-protein pathway, while terbutaline and salbutamol activate both the β_2AR-G_s- *and* β_2AR-G_i-protein pathway.

Although in the failing human heart, apoptosis and necrosis has been demonstrated (14, 15), it is not known whether β_1AR are involved in regulation of cardiomyocyte apoptosis. Similarly, it is still a matter of debate whether or not in the human heart, β_2AR may couple to the G_i-protein. Thus, in one study, in human right atrial membranes, it was shown by the use of photoaffinity labeling techniques followed by immunoprecipitation with an antibody specific for $G_{\alpha i}$ that isoprenaline in the presence of 100 μM of the β_1AR-selective antagonist CGP 20712A, but not in the presence of 100 μM of the β_2AR-selective antagonist ICI 118,551, stimulates $G_{\alpha i}$; similar effects were obtained with 100 μM of the β_2AR-agonist zinterol. Moreover, PTX-treatment of these human right atrial membranes enhanced adenylyl cyclase activation mediated by β_2AR stimulation but not by β_1AR stimulation (16). However, it should be considered that concentrations of agonists and antagonists used in this study (all 100 μM) were rather high; for example, it has been shown that zinterol, even in a ten times lower concentration (10 μM), acts as a mixed β_1/β_2AR-agonist evoking β_2AR effects only in the presence of a β_1AR-antagonist (for references, see ref. 10). Moreover, studies on isolated human atrial and ventricular preparations have consistently shown that activation of β_2AR caused – very similar to stimulation

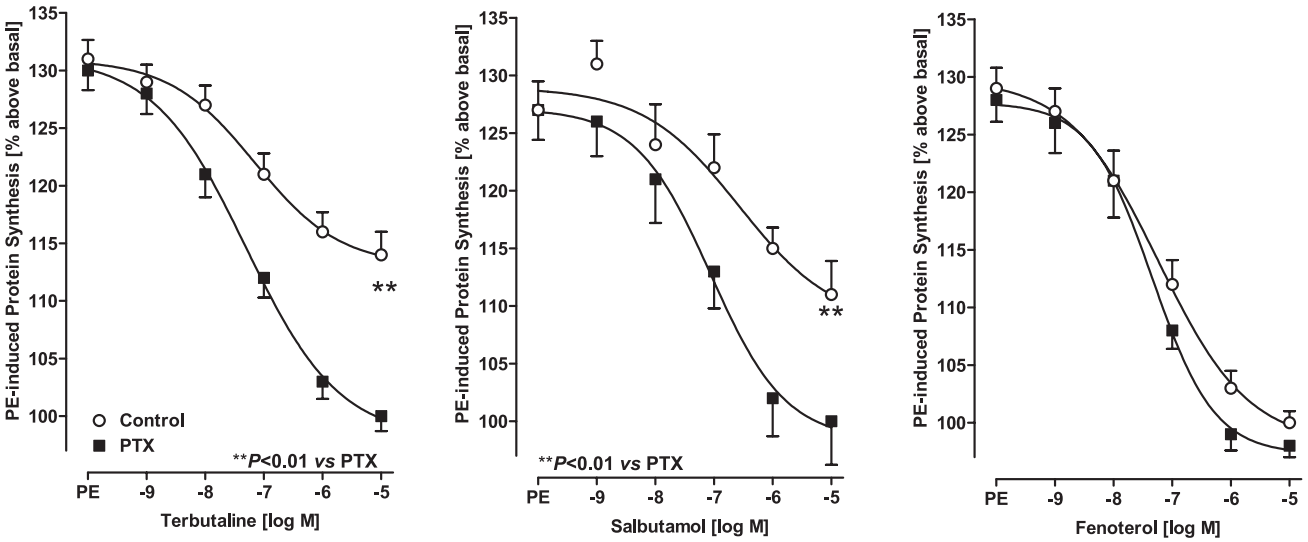

Fig. 1. Effects of pertussis toxin pretreatment (PTX, 250 ng/ml for 16 h) on terbutaline (left)-, salbutamol (middle)-, and fenoterol (right)-evoked inhibition of 1 μM phenylephrine (PE)-induced increase in protein synthesis (assessed as [^{3}H]-phenylalanine incorporation) in ventricular cardiomyocytes of 12-week-old male Wistar rats. Ordinate: 1 μM PE-induced [^{3}H]-phenylalanine incorporation as % of basal (= 100%). Abscissa: molar concentrations of terbutaline, salbutamol, and fenoterol. Means ± S.E.M. Modified from Pönicke et al. (ref. 13).

of β_1AR – increases in contractile force, hastened relaxation, and phosphorylation of phospholamban and troponin I (17 – 19). This strongly argues for a coupling of β_1- and β_2AR in the human heart to G_s-protein. In this context, it is also worth noting that preliminary data from our laboratory show that in healthy subjects, infusion of terbutaline and fenoterol causes increases in heart rate (that are predominantly mediated by β_2AR stimulation, see ref. 2) that were not considerably different between these two β_2AR-agonists (20). However, if in the human heart, β_2AR would couple to G_s- and G_i-protein, the heart rate-increasing effect of fenoterol should be significantly larger than that of terbutaline because, as discussed above, fenoterol activates only the β_2AR-G_s-protein pathway (that increases heart rate), while terbutaline activates besides the β_2AR-G_s- also the β_2AR-G_i-protein pathway (that should inhibit increases in heart rate). Thus, taken together, evidence accumulated so far makes it rather doubtful whether in human heart, β_2AR can couple to, besides G_s-protein, G_i-protein. In this context, however, it is interesting to note that Gong et al. (21) have found that in ventricular cardiomyocytes from patients with heart failure (who exhibit increased ventricular G_i-protein activity, see below), but *not* in cardiomyocytes from non-failing human hearts (with normal G_i-protein), the β_2AR-antagonist ICI 118,551 exerted agonistic effects evoking direct negative inotropic effects.

Whether or not β_3AR might exist in the human heart, is also still an open question. Several groups did not find any evidence for β_3AR mediated effects (for reviews, see refs. 2, 22, and 23), whereas Gauthier and associates recently found in ventricular endomyocardial biopsy samples of heart transplant recipients, β_3AR that obviously couple to a G_i/nitric oxid (NO) pathway and mediate negative inotropic effects (24).

In addition to β_1-, β_2-, and β_3AR, it had been postulated, mainly by the group of Kaumann and his coworkers, that in the heart of several species, including humans, an additional "putative β_4AR" may exist that couples to the G_s-protein pathway and mediates, upon stimulation, positive inotropic effects (for a review see ref. 22). This "receptor" was characterized by insensitivity against classical βAR-antagonists such as propranolol, and it was potently activated by CGP 12177 (a potent antagonist at classical β_1- and β_2AR). However, it is now clear that this "β_4AR" is not an additional βAR subtype, but is a low affinity state of the β_1AR (for references see ref. 25). Accordingly, it has been recently suggested to define two distinct states of the β_1AR: the classical β_1AR as "β_{1H}" and the "low affinity state" of the β_1AR (the previous "putative β_4AR") as "β_{1L}" (26).

2.2. α_1-Adrenoceptors

At present, three α_1AR subtypes have been identified: α_{1A}-, α_{1B}-, and α_{1D}AR (for a review, see ref. 1). The existence of α_1AR in the human heart has been demonstrated by molecular biology and biochemical methods as well as in functional studies; however, the α_1AR subtype present in the human heart is not well characterized (2).

The density of human cardiac α_1AR is only 10% – 15% of that of βAR; they couple presumably via $G_{q/11}$-protein to inositol phosphate formation (27) and mediate positive inotropic effects (for reviews, see refs. 2, 28 – 30). The maximum positive inotropic effect following α_1AR stimulation is, however, by far less than that evoked by βAR stimulation in the human heart. In rat heart, stimulation of cardiac α_1AR evokes, besides inotropic effects, the development of a hypertrophic phenotype (31, 32) that in adult rat cardiomyocytes is mediated by α_{1A}AR (12). Whether or not activation of α_1AR in the human heart might also induce a hypertrophic response is not known at present.

2.3. α_2-Adrenoceptors

At present, three α_2AR subtypes have been identified: α_{2A}-, α_{2B}-, and α_{2C}AR (for a review see ref. 1). The existence of (small amounts of) α_2AR in the human heart has been demonstrated by molecular biology methods, while almost all attempts have failed to demonstrate the existence of human cardiac α_2AR on the protein level; accordingly, it is not very surprising that the α_2AR subtype present in the human heart is not well characterized (2).

However, several groups have clearly demonstrated that the α_2AR plays functionally a role in presynaptic regulation of noradrenaline release in the human heart. Thus, the existence of presynaptic α_2AR inhibiting noradrenaline release have been directly demonstrated ex vivo in isolated human right atria (33, 34) and indirectly in vivo by systemic and intracoronary application of phentolamine (35, 36) that caused a marked increase in plasma noradrenaline levels. In this context, it is interesting to note that phentolamine effects on plasma noradrenaline levels were much more pronounced in patients with heart failure (who have increased sympathetic activity, see below) than in healthy subjects. It is, however, still a matter of debate whether the presynaptic α_2AR present in the human heart is of the α_{2A}AR subtype (as in several other species, see ref. 37) or of the α_{2C}AR (2).

3. Changes of α- and β-adrenoceptors with aging and in chronic heart failure

Changes of human cardiac α- and βAR in aging and in chronic heart failure (CHF) have been recently reviewed in great detail (2, 23, 25, 38, 39) and are only briefly summarized here.

3.1. Changes in β-adrenoceptor function

Both aging and CHF are clinical settings that are characterized by an increase in sympathetic activity. However there are distinct differences: in aging, the increase in sympathetic activity develops slowly [plasma noradrenaline levels (an index for sympathetic activity) increase only 10% to 15% per decade (40)]. In CHF, on the other hand, sympathetic activity increases much more rapidly (41); in addition, increases in plasma noradrenaline levels are by far higher and reach within short time levels between 600 and 1300 pg/ml (42). Nevertheless, in both settings, functional responsiveness of cardiac βAR is diminished. In CHF, this reduction is due to a decrease in β_1AR density and an uncoupling of β_2AR from the G_s-protein-adenylyl cyclase pathway. Moreover, amount and activity of the inhibitory G_i-protein that further dampens βAR mediated effects is enhanced. Finally, the amount and activity of the neuronal uptake transporter (uptake-1) that causes reuptake of neuronally released noradrenaline into the sympathetic nerve endings is decreased. This decrease in uptake-1 results in increased noradrenaline concentrations at the receptor site that can further contribute to βAR desensitization and down-regulation. In aging, it is not clear whether the reduced βAR function is due to a decrease in β_1AR density [as found in ventricular myocardium (43)] or due to a decrease in the catalytic unit of the adenylyl cyclase [as found in atria (44)]; however, both settings finally result in a diminished cyclic AMP formation upon βAR stimulation. In addition, also in the aging human heart, uptake-1 is reduced and the resulting increase in noradrenaline concentration at the receptor site might contribute to βAR desensitization. Controversial data exist on changes in G_i-protein: one group found that, as in CHF, G_i-protein increases with age [atria (44)], but another group failed to find any G_i-protein changes with age [ventricular myocardium (43)]. In ventricular myocardium of aged rats also, no increase in G_i-protein was found (45).

3.2. Role of changes in G_i-protein

In general, the role of the increase in G_i in CHF is still a matter of debate. On the one hand, it could cause impairment of cyclic AMP formation and by this attenuate positive inotropic effects evoked by stimulation of β_1- and β_2AR. In fact increases in G_i-protein can suppress receptor-mediated activation of adenylyl cyclase [for references see (46)]. This might be detrimental to the heart because the βAR-adenylyl cyclase-cyclic AMP pathway is the most powerful physiologic mechanism in the human heart to acutely increase myocardial performance (47). On the other hand, it could be protective to the heart because it could augment antiapoptotic effects. As mentioned above, at least in rat cardiomyocytes, cardiac βAR stimulation can affect apoptosis with β_1AR (via the G_s-protein) inducing proapoptotic effects and β_2AR (via the G_i-protein) inducing antiapoptotic effects. If this would occur also in human heart, the increase of G_i-protein in CHF should promote antiapoptosis. Accordingly, it has been suggested that chronic treatment with β_2AR-agonists might exert beneficial effects in CHF (48). In fact, Ahmet et al. have recently shown that in rats with ischemic cardiomyopathy (ICM) due to myocardial infarction, long-term treatment with the β_2AR-agonists zinterol and fenoterol exerted beneficial effects (attenuation of disease progression) that were mainly due to their antiapoptotic effects (49). However, before extrapolating from these data obtained in rats with experimental heart failure to the human situation, the following caveats should be considered: a) while zinterol appears to belong to those β_2AR-agonists that can signal via the G_s- *and* G_i-protein pathway, fenoterol has been shown by two different groups to signal selectively via the G_s-protein pathway (see above). Since activation of the G_s-protein pathway is considered to be linked to proapoptotic effects, fenoterol should not evoke antiapoptotic effects; thus, its beneficial effects observed in rats with ICM are difficult to understand; b) various in vivo animal studies in the 1990's have clearly shown that following chronic βAR-agonist stimulation, cardiac β_2AR undergo rapid desensitization and down-regulation, whereas cardiac β_1AR appear relatively resistant to down-regulation, and that held true for treatment with β_1AR-, β_2AR-, and non-selective β_1- and β_2AR-agonists (for references see ref. 47). This again would lead to the question of how long-term treatment with β_2AR-agonists can be beneficial because their effects should rapidly wane because of rapid desensitization; c) early attempts to treat patients with CHF with the β_2AR-agonist fenoterol were not very successful (50).

Studies in rats had shown that G_i-protein appears to be involved in catecholamine-induced arrthythmias. Thus, inactivation of G_i-protein by PTX-treatment in rats led to a marked increase in the arrhythmogenic effect of isoprenaline (51). On the other hand, chronic treatment of rats with isoprenaline caused decreases in βAR and increases in G_i-protein, and this was accompanied by a

marked decrease in isoprenaline- or forskolin-induced arrhythmias (52). Taken together, these data are in favor of the idea that the increase in cardiac G_i-protein in CHF-patients might protect the heart against catecholamine-induced arrhythmias.

3.3. Role of changes in G-protein-coupled receptor kinases

In addition to down-regulation of β_1AR, uncoupling of β_2AR, and increases in G_i-protein, upregulation of the β-adrenergic receptor kinase (βARK1, also known as GRK2) appears to be a major factor contributing to βAR desensitization in the failing human heart. G-Protein-coupled receptor kinases (GRK) are a family of serine/threonine kinases that phosphorylate only agonist-occupied receptors, thereby facilitating binding of arrestins to the phosphorylated receptor. This leads to an uncoupling of the receptor from the $G_{\alpha s}$-adenylyl cyclase system and finally to a decrease of βAR responses to agonist stimulation. In the heart, three isoforms of the GRK are expressed: GRK2 (or βARK1), GRK3, and GRK5 whereby GRK2 is the most abundant isoform (53, 54). Several studies have demonstrated upregulation in expression and activity of GRK2 in patients and animal models of heart failure and hypertrophy (for recent reviews, see refs. 23, 55, 56). In animal models also, increases in expression and activity of GRK5 have been found, but its role in human heart failure remains unclear. GRK3 appears not to be altered in human and animal models of heart failure (55).

In the human heart, GRKs have been mainly assessed in myocardial samples from patients with end-stage heart failure undergoing heart transplantation. In these samples from explanted hearts, expression and activity of GRK (mainly GRK2) was markedly enhanced (57 – 59). However, it should be considered that one of the strongest stimuli to activate GRK2 is increased sympathetic activity, and by this, βAR become activated, whereas stimulation of αAR appears not to activate GRKs (60). Patients with end-stage heart failure often are treated with βAR inotropic support for "bridging to transplant". Hence, it cannot be completely excluded that the increase in cardiac GRK2 observed in explanted hearts from patients with end-stage heart failure might be, at least partly, due to exogenous βAR stimulation. Two studies have recently investigated changes in GRK2 in relation to the course of heart failure. We have determined GRK2 activity in right atrial appendages from patients undergoing open heart surgery with different degrees of heart failure, NYHA class I – IV (59). In these patients, GRK2 increased significantly only in early stages of heart failure (with the maximum increase in patients with NYHA class II), while in patients with more severe heart failure (NYHA class III – IV) GRK2 activity was not significantly different from control levels in atrial appendages from non-failing hearts (Fig. 2). On the other hand, in the same study GRK2 activity in explanted hearts from heart transplantation-patients was significantly enhanced, whereby these patients had been treated with dobutamine/dopamine for several days before transplantation. Iaccarino et al. (61) assessed in 55 patients with different degrees of heart failure (NYHA class I – IV) GRK2 activity in circulating lymphocytes. They found, in contrast to our data, that with increasing NYHA class, activity of soluble GRK2 in the lymphocytes increased. The reason for this discrepancy – in atria GRK-increases only in early heart failure, in lymphocyte GRK-increases related to the severity of heart failure – is not completely understood at present. However, it should be noted that lymphocytes, which contain functional β_2AR and have been frequently used in the 1980's as marker for βAR changes in solid human tissue like the heart (62), are composed of several subsets that all contain different amounts of β_2AR, making it difficult to judge whether changes in βAR are real receptor changes or are due only to redistribution of subsets; this might be also true for expression and/or activity of GRK2 in circulating lymphocytes, although the final experimental proof for that is lacking at present. In addition, it has been clearly shown that changes in lymphocyte β_2AR mirror much more precisely changes of β_2AR in solid human tissues, whereas in the human heart, β_1AR are the predominant βAR subtype (63).

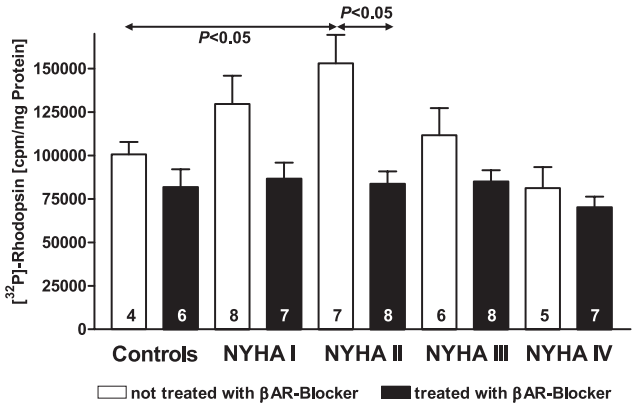

Fig. 2. Effects of long-term βAR blockade (with metropolol or bisoprolol) on right atrial GRK-activity in patients with different degrees of heart failure (judged by NYHA functional class). Ordinate: Total GRK-activity (sum of soluble and membraneous) in cpm [^{32}P]rhodopsin/mg protein. Numbers at the bottom of the column = number of patients studied. Modified from Leineweber et al. (ref. 59, ©2005) with permission from the European Society of Cardiology.

Since GRK2 plays a critical role in regulation of myocardial function, heart failure is accompanied by increased activity of GRK2 and genetically engineered mice with cardiac specific overexpression of GRK2 exhibit blunted inotropic responses to βAR stimulation (for reviews, see refs. 55 and 56), this has led to the hypothesis that inhibition of GRK2 activity might reverse cardiac dysfunction. A specific inhibitor of GRK2 is not known at present. However, GRK2 is a cytosolic enzyme that must translocate to the membrane to phosphorylate the receptor. This translocation is due to an interaction between the approximately 195 amino-acid C-terminus of GRK2 and the $\beta\gamma$ heterodimer ($G_{\beta\gamma}$) that is released from the G-protein upon activation. In several animal models it could be shown that this C-terminal domain of GRK2 (named βARKct) can inhibit GRK2 activation by competing for free $G_{\beta\gamma}$-subunits, and over-expression of βARKct in several animal models of heart failure was found to reduce cardiac hypertrophy and to attenuate the progressive deterioration of heart function (60, 64). These results are compatible with the view that the beneficial effects of βARKct are due to its inhibition of GRK2 activation. However, it should be mentioned that βARKct acts via binding to the $G_{\beta\gamma}$-subunit, thereby preventing translocation of GRK2 to the membrane. $G_{\beta\gamma}$-proteins, however, exert their own signaling properties including modulation of activity of phospholipase C, potassium channels, and other second messenger systems (for a recent review see ref. 65). Thus, it might be also possible that the beneficial effects of βARKct might be due to its inhibition of $G_{\beta\gamma}$-signaling. In fact, Li et al. (66) have recently shown that in rabbits with rapid ventricular pacing-induced heart failure, phosducin (a known $G_{\beta\gamma}$-subunit binding protein) exerted very similar beneficial effects on heart failure progression as did βARKct, but phosducin did not restore βAR responsiveness as did βARKct. Thus, it is still an open question whether or not beneficial effects of βARKct in heart failure models are due to inhibition of GRK2 or to inhibition of $G_{\beta\gamma}$-signaling.

As mentioned above, one of the strongest stimuli to activate GRK2 is activation of βAR. Thus, it is rather plausible that βAR-blockers by preventing βAR activation (either due to exogenously applied agonist or endogenously increased agonists, i.e., catecholamines) may reduce GRK2 activity. In fact, in several animal models of heart failure, increased GRK2 activity could be reduced by application of βAR-blockers (for reviews, see refs. 2, 60, 64). We have recently shown that this appears to be true also for patients with CHF: in patients with different degrees of CHF treated with the β_1AR-blockers metoprolol or bisoprolol, right atrial GRK2 activity was in each NYHA class significantly lower than in those patients not treated with βAR-blockers (Fig. 2) (59). Thus, it is tempting to speculate that, at least in part, beneficial effects of βAR-blockers in treatment of heart failure are due to their ability to prevent activation of GRK2.

Finally, it should be noted that in contrast to the failing human heart, in the aging human heart activity of GRKs is *not* altered (58). A similar lack of changes in GRK activity has been also found in the aging rat heart (45). The reason for this different behavior of GRK in the failing versus the aging heart is not completely understood. As mentioned earlier, stimulation of βAR is a strong stimulus to activate GRK; and in heart failure as well as in aging, sympathetic activity is increased. However, increase in sympathetic activity is much more vigorous in heart failure versus aging (see above); it might be possible, therefore, that these differences in intensity of sympathetic activation is one explanation for the different changes in GRK activity in the aging versus failing human heart.

4. β-Adrenoceptor polymorphisms

4.1. Functional importance of β_1-adrenoceptor polymorphisms

Recent studies have shown that β_1AR are polymorphic. There are at least two functionally important single nucleotide polymorphisms (SNPs) in the β_1AR gene (67 – 69): at position 49 in the extracellular amino-terminus of the β_1AR a serine is substituted by a glycine (Ser49Gly). When expressed in Chinese hamster fibroblasts (CHW) the Gly49 variant exhibited much more rapid down-regulation following long-term agonist activation than the Ser49 variant (70, 71). At position 389 in the intracellular carboxy-terminus (a β_1AR region important for G-protein coupling), an arginine is substituted by a glycine (Arg389Gly); expressed in CHW-cells, the Arg389 β_1AR variant had a slightly higher basal and 3 – 4-fold higher isoprenaline-stimulated adenylyl cyclase activity than the Gly389 β_1AR variant (72). This was due to a better coupling of the Arg389 β_1AR variant to the G_s-protein than the Gly389 β_1AR variant. It is interesting to note that the Gly389 β_1AR variant was initially considered the wild-type β_1AR (73), but it is now clear that it is the minor allele.

Between codon 49 and 389 SNPs of the β_1AR, a strong linkage disequilibrium exists. Thus, individuals homozygous Gly49 are nearly always homozygous Arg389; and vice versa, individuals homozygous Gly389 are nearly always homozygous Ser49; the haplotype Gly49Gly389 appears not to occur natively (74). On the other hand, codon 49 SNP appears to

modulate functional responsiveness of codon 389 SNP: in vitro in HEK293 cells stably transfected with different β_1AR haplotypes (particular combinations of the 49 and 389 SNPs), isoprenaline evoked maximal cyclic AMP generation with an haplotype order Gly49Arg389 > Ser49Arg389 ≫ Ser49Gly389 (75).

Divergent results have been described for the functional consequence of the Arg389Gly β_1AR polymorphism in humans: ex vivo on isolated electrically driven right atrial preparations obtained from patients undergoing coronary artery bypass grafting, Molenaar et al. (76) did not find any influence of the Arg389Gly (or the Ser49Gly) β_1AR polymorphism on positive inotropic effects of noradrenaline; similarly, the lipolytic response to β_1AR stimulation in human adipocytes natively expressing the Arg389 or Gly389 variant of the β_1AR was not different between the two genotypes (77). In contrast, Sandilands et al. (78) demonstrated in human right atrial preparations, greater inotropic and cyclic AMP responses to noradrenaline at the Arg389 versus the Gly389 β_1AR variant. In vivo, several groups have studied the effects of dynamic exercise on heart rate [the "classical" test for cardiac β_1AR function (2, 79)] in subjects homozygous for the Arg389 or Gly389 β_1AR. All groups so far found that increases in heart rate were *not* genotype-dependent (80 – 83). Moreover, also exercise-induced increases in contractility and plasma-renin-activity (PRA), a renal β_1AR mediated effect in humans, were not different in Arg389 versus Gly389 subjects (Fig. 3) (81). This holds true even

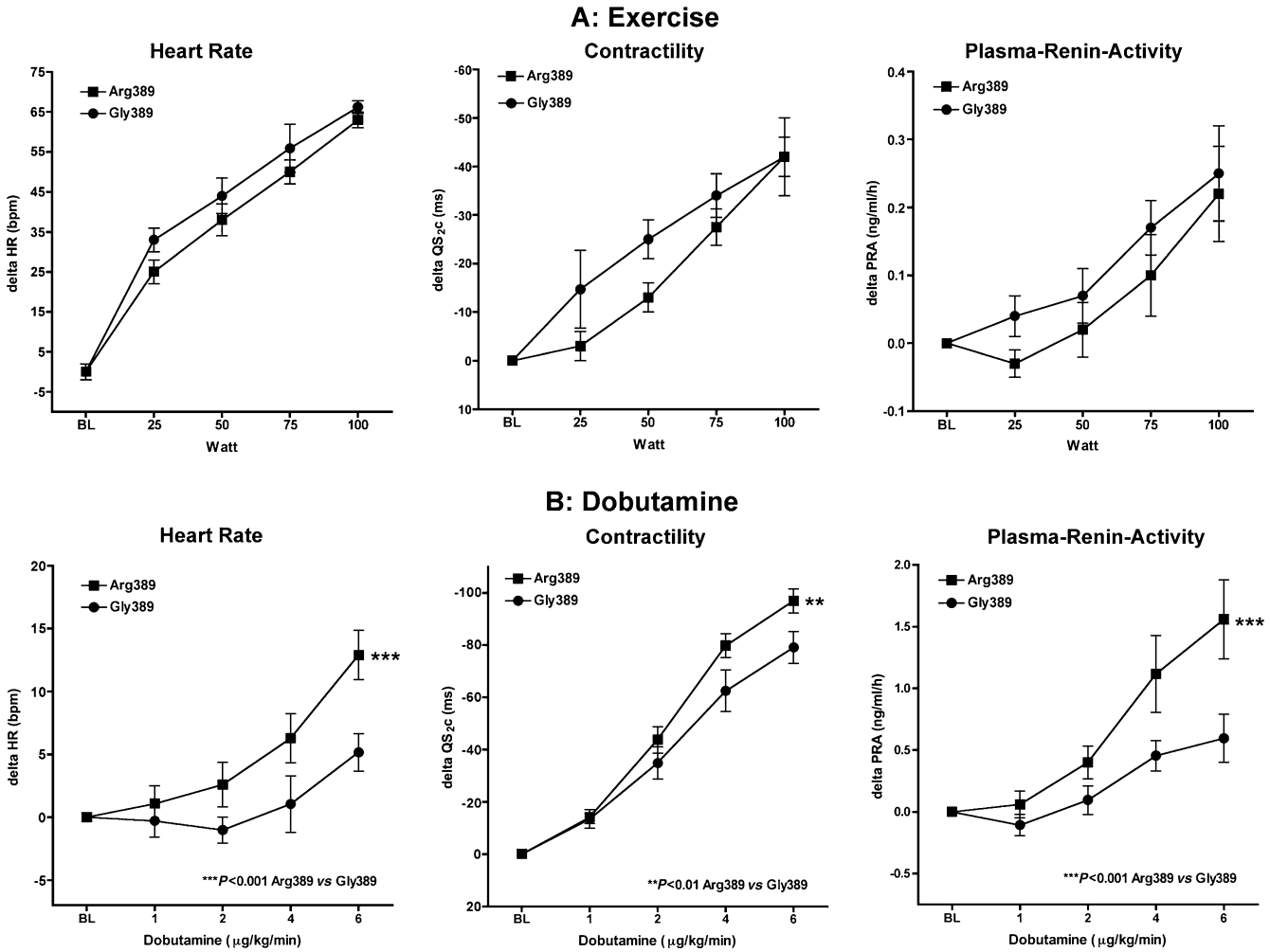

Fig. 3. Exercise (bicycle-exercise in supine position, A)- and dobutamine-infusion (lower panel, B)-induced increases in heart rate (left), contractility (middle, assessed as heart rate-corrected duration of the electromechanical systole - QS_2c), and plasma-renin-activity (PRA) (right) in male subjects homozygous for Arg389 β_1AR or homozygous for Gly389 β_1AR. Ordinates, left: changes in heart rate in beats/min (bpm); middle: changes in contractility (shortening of QS_2c in -ms); right: increases in PRA in ng angiotensin I formed/ml/h. Abscissa: panel A: Work-load in Watt for 5 min each; panel B: dose of dobutamine in μg/kg/min for 15 min each. Modified from Büscher et al. (ref. 81, ©2001) and Bruck et al. (ref. 86, ©2005) with permission from Lippincott Williams & Wilkins and American College of Cardiology Foundation, respectively.

when in the subjects, possible counter-regulatory parasympathetic effects were blocked by atropine: also under these conditions, exercise-induced increase in heart rate, contractility, and PRA were not different between homozygous Arg389 and Gly389 β_1AR subjects (84). On the other hand, using a modified dobutamine stress echocardiography protocol, LaRosee et al. (85) demonstrated recently that in subjects pretreated with atropine in order to exclude possible parasympathetic effects, those homozygous for the Arg389 β_1AR variant exhibited larger inotropic and blood pressure responses than those carrying one or two Gly389 alleles. However, the use of atropine appears *not* to be necessary to demonstrate differential responses of Arg389 versus Gly389 β_1AR to dobutamine. We have recently shown in healthy subjects that, also in the absence of atropine, dobutamine causes significantly larger cardiac effects in Arg389 than in Gly389 β_1AR subjects; the PRA response to dobutamine was also significantly larger in β_1AR subjects than in Gly389 β_1AR subjects (Fig. 3) (86). The reason for this discrepancy in cardiac- and PRA-responses to exercise versus dobutamine is not completely understood at present. However, it should be considered that 1) responses to dynamic exercise are strongly dependent on the physical fitness of the test subjects, and it is extremely difficult to precisely control this factor. Thus, it cannot be excluded that subjects participating in exercise studies are of different physical fitness, and that would evoke unpredictable results; 2) exercise may induce more physiologic responses, whereas dobutamine-infusion may induce more pharmacologic responses; 3) in all exercise studies published so far, subjects were not controlled for codon 49 SNP. As mentioned above, codon 49 SNP can modulate functional responsiveness of codon 389 SNP; accordingly, β_1AR haplotype-analysis could be more important than single SNP-analysis (75). Thus, divergent results obtained in exercise- versus dobutamine-studies could, at least partly, be due to the β_1AR haplotype inhomogeneity of study groups. However, our recent data show that codon 49 SNP might not play a very important role: we had studied only subjects homozygous for Ser49 and had, nevertheless, found that dobutamine evoked β_1AR-genotype-dependent effects, while exercise did not (84, 86).

Recently evidence has accumulated that subjects or hypertensive patients exhibited larger blood pressure and heart rate responses to β_1AR-blockers if they carry the Arg389 β_1AR variant (82, 83, 86, 87), and this could be modulated by codon 49 SNP. Thus, the haplotypes Gly49Arg389 and Ser49Arg389 exhibited much larger responses than the haplotype Ser49Gly389 (86 – 88). Similarly, in CHF-patients carrying the Arg389 β_1AR variant, chronic βAR-blocker treatment caused significantly larger improvement of left ventricular ejection fraction (LVEF) than in Gly389 β_1AR patients (89, 90); however, some researchers have reported no genotype-dependent differences in LVEF-improvement during chronic βAR-blocker treatment (91, 92). Recently, Magnusson et al. (93) suggested that in CHF-patients due to dilated cardiomyopathy, the influence of codon 49 SNP on outcome and effective βAR-blocker treatment appeared to be more pronounced than that of codon 389 SNP. Thus, Gly49 carriers treated with low dose of βAR-blocker had a significantly lower 5-year mortality rate than Ser49 carriers. This is, however, not in contradiction to the above described observation that the Arg389 β_1AR variant is the determinant of responses to βAR-blockade: it should be considered that patients with the Gly49 β_1AR variant do always carry Arg at codon 389 because of the linkage disequilibrium between codon 49 and 389 SNPs, while Ser49 carriers can have Arg or Gly at codon 389.

It should be noted that we recently showed that a β_1AR-blocker (bisoprolol) inhibited dobutamine-evoked PRA-increases to a significantly larger extent in Arg389 β_1AR subjects than in Gly389 β_1AR subjects. These results might have important clinical implications for treatment of hypertensive patients. βAR-blockers are first-line drugs for the treatment of hypertension. Although experience of antihypertensive treatment with βAR-blockers exists for more than 40 years, the mechanism of blood pressure lowering effect of βAR-blockers is still not completely understood (94). However, the renin-angiotensin-aldosterone system (RAAS) plays an important role in blood pressure regulation and is certainly one target, out of several, for the blood pressure lowering effect of βAR-blockers (95). Thus, various authors could show that the blood pressure lowering effect of βAR-blockers was related to PRA: with high PRA levels, βAR-blockers evoked a large effect, while with low PRA levels, the effect of βAR-blockers was small (94). Taken together, it is tempting to speculate that β_1AR polymorphisms might predict hemodynamic response to βAR-blocker treatment: patients with the Arg389 β_1AR variant should be good responders, while those with the Gly389 variant should be poor- or non-responders.

4.2. Role of β_1-adrenoceptor polymorphisms in chronic heart failure

Because of the great importance of β_1AR in the regulation of heart rate and contractility (2), attempts have been made to identify possible associations between the Ser49Gly and Arg389Gly β_1AR polymorphisms and

CHF. However, the studies published so far have not found an association between Ser49 or Gly49 alleles and CHF (96) or Arg389 or Gly389 alleles and CHF (97 – 101). Wagoner et al. (99), however, found that peak oxygen consumption (VO_2) during exercise (i.e., a clinically relevant measure of the capacity of the heart to increase cardiac output) was significantly lower in CHF-patients homozygous for Gly389 than in those homozygous for Arg389. Interestingly, however, Small et al. (100) recently found that, in African Americans, the Arg389 genotype in combination with a deletion polymorphism of the α_{2C}AR, significantly increases the risk of heart failure. Two studies in patients with dilated cardiomyopathy found that the Gly49 β_1AR variant is associated with a lower risk of heart failure (102) or a decreased 5-year mortality risk (96).

4.3. Functional importance of β_2-adrenoceptor polymorphisms

There are at least three functional important SNPs in the β_2AR gene: Arg16Gly, Gln27Glu, and Thr164Ile (67 – 69, 103). In vitro, expressed in CHW-cells, the functional properties of Gly16 and Glu27 β_2AR variants do not differ from those of wildtype β_2AR (WT, Arg16Gln27Thr164) (104). In contrast, the Ile164 β_2AR variant exhibited extensive signaling defects (105). Thus, isoprenaline, adrenaline, and noradrenaline had a 4-fold lower affinity for Ile164 β_2AR expressed in CHW-cells than for WT β_2AR. Moreover, the Ile164 variant exhibits reduced basal and agonist-induced activation of adenylyl cyclase and a rightward shift of agonist concentration-effect curve, suggesting diminished β_2AR-G-protein interaction (105). In vivo, in transgenic mice, expressing Ile164 β_2AR specifically in cardiomyocytes, myocardial basal and isoprenaline-stimulated adenylyl cyclase activity was lower than in mice overexpressing Thr164 β_2AR (106). In humans, heterozygous for Thr164Ile β_2AR [homozygous Ile164 variants are not found in the human population (67 – 69, 103)], cardiac and venous β_2AR responses were blunted compared to subjects carrying homozygous Thr164 β_2AR (Fig. 4) (107 – 110).

In humans, β_2AR-agonists increase heart rate and contractility (2). In general, these β_2AR mediated increases in heart rate and contractility did not differ between WT β_2AR subjects and subjects with Arg16Gly and Gln27Glu β_2AR polymorphisms (for a review, see ref. 103), thus confirming the in vitro data (see above).

In contrast, data obtained on impact of Arg16Gly and/or Gln27Glu β_2AR polymorphisms on vascular responsiveness are rather controversial. In studies with healthy subjects, "systemic" application of β_2AR-agonists resulted in larger vasodilation in subjects homozygous for Arg16 (111 – 113), whereas "local" application into brachial artery or hand vein evoked larger vasodilatory responses in subjects homozygous for Gly16 (114 – 116); however, in one study, terbutaline evoked larger venodilatory effects in subjects homozygous Arg16 (110). The reason for this discrepancy is not well understood; it might be tissue- or agonist- (isoprenaline or terbutaline) dependent.

Arg16Gly and Gln27Glu β_2AR have been suggested

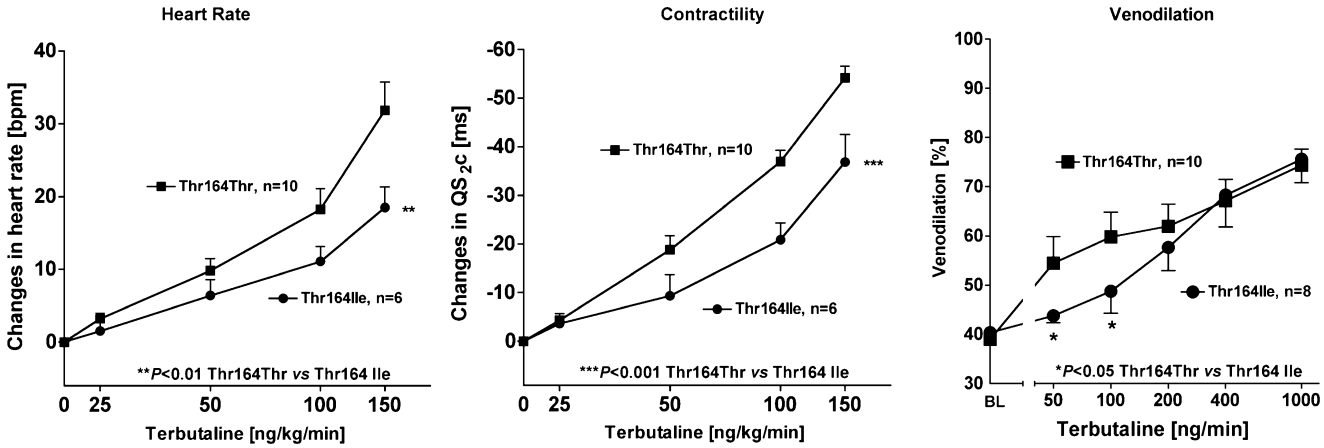

Fig. 4. Terbutaline-infusion-induced increase in heart rate (left) and contractility (middle, assessed as heart rate-corrected duration of the electromechanical systole - QS_2c) and terbutaline-induced venodilation in phenylephrine-preconstricted dorsal hand veins (right) in subjects homozygous for Thr164Thr β_2AR or heterozygous for Thr164Ile β_2AR. Ordinates: left: changes in heart rate in beats/min (bpm); middle: changes in contractility (shortening of QS_2c in −ms); right: reversal of phenylephrine-induced preconstriction expressed as percentage venodilation Abscissa: dose of terbutaline in ng/kg/min for 15 min each (left and middle) and in ng/min (right). Means ± S.E.M. Modified from Bruck et al., ref. 108 ©2003 and ref. 110 ©2005, with permission from the American Physiological Society and American Society for Clinical Pharmacology & Therapeutics, respectively.

to differ in their susceptibility to agonist-induced receptor down-regulation. Thus, in vitro studies showed that the Gly16 β_2AR variant undergoes significantly enhanced agonist-promoted down-regulation compared with Arg16 (WT) β_2AR. In contrast, the Glu27 β_2AR variant seems to be resistant against agonist-promoted β_2AR down-regulation (104). Gly16Glu27 double mutant receptors, however, demonstrated that Gly16 effects dominate over Glu27 effects. These receptors underwent even greater agonist-promoted down-regulation than Gln27 β_2AR. In contrast, Arg16Glu27 double mutant β_2AR were completely resistant to down-regulation. However, because of the strong linkage disequilibrium between codon 16 and codon 27 (117), subjects homozygous for Glu27 are nearly always homozygous Gly16; haplotype Arg16Glu27 occurs naturally, extremely rare, being present in less than 1% of the population (67 – 69, 103). Thus, in fact position 16 determines the phenotype of the three haplotypes Arg16Gln27, Gly16Gln27, and Gly16Glu27 with regard to agonist-induced receptor down-regulation.

We have recently investigated whether in healthy subjects, cardiac responses to the β_2AR-agonist terbutaline might desensitize in a β_2AR genotype-dependent manner. For this, we treated subjects for 2 weeks with 3×5 mg/day terbutaline orally and determined before and at the end of treatment terbutaline infusion-induced increases in heart rate and contractility. Under these conditions, cardiac β_2AR responses to terbutaline were desensitized; however, the extents of desensitization in subjects with the three β_2AR haplotypes (Arg16Gln27 [WT], Gly16Gln27, or Gly16Glu27) were not significantly different (118). Similarly, oral terbutaline-treatment caused also down-regulation of lymphocyte β_2AR densities in a β_2AR genotype-independent manner (119). These results clearly show that Glu27 resistance against agonist-promoted down-regulation, initially observed in vitro (104), does not occur in vivo, very likely because of the linkage disequilibrium between codon 16 and 27 (117), haplotype Arg16Glu27 (that is in vitro resistant against down-regulation) is nearly not natively expressed in humans (see above).

4.4. Role of β_2-adrenoceptor polymorphisms in chronic heart failure

Several association studies have investigated a possible role of the three β_2AR polymorphisms in CHF. Regarding the Thr164Ile β_2AR variant, three studies showed that the allele frequency of the Ile164 was not different between CHF-patients and healthy controls (92, 102, 120). However, Thr164Ile CHF-patients exhibited during exercise-capacity tests lower peak VO_2 than Thr164 CHF-patients (121). Liggett et al. (120) had previously shown that CHF-patients heterozygous for the Thr164Ile β_2AR variant have a worse prognosis than CHF-patients homozygous for the Thr164 β_2AR: 1-year survival, calculated as the proportion of patients who have not died or have not undergone heart transplantation, was with 42%, significantly lower in Thr164Ile β_2AR patients than in Thr164Thr β_2AR patients with 76%. Because of the low frequency of the Thr164Ile β_2AR variant in the general population, these findings were obtained in only 10 out of 259 CHF-patients. We have recently tried to further evaluate the (patho)physiologic role of the Thr164Ile β_2AR in CHF. For this purpose we hypothesized that, since Thr164Ile β_2AR patients undergo rapid progression to death or heart transplantation (120), the prevalence of the Thr164Ile β_2AR variant and the frequency of the Ile164 allele should be much higher in heart-transplanted patients than in patients with stable CHF or healthy controls. However, we found that the prevalence of the Thr164Ile β_2AR variant was in 309 heart-transplanted patients (2.3%), not significantly different from that in 520 patients with stable CHF (1.9%) or 328 healthy controls (2.1%); similarly, the frequency of the minor Ile164 allele was in heart-transplanted patients ($f(-) = 0.0106$) not significantly different from that in CHF-patients ($f(-) = 0.0096$) or healthy controls ($f(-) = 0.0113$). Hence, the role of the Thr164Ile β_2AR in CHF remains to be questionable.

Divergent results have been described for Arg16Gly and Gln27Glu β_2AR variants and CHF. Forleo et al. (102) found that patients with dilated cardiomyopathy and homozygous for the Glu27 β_2AR variant (and because of the linkage disequilibrium between codon 16 and codon 27 SNP, homozygous Gly at codon 16) had a significantly higher risk for heart failure than patients with the Arg16 and Gln27 β_2AR. In this context, it is also interesting to note that Kaye et al. (122) recently found that in CHF-patients carrying one or two Glu27 alleles, significantly more patients (86%) exhibited beneficial responses to carvedilol (defined as LVEF-improvement by 10%) than patients homozygous for the Gln27 β_2AR variant (only 50%); thus, those patients carrying the Glu27 allele, which is speculated to be associated with heart failure (see above), exhibited much greater beneficial effects than those patients carrying the Gln27 allele, which is obviously protective against heart failure. On the other hand, three studies (92, 101, 120) failed to find any association between Arg16Gly or Gln27Glu β_2AR polymorphisms and CHF.

5. Conclusion

There can be no doubt that in the mammalian heart,

several AR-subtypes exist that regulate cardiac function. Among those in the human heart, β_1- and β_2AR are the most powerful physiologic mechanism to acutely increase cardiac performance. In the last several years, great progress has been made in more precisely defining the role of βAR in heart failure. Thus, stimulation of β_1AR in heart failure is initially beneficial to the heart by increasing short-term cardiac performance, but long-term stimulation (similarly to transgenic overexpression) of β_1AR is detrimental to the heart. While this seems to be generally accepted, several questions remain to be resolved: What is the role of β_2AR in heart failure? Can stimulation of cardiac β_2AR evoke beneficial effects? Is the increase in activity of cardiac G_i-protein protective or detrimental to the heart? Are changes in GRK2 consequences of the chronic βAR stimulation in heart failure or are they caused by an yet unknown mechanism? Is the increase in GRK2 detrimental to the heart or is it a somewhat protective mechanism that, by further desensitizing cardiac β_1AR, may attenuate harmful effects of chronic β_1AR stimulation? Is the reduction in GRK2-activity evoked by βAR-blocker treatment part of the beneficial effects of chronic βAR-blocker treatment in CHF-treatment, or do these GRK2-changes only secondarily accompany the βAR-blocker evoked inhibition of βAR-signalling?

During the last decade, growing evidence has accumulated that β_1- and β_2AR genes have genetic polymorphisms that are of functional importance. Many studies investigating possible associations between βAR genotypes and cardiovascular diseases have been performed; the results are, however, rather inconsistent. At present, it seems to be clear that βAR polymorphisms do not play a role as disease-causing genes; however, they might be risk factors, might modify disease, and/or might influence progression of disease. In addition, βAR polymorphisms might influence drug responses. Thus, evidence has accumulated that in patients with chronic asthma, β_2AR polymorphisms appear to influence drug response (β_2AR-agonist treatment), and in long-term studies, development of β_2AR-desensitization (123). In addition, several studies have recently shown that the Arg389Gly β_1AR polymorphism determines cardiovascular responses to βAR-blocker treatment (124). Further studies have to show whether in fact βAR polymorphisms might affect drug responsiveness because this could lead to a more individualized drug therapy.

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