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Invited review

Signal transduction mechanisms controlling cardiac contractility and their alterations in chronic heart failure

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1. Introduction

The contractility and rate of the human heart are regulated by numerous neurotransmitters and hormones which act via specific membrane-bound receptors which in turn activate various intracellular signalling pathways. This article will focus on those receptor systems and their alterations in heart failure which signal via heterotrimeric GTP-binding regulatory proteins (G-proteins). Three types of receptor/G-protein complexes appear to be important in the human heart: Some receptor systems such as the β-adrenoceptors couple via G_s to the adenylyl cyclases to increase intracellular levels of cyclic AMP and to activate cAMP-dependent protein kinase (PKA); this may promote influx of extracellular Ca^{2+} through phosphorylation of calcium channels [1], thereby enhancing contraction, but may also lead to enhanced relaxation through increased calcium uptake in the sarcoplasmic reticulum via phosphorylation of phospholamban and by decreasing calcium sensitivity of troponin through phosphorylation of troponin I [2]; additionally $G_{s\alpha}$ has been shown to be capable of directly activating L-type Ca^{2+} channels [3] and Na^+-channels [4] without involvement of cyclic AMP, but this is still a matter of controversy [5,6] and the physiological importance of these pathways for regulation of contractile force in the human heart remains to be determined. Other receptor systems such as the muscarinic acetylcholine receptors couple via G_i to the adenylyl cyclases to inhibit cyclic AMP formation and to activate certain potassium channels [7]. Finally, some receptor systems act independently of adenylyl cyclases by stimulating a phospholipase C via G-proteins of the $G_{q/11}$ family. In the following we will first discuss the signalling properties of each of these systems in human heart and then their alterations in heart failure. We will largely focus on β-adrenoceptors since they are the best-investigated receptor system in the human heart; however, alterations of β-adrenoceptor signalling in animal models of chronic heart failure will also discussed briefly.

2. Receptor systems in the non-failing human heart

2.1. G_s-coupled receptors

In the human heart both isoforms of the α-subunit of G_s, $G_{s\alpha\,long}$ ($G_{s\alpha L}$) and $G_{s\alpha\,short}$ ($G_{s\alpha S}$) exist; while $G_{s\alpha L}$ predominates in the human heart [8], the specific function of both isoforms is not fully understood, but both may undergo differential regulation [9]. In the human heart many receptors couple via G_s to the adenylyl cyclase/cyclic AMP system including β-adrenergic, H_2-histamine, 5-HT_4-serotonin, VIP-, glucagon- and prostaglandin E_2-receptors. Whether each of these receptors is located on cardiomyocytes or whether some at least partly reside on non-myocyte cells of the heart is not fully clear. Thus, stimulation of β-adrenergic, H_2-, 5-HT_4- and VIP-receptors can evoke positive inotropic effects (at least in isolated human myocardial preparations), but a clear-cut positive inotropic effect of glucagon and prostaglandin E_2 in the human heart has not been demonstrated.

In the human heart both β_1- and β_2-adrenoceptors coexist; this has first been demonstrated by radioligand binding studies, and was subsequently confirmed in functional experiments [for reviews, see Refs. 10–12]. The number of β-adrenoceptors is quite evenly distributed in right and left atrial and ventricular tissue; however, the proportion of β_2-adrenoceptors is somewhat higher in the atria (approximately 30% of the total β-adrenoceptor pop-

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ulation) than in ventricular myocardium (about 20% of the total β-adrenoceptor population) [see Refs. 12 and 13], and may be even higher (up to 50%) in the atrioventricular conducting system [14]. On the other hand, β_3-adrenoceptors have not been found in the human heart, either in functional [15] or in mRNA distribution studies on β_1-, β_2- and β_3-adrenoceptors [16,17].

Both β_1- and β_2-adrenoceptors couple to adenylyl cyclase and increase the intracellular amount of cyclic AMP [18–21]. Interestingly, in the human heart—in atria as well as in ventricular myocardium—adenylyl cyclase is preferentially activated by β_2-adrenoceptor stimulation although β_1-adrenoceptors predominate [for review, see Ref. 12]. The mechanism underlying these different coupling efficiencies of human cardiac β_1- and β_2-adrenoceptors to adenylyl cyclase is not known at present. However, when β_1- and β_2-adrenoceptors are transfected into the same cell line, the β_2-adrenoceptors exhibit a much greater functional coupling to adenylyl cyclase than the β_1-adrenoceptors [22,23]. Thus, it might be a general phenomenon that β_2-adrenoceptors couple more efficiently to adenylyl cyclase than β_1-adrenoceptors. It might, however, also be possible that the differences between efficiencies of β_1- and β_2-adrenoceptors to increase cyclic AMP in the human heart are due to compartmentalization of cyclic AMP production within the cardiomyocytes, since compartmentalization of cyclic AMP production has been demonstrated in various other mammalian cardiomyocytes [24–27].

In vitro experiments have convincingly shown that both β_1- and β_2-adrenoceptors can mediate positive inotropic effects of β-adrenoceptor agonists in isolated electrically driven atrial and ventricular preparations [for references, see Refs. 10–12, 20 and 28]; this has recently also been demonstrated in single myocytes from human ventricle [29]. In right and left atria β_1- *and* β_2-adrenoceptor stimulation can evoke maximum positive inotropic effects, while on right and left ventricles only β_1-adrenoceptor stimulation can evoke maximum positive inotropic effects, β_2-adrenoceptor stimulation only submaximal positive inotropic effects [20,30,31].

In vivo experiments in humans have confirmed that β_2-adrenoceptors can mediate positive chronotropic and inotropic effects of β-adrenoceptor agonists. Several studies have shown that isoprenaline-induced tachycardia in humans is mediated by both β_1- and β_2-adrenoceptors to about the same degree, while exercise-induced tachycardia (which is mainly due to neuronally released noradrenaline), is mediated solely by β_1-adrenoceptor stimulation [for references, see Refs. 12 and 32]—in close agreement with in vitro data on isolated human right atria [20,31].

Moreover, in healthy volunteers the positive chronotropic effect caused by intravenous infusions of terbutaline was only marginally affected by the β_1-adrenoceptor selective antagonists atenolol and bisoprolol given in doses that markedly inhibited β_1-adrenoceptor-mediated effects [33–35]. Finally, Hall et al. [36] have demonstrated that the positive chronotropic effect of salbutamol upon injections into the right coronary artery of patients with chronic stable angina (thereby avoiding any systemic effects) was not affected by the β_1-adrenoceptor selective antagonist practolol, but was significantly antagonized by propranolol, indicating that it is mediated exclusively by (cardiac) β_2-adrenoceptor stimulation. It is interesting to note, however, that—in contrast to the in vitro data [20,31]—adrenaline appears to cause its positive chronotropic effect in vivo solely via (cardiac) β_2-adrenoceptor stimulation. Thus, several authors have shown that adrenaline-induced tachycardia is not affected by β_1-selective antagonists such as metoprolol [37], atenolol [38] or bisoprolol [39], but is completely abolished by the β_2-selective antagonist ICI 118,551 [40], or by the non-selective β-adrenoceptor antagonist propranolol [39].

Using the β_2-adrenoceptor agonist terbutaline, two groups have convincingly shown that cardiac β_2-adrenoceptors can also mediate positive inotropic effects in vivo [34,35]. Moreover, Schäfers et al. [35] recently compared in healthy volunteers the positive chrono- and inotropic effects induced by infusions of isoprenaline and terbutaline; they found that at doses that caused the same increase in heart rate isoprenaline caused larger positive inotropic effects than did terbutaline—in close agreement with the in vitro observation (see above) that in human right atrium both β_1- and β_2-adrenoceptors cause maximal positive inotropic effects while in the ventricular myocardium only β_1-adrenoceptor stimulation caused maximal positive inotropic effects, β_2-adrenoceptor stimulation evoked only submaximal positive inotropic effects.

In this context it is interesting to note that in transgenic mice overexpression of the β_2-adrenoceptor specifically in the heart resulted in marked elevation of baseline heart rate and contractility [41]; the extent of this increase was comparable with that induced by maximal isoprenaline stimulation in control mice; in addition, increases in baseline heart rate and contractility persisted in transgenic mice pretreated with reserpine [41], thus depleting endogenous catecholamine stores. On the other hand, overexpression of the human β_1-adrenoceptor in atria of transgenic mice did not show considerable changes in contractility [42]. These differences could be due to the amount of overexpression; they could, however, also be due to the lower efficiency of the β_1-adrenoceptor in coupling to adenylyl cyclase when compared with the β_2-adrenoceptor (see above). Moreover, in the transgenic mice with overexpressed β_2-adrenoceptors, but not in the control mice, the β_2-adrenoceptor antagonist ICI 118,551 significantly decreased basal heart rate and contractility, demonstrating for the first time in vivo that part of the β-adrenoceptor is constitutively active, even in the absence of β-adrenoceptor agonists. Such agonistic activity of "empty" β-adrenergic receptors and its reversal by certain β-adrenoceptor antagonists has been recently shown in several cell culture systems [43–45] including human cardiomyocytes [46], a phenomenon named "inverse agonism" [47].

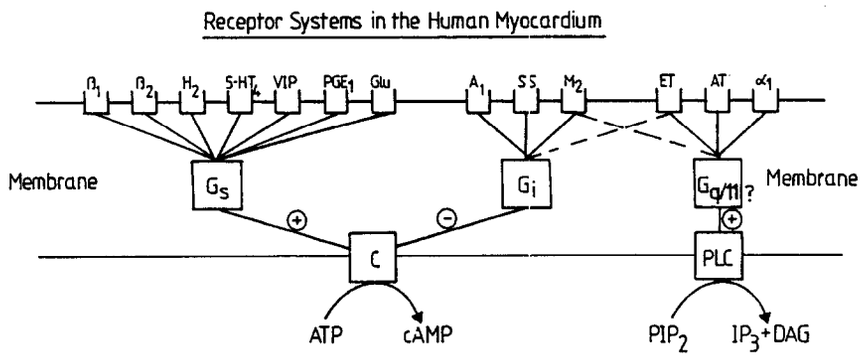
In addition to β-adrenoceptors at least three other receptor systems exist in the human heart that couple via G_s to adenylyl cyclase and can mediate positive inotropic effects: the histamine H_2-receptor, the serotonin 5-HT_4-receptor and the VIP-receptor. Compared with β-adrenergic stimulation, however, stimulation of these three receptors causes only submaximal activation of adenylyl cyclase in atrial (H_2, 5-HT_4) and ventricular (H_2, VIP) membrane

preparations from non-failing human heart [48–54]. Similarly, positive inotropic responses of isolated electrically driven ventricular preparations from non-failing human heart to histamine and VIP were only 30–40% of those evoked by isoprenaline [49,54–56] while in human right atrial tissue the histamine response was only slightly less than that of isoprenaline [50]. Interestingly, the 5-HT_4-receptor appears to mediate (submaximal, about 25–55% of that of isoprenaline) positive inotropic effects only in human right [50–52,57,58] and left atria [53], but not in ventricular myocardium [57,58], indicating that the ventricular myocardium may lack functional 5-HT_4-receptors.

Thus, in the human heart multiple receptor systems increase cyclic AMP concentrations and activate PKA, but the β-adrenoceptor is by the far the most effective receptor in inducing positive inotropic effects with all other G_s-coupled receptors causing only 30–60% of the maximal positive inotropic effect induced by β-adrenergic agonists (Fig. 1). It should be noted, however, that in the human heart there exists only a small receptor reserve for β-adrenoceptor-mediated positive inotropic effects. This was initially suggested by Kaumann et al. [59] and Bristow et al. [60] based on the comparison of affinity estimates and inotropic potency estimates for catecholamines and has been subsequently confirmed and extended by Schwinger et al. [61] and Brown et al. [62]. Interestingly, we recently observed [50] that in human right atrium histamine (acting at H_2-receptors) and serotonin (acting at 5-HT_4-receptors) stimulated adenylyl cyclase activity with a potency that was nearly identical to their respective positive inotropic potencies, indicating that also for these (G_s/adenylyl-cyclase-coupled) receptors only a few spare receptors exist although their true affinities (because of lack of suitable radioligands) have not been assessed in the human heart. Thus, it might be speculated that in the human heart G_s-adenylyl-cyclase-coupled receptors might have in general only a small receptor reserve, and hence alterations of their signal transduction in heart failure can be expected to directly affect their ability to elicit inotropic responses.

2.2. G_i-coupled receptors

The major isoform of human cardiac G_i α-subunits is $G_{i\alpha\text{-}2}$; in addition, $G_{i\alpha\text{-}3}$ has been demonstrated while $G_{i\alpha\text{-}1}$ appears to be absent, at least at the mRNA level [for a recent review, see Ref. 63]. At least three receptor systems exist in the human heart that couple to G_i: muscarinic M_2-, adenosine A_1- and somatostatin-receptors. All three receptor systems have been shown to inhibit adenylyl cyclase activity [64–68] and at least M_2- and A_1-receptors



Positive Inotropic Effect (% of Isoprenaline-Maximum)

	Right Atrium	Ventricular Myocardium
β_1	100	100
β_2	90-100	30-50
H_2	80-85	30-40
5-HT_4	25-55	0
VIP	???	40
PGE_1	???	???
Glu	???	???
α_1	15-25	15-45
ET	35-45	20
AT	30-45	0

Fig. 1. Receptor systems and their signal-transduction mechanisms in the non-failing human heart. For details, see text. Abbreviations: β_1, β_2, $\alpha_1 = \beta_1$-, β_2- and α_1-adrenoceptors; H_2 = histamine H_2-receptors; 5-HT_4 = 5-HT_4-serotonin receptors; VIP = vasoactive intestinal peptide receptors; PGE_1 = prostaglandin E_1 receptors; Glu = glucagon receptors; A_1 = adenosine A_1-receptors; SS = somatostatin receptors; M_2 = muscarinic M_2-receptors; ET = endothelin receptors; AT = angiotensin II receptors; G_s = stimulatory guanine nucleotide binding protein; G_i = inhibitory guanine nucleotide binding protein; $G_{q/11(?)}$ = the guanine nucleotide binding protein that presumably couples ET-, AT- and α_1-adrenergic receptors to PLC; C = catalytic unit of adenylyl cyclase; PLC = phospholipase C; PIP_2 = phosphatidylinositol 4,5-bisphosphate; DAG = 1,2-diacylglycerol; IP_3 = inositol-1,4,5-triphosphate; + = activation; − = inhibition. Right atrium: positive inotropic effects were determined on isolated electrically-driven right atria from patients without apparent heart failure undergoing coronary artery bypass grafting. Ventricular myocardium: positive inotropic effects were determined on isolated electrically-driven right and left ventricular preparations obtained from would-be cardiac transplant donors.

can cause negative inotropic effects. However, differences exist in their mode of action in atria and ventricles: in atria acetylcholine and adenosine can cause negative inotropic effects in the absence of other transmitters, presumably by directly opening a potassium channel to cause hyperpolarization that leads to negative inotropic effects, and can also reduce inotropic effects elicited by cyclic-AMP-elevating agents [69–71]. In ventricular myocardium, however, they can only reduce the force of contraction elicited by cyclic-AMP-elevating agents [65,66,72–76]. The reason for these different effects in atrial and ventricular myocardium is not known at present but could possibly be related to the existence of different potassium channels in atria and ventricles and/or to the fact that the densities of M_2- and A_1-receptors in human atria is about twofold higher than in the ventricles [74,75,77]. In addition, it is still a matter of debate whether the reduction of inotropic effects of cyclic-AMP-elevating agents by acetylcholine and adenosine is mediated via G_i-mediated inhibition of cyclic AMP elevation [for references, see Refs. 63, 69 and 71].

Somatostatin can inhibit human cardiac adenylyl cyclase activity [68]. However, somatostatin does not exert a negative inotropic effect on human atrial and ventricular myocardium pre-stimulated with noradrenaline but was found to significantly reduce basal force of contraction in atria, but not in ventricles [76]. Thus, further studies have to show whether somatostatin may play a physiological role in regulation of human cardiac contractility and/or heart rate.

2.3. Receptor systems signalling independent of the cyclic AMP system

In addition to the receptor systems that cause positive inotropic effects through elevation of intracellular cyclic AMP, some receptor systems in the human heart can elicit positive inotropic effects independently of cyclic AMP such as α_1-adrenoceptors, endothelin receptors and angiotensin II AT_1-receptors; moreover, under certain experimental conditions M_2-receptors increase force of contraction. Among these the most intensively studied receptor is the α_1-adrenoceptor. Numerous studies have clearly demonstrated that in the heart of various species including man α_1-adrenoceptors exist that can cause positive inotropic effects without changes in the intracellular levels of cyclic AMP in vitro [78–81] and in vivo [82,83]. The number of cardiac α_1-adrenoceptors varies markedly between species, being quite high in rat and very low in man [84–86]. Nevertheless, in the human heart α_1-adrenoceptor-mediated positive inotropic effects have been demonstrated in atrial [87–89] and ventricular preparations [86,90–92]. The mechanism of α_1-adrenoceptor-mediated inotropic effects is still a matter of debate: α_1-adrenoceptors couple via a pertussis toxin (PTX)-insensitive G-protein (possibly $G_{q/11}$) to phospholipase C [93,94]. This causes formation of 1,4,5-inositoltrisphosphate (IP_3) and diacylglycerol (DAG) with the former mediating the release of Ca^{2+} from intracellular stores which might be involved in increases in force of contraction. In addition, α_1-adrenoceptor stimulation increase the Ca^{2+}-sensitivity of myofilaments, transsarcolemmal Ca^{2+}-influx and intracellular alkalinization via activation of the Na^+/H^+-antiporter and it has been suggested that these effects are—at least partly—due to DAG-induced activation of PKC [for recent reviews, see Refs. 81, 95 and 96]. Among the three known α_1-adrenoceptor subtypes (α_{1A}, α_{1B} or α_{1D} [97]) the α_{1A}-adrenoceptor (formerly called α_{1C}) appears to dominate at the mRNA level [98,99], but whether this reflects the situation at the protein level is unclear.

Recently endothelin and angiotensin II have also been found to couple to the phospholipase C pathway and cause positive inotropic effects in the human heart. Based on radioligand binding studies the human heart contains endothelin receptors of the ET_A- and ET_B-subtype [100,101] and angiotensin II receptors of the AT_1-and AT_2-subtypes [102]. However, the endothelin- and angiotensin II-induced formation of inositol phosphates in human right atrial slices appears to involve only ET_A- and AT_1-receptors because endothelin-1 was 100-fold more potent than endothelin-3 [103] and because the angiotensin II effect was completely abolished by the AT_1-receptor antagonist losartan [50], respectively. Endothelin causes positive inotropic effects in atrial and ventricular preparations of the human heart [50,104–108]; at least in human right atrium this effect appears to result from activation of PKC with a subsequent activation of the Na^+/H^+-antiporter [108]. On the other hand, positive inotropic effects evoked by angiotensin II have consistently been found only in atria [50,76,109,110] while ventricular preparations showed no [76,110] or only inconsistent positive inotropic effects [109], possibly due to the fact that in ventricular myocardium the number of AT-receptors is rather low and the AT_2-subtype predominates [102].

Interestingly ET_A-receptors in adult rat cardiomyocytes [111] and human right atrium [103] but not in human left atrium and left ventricular myocardium [Brodde, unpublished observations] couple also to inhibition of adenylyl cyclase—very likely via G_i. This might explain the transient small negative inotropic effect preceding the positive inotropic effect that is observed with endothelin on isolated cardiac preparations [103,108,112,113]. In the heart of various mammalian species including humans muscarinic M_2-receptors (similar to ET_A-receptors) can couple to inhibition of adenylyl cyclase (see above) and to activation of phospholipase C. Thus, high concentration of carbachol ($> 10^{-6}$ M) can increase formation of inositol phosphates and cause positive inotropic effects; this effect is PTX-insensitive, indicating that it does not occur via G_i, and the positive inotropic effects are enhanced after PTX-pretreatment [76,114–116].

Finally, besides mediating positive inotropic effects some of the receptor systems present in human heart can also induce cardiac growth and may be, therefore, involved in hypertrophic responses. On isolated cardiac myocytes a direct effect on protein synthesis has been demonstrated for the phospholipase-C-coupled α_1-adrenergic, endothelin ET_A- and angiotensin II AT_1-receptors and it has been suggested that activation of phospholipase C and PKC may be essential for these responses [117–122]. Moreover, myocardial overexpression of a constitutively active α_{1B}-adrenergic receptor mutant (a mutant that was capable of activating PLC in an agonist-independent manner) in trans-

genic mice caused marked cardiac hypertrophy [123]. Whether these effects might also occur in the human heart remains to be elucidated, but the fact that angiotensin converting enzyme inhibitors reduce cardiac hypertrophy more effectively than other antihypertensive drugs in hypertensive patients [124] indicates this may also be the case in vivo.

3. Changes in human cardiac receptor systems in chronic heart failure

3.1. G_s-coupled receptors

Heart failure is a disease state that is primarily characterized by inadequate perfusion of peripheral organs and pulmonary and venous congestion. This is associated with activation of various neurohumoral systems including the renin–angiotensin system and the sympathetic nervous system. On the other hand, the cardiac responsiveness to neurohumoral stimulation is markedly altered in heart failure. Among receptor systems present in the human heart, this has been best investigated for the β-adrenoceptor–G-protein–adenylyl cyclase system. Bristow et al. [60] were the first to report that β_1-adrenoceptor number is decreased in patients with chronic failure of different etiology. Numerous studies have confirmed and extended this finding and demonstrated that it occurs with all etiologies of heart failure and that the extent of reduction in β-adrenoceptor number is directly related to the severity of the disease, often judged by NYHA classification [for recent reviews, see Refs. 116, 125 and 126]. While there is general agreement that β_2-adrenoceptor function (i.e., induction of positive inotropic effects) is also decreased in patients with chronic heart failure, it is still controversial whether β_2-adrenoceptor number decreases [10–12, 116,125,126]. Thus, reduced β_1-adrenoceptor numbers have been reported in all studies, whereas decreased β_2-adrenoceptors were found in some and unchanged numbers in others. Some data indicate that these differences may possibly depend on the etiology of the disease.

Due to the high sympathetic tone in chronic heart failure, it has frequently been assumed that cardiac β-adrenoceptor desensitization is a form of agonist-induced desensitization. Studies on agonist-induced desensitization of β-adrenoceptors in other models [for a recent review, see Ref. 127] have indicated various possible molecular mechanisms:

(1) Phosphorylation of the receptor by the enzyme β-adrenoceptor kinase (βARK) which recognizes receptors only if they are occupied by agonists and enhances the binding of the inhibitor protein β-arrestin which inhibits interaction of the receptor with the G_s-protein; since this can occur only with agonist-occupied receptors, this mechanisms is mainly implicated in homologous desensitization.

(2) Phosphorylation of the receptor by PKA and possibly by PKC; the desensitizing effect of phosphorylation by PKA or PKC does not require the binding of β-arrestin and may be involved in homologous and heterologous (PKA) or in heterologous desensitization only (PKC). There are two major differences between βARK- and PKA-induced desensitization: βARK-induced phosphorylation is considerably faster than PKA-induced effects, and βARK-induced phosphorylation requires much higher agonist concentrations than does PKA phosphorylation.

(3) Transient internalization of the receptors into a still unknown intracellular compartment where they are not accessible to hydrophilic ligands such as catecholamines. These internalized receptors remain functionally intact and can be recycled.

(4) Down-regulation of the receptors (i.e., a decrease in the total number of receptors). This can be caused either by enhanced degradation of the receptors and/or by diminished synthesis.

(5) Uncoupling of the receptors from their G-proteins, which may be a consequence of one of the above events.

(6) Altered expression of G-proteins (e.g., decreased G_s or increased G_i) and/or isoforms of adenylyl cyclases which also may be a result of one of the above events, particularly of PKA or PKC activation.

A role for βARK in cardiac β-adrenoceptor desensitization in heart failure has recently been suggested by Ungerer et al. [128,129]. They demonstrated that βARK mRNA levels and activity are increased in hearts of patients with end-stage dilated or ischemic cardiomyopathy. Interestingly this did not involve altered amounts of β-arrestin at the mRNA or protein level [129]. Increased βARK activity might be more important for β_2- than for β_1-adrenoceptor desensitization since it has been shown in various cell line experiments that the β_2-adrenoceptor undergoes more extensive phosphorylation by βARK than the β_1-adrenoceptor, possibly because the β_2-adrenoceptor contains more potential βARK phosphorylation sites than the β_1-adrenoceptor [130]. Some authors have even doubted that βARK plays a physiological role at all in β_1-adrenoceptor phosphorylation [131].

PKA is also likely to contribute to β-adrenoceptor desensitization in chronic heart failure, since PKA will be chronically activated by enhanced endogenous noradrenaline in this setting. Böhm et al. [132] recently reported that the potency and efficacy of cyclic AMP in activating PKA is not changed in the failing human heart, indicating that PKA itself may not be altered, but this conclusion awaits confirmation by direct assessment of cardiac PKA activities. While PKA can clearly phosphorylate and desensitize β_1-adrenoceptors (e.g., in the human neuroblastoma cell line SK-N-MC [131]), β_2-adrenoceptors are much more susceptible to PKA-phosphorylation than β_1-adrenoceptors possibly because β_2-adrenoceptors contain two potential PKA-phosphorylation sites, but β_1-adrenoceptors contain only one [130].

Internalization seems not to play an important role in chronic heart failure since several groups have shown no differences in the percentage of β-adrenoceptors in a light vesicular fraction of non-failing and failing human hearts [133–135].

Down-regulation of β_1-adrenoceptor number has been demonstrated in all forms of chronic heart failure in man (see above). This down-regulation of β_1-adrenoceptors could be due to the decrease in mRNA levels that have been recently demonstrated in the hearts of patients with

dilated and ischemic cardiomyopathy [128,136]. mRNA levels for β_2-adrenoceptors were not altered in the failing human heart in these studies, but whether β_2-adrenoceptors are down-regulated at the protein level is still controversial (see above).

Despite these data many questions remain open to clarify the mechanism underlying β_1-adrenoceptor down-regulation and desensitization and β_2-adrenoceptor desensitization in chronic heart failure. For example, it is not known what mechanism triggers the decrease in β_1-adrenoceptor mRNA and why this process is selective for β_1-adrenoceptors (relative to β_2-adrenoceptors). This question is especially intriguing since they do not fit well into the general concepts of β_1- and β_2-adrenoceptor desensitization. Thus, it has been hypothetized that cardiac-derived noradrenaline is responsible for β_1-adrenoceptor down-regulation in chronic heart failure [137] because noradrenaline is a selective β_1-adrenoceptor agonist [138] and locally sympathetic drive is markedly enhanced in the heart [139]. This could lead to a selective down-regulation of β_1-adrenoceptors. Selective β_1-adrenoceptor down-regulation has been demonstrated in several tissues including the heart of rats harboring a noradrenaline-secreting pheochromocytoma [140,141]. On the other hand, it has been observed in various in vivo animal studies that following chronic β-adrenoceptor stimulation the cardiac β_2-adrenoceptor undergoes rapid desensitization and down-regulation while β_1-adrenoceptors were relatively resistant to down-regulation. This holds true not only for isoprenaline [142–146] and adrenaline [147], which are non-selective β_1- and β_2-adrenoceptor agonists, but also for noradrenaline [147] which is a rather selective β_1-adrenoceptor agonist [138]. This differential susceptibility to desensitization is also found in isoprenaline-treated rats and guinea-pigs in which sympathetic nerve endings had been destroyed by 6-hydroxydopamine pretreatment [146,148]. Thus, chronic exposure of β_1- but not β_2-adrenoceptors to continuously released endogenous noradrenaline from sympathetic nerve endings cannot explain the differential regulation of the two subtypes. Recently, similar differences in β_1- and β_2-adrenoceptor desensitization have been also observed in human coronary arteries containing both β-adrenoceptor subtypes [149]. In this preparation a 16-hour in vitro incubation with noradrenaline did not affect β_1-adrenoceptor-mediated relaxation, but markedly desensitized β_2-adrenoceptor relaxation [150]. Thus, β-adrenoceptor regulation in the human heart with consistent β_1-adrenoceptor down-regulation but inconsistent β_2-adrenoceptor down-regulation appears to be quite unique and it is still not known why only β_1-adrenoceptors are so dramatically down-regulated in chronic heart failure.

Desensitization of cardiac β-adrenoceptor responses in human heart failure can also occur by altered G-protein expression. There is general agreement that α-subunits of cardiac G_s are not quantitatively altered in chronic heart failure, either when determined on the protein level by cholera-toxin (CTX)-catalyzed ADP-ribosylation or quantitative Western-blotting, or on the mRNA levels (Table 1) or functionally in a reconstitution assay using cyc^- cells

Table 1
Changes in human cardiac G-proteins in chronic heart failure

Diagnosis	Region	Technique	Result		Reference
DCM	LV	PTX	$G_{i\alpha}/G_{o\alpha}$	↑36%	Feldman et al. 1988 [151]
	LV	CTX + cyc^-	$G_{s\alpha}$	–	
DCM	RV	PTX	$G_{i\alpha}/G_{o\alpha}$	↑40%	Neumann et al. 1988 [152]
DCM	LV	PTX	$G_{i\alpha}/G_{o\alpha}$	↑37%	Böhm et al. 1990 [65]
ICM			$G_{i\alpha}/G_{o\alpha}$	–	
DCM	LV	CTX	$G_{s\alpha}$	–	Schnabel et al. 1990 [214]
DCM	LV	Western	G_α-37 kDa	–	Feldman et al. 1991 [153]
			G_α-38 kDa	–	
			G_α-42 kDa	–	
		PTX	$G_{i\alpha}/G_{o\alpha}$	↑	
DCM	LV	PTX	$G_{i\alpha}/G_{o\alpha}$	↑26%	Hershberger et al. 1991 [66]
DCM	LV	PTX	$G_{i\alpha}/G_{o\alpha}$	↑103%	Bristow et al. 1991 [154]
ICM	LV	PTX	$G_{i\alpha}/G_{o\alpha}$	↑91%	
DCM/ICM	LV	CTX	$G_{s\alpha}$	–	
DCM	LV	RIA	$G_{i\alpha}$	↑138%	Böhm et al. 1994 [158]
ICM	LV	RIA	$G_{i\alpha}$	↑58%	
DCM/ICM	RV	RIA	$G_{i\alpha}$	–	
DCM	LV	Northern	$G_{i\alpha\text{-}3}$-mRNA	↑92%	Feldman et al. 1989 [155]
			$G_{o\alpha}$-mRNA	↑36%	
			$G_{s\alpha}$-mRNA	↑63%	
DCM	LV	PCR	$G_{i\alpha\text{-}3}$-mRNA	–	Feldman et al. 1991 [156]
			$G_{s\alpha}$-mRNA	–	
DCM	LV	Northern	$G_{i\alpha\text{-}2}$-mRNA	↑103%	Eschenhagen et al. 1992 [157]
ICM			$G_{i\alpha\text{-}2}$-mRNA	↑77%	
DCM/ICM			$G_{i\alpha\text{-}3}$-mRNA	–	
DCM/ICM			$G_{s\alpha}$-mRNA	–	

Only studies comparing G-protein expression in failing human hearts as compared to non-failing control hearts are listed. Values represent percent changes from non-failing hearts. ↑ = increase, – no significant change. DCM = idiopathic dilated cardiomyopathy; ICM = ischemic cardiomyopathy; LV/RV = left/right ventricle; cyc^- = assessment of $G_{s\alpha}$ activity by reconstitution in cyc^- membranes. PTX/CTX = pertussis or cholera, respectively, toxin-catalyzed ADP-ribosylation; RIA = radioimmunoassay; PCR = polymerase chain reaction. Modified from Eschenhagen [63] with permission.

[151]. On the other hand, the majority of studies have found cardiac expression of α-subunits of G_i to be increased (Table 1). This has been initially demonstrated by pertussis-toxin-catalyzed ADP-ribosylation [65,66,151–154] which measures all forms of G_i as well as of G_0. However, the use of quantitative Western and Northern blotting to differentially assess expression of G_i isoforms at the protein and mRNA levels, respectively, has yielded controversial results with regard to the three $G_{i\alpha}$ isoforms in end-stage dilated and ischemic cardiomyopathy. Thus, Feldman et al. [153,155,156] found $G_{i\alpha\text{-}3}$ to be the predominant form in human heart which was unchanged in heart failure at the protein and mRNA level as determined by quantitative Western and Northern blotting; functionally decreased Gpp(NH)p- but not NaF-induced adenylyl cyclase activation, however, indicated some form of G-protein alteration. On the other hand, two other groups showed $G_{i\alpha-2}$ to be the predominant form in human heart and this was found to be increased at both protein and mRNA levels [157,158]. Thus, it appears at present that cardiac PTX-substrates are increased in human heart failure, which may be related to a selectively enhanced expression of $G_{i\alpha-2}$.

Increased G_i expression could mitigate cyclic AMP formation, and thus contribute to the diminished response of β_2- or β_1-adrenoceptors in chronic heart failure. Three lines of evidence favor this hypothesis. First, in ventricular membranes obtained from end-stage heart failure, activation of adenylyl cyclase by GTP or its non-hydrolyzable analogue Gpp(NH)p (involving G_s and G_i) and forskolin is diminished, while that induced by NaF (involving only G_s) and Mn^{2+} (activating directly the catalytic unit of the adenylyl cyclase) are unchanged [for references, see Ref. 12]. Second, Feldman et al. [151] showed that pretreatment of cardiac membranes from severely failing hearts with PTX restores the previously decreased adenylyl cyclase response to isoprenaline, and third Brown and Harding (159) have recently shown, in isolated human cardiomyocytes, that pretreatment of PTX restored the previously reduced maximal inotropic response to isoprenaline. Moreover, PTX pretreatment also enhanced cyclic AMP responses in lymphocytes from heart failure patients but not in those from control subjects [160].

The mechanisms underlying the increase in G_i are not fully understood, but it has been speculated that it is due to the increased activity of the sympathetic nervous system and hence increased noradrenaline levels. This hypothesis is based on findings that chronic exposure of noradrenaline increases G_i in guinea-pig cardiomyocytes [159] and in rat neonatal cardiomyocytes [161] and chronic treatment of rats with isoprenaline increased myocardial G_i protein [162] and $G_{i\alpha\text{-}2}$ and $G_{i\alpha\text{-}3}$ mRNA [163,164]. Thus, it might well be that noradrenaline via increasing cyclic AMP and activating a cyclic AMP-response element in a G_i-gene might increase expression in chronic heart failure. Since the gene for $G_{i\alpha\text{-}2}$ contains a possible consensus sequence of cyclic AMP-response element [165,166] while the $G_{s\alpha}$-gene does not contain a cyclic AMP response element [167], this would also explain why in chronic heart failure G_i, but not G_s, increases. However, similarly to β-adrenoceptor regulation in the human heart (see above) some open questions still exist for G_i-regulation in chronic heart failure. If the cyclic AMP/cyclic AMP response element hypothesis is correct, one could expect that following chronic activation of β-adrenoceptors G_i increases in all cell types. We have recently tested this hypothesis in three different settings. First, we assessed β-adrenoceptor number and G_i-expression in myometria from pregnant women undergoing long-term β_2-adrenergic therapy to prevent pre-term labor. This treatment led to a marked decrease in β_2-adrenoceptor number but G_i-levels were unchanged, independently of whether assessed by PTX or quantitative Western blotting [168]. Secondly, we treated healthy volunteers for 2 weeks with the β_2-adrenergic agonist terbutaline and assessed β_2-adrenoceptor number and Gi_i-levels in circulating lymphocytes. Again, treatment caused a significant decrease in β_2-adrenoceptor number while G_i—assessed by PTX—was unchanged [169]. Thirdly, we have treated the human neuroblastoma cell line SK-N-MC containing a homogeneous population of β_1-adrenoceptors for 24 hours with 10 μM isoprenaline; under these conditions β_1-adrenoceptors were down-regulated and the adenylyl cyclase response to isoprenaline was markedly desensitized, but again G_i was not changed [170]. Extending the incubation to 4 days also did not lead to any changes in G_i [Michel and Brodde, unpublished observations]. Thus, it appears that chronic activation of β-adrenoceptors is not the sole explanation for the increase in G_i in chronic heart failure, or at least that the underlying mechanism is not operative in all cell types and that possibly other factors may be involved as well. In this context it is interesting to note that in isolated rat neonatal cardiomyocyte tumor necrosis factor (TNFα) can increase G_i [171]—and circulating levels of TNFα have recently been found to be markedly elevated in patients with severe chronic heart failure [172]. However, it might also be possible that the increases in human cardiac G_i observed in chronic heart failure are more an organ (cardiac)-specific than a general phenomenon, as the pronounced β_1-adrenoceptor down-regulation in chronic human heart failure is also somewhat atypical.

Relatively little is known about changes in cardiac adenylyl cyclase in chronic human heart failure. In the majority of studies Mn^{2+} has been used to assess the activity of the catalytic unit of the enzyme; nearly all studies have shown unchanged activity in different forms of heart failure [for review, see Ref. 12], with the exception of a decreased activity of the catalytic unit in right ventricular preparations from hearts subjected to pressure overload [173]. However, at least eight isoforms of membrane-bound adenylyl cyclase have been cloned [174,175]; among these the heart, including human heart, seems to contain mainly type V and VI adenylyl cyclase, i.e. those isoforms that are inhibited by G_i and submicromolar Ca^{2+} concentrations in a calmodulin-independent manner [176]. It has been recently shown that in dogs rapid-pacing-induced heart failure caused a reduction in steady-state mRNA-levels of type V and VI adenylyl cyclase [177]; moreover, in rats, steady-state mRNA levels of type V and VI adenylyl cyclase showed age-dependent changes but in an opposite direction: while type V increased with age, type VI decreased with age and this decrease paralled the

decrease in adenylyl cyclase activity [178]. Thus, the possibility might be considered that cardiac adenylyl cyclase activity is—at least partly—determined by the ratio of the isoforms of the enzyme. However, at present nothing is known on possible adenylyl cyclase isoform switches in the failing human heart.

Only a few data exist on changes of the other G_s-coupled cardiac receptors in chronic human heart failure. VIP-receptor number and the contractile response of left ventricles of severely failing hearts were found to be markedly reduced [54] while interestingly affinity of the receptors to VIP was enhanced. Nothing is known on changes of cardiac 5-HT_4-receptors in chronic heart failure. Controversial data have been reported on H_2-receptor changes in chronic heart failure. One study described unchanged receptor number in left ventricular membranes from patients with mild to moderate heart failure because of mitral and aortic valve disease [49]; in this study the positive inotropic effect of the H_2-receptor agonist impromidine was unchanged while that of isoprenaline was markedly reduced. Similarly, Bristow et al. [48] found an unchanged histamine-induced positive inotropic effect in left ventricles from patients with severe heart failure compared with the effects on ventricles from non-failing hearts. On the other hand, Näbauer et al. [56] found the positive inotropic effect of histamine markedly reduced in severely failing hearts. However, all receptor systems involving the G_s-adenylyl cyclase pathway (and hence cyclic AMP as one of the most important second messengers) in the human heart appear to have a low spare receptor capacity for positive inotropic effects (see above), and therefore any decrease in receptor number and/or any impairment in coupling receptor to the adenylyl cyclase/cyclic AMP system should reduce their positive inotropic effects. As discussed above, in chronic heart failure the functional activity of G_i is increased, which leads to inhibition of cyclic AMP formation. Therefore, it might be expected that all cyclic AMP-dependent positive inotropic responses are reduced under these conditions, as this has been clearly shown for β-adrenergic agonists and cyclic AMP-dependent phosphodiesterase inhibitors [179; for further references, see Ref. 12]. Certainly additional experiments are needed to clarify this point.

In this context it is interesting to note that the ageing human heart shows some similarities to the failing human heart: it is well known that the ageing human heart has reduced responses to β-adrenergic stimulation [for references, see Refs. 180 and 181]. Two groups have recently studied the mechanisms underlying this effect in more detail. We determined in right atria from 52 patients undergoing open-heart surgery without apparent heart failure of different ages (7 days–83 years) β-adrenoceptor number and subtype distribution, G_s- and G_i-proteins and adenylyl cyclase activity [182]. We found significant negative correlations between GTP-, isoprenaline-, histamine-, 5-HT-, forskolin-, NaF- and Mn^{2+}-activated adenylyl cyclase and the age of the patients. In addition, $G_{i\alpha}$ increased with age; β-adrenoceptor number and subtype distribution, however, were unchanged. These results indicate that in the human right atrium the reduction in β-adrenergic responsiveness with age might involve a reduction in the activity of the catalytic unit of the adenylyl cyclase, which leads to impairment of cyclic AMP formation. An increase in G_i might enhance that effect. White et al. [183] studied the β-adrenoceptor-G-protein-adenylyl cyclase system in ventricular myocardium obtained from potential organ donors whose heart could not be used for transplantation for several reasons. They found—similarly to the right atrium—an age-dependent decline in adenylyl cyclase activity in response to isoprenaline, Gpp(NH)p, NaF and forskolin but not to Mn^{2+}. In addition, in contrast to the right atrium, in aged ventricular myocardium $G_{s\alpha}$ was decreased, whereas $G_{i\alpha}$ was unchanged; β-adrenoceptor number decreased with age and this was due to a selective decrease in β_1-adrenoceptors. Although the reason for these (chamber-specific?) differences between aged right atria and aged ventricular myocardium is not known at present, these data clearly indicate that in both settings—chronic heart failure and advanced age—β-adrenoceptor-mediated effects and all other cyclic-AMP-dependent effects are depressed.

3.2. G_i-coupled receptors

Much less is known at present about G_i-coupled cardiac receptors and cardiac receptor signalling through the phospholipase C pathway in chronic heart failure. The number of adenosine A_1- [65] and muscarinic M_2-receptors [65,67,184] is unchanged like the negative inotropic effect of adenosine and carbachol which surprisingly is not changed in patients with severe heart failure [65–67] despite the increase in G_i (see above). These findings indicate (a) that either the G_i-pathway might be not the most important signalling pathway for these receptors or (b) considering the receptor/G-protein ratio of 1 : 10 to 1 : 100 [185] that the small increases in G_i seen in chronic heart failure (about 30–50%) may not significantly contribute to the extent of the functional responses.

3.3. Receptor systems signalling independent of the cyclic AMP system

The human cardiac α_1-adrenoceptor is the most intensively studied among the phospholipase-C-coupled receptors in chronic heart failure. There appears to be general agreement that the number of α_1-adrenoceptors (although very low in the human heart) is increased in patients with chronic heart failure [92,116,186]; the mechanism underlying this phenomenon is not completely understood but may be due to the fact that it has been shown in a cell line that chronic β-adrenergic stimulation increases the amount of α_1-adrenoceptor mRNA [187]. On the other hand, in rats chronically treated with the β-adrenoceptor antagonist propranolol, α_1-adrenoceptors also increase [188,189], which might argue against the cross-regulation hypothesis. Despite the increased number, α_1-adrenoceptor-mediated inositol phosphate accumulation is unchanged in the failing human heart [85,116]. Controversial data exist on the positive inotropic effect induced by α_1-adrenoceptor activation. While Böhm et al. [86] found it to be unchanged in left ventricular preparations from patients with severe

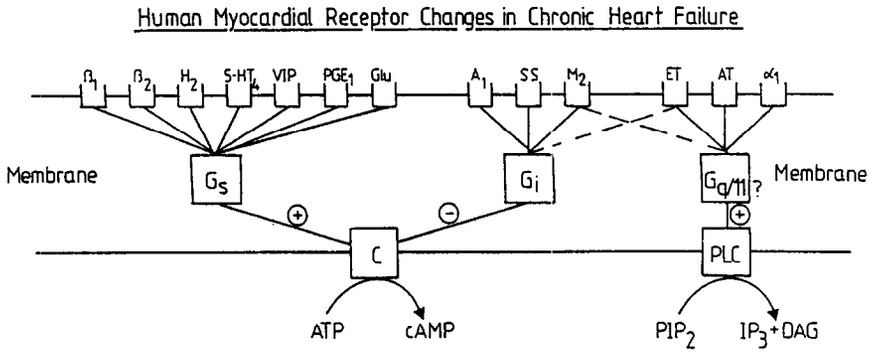
chronic heart failure, Steinfath et al. [92] demonstrated in this preparation a marked decreased response to phenylephrine and noradrenaline; these in vitro data have been recently confirmed in an in vivo study by Landzberg et al. [83] who demonstrated that the positive inotropic effect of phenylephrine was markedly reduced in patients with severe heart failure when compared with healthy controls following intracoronary injections. Thus, taken together, the increased number, the unchanged inositol phosphate response and the (presumably) decreased inotropic response indicate that cardiac α_1-adrenoceptors in chronic heart failure are uncoupled from the response.

Similar to α_1-adrenoceptors, M_2-receptor [116] and ET_A-receptor [Brodde, unpublished observations] induced inositol phosphate accumulation seems not to be different in failing and non-failing human hearts. Preliminary data suggest that the endothelin-induced positive inotropic effect in left ventricular preparations of severely failing human hearts is decreased [107,190]. However, this remains to be confirmed in other studies.

Finally, controversial data have been published on changes in AT-receptor number in the failing human heart. Urata al. [191] described AT-receptors to be unchanged while Regitz-Zagrosek et al. [102] recently found in atria and ventricular myocardium obtained from explanted hearts of patients with end-stage heart failure markedly decreased AT-receptors assessed on both a protein and a mRNA-level. Whether positive inotropic responses to angiotensin II in heart failure are changed is not known at present.

4. Animal models of chronic heart failure

As discussed above, a general feature of human heart failure seems to be a decrease in cardiac β-adrenoceptors accompanied by increases in the functional activity of cardiac G_i resulting in a marked attenuation of adenylyl cyclase activation. To investigate the mechanisms underlying these alterations in more detail, numerous studies have been performed in animal models of heart failure, but the



β_1	Number decreased; functional response decreased
β_2	Number unchanged (?) or decreased (?); functional response decreased
H_2	Number unchanged (?); functional response unchanged (?) or decreased (?)
$5\text{-}HT_4$	???
VIP	Number decreased; functional response decreased
PGE_1	???
Glu	???
α_1	Number increased; functional response decreased (?) or unchanged (?)
ET	Number ???; functional response decreased (?)
AT	Number unchanged (?); functional response ???
M_2	Number unchanged; functional response unchanged
A_1	Number unchanged; functional response unchanged
SS	???
G_s	Amount unchanged; functional activity unchanged
G_i	Amount increased (?) or unchanged (?) functional activity increased
$G_{q/11}$	???
C	functional activity unchanged
PLC	???
PKA	functional activity unchanged (?)
PKC	???

Fig. 2. Changes in human cardiac receptor systems and their signal-transduction mechanisms in chronic heart failure. For details, see text. For explanation of abbreviations, see legend to Fig. 1. PKA = protein kinase A; PKC = protein kinase C.

results were quite different from those obtained in human heart failure. Thus, a decreased β-adrenoceptor density (mainly β_1-adrenoceptors) has been found in dogs with right heart failure produced by progressive pulmonary artery constriction and tricuspid avulsion [192], in dogs [193,194] and pigs [195,196] with congestive heart failure due to rapid pacing, in pigs with chronic volume-overload hypertrophy and circulatory congestion [197], and in rabbits with chronic heart failure due to pressure and volume overload [198]. On the other hand, β-adrenoceptor density was found to be unchanged in rats [199] and dogs [200] with heart failure due to myocardial infarction as well as in the genetically-linked model of cardiomyopathy, the Syrian hamster [201,202]; and finally, increased β-adrenoceptor density was found in guinea-pigs [203] and dogs [204,205] with heart failure due to pressure overload. However, in all these models adenylyl cyclase activation by GTP, isoprenaline and forskolin was attenuated, indicating that disturbances in coupling β-adrenoceptors to the adenylyl cyclase also occur in animal models of heart failure.

Several studies have addressed the question of whether cardiac G-proteins might be changed in animal models of heart failure. Again, the data obtained were quite different from those obtained in human heart failure. Thus, while it is generally agreed that $G_{s\alpha}$ is not changed in human heart failure, in the animal models of heart failure in the majority of studies a decrease in either the amount (assessed by CTX-catalyzed ADP-ribosylation or Western blotting) or function (assessed by the cyc^- reconstitution assay) or mRNA-levels for $G_{s\alpha}$ was found in the pressure overload model of the dog [206], in the volume-overload model of the pig [197], in the rapid pacing model of the pig [195,196] and in the genetic model of cardiomyopathy, the Syrian hamster [201,207], although Sehti et al. [202] recently found increased amounts of $G_{s\alpha}$ in this model. Furthermore, in contrast to human heart failure $G_{i\alpha}$ (assessed by PTX-catalyzed ADP-ribosylation or Western-blotting) and mRNA levels for $G_{i\alpha}$ were in general not found to be increased (with the exception of a recent study by Sehti et al. [202] in the Syrian hamster) but were either unchanged (in the pressure overload model of the dog [208], in the volume-overload model of the pig [197], and in the Syrian hamster [201]) or decreased (in the rapid pacing model of the pig [195,196] and in the Syrian hamster [207]) in the animal models of heart failure. Taken together, these results show that at present it is quite difficult to extrapolate from results obtained in animal models of chronic heart failure to human heart failure.

5. Conclusion

In the human heart many receptor systems exist that regulate contractility and heart rate. Among these the β-adrenoceptor–G_s-protein–adenylyl cyclase system is the most powerful physiologic mechanism to acutely augment cardiac contractility (cf. Fig. 1). In chronic heart failure this pathway exhibits two marked alterations: (a) a decrease in β_1-adrenoceptor number and (b) an increase in the functional activity of G_i. Both will lead to reduced physiologic responses of the failing human heart to β-adrenergic stimulation. In addition, the increase in G_i—which reduces cyclic AMP formation—might also cause diminished physiologic responses of the failing human heart to activation of all receptor systems signalling through the G_s-adenylyl cyclase/cyclic AMP pathway (cf. Fig. 2). Interestingly, the functional activity of receptor systems signalling via G_i is unchanged in chronic heart failure despite the increase in G_i (cf., Fig. 2). On the other hand, very little information is available on changes in receptor systems signalling through the PLC/IP_3/DAG pathway in the human heart although some preliminary data might suggest that these receptor systems are also desensitized from their functional response.

Thus, although during the last decade much has been learned about alterations in cardiac signal transduction mechanisms in chronic heart failure, many open questions remain, for example: (i) Why is it predominantly the β_1-adrenoceptor that is down-regulated in chronic heart failure? (ii) What is the mechanism underlying the increase in (the functional activity of) G_i? (iii) What is the physiologic role of PLC/IP_3/DAG-coupled receptors in the human heart and how are the components of this system altered in chronic heart failure? (iv) Since cross-talk between different signalling pathways has been demonstrated in many cell culture systems [209], how does cross-talk between the different signalling pathways in the human heart (cf. Figs. 1 and 2) contribute to alterations in signal-transduction mechanisms observed in chronic human heart failure? Cross-talk between β_1-adrenergic, β_2-adrenergic and M_2-muscarinic receptors may in fact exist in the human heart: in patients with coronary artery disease chronic treatment with selective β_1-adrenoceptor antagonists such as metoprolol, bisoprolol and atenolol caused selective up-regulation of right atrial β_1-adrenoceptor number and down-regulation of muscarinic M_2-receptor number; right atrial β_2-adrenoceptor number was unaltered [210,211]. On the other hand, inotropic responses to noradrenaline (acting solely via β_1-adrenoceptors, see above) were unchanged while β_2-adrenoceptor-mediated positive inotropic effects of procaterol and salbutamol were markedly enhanced [211,212]; similarly, in vivo in β_1-adrenoceptor-antagonist-treated patients the positive chronotropic effect of salbutamol was significantly increased [213].

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