Regulation of β-Adrenoceptor Signaling in Cardiac Function and Disease

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I. Introduction

In the past two decades, a plethora of information has accumulated on β-adrenoceptor (AR)[2] functional expression in both normal cardiac physiology and disease. The paradigm of this expression is that catecholamine binding to the β-AR on the cell surface initiates a cascade of intracellular biochemical and molecular responses,

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[2] Abbreviations: AR, adrenoceptor; ANP, atrial natriuretic peptide; AC, adenylyl cyclase; ACE, angiotensin-converting enzyme; AT_1, type 1 angiotensin II receptor; βARK1, β-adrenergic receptor kinase 1; Erk, extracellular response kinase; GC, guanylyl cyclase; Gpp(NH)p, guanosine-5′-(β,γ-imido)triphosphate; GRK, G protein-coupled receptor kinase; GPCR, guanine nucleotide-binding protein-coupled receptor; HEK, human embryonic kidney; I_{Ca}, Ca^{2+} channel current; IP_3, inositol triphosphate; JNK,

c-Jun amino-terminal kinase; MAP, mitogen-activated protein; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/Erk kinase; MEKK, mitogen-activated protein kinase/Erk kinase kinase; NaF, sodium fluoride; PDE, phosphodiesterase; PI_3, phosphoinositide; PMA, phorbol 12-myristate 13-acetate; PK, second messenger-activated protein kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKG, protein kinase G; PLA_2, phospholipase A_2; PLC, phospholipase C; PP, protein phosphatase; PTX, pertussis toxin; TD, transmembrane domain.

leading ultimately to the stimulation of a number of cellular activities. At the onset, the activation of the β-ARs directly triggers interactions of guanine nucleotide-binding proteins (G proteins) with adenylyl cyclases (ACs) to synthesize the second messenger cAMP from ATP (Gilman, 1987, 1990). The cascade of events progresses as cAMP in turn activates certain serine (Ser)/threonine (Thr) protein kinases (PKs), particularly protein kinase A (PKA), resulting in either an enhancement or inhibition of certain cellular functions. The ensuing responses are mediated by alterations in gene expression induced by the phosphorylation of specific transcription factors (Gilman, 1990; Hadcock and Malbon, 1991). The β-AR-positive inotropism involves cAMP-mediated increases in intracellular Ca^{2+} concentration, resulting in part from the phosphorylation of L-type Ca^{2+} channels by PKA (Sperelakis et al., 1994; Hove-Madsen et al., 1996). This receptor system probably is the most extensively studied and best understood signaling pathway to date, yet it continues to attract great research interest, particularly with regard to its mode of signaling in cardiac disease. This is attributable mainly to the fact that in humans, although there are several receptor signaling systems capable of exerting positive inotropic effects via various mechanisms, the β-AR pathway remains the most powerful tool by which heart rate and contractility are physiologically regulated and maintained (Brodde et al., 1992). On the other hand, the human heart possesses only a few spare receptors for β-AR-mediated positive inotropy, requiring the mobilization of virtually all available cardiac β-ARs to produce maximal inotropic effects at all times (Brodde et al., 1992). This phenomenon becomes particularly important in heart failure, where $β_1$-ARs are desensitized, often accompanied by the uncoupling of the $β_2$-AR subtypes (Bristow et al., 1986; Brodde, 1991). This scenario implies, among others, that any alteration in the cardiac contractile machinery capable of triggering down-regulation of β-AR will automatically lead to a malfunction of this signaling pathway and is likely to prove detrimental to the cardiac circulatory function. Naturally, by virtue of its pivotal role to sustain life, the heart should have at its disposal the ability to regulate its receptor signaling pathways in a fashion that ensures continuity of this vital function, if and when the β-AR system should fail. Several receptor systems, including the $α_1$-ARs, probably are capable of providing a compensatory mechanism against loss of the $β_1$-AR-positive inotropism in heart failure. However, although this notion has been entertained for almost a decade, no compelling evidence has come to surface to substantiate it. A further point of interest is the likelihood that the prevalence of certain genotypes and inherent genetic defects in some signaling components of the β-AR pathway are potential markers for cardiac disease. This notion only adds a twist to the already complex and challenging task of defining the actual extent to which β-AR signaling is

involved in cardiac function in both the healthy and disease states. Even more exciting are data suggestive of a cross-talk at various signaling levels among the cardiac receptors, such as the renin-angiotensin-aldosterone (RAS) and atrial natriuretic peptide (ANP) systems, that contribute to cardiac circulatory function as well as receptors for growth factor systems. Such a cross-talk probably constitutes an integral regulatory network of the cardiac circulatory signaling complex, the essence of which is likely to be more eminent in cardiac disease than in normal physiological signaling. Our knowledge on this subject promises to increase exponentially in the foreseeable future. Indeed, the functional expression of the cardiac β-AR pathway will soon be inconceivable without intimate linkage with other signaling systems contributing to cardiac circulatory function. This review thrives to integrate the current concepts on β-AR signaling with the view of forging a basis to recognize this system primarily as an integral component of the complex machinery regulating cardiac circulatory function rather than an isolated regulator of the cardiac contractile apparatus.

II. The Cardiac β-Adrenoceptor Signaling Pathway

The β-ARs form part of a large superfamily of G protein-coupled, heptahelical, membrane localized receptors for drugs, neurotransmitters, and hormones. These G protein-coupled receptors (GPCRs) activate a small but diverse subset of effectors, including ACs, phospholipases, and various ion channels (reviewed in Karoor et al., 1996; Gudermann et al., 1997). Their expression is highly regulated and controlled largely by the activation or repression of genes encoding receptors, balanced by post-transcriptional mechanisms, such as the destabilization of receptor mRNA (Hadcock and Malbon, 1988). The β-AR family transduces catecholamine signals by coupling to the large G proteins composed of $G_{αβγ}$ heterotrimers.[3] In the basal state, the heterotrimeric G proteins have GDP bound to the catalytic site of GTPase on their $G_α$ subunit. After interactions with the receptor, their activation requires association of GTP to the $G_{αβγ}$ in exchange for GDP, leading to the dissociation of the complex into GTP $G_α$ and $βγ$ subunits. The dissociated

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[3] The $G_α$ subunits have been classified into four major groups based on their amino acid sequence similarities: 1) the ubiquitous stimulatory G subunit ($G_{sα}$), which was first recognized by its ability to activate AC, and the subunit from olfactory epithelium (G_{olf}); 2) the inhibitory G subunit ($G_{iα}$), which includes the subtypes $α_{i1}$, $α_{i2}$, and $α_{i3}$, so called because of the ability to inhibit AC; the neural subunit $G_{oα}$; and the two retinal subunits $α_{r1}$ and $α_{r2}$, 3) the $G_{αq}$ consisting of $α_q$, $α_{11}$, $α_{14}$, $α_{15}$, and $α_{16}$, which activate PLC, and 4) the $G_{α12}$ composed of the $α_{12}$ and $α_{13}$ whose main function is to regulate Na^+/K^+ exchange. Most subunits are ubiquitous. The table lists only the organs in which they are highly expressed. The following reviews are recommended for further reading on the subject: Kaziro et al., 1991; Simon et al., 1991; Clapham and Neer, 1997; Kalkbrenner et al., 1997.

G_α and $G_{\beta\gamma}$ subunits then either positively or negatively regulate a variety of effector systems, resulting in changes in intracellular second messenger level and/or conductance (Northup et al., 1983; Birnbaumer, 1992). Hydrolysis of GTP to GDP by the complex results in the reassociation of the G_α with the $\beta\gamma$ subunits to commence the next activation cycle (Helper and Gilman, 1992). The affinity of the GDP- and GTP-binding sites of the G_α subunit varies with the occupation of the receptor, and both the G_α and $\beta\gamma$ complexes are known to determine the G protein specificity. The classic route leading to stimulation of the contractile function results from the activation of AC catalytic activity by the stimulatory G protein (G_s), leading to increased intracellular cAMP levels (Birnbaumer, 1992). This in turn stimulates PKA, which then mediates phosphorylation of target proteins such as the cardiac Ca^{2+} channels leading to various metabolic and physiological responses in the cells. Apart from this classic pathway, some evidence suggests that the heart may use other pathways in regulating its Ca^{2+} metabolism and therefore its contractile function by coupling β-AR to G_q or G_i-G_o (Xiao and Lakatta, 1993; Zhou et al., 1997).

A. Structure, Localization, and Function of β-Adrenoceptors

At least three human genes that express the individual β-AR subtypes β_1-, β_2-, and β_3-AR have been identified so far, and unequivocal evidence for the existence of a putative fourth β_4-AR awaits its cloning and sequencing. The three known genes are characterized by an extracellular glycosylated amino (N) terminus, an intracellular carboxyl (C)-terminal region, and seven transmembrane domains (TDs) linked by three extracellular and three intracellular loops. The β_1- and β_2-ARs show 48.9% homology, whereas β_3 exhibits 50.7 and 45.5% homology with the other two receptors, respectively (Emorine et al., 1989). Several amino acids are conserved in all three proteins (Dohlman et al., 1987). Valuable information on the β-AR functional domains, and indeed those of GPCRs in general, has been derived mainly from studies using the prototypic β_2-AR involving mutant receptors in which certain amino acids or regions were deleted or substituted, as well as synthetic chimeric receptors subtypes. Neither of the two receptor termini nor the hydrophilic loops are essential for ligand binding (Dixon et al., 1987a,b); rather, the catecholamine binding domain is a pocket lined by residues belonging to the hydrophobic TDs, which are also essential for protein folding involved in receptor-ligand interactions (Tota et al., 1991). The amino acid residues essential for agonist binding are different from those that are important in their interactions with antagonist (Dohlman et al., 1987; Tota et al., 1991). In particular, the Ser^{204} is presumably responsible for the receptor interactions with the parahydroxyl groups of the catecholamine ligands (Tota et al., 1991) and may be rele-

vant for agonist high-affinity binding. Furthermore, the Asn^{293} has been associated with both stereospecificity and intrinsic activity of agonists in their interactions with the receptor (Wieland et al., 1996). For antagonists, on the other hand, the regions VI and VII seem to be essential in determining their specificity (Frielle et al., 1988; Kobilka et al., 1988). This differentiation in residues responsible for agonist and antagonist binding may provide a basis for delineating the specificity of ligand binding to β-AR (Kikkawa et al., 1997, 1998). The region responsible for receptor interactions with the G_s protein is composed of residues belonging to parts most proximal to the membrane side of the third intracellular hydrophobic loop and C-terminal region (Strader et al., 1987; O'Dowd et al., 1988; Cheung et al., 1989; Strosberg, 1995). The first loop may be important for receptor expression, and Asp^{130} in loop 2 is probably involved in the $G_{s\alpha}$ coupling.

All four β-AR subtypes are integral membrane proteins present in the human heart (Bylund et al., 1998). The β_1-AR is a protein of 477 amino acids found on chromosome 10 (Frielle et al., 1987), and it is distributed in all parts of the heart (Brodde, 1991). Stimulation of the cardiac β_1-AR leads to an increase in automaticity, conduction velocity (chronotropy), excitability, and contraction force (inotropy) of the cardiac muscle (Kaumann et al., 1989; Bristow et al., 1990). In the nonfailing myocardium, the β_1-AR population mediates the majority of the tensile responses to nonselective agonists (Brodde, 1991). The β_2-AR subtype consists of 413 amino acids and is located on chromosome 5 (Kobilka et al., 1987). It is concentrated mainly in the ventricles and atria, where it is similarly coupled to the myocardial contractile system (Bristow et al., 1986). In these tissues, the human β_2-ARs are also functionally linked to the cardiac positive inotropic responses to agonists (Summers et al., 1989). In both atria and ventricles, the β_1- and β_2-subtypes exist in a ratio of approximately 2:1. High proportions of the β_2-AR are apparently also found in the pacemaker and conducting regions, where they may be important in controlling heart rate and rhythm. The β_3-AR, on the other hand, is found mainly in the coronary vascular bed (Strosberg, 1995). It is 402 amino acids long and is located on chromosome 8 (Emorine et al., 1989). Although the cardiac β_3-AR is capable of exerting positive inotropic effects in isolated atria (Emorine et al., 1994), its actual contribution to the cardiac contractile function has yet to be defined more precisely. Recent studies have shown that the pharmacology of the human β_3-AR differs significantly from that of the other two human subtypes as well as the β_3-AR found in other species (Kaumann and Molenaar, 1996). The most striking difference is its recognition as agonists, several compounds acting as potent β_1-AR and β_2-AR antagonists, and its down-regulation by derivatives of various compounds, such as dexamethason butyrate or insulin, which up-regulate the other two subtypes (Silence et al.,

1993). Hence, the existence of β_3-AR in the human heart initially led to the speculation that it may be responsible for the unexpected negative inotropic effects of catecholamines and that it may be involved in the pathophysiological mechanisms leading to heart failure (Gauthier et al., 1996). Although the notion of a putative fourth β_4-AR is about 20 years old, information on its pharmacological properties is just beginning to emerge. The receptor has been shown to mediate positive inotropic and chronotropic effects in mammalian hearts (Kaumann and Molenaar, 1997; Molenaar et al., 1997). In humans and a variety of other species, this receptor apparently mediates cardiostimulant effects of nonconventional partial agonists (i.e., high-affinity β_1- and β_2-AR blockers that cause agonist effects at considerably higher concentrations than those that block the receptors; Sarsero et al., 1998). The receptor is expressed in several heart regions, including the sinoatrial node, atrium, and ventricle. It has also been reported to mediate increases in Ca^{2+} transients in ventricular myocytes and to induce arrhythmias, probably via a mechanism that is different from that of β_1-AR (Kaumann and Molenaar, 1997, Lowe et al., 1998). All four β-ARs are involved in the regulation of energy expenditure and lipolysis (Lafontan et al., 1997). The β_4-AR mediates lipolysis like the β_3-AR by interacting with the nonconventional agonists. However, it is not activated by selective β_3-AR agonists (Kaumann and Molenaar, 1996) and maintains its function in the hearts of β_3-AR knockout mice (Kaumann et al., 1998).

All four β-AR subtypes are coupled to their effector systems by G proteins (Bylund et al., 1998). The human β_1-, β_2-, and β_4-ARs are coupled mainly to the stimulatory G_s-AC system, whereas the ventricular β_3-AR is coupled to G_i (Kaumann and Molenaar, 1997). Interestingly, although most of the β_2-AR actions are mediated by the G_s protein coupling via the cAMP-dependent PKA system, at least three other signal transduction cascades have been described by which it can mediate various agonist effects. In 1993, Xiao and Lakatta described a β_2-AR-stimulated cAMP production that was dissociated from the regulation of myofilament and sarcoplasmic reticulum functions. Because β_2-AR modulation of cardiac excitation-contraction coupling requires cAMP, a functional compartmentalization of cAMP signaling was postulated that is due to the activation of β_2-AR coupled to G_i and/or G_o to explain this phenomenon (Zhou et al., 1997). There also is compelling evidence indicating that the PKA-mediated heterologous desensitization of the β_2-AR pathway may actually serve an independent physiological signaling route rather than simply an escape circuit for the receptor from unabated stimulation by agonists. This is based on a recent finding by Daaka et al. (1997a) describing a switching of the β_2-AR coupling, in which the phosphorylated and internalized β_2-ARs tend to lose affinity for G_s and gain affinity for G_i. Besides, there is some evidence to suggest that certain

physiological functions of the β_2-AR do not depend entirely on G protein activation but may be a result of protein-protein interactions mediated by conserved protein modules known as PDZ domains (derived from the three proteins PSD-95, Dlg, and ZO-1). These are protein-recognition modules of approximately 90-residue repeats found in a number of proteins implicated in ion-channel and receptor clustering, and the linking of receptors to effector enzymes and are specific for the postsynaptic scaffolding protein of 95 kDa (PSD-95), *Drosophila* discs-large tumor-suppressor gene (Dlg), and zonula occludens protein (zo)-1. Hall et al. (1998) reported that β_2-AR controls the Na^+/H^+ exchange via its direct agonist-promoted association with the Na^+/H^+ exchanger regulatory factor (NHERF) type 3 as a result of the NHERF binding to the last C-terminal cytoplasmic residues of the receptor by means of PDZ domain-mediated interactions. These observations indicate that β-AR signaling is finely tuned by an interplay of several regulatory mechanisms, notably at the G protein coupling level, and that further diversity is likely to surface soon, which should enhance our understanding of the different modes by which their regulation contributes to cardiac function.

B. Guanine Nucleotide-Binding Proteins

The heterotrimeric G proteins are a family of proteins composed of α, β, and γ subunits of 45 to 52, 35 to 37, and 8 to 10 kDa, respectively, that play a key regulatory role as transducers of various signaling pathways in different cell types (Gilman, 1987). To date, at least 20 G_α, 5 G_β, and 11 G_γ subtypes of the G protein have been identified (Table 1). The G_α subunits differ significantly from one another, whereas the G_β and G_γ subunits do not vary remarkably among the G proteins, with the G_β subunits exhibiting higher sequence similarities than the G_γ group. Among the G_α proteins, the stimulatory $G_{s\alpha}$ and inhibitory $G_{i\alpha}$ are highly homologous, but they differ profoundly with respect to their effector, regulator, and receptor specificities. At least seven of the G_α proteins (four $G_{s\alpha}$ gene splice variants and one each of $G_{i\alpha1}$, $G_{i\alpha2}$, and $G_{i\alpha3}$) are involved in the regulation of the AC signaling mechanisms. Particularly relevant for cardiac β-AR signaling is the fact that $G_{s\alpha}$ activates all AC isoforms, whereas the $G_{i\alpha}$ proteins inhibit only certain isoforms, notably AC IV and V, depending on the nature of the enzyme activators (Tang and Gilman, 1991; Taussig et al., 1993). The $G_{s\alpha}$ is abundantly expressed in the myocardium, whereas among the $G_{i\alpha}$ subunits, $G_{i\alpha2}$ is predominant in the heart with a little of $G_{i\alpha3}$ (Luetje et al., 1988; Holmer et al., 1989). However, the relevance of not only their presence in the heart but also their diversity with regard to cardiac β-AR signaling under physiological conditions remains somewhat elusive. Apparently, β_1-AR and β_2-AR share at least one coupling domain within the $G_{s\alpha}$ for its activation (Novotny et al., 1996). The most significant role for the $G_{s\alpha}$ coupling has

TABLE 1

Heterotrimeric G protein subtypes and their coupling to some effector systems

Family	Subtype	Action on Effectors	Distribution	Effects
$G_{s\alpha}$	α_s	Stimulate all AC isoforms; synergism with forskolin (AC IV, AV, and VI); synergism with Ca^{2+}/CaM (AC I, III, and VIII)	Heart, brain, lung neurons, kidney liver, adrenal gland, cerebral cortex	Activate Ca^{2+} channels, cardiac positive inotropy Activate Ca^{2+} channels, contractile proteins
	α_{olf}	Stimulate AC V, VI, and III	olfactory epithelium	activate Ca^{2+} channels
$G_{i\alpha}$	α_{i1}	$G_{i\alpha1}$–$G_{i\alpha3}$ inhibit AC I, V,	Brain, olfactory	Regulate K^+, Ca^{2+} channels
	α_{i2}	AC VI (AC I modestly with	(α_{i1}–α_{i3}); heart, all	(α_{i1}, α_{i3}); inhibit cAMP
	α_{i3}	Ca^{2+}/CaM, poor with $G_{s\alpha}$);	tissues (α_{i1}–α_{i3}); liver;	synthesis (α_{i1}–α_{i3});
	α_o	inhibit AC I as above	olfactory, neurons	inhibit Ca/CaM actions
	$\alpha_{\tau1}$	Activate cGMP PDE	Retina rod	Phototransduction
	$\alpha_{\tau2}$	($\alpha_{\tau1}$, $\alpha_{\tau2}$)	Retina cone	($\alpha_{\tau1}$, $\alpha_{\tau2}$)
	α_z	Inhibit AC I and V	brain, neurons	inhibit cAMP synthesis
$G_{q\alpha}$	α_q	Activate PLC-β_1	Neurons, heart	Phototransduction
	α_{11}	Activate PLCs	ubiquitous (α_q, α_{11})	Mobilize Ca^{2+}, IP_3 formation
	α_{14}	(all $G_{q\alpha}$)	stroma, epithelium	Activate ion channels
	α_{15}	Activate PLC-β_1	hematopoietic cells	Mobilize Ca^{2+}
	α_{16}	Activate PLC-β_1, PLC-β_2	(α_{15}, α_{16})	Mobilize Ca^{2+}
$G_{\alpha12}$	α_{12}	Inhibit ACI	Ubiquitous	Regulate Na^+/K^+ exchange
	α_{13}	Activate AC	Brain	(α_{12}, α_{13})
$G_{\beta\gamma}$ complex		Inhibit AC I, stimulate AC II, IV, and VII (with $G_{s\alpha}$ activation), GRKs, PLC-β_1, PLC-β_2, PLC-β_3, PLA$_2$	Heart, brain retina, ubiquitous	Activate cardiac K^+ channel
$G_{\beta1}$–$G_{\beta5}$		Combinations with G_γ inhibit AC I and II	Heart (except β_4, β_6) ubiquitous	Additional specificity and selectivity versus $G_{\beta\gamma}$ complex?
$G_{\gamma1}$–$G_{\gamma11}$		Combinations with G_β inhibit AC I and II	Brain ($\gamma2$, $\gamma3$, $\gamma5$, $\gamma7$), heart ($\gamma5$, $\gamma7$) kidney ($\gamma5$), liver ($\gamma5$), lung ($\gamma5$, $\gamma7$)	Additional specificity, selectivity versus $G_{\beta\gamma}$ complex?

been assigned to the third loop, which, among others, accommodates the sites involved in the guanylyl cyclase (GC) coupling (Summers and MacMartin, 1993). Basic amino acids, such as His[269] and Lys[270] in this region and Pro[138] in the III/IV segment, are thought to be particularly important for the receptor/G protein coupling (Strader et al., 1987).

It was not until recently that the $G_{\beta\gamma}$ complex was recognized as a signal transducing molecule in its own right that directly regulates just as many different protein targets as the G_α. Apart from its differential regulation of the various AC isoforms (Tang and Gilman, 1991), the $\beta\gamma$ complex also directly stimulates several effector systems, including PLC, a cardiac potassium channel, a retinal PLA$_2$, and a specific receptor kinase (Clapham and Neer, 1997). Its synergistic enhancement of the GPCR-mediated activation of GRK2 has led to the proposition of a pleiotropic function in its regulation of GPCR signal transduction (Haga et al., 1994). Of the 12 G_γ subunits, at least 5 ($\gamma1$, $\gamma2$, $\gamma3$, $\gamma5$, and $\gamma7$) are expressed in the rat heart (Hansen et al., 1995), displaying a huge potential for diversity in the coupling of the G protein subunits to transduce cardiac GPCR signaling in particular.

C. Adenylyl Cyclases

In cardiac myocytes, the Ca^{2+} required for the activation of the contractile proteins is furnished by the Ca^{2+} current (I_{Ca}) emitted via the slow (L-type) Ca^{2+} channel (Sperelakis et al., 1994). The availability of these chan-

nels for voltage activation during excitation is regulated, at least in part, by cAMP through both direct and indirect pathways. An elevation in cAMP levels produces a very rapid increase in the number of channels available for activation, thereby augmenting also the probability of a Ca^{2+} channel opening and its mean opening time. ACs constitute the effector enzymes that catalyze the conversion of ATP to cAMP, a ubiquitous second messenger that mediates diverse cellular responses primarily by activating Ser/Thr PKs. At least 10 mammalian AC isoforms have been identified so far, all of which are membrane-bound enzymes, with molecular masses of 115 to 150 kDa exhibiting overall homology of roughly 60% and shared identity of 50 to 90% within the two cytoplasmic regions (Krupinski et al., 1989; Iyengar, 1993; Sunahara et al., 1996). The cytosolic regions represent the catalytic sites of the ACs, exhibiting approximately 50% similarity and 25% identity (Sunahara et al., 1997). The isoforms share several regulatory features, including activation by the $G_{s\alpha}$ and forskolin and inhibition by a class of adenosine analogs known as P-site inhibitors (Iyengar 1993). They appear to fall into three broad categories. The first group represents Ca^{2+}-calmodulin (CaM)-stimulated enzymes that are activated synergistically by $G_{s\alpha}$ and Ca^{2+}-CaM (types AC I, III, and VIII). The second group consists of isoforms that are activated synergistically by $G_{s\alpha}$ and $G_{\beta\gamma}$ (types AC II, IV, and VII), and the third is composed of the isoforms that are inhibited by $G_{i\alpha}$ and Ca^{2+} (types AC V and VI; Sunahara et al., 1996). The isoforms can also be

individually regulated by Ca^{2+}-CaM, Ca^{2+}, or phosphorylation processes (Yu et al., 1990; Xia and Storm, 1997). Incidentally, AC V and VI, which are inhibited by Ca^{2+}, are also distinctly expressed in excitable tissues, particularly in the heart and the brain. In cardiac myocytes, where AC V and VI are most abundant, inhibition of their catalytic activity results primarily from the activation of L-type Ca^{2+} channels (Iwami et al., 1995). The diversity in the way by which β-AR signaling regulates Ca^{2+} metabolism is already evident at the G protein coupling level. At least three pathways for enhancing cAMP synthesis by β-ARs have been delineated involving signaling coupling via the G_s, G_q, and G_i/G_o proteins, respectively. The G_s-coupled β-AR signal transduction directly enhances cAMP synthesis. The G_q does so via Ca^{2+} and protein kinase C (PKC), whereas the G_i and G_o do so via the βγ complex. On the other hand, the most obvious mechanism for the inhibition of cAMP synthesis in the heart probably is the direct interaction of $G_{i\alpha}$, notably $G_{i\alpha2}$, with AC after the activation of the G_α subunit and its dissociation from the βγ complex. Moreover, the different AC isoforms are phosphorylated by different types of PKCs in a fashion that is synergistic to that of forskolin or $G_{s\alpha}$ (Jacobowitz and Iyengar, 1994; Kawabe et al., 1994). AC V is a substrate for phosphorylation by PKC-α and PKC-ζ in vitro, whereas AC II and IV are phosphorylated in vivo in response to phorbol 12-myristate 13-acetate (PMA). This regulatory diversity allows each AC isoform to respond to intercellular and intracellular signals in a manner appropriate for the individual cellular and environmental requirements, which might be particularly important in receptor crosstalk in cardiac disease. This renders the ACs as important targets for direct or PKC-mediated modulatory effects of Ca^{2+} and therefore as essential nodes for integrating the crucial cAMP- and Ca^{2+}-regulated signaling systems (Sunahara et al., 1997). Similarly, members of the G_q protein family also should be capable of modulating the AC function indirectly via their effects on phospholipase C (PLC)-β and inositol-1,4,5-triphosphate (IP_3) synthesis by coupling to PKC. Furthermore, both Ca^{2+}-mediated inhibition of the cardiac AC V and VI and inhibition of their phosphorylation by PKA may provide a suitable negative feedback loop for the events leading to cAMP-mediated increases in intracellular Ca^{2+} (Iwami et al., 1995; Tang and Gilman, 1995).

D. Protein Kinases and Phosphatases

PKs are phosphotransferases that catalyze the transfer of the γ-phosphoryl group of ATP to an amino acid side chain in basic sequences in the presence of Mg^{2+} in their post-translational regulation of receptors and in directing the downstream trafficking of receptor-mediated signaling messages. The PKs involved in signal transduction are divided into two major classes: those that phosphorylate Ser/Thr and those that phosphorylate Tyr. PKC and PKA are the two important Ser/Thr

PKs involved in β-AR signal transduction mechanisms. PKA is a tetramer of two regulatory subunits, associated with catalytic subunits termed C_α, C_β, and C_γ exhibiting different substrate and inhibitor specificity, as well as requiring different cAMP levels for activation (Tasken et al., 1997). In cardiac myocytes, this enzyme is found both in the cytosol and in association with the intracellular membrane, a fact that may be important with respect to the postulated compartmentalization of cAMP-mediated signal transduction discussed below. PKCs, on the other hand, comprise a family of at least 12 isoforms of Ca^{2+}- and phospholipid-dependent kinases encoded by nine genes (Nishizuka, 1988; Asaoka et al., 1992). They constitute key enzymes for growth regulation as well as tissue and cell differentiation and are extensively involved in cross-talk among cardiac receptor signaling pathways. Like PKA, the PKCs have four conserved domains (C1–C4). They are classified into three groups based on their structure and cofactor regulation. The best characterized are the conventional PKCs (cPKCs), which consist of the α, two alternatively spliced β variants (βI, βII), and the γ isoenzymes. This group distinguishes itself from the others in that its C2 domain contains a putative binding site for Ca^{2+} that regulates its function. The second class are the novel PKCs (nPKCs) δ, ε, η(L), θ, and μ isoenzymes, which are structurally similar to the cPKCs, except that the C2 domain does not have the functional groups to mediate Ca^{2+} binding. The third class consists of the atypical PKCs (aPKCs) π, τ, and ζ isoenzymes, which contain only one Cys-rich motif in C1 and lack the key residues that maintain the C2 fold. The heart contains a large amount of Ca^{2+}-dependent isoforms α, βI, and βII but not γ or the Ca^{2+}-independent isoforms δ and ζ (Mochly-Rosen et al., 1990; Inoguchi et al., 1992). The relevance of this diversity and the Ca^{2+}-independent isoforms for the regulation of cardiac function is not yet fully understood. However, there is mounting evidence showing that both PKA and PKC act as hubs for sorting adrenoceptor signaling routes involving cross-talk with other cardiac signaling systems as well as tyrosine receptor kinase pathway to stimulate the mitogenesis (Fig. 1).

Protein phosphatases (PPs) are phosphotransferases that catalyze the transfer of phosphoryl groups from the phosphorylated side chain of an amino acid to water molecules, thereby controlling a variety of physiological events such as cell proliferation, cell cycle, and receptor recycling. Unlike PKs, the PPs belong to several protein and/or gene families. Three prominent types of phosphatase catalytic subunits have been identified, termed PP-1, PP-2A, and PP-2B based on their substrate preferences, mechanisms of activation, and sensitivity to inhibitor proteins or naturally occurring toxins (Faux and Scott, 1996). The regulation of PPs appears to involve protein-protein interactions controlled by phosphorylation and to be independent of second messengers (Girault, 1993; Hubbard and Cohen, 1993). Several Ser/
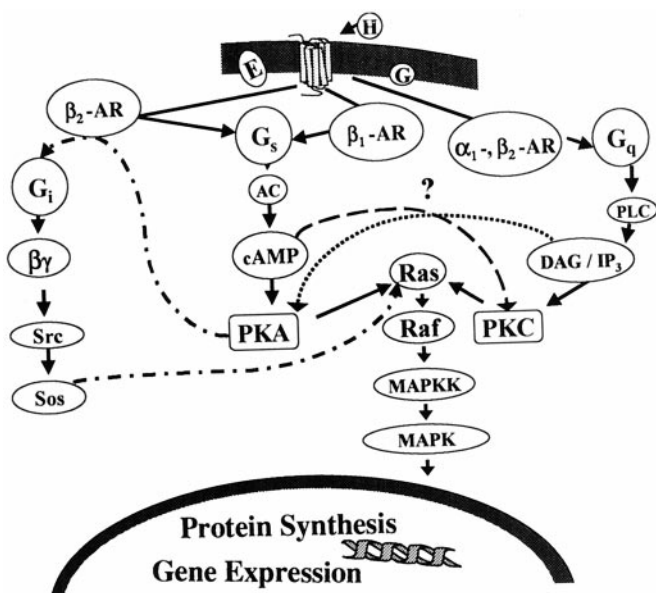
FIG. 1. Regulation of the Ras/Raf/MAP kinase pathway by adrenoceptors. Catecholamine stimulation of β_1-AR activates this system via the cAMP-PKA, and stimulation of α_1-AR activates this system via the 1,2-diacylglycerol/IP$_3$-PKC pathway. The β_2-AR can use both classic pathways. Both PKC and PKA serve as hubs for the cross-regulation of the adrenoceptors, but cross-talk may occur already at the second messenger levels. The PKA-mediated β_2-AR desensitization pathway may also stimulate the Ras/Raf/MAP kinase pathway by coupling to G_i/G_o via Src/Sos pathway. The various PK isoforms may act specifically to sort and sieve the individual signaling messages.

Thr phosphatases and kinases are associated with targeting subunits that contribute to the organization and specificity of signal transduction pathways by favoring the accessibility of their enzymes to certain substrate proteins (Faux and Scott, 1996). For this purpose, compartmentalization has been suggested as a means to attain some measure of selectivity toward the physiological substrates at the plasma membrane and is thought to be particularly important with respect to the function of PKs and PPs (Newton, 1995).

E. The G Protein-Coupled Receptor Kinase Family

In 1986, Lefkowitz and associates identified a cAMP-independent kinase that specifically phosphorylates the agonist-occupied form of the β-ARs, which they called β-adrenergic receptor kinase (βARK1; Benovic et al., 1986). This discovery was immediately followed by the realization that βARK1 was only a member of a family of proteins that specifically phosphorylate GPCRs leading to their desensitization (Benovic et al., 1989). Hence, these proteins are now more commonly known as the G protein-coupled receptor kinases (GRKs). At least six members of this family (GRK1–6) sharing 53 to 93% overall sequence homology have been identified and sequenced to date, and more may be in the pipeline (Premont et al., 1995). They have been subdivided into three groups according to their sequence homology and functional similarities. Group 1 consists of the rhodopsin kinase (GRK1), which is predominantly localized to the

retina. Group 2 consists of the β-AR kinase (referred to as either βARK1 or GRK2) and βARK2 (also known as GRK3), which exhibit a more ubiquitous tissue distribution, and the third subfamily is composed of GRK4, GRK5, and GRK6 (Premont et al., 1995). GRK4 is localized primarily to the testis, whereas GRK5 and GRK6 are more ubiquitously expressed. The common structure of GRKs consists of three main domains. One of these is a centrally localized highly conserved catalytic domain of approximately 240 amino acids, which shares significant amino acid identity (46–95%). This domain is flanked by a conserved N-terminal sequence of 161 to 197 amino acids (except for the *Drosophilia* kinase 2) and a variable length of C-terminal domain of 100 to 263 amino acids (Benovic and Gomez, 1993). The N-terminal domain is considered important for the recognition of activated receptor substrates, whereas the C-terminal domain probably is important for their membrane targeting (Palczewski et al., 1992) and harbors the autophosphorylation sites of GRK1 and GRK5 and the putative sites of GRK4 (Premont et al., 1996).

The most explicit functional role of GRKs is their preferential phosphorylation of several Ser/Thr residues of an agonist-occupied GPCR in either the C-terminal tail (e.g., rhodopsin and β_2-AR) or the third intracellular loop of the receptor (e.g., M$_2$ muscarinic acetylcholine receptor) in an agonist-dependent manner (Fredericks et al., 1996). Several GPCRs have been shown to act as substrates both in vitro and in vivo for various GRKs. Notably, although GRK2 is capable of using primarily the β_2-AR and muscarinic receptors and, to lesser extent, rhodopsin (Kwatra et al., 1989; Roth et al., 1991; Pippig et al., 1993), it prefers β-AR as substrate to the other receptors (Lohse et al., 1990; Müller et al., 1997). For the GRKs to mediate phosphorylation of their substrates, they have to be associated with the plasma membrane. The different subfamilies use different mechanisms to achieve this. The first subfamily, GRK1, GRK2, and GRK3 which are primarily cytosolic proteins, translocate to the plasma membrane on receptor stimulation (Strasser et al., 1986; Inglese et al., 1993; Freedman and Lefkowitz, 1996). In contrast, GRK4, GRK5, and GRK6 are not isoprenylated, do not bind to $G_{\beta\gamma}$, and do not show agonist-dependent membrane association (Premont et al., 1996). Despite these differences among the three subfamilies, the membrane association of all of them appears to be mediated at least in part by residues in their C-terminal domains, such as the CAAX motif of the GRK1 (Inglese et al., 1992). This motif is supposed to direct its isoprenylation and caboxymethylation, whereas protein-protein interactions of the $G_{\beta\gamma}$ complex probably take place with the C-terminal Pleckstrin homology domain of GRK2 (Koch et al., 1993; Müller et al., 1993; Parruti et al., 1993). For the first group, isoprenylation is believed to play a central role in mediating their membrane association either through direct covalent modification of the kinase (GRK1) or through

protein-protein interactions between the kinase and the isoprenylated $G_{\beta\gamma}$ (GRK2 and GRK3). It is postulated that after the activation of the G protein and its dissociation into G_α and $G_{\beta\gamma}$, the latter subsequently interacts with the GRK and serves to target these enzymes to their membrane-incorporated substrates (Daaka et al., 1997b). Although it is generally accepted that $G_{\beta\gamma}$ stimulates GRK2/3, the exact mechanism of these actions has yet to be elucidated. Pitcher et al. (1992) originally proposed that the $G_{\beta\gamma}$ primarily translocates the GRK from the cytosol to the plasma membrane to permit GPCR phosphorylation. In contrast, Inglese et al. (1992) suggested that the GRK binds to $G_{\beta\gamma}$ to access its isoprenyl group for the membrane localization because it lacks this group in its C terminus. Besides GRK2 translocation, the $G_{\beta\gamma}$ is associated with an additional role of facilitating its interactions with the activated receptor substrates resulting in the allosteric activation of the kinase (Kim et al., 1993; Haga et al., 1994). Unlike the members of the first subfamily, which engage isoprenylation in their membrane association, GRK4 and 6 probably use palmitoylation for this purpose (Stoffel et al., 1994; Premont et al., 1996). Site-directed mutagenesis studies have revealed a cluster of three Cys residues (Cys^{561}, Cys^{562}, and Cys^{565}) in the C terminus of the GRK6 as a region of palmitate attachments, consistent with the location of membrane targeting domains of GRK1, GRK2, GRK3, and GRK5 (Stoffel et al., 1994). The palmitoylation of these Cys residues may function as a link between its activity toward its receptor substrates and its membrane association (Loudon and Benovic, 1997). By analogy with GRK6, the C-terminal Cys^{561} and Cys^{578} of GRK4 are the probable sites of its palmitoylation (Stoffel et al., 1997). GRK5, on the other hand, is apparently constitutively associated with the membrane (Premont et al., 1995, 1996), an observation that might explain its higher basal activity compared with GRK2, for example (Premont et al., 1994). Two distinct lipid-binding domains of GRK5 have been identified, one in the N terminus and another in the C terminus, that may be involved in its receptor association (Stoffel et al., 1997). Besides, all five nonisoprenylated GRKs seem to require the presence of lipid cofactors for their interactions with their receptor substrates (Stoffel et al., 1997). Both the enhancement of GRK-mediated GPCR phosphorylation, by targeting the GRK to the membrane and locating the enzyme into close proximity with its receptor substrate, and the direct enhancement of GRK catalytic activity have been associated with the lipid-dependent nature of the GRKs. Lipids such as phosphatidylinositol-4,5-bisphosphate (PIP_2) have been shown to promote GRK2/$G_{\beta\gamma}$ complex formation by binding to the N terminus of GRK2 (DebBurman et al., 1996). This led to the notion that the coordinated binding of PIP_2 to this N terminus and $G_{\beta\gamma}$ to the C-terminal Pleckstrin homology is necessary for its effective membrane localization and function. In con-

trast, negatively charged phospholipids such as phosphatidylserine enhance GRK2-mediated β-AR phosphorylation, presumably as a direct result of increasing the GRK2 catalytic activity (DebBurman et al., 1995). Therefore, the receptor regions responsible for GRK activation are probably distinct from the sites of GRK-mediated phosphorylation. The current view is that GRKs not only phosphorylate, bind, and uncouple the agonist-activated receptor but also play a pleiotropic role in their regulation of receptor responsiveness by providing the signal and serving as the molecular intermediates directing the agonist-promoted sequestration of the β_2-AR (Ferguson et al., 1997). Also noteworthy is the recent observation that PKC-mediated phosphorylation of certain C-terminal residues of GRK2 not only upregulates its own activity but also may influence its targeting to the plasma membrane, implicating PKC as a direct regulator of GRK-mediated receptor desensitization (Winstel et al., 1996). Therefore, apart from communicating with each other via the normal physiological route, these two kinases may do so under conditions leading to β-AR desensitization. Furthermore, the GRK and PKC are engaged in cross-talk in their activation of several receptor systems, notably those that are involved in growth regulatory mechanisms, adding yet another twist to our understanding of the role of phosphorylation in GPCR signaling, which should command great future research interest.

F. The Arrestin Family

Soon after the discovery of GRKs, it became apparent that although homologous desensitization of GPCRs is triggered by their specific phosphorylation, this alone was insufficient to inhibit receptor function. It was realized that under physiological conditions, GRKs required the presence of a protein cofactor to accomplish receptor phosphorylation. The existence of such an arresting protein (visual arrestin) was postulated by Benovic et al. (1987) almost accidentally as a result of their experiments demonstrating the binding of a 48-kDa soluble retinal protein to GRK2-phosphorylated rhodopsin. The observations from this study led the investigators to suggest that a protein analogous to retinal arrestin may exist in other tissues and function in concert with GRK2 to regulate the activity of AC-coupled receptors. This finding was succeeded by experiments of Lohse et al. (1990) suggesting that appropriate homologous β_2-AR desensitization by GRK2 in vitro also required an "arrestin-like" protein (β-arrestin). It was also shown that in Chinese hamster ovary cells expressing high levels of β_2-ARs, β-arrestin, and GRK2 become limiting for homologous receptor desensitization, providing support for their involvement in the regulation of β_2-AR (Pippig et al., 1993). To date, six members of the family have been identified, and they exhibit 44 to 84% sequence homology: 1) visual arrestin (S antigen) localized primarily to the retina but also found in the pineal

and primary blood leukocytes; 2) β-arrestin-1 (bovine arrestin) and β-arrestin-2, which are more ubiquitously expressed but most highly concentrated in nervous and lymphatic tissues; 3) cone arrestin (also known as C- or X-arrestin), which is found primarily in the cones but also is localized to the pineal, pituitary, and lung tissues; and 4) D-arrestin and E-arrestin, which are partial arrestin clones that have not been characterized but exhibit specific tissue distribution (Attramadal et al., 1992; Calabrese et al., 1994). Arrestins have several functional domains that contribute to the multisite binding of their receptor substrates: 1) a C-terminal acidic region that serves a regulatory role in controlling arrestin binding selectivity toward the phosphorylated and activated form of the receptor without participating in receptor interaction, 2) a basic N-terminal domain that directly participates in receptor interactions and serves a regulatory role via intramolecular interactions with the C-terminal acidic region, and 3) two centrally localized domains that are directly involved in determining receptor binding specificity and selectivity (Gurevich et al., 1995). Overall, β-arrestin-2 exhibits 78% amino acid identity with β-arrestin-1, both of which preferentially interact with GRK1, GRK2, and GRK5 in their regulation of β-AR phosphorylation to other GPCRs as substrates (Attramadal et al., 1992; Freedman et al., 1995). Besides their apparently dual regulatory role in the GPCR life cycle in controlling both their desensitization and internalization, there are strong indications that both GRKs and arrestins might have a greater role to play in, for example, cardiac development or their regulation of the β-AR signaling (Jaber et al., 1996; Rockman et al., 1998).

III. The β-Adrenoceptor Signaling Circuits

The mechanisms by which GPCRs interact with their downstream effector molecules in transmitting signals of their primary messenger is only beginning to be deciphered, thanks to the arrival of molecular techniques that have allowed us to examine these individual processes at single-amino acid functional levels. These techniques have revealed that the interactions of GPCRs and their effector molecules are a dynamic process requiring various coordinated interactive cycles. Because these interactions take place in specific cellular compartments or organelles under stringent conditions, both the receptors and their downstream signaling components are subject to structural transformations and translocations by stringently coordinated mechanisms to ensure proper signal propagation. To initiate the signaling, the formation of the receptor active state may depend on agonist binding promoting a conformational change in the receptor, constituting a post-translational acylation by natural fatty acids in the form of palmitoylation, myristoylation, or prenylation (Rando, 1996; Stoffel et al., 1997). Also, the hormone-sensitive ACs have been localized to a specialized subdomain of the plasma membrane

called caveolae that is believed to contain G proteins to optimize their signal transduction efficiency and specificity (Huang et al., 1997). This means that the cytosolic signaling components must be brought into close proximity with the plasma membrane-bound receptors and the AC. The G proteins also undergo several modifications, such as isoprenylation/methylation, possibly to enhance their association with the plasma membranes and therefore facilitate their signaling capability. Myristoylation of the N termini of the G_α proteins and/or binding to $\beta\gamma$ (and some other factors) probably directs them to a membrane location where palmitoylation takes place. The geranylgeranylation of the G_γ subunits (or farnesylation as in the case of retinal G_γ subunit) probably is required for correct targeting of the $G_{\beta\gamma}$ dimer (Wedegaertner et al., 1995), whereas prenylation of the complex is thought to be a prerequisite for productive interactions of the complex with the G_α subunits, receptors, and effectors (Casey et al., 1994). On the other hand, the G_β subunits are predicted to form coiled-coil structures in their N-terminal region signifying stabilized α helices important for protein-protein interactions, especially with the G_γ subunit. There also is some evidence to suggest that caveolins, the structural components of caveolae, play pivotal roles in the transportation of molecules and cellular signaling by interacting directly with and regulating the function of G proteins (Li et al., 1995). In the heart, the expression of caveolin subtypes is apparently regulated by β-AR stimulation (Oka et al., 1997). However, there is much to be elucidated regarding the regulators of the mechanisms that switch on GPCR signaling.

A. Receptor-G Protein-Adenylyl Cyclase Circuit

The β-AR-G protein-AC circuit consists of three independent but tightly interlinked signaling cycles: 1) the formation of a complex by the agonist-occupied receptor with the G protein and AC to initiate the signaling, 2) the G protein cycle to stimulate and regulate the coupling mechanism, and 3) the AC-G protein cycle regulating the AC catalytic function. Originally, a "ternary complex" model was suggested by De Lean et al. in 1980 to explain the agonist-specific binding properties of the AC-coupled β-AR. In essence, this model suggests an agonist-promoted formation of high-affinity ternary complex composed of the hormone, receptor, and AC at the onset of the signal transduction, which is destabilized by the addition of a guanine nucleotide, leading to the dissociation of both the hormone and AC. The model was initially found to accurately fit data obtained with a full or partial agonist, situations in which a system was altered by the addition of a guanine nucleotide, or after treatment with agents specific for a particular group. It was then discovered that many GPCRs have the spontaneous capacity to activate their cognate G proteins and regulate downstream effectors in the absence of an agonist ligand and that designed mutational

modifications of GPCRs known as constitutively active mutant (CAM) receptors could increase their extent of agonist-independent signal transduction (Lefkowitz et al., 1993; Samama et al., 1993, 1997). The CAM β_2-AR exhibits not only agonist-independent activation of AC but also increased affinity for agonists (even in the absence of G proteins) but not antagonists, whereby the increase in affinity correlates with the intrinsic activity of the ligand, including partial agonists (Samama et al., 1993). These authors noted that the ternary complex model previously suggested by De Lean and colleagues was not sufficient to adequately explain such phenomena; rather, an explicit isomerization of the receptor into an active state would conform to conditions for both the mutant and the wild-type receptors. Recently, it was reported that the mutation conferring constitutive activity to β_2-AR removes some stabilizing conformational constrains, allowing the CAM to undergo transitions more readily between the inactive and the active states and thereby making the receptor more susceptible to denaturing (Gether et al., 1997a). For the β_2-AR, the movements around Cys^{125} in the TD III and Cys^{285} in TD VI are thought to be involved in this activation (Gether et al., 1997b). More recently, it was suggested that the association of, and equilibrium among agonist, antagonist, and G protein, as well as other factors within the membrane environment, consists of two events. First, an association of two domains, the TD helices 1 through 5 (domain I) and TD helices 6 and 7 (domain II), probably is necessary for signal transduction. Specific membrane perturbations of the conformational state of receptor side chains in the vicinity of the binding site of the agonist followed by G protein association may also be required for agonist activity (Underwood and Prendergast, 1997). However, it is still not known how and where in the cell such complexes are assembled and disassembled. Regardless of the way by which the β-AR, G protein, and AC complexes are initiated, it is well established that the conformational change in the receptor induces the release of GDP in exchange for GTP on the G_α subunit, leading to the stimulation of AC catalytic activity. Two independent views are being entertained with respect to the activation of the AC by the β-AR after the complex formation. One line of thinking purports that GPCRs govern their effectors indirectly via a two-step shuttling mechanism that involves the exchange of G proteins or their components between ephemeral GPCR-G protein and G protein-effector complexes. This may require the stimulation of AC by β-AR involving, first, the activation of G_s independent of the cyclase, followed by $G_{s\alpha}$ activation of the cyclase independent of the receptor. The second notion envisages the activation of a preoccupied G_s-AC complex by the receptor in a single step. Several experimental models have been put forward in favor of both mechanisms. Arguments for the shuttle mechanism are based on experiments pointing to changes in second messenger production, GTPase activity, and the binding characteristics of agonists, antagonists, and guanine nucleotides, as well as experimental evidence for the occurrence of the receptor-G protein-effector complexes. Although there is no direct documentation for the cyclical formation and dissociation of these complexes during signaling, it has been argued that they nevertheless reflect on perturbations in the equilibrium between the G protein and the other two components (Krumins et al., 1997). On the other hand, some investigators argue that the random, transient association of the G protein and the receptor is largely inconsistent with the binding of agonists to receptors and the allosteric regulation of that binding by guanine nucleotides. Furthermore, this paradigm does not readily account for receptor-effector coupling specificity, because the promiscuous interaction of most G proteins with both receptors and effectors in vitro is at odds with the general inability of the G proteins to be shared among highly congruous signal transduction pathways in vivo (Chidiac, 1998).

B. Adenylyl Cyclase Catalytic Circuit

The cardiac-specific AC function is predominantly under the dual stimulatory and inhibitory regulation by receptors acting through the G_s and G_i proteins, respectively (Helper and Gilman, 1992; Spiegel et al., 1992; Eschenhagen, 1993). For the β-AR-mediated stimulation of cAMP synthesis, the interaction between $G_{s\alpha}$ and the two AC cytoplasmic domains (C1 and C2) constitutes a key step. This action is initiated by the dissociation of the $\beta\gamma$ complex as a result of the conformational changes after the catalysis of GDP-GTP exchange by the agonist occupation of the GPCR. Termination of the AC activation by $G_{s\alpha}$-GTP is rapidly achieved by the intrinsic GTPase activity of the G_α subunits initiating the hydrolysis of $G_{s\alpha}$-bound GTP to GDP. Mutation analysis reveals three discrete regions in the C2 primary sequence of AC that are close together but are distal to the catalytic site and probably function as the affinity determinants of the $G_{s\alpha}$ protein. At present, a number of ideas are being entertained as potential mechanisms for the stimulation of AC activity by the $G_{s\alpha}$, including the possibilities that 1) the $G_{s\alpha}$ directs the productive formation of a complex interface between the conserved units, 2) the $G_{s\alpha}$ acts primarily as an allosteric activator of AC, and 3) it activates AC primarily by stabilizing a catalytically competent form of the enzyme (Tesmer et al., 1997). All of the residues that interact with $G_{s\alpha}$ are conserved among AC isoforms (Yan et al., 1997). Only two of the $G_{s\alpha}$ residues involved in the interfaces with AC, Gln^{236} and Asn^{239}, are significantly different from the analogous residues His^{213} and Glu^{217} in the $G_{i\alpha}$ subunit (Sunahara et al., 1996). The Phe^{379} in the conserved domains may be important for the activation of AC by the G proteins, whereas Arg^{484} probably is involved in $G_{s\alpha}$ activation (Tang and Gilman, 1991; Taussig et al., 1994). The latter is a pyrophosphate-binding

residue whose proximity to other residues in the active site may be essential for positioning other residues such as the Arg^{1029} to interact with the perivalent transitional state of the substrate. By so doing, $G_{s\alpha}$ can improve the stabilization of this transition state and potentiate a chemical step in the reaction mechanism (Tesmer et al., 1997). The binding site for the $G_{i\alpha}$ is distinct from that of the $G_{s\alpha}$ subunit. It is most likely situated between the $\alpha1$-$\alpha2$ loop and the $\alpha3$ helix on the C1 subunit directly opposite to the binding site of $G_{s\alpha}$. This region contains many residues such as Glu^{398} and Leu^{472} that are invariant in AC V and VI but not in $G_{i\alpha}$-insensitive cyclases (Tesmer et al., 1997). These differences suggest that the specificity of $G_{s\alpha}$ and $G_{i\alpha}$ subunits for AC is dictated primarily by the backbone conformation of the residues that form the interface with the enzyme rather than their primary structure (Sunahara et al., 1996).

The mechanism by which the ACs catalyze the conversion of ATP to cAMP is still not fully understood. One line of thinking implicates its cytosolic conserved C1 and C2 domains singly or in combination as representing the AC catalytic sites for this function (Coleman et al., 1994; Zhang et al., 1997a,b). Their notion is based on the argument that the sequence homology of these AC domains is similar to GC domains believed to perform the same functions. A completely different school of thought advanced by Tesmer et al. (1997) suggests that both conserved domains provide the binding sites for ATP, and therefore the individual cytosolic domains are unlikely to be catalytic. Rather, a direct in-line attack of the O3′-hydroxyl on the 5′-phosphate of ATP without the formation of a phosphor-enzyme intermediate may account for the observation that AMP cyclization proceeds with an inversion of the configuration at the α-phosphate (Tesmer et al., 1997).

C. cAMP-Protein Kinase-Effector Circuit

The ultimate product of AC stimulation by the cardiac β-AR pathway is the activation of the slow Ca^{2+} channel to regulate cardiac positive inotropy. This channel is a complex structure of several regulatory proteins whose function is controlled by a number of factors that are intrinsic and extrinsic to the cell. After its AC-mediated synthesis from ATP, cAMP regulates a series of cellular functions mediated by PKA and phosphorylation of the Ca^{2+} channel or an associated stimulatory type of regulatory protein. The cAMP-dependent PKA mediates most cAMP actions by phosphorylation. It is thought that in absence of cAMP, the two regulatory subunits associate with two catalytic units in the form of a tetramer in which the PKA catalytic activity is inhibited. Binding of the cAMP to the regulatory domain relieves this internal inhibitory effect, presumably by means of a conformational change causing the complex to dissociate into a dimer and two free active catalytic subunits. Phosphorylation of the catalytic domain is often required for

the enzymatic activity of the PKA. It can be either constitutive, as in the autophosphorylation of Thr^{197} in PKA, or provided by a regulatory kinase. The phosphorylations that occur on a loop in the vicinity of the catalytic site may play a critical role in the proper positioning of ATP and catalytic residues, as well as in the accessibility of the substrate (Knighton et al., 1991). The fine tuning of the cAMP cascade is provided by cAMP phosphodiesterases (PDEs) that break down cAMP and thus limit the degree of cAMP-dependent phosphorylation. Because PDE activity is controlled by the intracellular cGMP, both cAMP and cGMP are likely to determine the degree of cAMP-dependent phosphorylation of the cardiac Ca^{2+} in a competitive antagonist fashion. In ventricular myocytes from different species, I_{Ca} is probably deregulated by cGMP via a PDE-independent mechanism mediated by protein kinase G (PKG) and phosphorylation of possibly an inhibitory type of a regulatory protein associated with the Ca^{2+} channel (Sperelakis et al., 1994). Hence, the cGMP-PKG system stimulates a phosphatase that dephosphorylates the Ca^{2+} channel to provide a recycling mechanism for the channel. In addition to the slower indirect pathway exerted via cAMP-PKA, there apparently is a faster, more direct and efficient pathway for the stimulation of the Ca channels by β-ARs that involves the direct modulation of the channel activity by the $G_{s\alpha}$ protein (Knighton et al., 1991). The superiority in the efficiency of β-AR over other systems in coupling to the regulation of cardiac I_{Ca} is thought to be embedded in the difference in the degree of cAMP accumulation near the Ca^{2+} channels due to colocalization of AC with the channels (Jurevicius and Fischmeister, 1996).

IV. Cardiac Receptor Cross-Talk and β-Adrenoceptor Signaling

It is mandatory for an essential organ, such as the heart, to be endowed with the ability to fend off some of the potentially fatal consequences arising from possible failure or total loss of β_1-AR positive inotropism to sustain its very vital circulatory function in disease. The heart can achieve this by maintaining reserve signaling systems under its own humoral regulatory control or by the ability to adapt itself appropriately and rapidly to its altered circulatory demands. Moreover, several cardiac GPCR signaling components, including the G proteins, ACs, and PKs, exist in multiple isoforms and subtypes, of which different combinations determine the specificity of their actions. For example, different combinations of the G protein subunits often produce antagonistic signaling products when interacting with the various ACs. Thus, the AC V–VI group has $G_{s\alpha}$ as their activator and $G_{i\alpha}$ and Ca^{2+} act as inhibitors, whereas the $\beta\gamma$ complex appears to be uninvolved in this signaling process (Tesmer et al., 1997). Furthermore, $G_{s\alpha}$ and $G_{o\alpha}$ do not seem to inhibit certain ACs directly, apparently because this effect would be in opposition to those of their

associated $\beta\gamma$ subunits (Tesmer et al., 1997). This diversity in the key components of GPCR signaling demonstrates the variability by which the different isoforms channel, refine, or divert signaling messages to meet the needs of the cardiac circulatory function according to demand.

One of the most intriguing phenomenon about β-AR signaling is the manifestation that the cardiac contractile tissue harbors functionally viable β_2-AR in addition to the β_1-AR. Equally perplexing is the recent observation that although most of the β_2-AR actions are mediated through G_s proteins and their cAMP-dependent PKA system, this receptor also couples to G_i-G_o proteins to stimulate the mitogen-activated protein (MAP) kinase pathway (Daaka et al., 1997a; Zhou et al., 1997). This actions is mediated by $\beta\gamma$ subunits of pertussis toxin (PTX)-sensitive G proteins through a pathway involving nonreceptor tyrosine kinase c-Src and the G protein Ras, probably serving as a switch for the β_2-AR to regulate its G protein coupling specificity to initiate new signaling events after heterologous desensitization. The important question remains, however, of why β_2-AR coupling should shuttle between two pathways that are otherwise effectively antagonistic to each other on their effector molecules, such as the AC. All indications are that the presence of β_2-AR in the cardiac tissue may serve a particular signaling function that has evaded recognition until now. This may point to cross-regulation of cardiac β-ARs as a form of dictating the direction of the catecholamine messages to meet the demands of different functional conditions by providing an additional way for channeling certain signaling messages and further refining their cross-regulation by other cardiac receptor systems. It is interesting that Daaka et al. (1997a) suggested that the switching of the β_2-AR from G_s to G_i-G_o serves the purpose of protecting the heart in disease. In this regard, it is not only the existence of a β-AR subtype cross-regulation that is fascinating but also their interaction with the other cardiac receptor systems such as the α_1-AR and angiotensin II, endothelin 1, muscarinic, thrombin, adenine nucleotide, and opioid peptide receptors. All of these systems present a huge inotropic potential that can theoretically be tapped whenever necessary to support a defunct or ailing contractile apparatus. There are several lines of evidence in the literature that strongly advocate for the existence of such a form of communication among these receptor systems. To begin with, the wide majority of these GPCRs transduce the signals of their primary messengers via the same route or their pathways converge at certain junctions, such as the G protein, second messenger, or PK levels. Thus, the G_s couples not only the β-AR but also histamine H_2 and prostaglandin E_2 receptors to AC in a stimulatory way, whereas, on the other hand, the G_i couples α_2-AR, M_2 cholinoceptors, and A_1 adenosine receptors to the same enzyme in an inhibitory fashion. This dual regulatory control of the AC activity by the G_s and G_i presents a

potential mechanism for the fine tuning of their signal transduction. Interestingly, these receptor systems are all characteristically domicile in the heart without displaying any evident physiological function, yet some of them, particularly the α_1-ARs, are altered in cardiac disease in association with the loss of β_1-AR function (De Bold et al., 1996; Dzimiri et al., 1996b; Li et al., 1997). Similarly, all of the above receptor systems are linked through GTP-binding proteins to PLC, which hydrolyzes PIP_2 in the myocardium, leading to the production of IP_3 and its phosphorylated derivative, inositol-1,3,4,5-tetrakisphosphate (Lamers et al., 1993). IP_3 releases Ca^{2+} from the sarcoplasmic reticulum, which is causally related to positive inotropism, whereas inositol-1,3,4,5-tetrakisphosphate is probably involved in the handling of intracellular Ca^{2+} and, consequently, inotropic responses, as well. The other product of PLC, 1,2-diacylglycerol activates Ca^{2+}-dependent PKC and potentially controls a wide array of cellular functions, including ion transport, myofibrillar Ca^{2+} sensitivity, protein synthesis, and hypertrophic cell growth (Lamers et al., 1993). These facts obviate the question as to why these GPCR systems are present in the cardiac tissue if they are not actively involved in the cardiac physiological function. For the AR subtypes, currently available evidence supports the view of a cross-regulation between the two systems at various signaling levels. It has been clearly demonstrated that α-AR agonists can transduce their signals via both α_1-AR and β_1-AR pathways (Yamazaki et al., 1997). In vivo stimulation of the β-AR pathway by isoproterenol has been found to stimulate an increase not only in $G_{i\alpha2}$-$G_{i\alpha3}$ but also in α_{2A}-AR expression (Lecrivain et al., 1998). Although the mechanisms of such a cross-talk are far from being elucidated, several recent observations point to its existence in the heart. Both the convergence of β_1-AR and α_1-AR systems at the AC circuit and cross-regulation between G_s- and G_i-mediated pathways controlling the AC activity have also been demonstrated. Stimulation of cardiac α_1-ARs inhibits the β_1-AR-mediated increase in cAMP, presumably by activating its PDE activity or by coupling to G_i protein (Li et al., 1996). Until recently, it was thought that GRK2 specifically phosphorylates coupled β-AR only. However, recent studies suggest that α_1-AR is also subject to phosphorylation by GRK2 under similar conditions. The activation of α_{1B}-AR by epinephrine in cell lines stably expressing the receptors and in human embryonic kidney (HEK) 293 cells transiently coexpressing this receptor and GRK2 results not only in stimulation but also in translocation of GRK2 immunoreactivity from the cytosol to the membrane fraction (Winstel et al., 1996). Therefore, apart from communicating with each other via the normal physiological route, these two receptor subtypes may do so under conditions leading to β-AR desensitization.

The regulation of α-AR and β-AR by their agonists can be traced along their respective downstream pathways

beyond the PK circuits. In cardiomyocytes expressing both α_1-AR and β_1-AR, their agonists activate the Ras/Raf/MAP kinase kinase (MEK)/MAP kinase (MAPK) pathway by stimulating either PKC or PKA activity (Post et al., 1996; Van Biesen et al., 1996; Diverse-Peirluissi et al., 1997; Ramirez et al., 1997; Yamazaki et al., 1997). Similarly, agonists of both receptor subtypes, individually or synergistically, enhance the activities of Raf and MAP kinases. The norepinephrine-induced activation of MAP kinases is partly inhibited by either an α_1-AR or a β_1-AR blocker and completely abolished synergistically by both types of receptor blockers (Yamazaki et al., 1997), suggesting that the α_1-AR– and β_1-AR–signaling pathways synergistically induce cardiomyocyte hypertrophy. The observation that these actions are mimicked by both PKA and PKC activators means that they are mediated via both PKs (Lazou et al., 1994; Post et al., 1996; Yamazaki et al., 1997). In the same cell lines in which both α_2-AR and β_1-AR stimulate the MEK/MAPK pathway, stimulation of MAP kinase by the β_2-AR is mediated by the $G_{\beta\gamma}$ subunits of PTX-sensitive G proteins through a pathway involving the nonreceptor tyrosine kinase C-Src and the small G protein Ras (Daaka et al., 1997a). The activation of this pathway by the β_2-AR requires that the receptor be phosphorylated by PKA, pointing to an involvement of a mechanism mediating the uncoupling of the β_2-AR from G_s and, thus, heterologous desensitization (Daaka et al., 1997a). The finding that GRKs and β-arrestins may be involved in the GPCR-mediated MAPK-signaling cascade suggests that these proteins are engaged in cross-talk with those signaling proteins for growth regulation.

Besides the cross-talk between the α-AR and β-AR subtypes, other cardiac receptor systems, such as the ANP and the type 1 angiotensin II receptor (AT$_1$), are also capable of interacting with both AR subtypes at various signaling levels. Sulakhe et al. (1997) suggested that chronic exposure of cardiomyocytes to β-AR, M$_2$ muscarinic, or A$_1$ adenosine receptor agonists can induce a desensitization of each other's pathway as well as AC activity. The implications are that AR agonists may stimulate these pathways, and conversely the agonists of these receptors stimulate ARs under certain conditions. It has also been suggested that angiotensin II decreases the responsiveness of the rat heart to β_1-AR stimulation by activating PKC (Schwartz and Naff, 1997). Thus, activation of AT$_1$, the mediator of angiotensin II actions, reduces and its inhibition increases β_1-AR activity. The regulation of one receptor by another may occur at various levels, including transcriptional, translational, or post-translational. There is a wealth of information showing that GPCRs can indeed influence each other at these levels. One such example is the increase in the synthesis rates and activities of various proteins and receptor systems, such as the *ANP* gene promoter, by both α_1-AR and β_1-AR agonists, which is believed to occur by stimulating cardiac signaling pathways other than their own, particularly the growth receptor systems. In neonatal rat ventricular myocytes, activation of genetic and morphological changes by the stimulation of the α_1-AR is characterized by transcription activation of the *ANF* gene and hypertrophy of the cells (Ramirez et al., 1997). Moreover, the α_1-AR agonist phenylephrine not only induces brain natriuretic peptide expression but also has been shown to stabilize its mRNA through both PKC and MAPK pathways (Hanford and Glembotski, 1996).

It has become clear in recent years that both α-AR and β-AR also have mitogenic properties, as demonstrated by their ability to stimulate the Ras/Raf/MAPK pathways and to transactivate cardiac-specific genes, such as ANP, myosin light chain, and fos promoters (Van Biesen et al., 1996). In fact, there is strong evidence suggesting that in general, GPCR-signaling pathways converge with those emanating from the receptors of the tyrosine kinases family at the level of Ras by coupling via the $\beta\gamma$ complex (Gutkind, 1998). The stimulation of the $\beta\gamma$-dependent regulation of the MAPK-signaling pathway by GPCRs is at least in part mediated by PI$_3$-kinase γ (PI2Kγ; Lopez-Ilasaca et al., 1997). Ramirez et al. (1997) showed that both α_2-AR stimulation and Ras activate the c-Jun NH$_2$-terminal kinase (JNK) in cardiomyocytes. Because Ras regulates hypertrophy by activating a kinase cascade involving Raf, MEK, and the extracellular signal-regulated extracellular response kinase (Erk), it was concluded that the α_1-AR effects on JNK occur through a pathway requiring Ras and MEK kinase (MEKK). This notion found support in the finding that a constitutively activated mutant of MEKK that preferentially activates JNK also stimulates ANF reporter gene expression, whereas a dominant negative MEKK mutant inhibits ANF expression induced by phenylephrine. Moreover, JNK activity is increased in the ventricles of mice overexpressing Ras, whereas Erk activity is not (Ramirez et al., 1997). It was therefore suggested that α_1-AR mediates *ANF* gene expression through a Ras-MEKK-JNK pathway and that this route is associated with hypertrophy both in vitro and in vivo. The involvement of α_2-AR in hypertrophic responses was supported by the finding that genistein, a tyrosine kinase inhibitor, prevents phenylephrine-induced activation of the Fos, ANF, and myosin light chain promoters, which are implicated in the hypertrophic responses. Genisten also inhibits phenylephrine-induced activation of the MAP kinases Erk1 and Erk2 and the GTP loading of the Ras protein (Thorburn and Thorburn, 1994). Although the physiological implications of these interactions are yet to be unraveled, it is evident that a new chapter in the understanding of mechanism regulating cardiac function is about to open. Cardiac receptor signaling is indeed a maze, which cannot be explained by looking at a single pathway. It is therefore only logical to hypothesize a regulatory system under the autonomous control of the heart itself to coordinate these three individual

pathways to ensure smooth circulatory function. The classic belief that the majority of cardiac receptor systems are simply harbored in the heart without being actively involved physiologically will soon have to undergo a revolutionary change. The simple explanation for this notion is our ignorance of the regulation of these signaling systems in normal cardiac function. The importance of cross-regulation among the cardiac receptor systems becomes even more evident in cardiac disease, where a malfunction or perturbation in any one of them is likely to adversely affect the function of the others two systems. Accordingly, the heart should be able to adapt itself, possibly by altering the otherwise inactive but functional pathways, to ensure continuity of its vital function in any given situation. This notion is bound to constitute an important research topic in the near future.

V. Factors Regulating β-Adrenoceptor Signaling

The cascade of events emanating from the activation of a GPCR by an agonist triggers both temporally and tightly coordinated generation of cellular responses and the fine tuning of responsiveness of the target molecules through various cellular and molecular mechanisms. The current view is that receptors are continuously translocating from particular cellular regions into others to permit specific compartmentalized interactions to take place. After their synthesis in the Golgi complex, GPCRs are delivered to the plasma membrane, where they reside predominantly and their signal transduction is turned on (Fig. 2). The translocation of the receptors from the plasma membrane into endosomes is usually slow but is dramatically increased in the presence of an agonist. From the endosomes, the receptors can be either recycled back to the plasma membrane or routed to lysosomes for degradation. Apart from these events, which take place under physiological conditions, cardiac disease may introduce other players into the equation, eventually inhibiting or eliminating altogether the functional efficiency of the signaling cycles. The impact of such defects is likely to be greatest if they occur at the initiation stage of the signaling pathway, inevitably causing an attenuation or even premature termination of the signal transduction. Because these initial stages involve several steps for transforming and activating the receptors, it can be reasonably assumed that malfunctional defects occur occasionally in the mechanisms regulating these events as a result of inefficient receptor recycling or transformation mechanisms. At present, very little, if anything, is known about such defects in β-AR signaling. Some of the structural modifications, such as palmitoylation of Cys^{341} in the C-terminal tail of the β_2-AR, regulate several signaling events, including receptor phosphorylation, desensitization, G protein membrane translocation, and, therefore, receptor turnover rate (Moffett et al., 1996; Morello and Bouvier, 1996). Because these processes do not occur simulta-
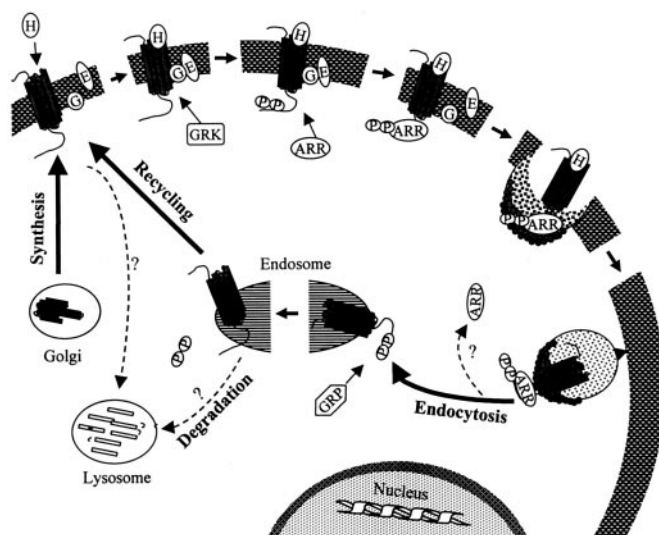

FIG 2. Regulation of β-adrenoceptor turnover via desensitization and sequestration mechanisms. At the onset, the constitutively phosphorylated β-arrestin-1 is recruited by the agonist stimulation of the receptor, followed by its rapid dephosphorylation at the plasma membrane. This process transforms the β-arrestin-1 into a clathrin adaptor, which then controls the endocytotic process. The β-arrestin binds to the GRK-phosphorylated β-AR bound to the G_s protein. The receptor is then sequestered to the endosomal compartment via clathrin-coated vesicles to be dephosphorylated and recycled back to the plasma membrane in their preligand exposed state. ARR, β-arrestin; H, hormone; G, heterotrimeric G protein; GRK, G protein-coupled receptor kinase; GRP, G protein-coupled receptor phosphatase; P, inorganic phosphate group.

neously, there should be mechanisms to sort them to avoid potentially detrimental conflict in the sequencing of the events. Moreover, the enzymes involved in the palmitoylation and depalmitoylation of the GPCRs are unknown, and there is no information as to whether these initiating steps may falter and what would happen if they did. These issues must be addressed to have a complete understanding of the factors that influence the regulation of β-AR signaling. In contrast, great strides have been registered in elucidating changes in β-AR functional expression associated with cardiac disease and in their potential role in receptor turnover processes. This is particularly true with respect to possible mechanisms underlying β-AR desensitization, sequestration, and resensitization discussed in this section.

A. Receptor Desensitization, Uncoupling, and Down-Regulation

The desensitization and the associated uncoupling or down-regulation of β-AR relate to their adaptational responses to various forms of stress. It has been suggested that the ability of adrenergic stimulation to elicit desensitization of the β-AR-AC–signaling cascade is not an inherent property of cells but rather is acquired during the period in which sympathetic innervation develops (Slotkin et al., 1996). Accordingly, during the perinatal transition period, early pioneer synapses are thought to provide a trophic signal that enables cells to increase their sensitivity to stimulation before physiological function is completely established. Thus, innervation provides a timing

signal for the onset of desensitization capabilities of sympathetic target cells but may not be absolutely required for the cells to learn how to desensitize (Slotkin et al., 1996). In principle, β-AR desensitization occurs as a result of two different alterations in their signaling, involving, first, diminished receptor numbers (receptor down-regulation) and followed by an impairment in the function of the remaining receptors (receptor uncoupling; Hausdorff et al., 1990). Traditionally, receptor desensitization has been classified as homologous (agonist dependent) triggered by GRK-mediated phosphorylation and heterologous (agonist independent), essentially resulting from receptor phosphorylation by PKA or PKC (Sibley and Lefkowitz, 1985). However, a large body of evidence indicates that both PK- and GRK-mediated receptor phosphorylation contribute to the agonist-dependent desensitization of GPCRs. The principle dividing line between the two is the observation that PKs exhibit the capacity to indiscriminately phosphorylate and desensitize receptors that have not been exposed to agonists, whereas GRKs specifically and solely phosphorylate the agonist-activated form of GPCRs (Pitcher et al., 1992a; Lefkowitz, 1993; Ferguson et al., 1996a, 1997). The GRK-mediated event apparently depends on the agonist coupling efficiency (January et al., 1997) but is independent of cAMP levels (Chuang et al., 1996). A second difference is that GRK-mediated receptor phosphorylation proceeds somewhat faster than the PK-dependent phosphorylation, which is probably the most efficient means of desensitizing GPCRs at low agonist concentrations (Lefkowitz, 1993). Moreover, unlike second messenger-dependent PKs, GRKs promote the binding of arrestins, which further uncouple the receptors by interdicting GPCR/G protein interactions (Benovic et al., 1987; Lohse et al., 1990, 1992). In essence, phosphorylation of an agonist-occupied receptor such as the β_2-AR by the specific GRK2 and GRK3 enhances the ability of the β-arrestin to bind and subsequently inhibit the phosphorylated receptor, ultimately leading to its uncoupling from the G_s protein and eventually its effectors (Bouvier et al., 1988; Lohse, 1992). However, although it is strongly believed that β-arrestins mediate the rapid desensitization by binding to phosphorylated GPCRs, deciphering the mechanisms by which they are targeted to the membrane to bind and uncouple them remains an interesting research challenge. According to current notion, the β-arrestins are cytoplasmic proteins, which occur after agonist stimulation of the receptor and translocate rapidly to the plasma membrane to bind their receptor targets, dependent on GRK activation (Barak et al., 1997). The phosphorylation of β_2-AR by the GRK on its C-terminal tail apparently causes the recruitment of the constitutively phosphorylated cytosolic β-arrestin by allowing them to bind to the third intracellular loop and C-terminal tail of the receptor to inhibit their signaling properties. Until recently, receptor desensitization was believed to fulfill the physiological role of a feedback mechanism limiting both acute and chronic overstimulation of GPCR signal transduction cascades. In

contrast to this notion, yet another line of thinking has recently surfaced pointing to desensitization as an independent physiological form of signal transduction serving to filter and integrate the multiple GPCR information inputs into a meaningful biological signal in a cell (Daaka et al., 1998). The finding of Daaka et al. (1997a) that desensitization may lay the basis for β-AR stimulation of the Ras-dependent MAPK pathway is a clear indication that these processes are much more complex than originally envisaged. The possibility of this double-pronged role of desensitization in regulating β-AR signaling is certainly interesting and demands further investigation. There are several issues that must be resolved to comprehend these mechanisms. The relative contribution of second messenger-dependent PKs and GRKs to the overall manifestation of desensitization is still unclear. Essential questions relating to the determinants of the decision by the cell as to which pathway and under what conditions the desensitization course proceeds have not been settled. This awaits the clarification and better understanding of the actual physiological roles of the different forms of desensitization.

If agonist-induced receptor desensitization persists for a period of hours to days, it may ultimately lead to a decrease in the total cellular complement of GPCRs commonly termed receptor down-regulation (Bouvier et al., 1989; Hadcock et al., 1989; Hausdorff et al., 1990). This decrease in receptor numbers is in part a product of elevated lysosomal degradation of preexisting receptors as well as decreased mRNA and protein synthesis (Hadcock and Malbon, 1988). The agonist-dependent component of β_2-AR down-regulation may require intact coupling of receptors to G_s and probably involves both transcriptional and post-transcriptional controls (Hadcock and Malbon, 1991; Suzuki et al., 1992). This event is characterized by the depletion of the cellular receptor content due to alterations in the receptor degradation and synthesis rates, in essence preceded by and in part attributed to a reduction in their steady-state mRNA levels probably mediated in part by changes in mRNA stability (Bouvier et al., 1988). Agonist-induced β_2-AR mRNA destabilization may be regulated by cAMP-dependent RNA-binding protein or proteins via a specific adenine/uracil (AU)-rich motif at positions 329 to 337 of its 3′-untranslated region as the responsible cis-acting element essential for the agonist sensitivity of the receptor mRNA (Tholanikunnel and Malbon, 1997; Danner et al., 1998). Other mechanisms for β-AR down-regulation include drug tolerance-induced processes and receptor degradation similarly characterized by depletion of the cellular receptor content and mediated by alterations in the rates of receptor degradation and synthesis (Bouvier et al., 1988). It has also been speculated that enhanced receptor degradation may occur in part as a result of PKA-dependent phosphorylation transforming the receptors into better targets for desensitization.

B. Regulation of β-Adrenoceptor Turnover

The β-AR turnover is regulated by two important features: phosphorylation and internalization, also known as sequestration. Once the receptors have completed their signaling circuit, regeneration of their functionality takes place through phosphorylation by specific PKs followed by dephosphorylation by PPs to reactivate them. Like phosphorylation, receptor dephosphorylation is tightly regulated. Physiological dephosphorylation of activated β-AR is mediated by a plasma and vesicular membrane-associated form of PP-2A by a mechanism, which is not fully understood yet (Pitcher et al., 1995). It appears to involve a multipoint attachment of the kinase and its substrate with the specificity being restricted by both the primary amino acid sequence and conformation of the substrate. Although PK-mediated receptor phosphorylation constitutes the first step in terminating and restoring GPCR signaling capacity, the recovery of their functionality after GRK-mediated phosphorylation seems to follow a more sophisticated route. It depends not only on appropriate interactions of multiple molecular events within the cytoplasmic region of the receptors but also on conformational limitations that may determine their orientation (Jockers et al., 1996). A number of suggestions have been discussed with regard to the role of GRK-mediated phosphorylation of GPCRs. Among others, it may serve to stabilize the receptor conformation required for internalization or to act as a signal-promoting receptor binding of some other intracellular components directly mediating receptor sequestration (Ferguson et al., 1996c). Sequestration describes the spatial removal and translocation of plasma membrane receptors to an intracellular compartment into endosomes in response to agonist stimulation and often varies among the different receptors. Although strong evidence points to an important role of GRK-mediated phosphorylation in β_2-AR desensitization and as a signal promoting β_2-AR internalization, the role of phosphorylation by GRK or PKC in GPCR sequestration is still quite controversial (Ferguson et al., 1996a,b). Early studies using mutant β_2-AR that lacked phosphorylation sites indicated that it is not prerequisite for this process (Lohse et al., 1990). On the other hand, when cells expressing endocytosis-deficient receptors were cotransfected with GRKs, the receptor was phosphorylated and their movement from the cell surface to internal compartments (endocytosis) is rescued, indicating that phosphorylation might in fact act as an endocytosis signal (Ferguson et al., 1995). Apparently, the phosphate groups added to β_2-AR in response to agonist stimulation do not have any signaling function in the endosomes. It is nevertheless possible that the activated state of the receptor may influence their fate on reaching the endosomes.

Besides contributing to GPCR desensitization by uncoupling their signal transduction processes, β-arrestins also play an integral role in targeting these receptors for internalization (sequestration) into endosomal vesicles in which receptor dephosphorylation and resensitization occur (Ferguson et al., 1996b; Goodman et al., 1996; Zhang et al., 1997c). This was demonstrated by experiments in which both β_2-AR desensitization was augmented and its sequestration was promoted by the overexpression of β-arrestin-1 or -2 (Ferguson et al., 1996b). Moreover, agonist-mediated sequestration was substantially attenuated by the reduction or removal of the β_2-AR ability to be phosphorylated by GRK2 or to interact normally with β-arrestin, whereas V53D, a dominant negative mutant of β-arrestin-1, was capable of impairing receptor endocytosis (Ferguson et al., 1996b). In general, GPCRs can use variable but distinct endocytotic pathways distinguishable by nonvisual arrestins, β-arrestin and arrestin-3, and dynamin, a GTPase that regulates the formation and internalization of clathrin-coated vesicles (Zhang et al., 1996). Based on the observation that β-arrestin-2 binds to phosphorylated nonactivated β_2-AR, albeit not as well as it does to phosphorylated activated receptor, it was postulated that although phosphorylation itself causes the recruitment of β-arrestin and hence endocytosis, agonist stimulation is required for maximal endocytosis. Thereby, β-arrestin-1 would function as a clathrin adaptor in receptor endocytosis, which is regulated by dephosphorylation at the plasma membrane. The recruitment of the constitutively phosphorylated cytoplasmic β-arrestin-1 to the plasma membrane by the agonist stimulation of the receptor, where it is rapidly dephosphorylated is a requirement for its clathrin binding and receptor endocytosis but not for its receptor binding and desensitization. It is this event that apparently transforms it into a clathrin adaptor and controls the process of receptor endocytosis (Lin et al., 1997). The rephosphorylation of the β-arrestin-1 occurs after its internalization. In addition, the β-arrestin mediates the interaction between the β_2-AR and clathrin (Goodman et al., 1996) via direct interaction with clathrin (Goodman et al., 1996, 1997). These findings led to the notion that the arrestins act as GPCR-trafficking elements that specifically target them to endocytotic organelles for clathrin-dependent endocytosis (Goodman et al., 1996; Ménard et al., 1996; Zhang et al., 1996; Ferguson et al., 1997). The predominant clathrin-binding domain of β-arrestin-1 and -2 is localized to C-terminal residues 371 to 379, and both the hydrophobic Leu[373], Ile[374], and Phe[376] and the acidic Glu[375] and Glu[377] residues are probably essential in the arrestin-3/clathrin interaction (Goodman et al., 1997; Krupnick et al., 1997). Apparently, the regulation of endocytosis by β-arrestin-1 is also distinguishable from its regulation of desensitization by its C-terminal Ser[412] phosphorylation, which is not a feature of the latter process (Lin et al., 1997). For β_2-AR, the second and third intracellular loops and the C-terminal dileucine (Leu[339] and Leu[340]) motif have been identified as the major determinants of agonist-promoted desensitization

and sequestration (Jockers et al., 1996; Gabilondo et al., 1997). Phosphorylation may also trigger a conformational change in the β_2-AR in common with the epidermal growth factor receptor, which either enhances interactions with intracellular adaptor proteins or exposes cryptic motifs that interact with intracellular adaptors. However, there is not much evidence to support this notion. Ferguson et al. (1997) have postulated that receptor sequestration is likely to be accomplished by both pathways, but the preferred mechanism of endocytosis used by a particular receptor depends on both receptor-specific structural factors and the cellular environment in which it is expressed. Put together, the modern view is that GRK-mediated phosphorylation and β-arrestin binding do not only serve to uncouple GPCRs from the heterotrimeric G proteins but also mediate GPCR internalization specifically via dynamin-dependent clathrin-coated vesicles (Fig. 2). The precise endocytotic mechanism by which GPCR internalization is attained remains, however, controversial because β_2-AR sequestration is also affected by clathrin-coated vesicles and perhaps caveolin (Ferguson et al., 1996b,c).

The events involved in the reestablishment of receptor responsiveness after endocytosis are less clear. It is currently thought that the receptor is dephosphorylated by specific phosphatases and subsequently mobilized back to the plasma membrane as fully functional receptors after its sequestration to the endosomal compartment (Ferguson and Caron, 1998). Nonrecycled receptors may be directed to lysosomes for degradation via distinct endocytotic mechanism (Bouvier et al., 1988; Zhang et al., 1996). A small number of sequestered receptors may be subject to endosomal sorting to lysosomes on each sequestration cycle (Fig. 2). It is not certain, however, whether receptor sequestration represents the first step in lysosomal degradation of GPCRs contributing to receptor down-regulation. Among others, a conformational change in the receptor induced by acidification of the endosomal compartment may play a major role in regulating receptor dephosphorylation and resensitization (Krueger et al., 1997). Interestingly, the same proteins, GRKs, and β-arrestins mediating the desensitization of GPCR responsiveness have been shown to contribute directly not only to sequestration but also to resensitization of several GPCRs, including the β_2-AR (Zhang et al., 1997c). Cell and tissue-type differences may exist in the GPCR resensitization depending on β-arrestin expression (Zhang et al., 1997c). The resensitization of second messenger-dependent PK-phosphorylated receptors also seems to require β-arrestin-dependent endocytosis. Presumably, both β_2-AR dephosphorylation and resensitization depend on an intact sequestration pathway. Because receptor sequestration is initiated within seconds to minutes of receptor activation, it was initially thought that this process contributes to receptor desensitization by limiting the number of plasma membrane-accessible receptor binding sites. However, according to

current understanding, the majority of the sequestered receptors are already desensitized as the consequence of phosphorylation. Instead, the dual role of both GRKs and β-arrestins in receptor regulation by mediating both receptor uncoupling and sequestration indicates that instead of playing a role in receptor desensitization, sequestration might be more important in mediating the resensitization of desensitized receptors (Ferguson et al., 1997).

VI. Regulation of β-Adrenoceptor Functional Expression in Cardiac Disease

A. β-Adrenoceptor Functional Expression in Heart Failure

Heart failure vindicates a state in which the heart becomes incapable of adequately meeting its circulatory demands without some form of assistance. It describes an end-stage scenario usually of a heart muscle disease that can be chronic or acute in terms of the time course. It became apparent as early as two decades ago that heart failure is always accompanied by an elevation in circulating catecholamines, particularly norepinephrine, with highest levels being associated with poorest prognosis (Packer et al., 1987). The elevated plasma norepinephrine levels in patients with chronic and congestive heart failure were perceived as being reflective of heightened sympathetic activity (Francis and Cohn, 1986; Prichard et al., 1991). It is therefore not surprising that in general, the functional expression of the β-AR in cardiac disease has classically been seen in light of the severity of heart failure. This notion led to a surge of studies in the late 1980s in which a dependence on the severity of heart failure was established in the reduction in β-AR density and responsiveness to agonists in chronic or end-stage heart failure associated with idiopathic dilated or congestive cardiomyopathy. The attenuation in receptor density was explained as a product of enhanced sympathetic drive to the heart and hence endogenous down-regulation by an elevated release of (cardiac-derived) norepinephrine (Ruffolo and Kopia, 1986; Bristow et al., 1988). Thus, although an elevated sympathetic function may initially furnish useful support for the failing heart, in the long run it is likely to deplete the heart of the functional responsiveness of its β-ARs to catecholamines that may similarly contribute to the loss of cardiac contractility (Brodde et al., 1991). In part, because of lack of substantial receptor reserve, this would augment the inability of the contractile apparatus to sustain adequate inotropic function (Brodde et al., 1992). These changes are particularly evident in the human ventricular myocardium, where the reduction in receptor density is selective for the β_1-AR subtype, with the levels of β_2-AR remaining unchanged (Bristow et al., 1986; Brodde, 1991). However, in dogs with right heart failure, the reduction in β_1-AR density was shown to occur only in the failing right ventricle, whereas cardiac

inotropic responses to receptor stimulation were reduced in both the right and left ventricles (Lai et al., 1996). This was interpreted as a result of the β_1-ARs being stimulated to a greater extent by norepinephrine released from the sympathetic nerve endings than β_2-ARs that are essentially stimulated only by circulating epinephrine in the failing human heart. It is, however, believed that the β_2-ARs become somewhat uncoupled (Brodde, 1991). The decrease in β_1-AR density in the failing human left ventricle is accompanied by a similar reduction in β_1-AR mRNA levels (Ihl-Vahl et al., 1996), which appears to correlate with the severity of heart failure (Engelhardt et al., 1996). This is not due to enhanced internalization but rather to a physiological loss of receptors (Pitschner et al., 1993). It is therefore the reduction in β_1-AR synthesis, as indicated by its reduced mRNA levels, that is probably responsible for its reduced function in heart failure. In contrast, the β_2-AR mRNA appears to be unaffected in concordance with the reported sustenance of the receptor density (Brodde, 1991).

The attenuated responsiveness of the β-AR system in heart failure resembles in many ways the phenomena observed in agonist-induced receptor desensitization and may contribute to contractile dysfunction in a chronic situation (Lohse, 1996). Although the manifestation of a reduction in both receptor density and mRNA implies that heart failure causes a down-regulation of the myocardial β_1-AR pathway, until recently, it has been difficult, if not impossible, to discern events leading to receptor desensitization from those that lead to their down-regulation in vivo. Both phenomena were ascribed partly to an elevated β_1-AR stimulation by norepinephrine released from the sympathetic nerves in an effort to restore normal cardiac inotropic function and, to a lesser extent, as being triggered by a stimulation of β_2-AR by circulating epinephrine (Bristow et al., 1986). A selective down-regulation of the β_1-AR population would therefore markedly reduce the ability of selective β_1-AR partial agonists to mediate a positive inotropic response, whereas selective β_2 agonists retain near-full positive inotropic activity mediated through a β_2-AR population that is not significantly decreased.

The notion that heart failure triggers β-AR down-regulation has obviated substantial interest in defining precisely the modes by which altered β-AR expression may influence the function of the downstream effector components and the end products of their signal transduction. The main focus has centered on those components operating in the immediate vicinity of the β-AR transduction circuit, such as the G proteins and the AC. Apart from β-AR down-regulation and uncoupling, several other signal transduction defects leading to a reduction in AC activity have also been described in heart failure, such as an increase in the G_i expression or a defect in the enzyme catalytic unit. It is now established that the basal, β-AR and guanosine-5'-(β,γ-imido)-triphosphate [Gpp(NH)p]-stimulated AC activities are significantly decreased in patients with severe heart failure (Feldman et al., 1988; Böhm et al., 1990; Bristow and Feldman, 1992; Brodde et al., 1998). In addition, the increase in AC due to isoprenaline infusions is depressed (Fan et al., 1987; Bristow et al., 1989). In patients undergoing heart transplant, the positive inotropic and cAMP-elevating effects of β-AR agonists and PDE inhibitors are diminished in the failing heart (Brodde et al., 1992). This notion has been supported by several studies in isolated cells and animal models of heart failure showing a reduction in both the receptor-dependent and -independent AC pathways in heart failure (Allen et al., 1989; Suzuki et al., 1992). Observations of prolonged down-regulation and diminished AC responses to sustained agonist stimulation of β-ARs in isolated cells suggest that the reduction in the enzyme activity is due to its increased exposure to adrenergic stimulation (Bristow et al., 1988, 1989; Kaumann et al., 1989; Suzuki et al., 1992). Thus, β-AR down-regulation in heart failure may explain in part at least the diminished inotropic and cAMP response to β-AR agonists (Brodde, 1996). However, other factors may be involved in the manifestation of these changes. For example, the fact that agonist-dependent β-AR down-regulation of AC can be dissociated from the attenuation in the enzyme activity led to the postulation of a postreceptor defect regulating some of the receptor-independent changes in the AC function in heart failure (Reithmann et al., 1997). Initially, a defect at the catalytic subunit of AC was postulated to contribute to the decreased effectiveness of cAMP-increasing agents observed in severely failing hearts of patients with congenital heart disease. This was supported by the observation that forskolin-stimulated AC activity that uncouples its catalytic subunits from G_s and G_i in the presence of Mn^{2+} is also markedly reduced by β-AR agonists in failing human hearts (Reithmann et al., 1997). Nevertheless, it remains doubtful whether the AC catalytic activity is altered in heart failure (Böhm, 1995). Some studies have suggested that in the failing myocardium, the effect of forskolin is reduced only in the presence of GTP (Böhm et al., 1990) or after the addition of Mn^{2+} (Feldman et al., 1988). It is therefore still debatable whether a postreceptor defect exists and, if it does, whether it has any particular role to play in cardiac function or disease.

The AC function may also be regulated at the G protein-coupling level. Thus, implicit in the antagonistic functions of the G_s and G_i proteins and their coexistence in the myocardium is that the latter may serve a feedback regulatory mechanism to control the stimulatory function of the G_s protein. Therefore, an alteration in the coupling efficiency particularly of the G_s would automatically trigger an attenuation of cAMP synthesis. Interestingly, although different methods used so far to quantify changes in $G_{s\alpha}$ function in human heart failure have produced somewhat inconsistent results, this protein

seems to remain unaltered in heart failure. The earliest experiments using the principle of ADP ribosylation of the $G_{s\alpha}$ catalyzed by cholera toxin indicated that heart failure exerts no influence on human $G_{s\alpha}$ function (Schnabel et al., 1990). The inability of this procedure to detect a significant change in $G_{s\alpha}$ expression was initially attributed to the rather low labeling efficiency of the ribosylation system. However, ensuing experiments using Western blotting (Böhm et al., 1992b), polyclonal rabbit antisera, and reconstitution experiments in cell membranes from S49 cyc⁻ mouse lymphoma cells confirmed the finding that $G_{s\alpha}$ is not influenced in human failing myocardium (Feldman et al., 1988). The steady-state $G_{s\alpha}$ mRNA levels also remained unchanged (Eschenhagen et al., 1992) or even slightly increased in some instances (Feldman et al., 1989), indicating that its coupling efficiency is at least not decreased in heart failure. In contrast, an increase in the $G_{i\alpha}$ in heart failure was first observed by Neuman et al. (1988) in failing human heart and later confirmed as a 40-kDa protein in idiopathic dilated cardiomyopathy by Feldman et al. (1989). Initially, some studies were unable to confirm this increase in immunoreactive $G_{i\alpha}$ in dilated cardiomyopathy (Feldman et al., 1991). These discrepancies may be due to species differences but can also be explained at least in part on the basis of the differences in the sensitivity of the methodologies used to quantify the proteins. The G_{α} subunits have GTPase activity, and most also have ADP ribosylation sites for cholera toxin or PTX, which have been used to study their functional expression (Gilman, 1990). The accuracy of this methodology depends on a number of factors such as biophysical membrane properties, post-translational modifications of $G_{i\alpha}$ proteins, and several cofactors required for the ADP ribosylation reaction. Moreover, PTX labeling is enhanced by GTP, GDP, and Gpp(NH)p derivatives, whereas Western blotting also has a lack of precision. Because the $\beta\gamma$ complex facilitates ADP ribosylation of the G_{α} subunits, preexisting covalent modifications at the C terminus of the G_{α} proteins could directly or indirectly influence the ADP ribosylation, just like its phosphorylation or lipid modifications of G_{α} or $\beta\gamma$ subunits (Schnabel and Böhm, 1995). It was later confirmed with the use of a radioimmunoassay with purified transducin-α as standard and its labeled C-terminal synthetic peptide as a tracer that the immunoreactive $G_{i\alpha}$ is increased in cardiac muscle disease associated with a manifestation of heart failure (Böhm et al., 1992b). Studies for animals further showed that G_i protein could be regulated at the transcriptional level by β-AR agonists. In neonatal rat cardiomyocytes, an increase in the PTX substrates was observed after their culture in the presence of norepinephrine, and treatment of the rats with isoprenaline resulted in an increase in the transcriptional rate of the $G_{i\alpha2}$ and $G_{i\alpha3}$ mRNA levels (Eschenhagen et al., 1992). Nuclear run-on assays also showed an increase in the transcriptional rate of $G_{i\alpha2}$

gene after isoprenaline treatment (Müller et al., 1993). An up-regulation of $G_{i\alpha2}$, but not of $G_{i\alpha3}$, mRNA was then proposed as the sole contributor to the increase in $G_{i\alpha}$ protein and therefore the pathophysiological process leading to reduced responsiveness to cAMP-increasing agents in end-stage heart failure. It is a paradigm now that in human heart failure, the reduction in the positive inotropic and cAMP-elevating effects of both β-AR agonists and PDE inhibitors is a product of an imbalance between the G_i and G_s proteins, triggered by an alteration in only one of them (Feldman et al., 1989, 1991; Eschenhagen, 1993). In dogs with right heart failure, the reduction in β_1-AR was associated with a nonselective reduction in G_s protein and none in the G_i protein (Lai et al., 1996). This would be an example of a perturbation in the balance of the two proteins as a result of a decrease in G_s rather than an increase in the G_i protein, effectively denoting an alternative route to the desensitization of the system. In theory, increased function of the G_i-mediated inhibitory pathway may compromise the ability of the failing heart to generate sufficient cAMP as a result of its inhibition of AC or by increased generation of dissociated $\beta\gamma$ subunits that might bind to and inactivate $G_{s\alpha}$ (Morello and Bouvier, 1996). This may further attenuate β-AR-mediated increases in cardiac contractility or heart rate and may explain why the positive inotropic effect of all other receptor systems acting by elevation of cAMP appears to be reduced in the failing human heart. A further point of interest is that the cAMP-responsive element binding proteins are also expressed and phosphorylated in human myocardium (Schnabel and Böhm, 1995; Monaco and Sassone-Corsi, 1997). This implies that an increase in cellular cAMP might increase the gene transcription of the $G_{i\alpha2}$ by cAMP-responsive element binding proteins that activate its promoter regions after cAMP-dependent phosphorylation, thereby rendering it a convenient feedback loop for AC activity. In addition, G protein function may theoretically be altered in disease states secondary to mutations in its gene, altered expression, post-translational modifications, or other mechanisms. Thus, increased cAMP production could result from constitutive activation of G_s by activating mutation or cholera toxin-catalyzed modification, PTX-catalyzed modification, as well as inactivation mutation of G_i protein, whereas inactivation mutations of G_s or activation mutations of G_i should result in decreased cAMP production. However, this remains only a theoretical possibility for the time being.

Studies using single myocytes revealed agonist-induced phosphorylation and desensitization of cardiac β-AR by GRK2 (Korzick et al., 1997), corroborating an important role for GRKs in modulating cardiac function. Transgenic mice with cardiac-specific overexpression of GRK2 exhibit attenuated isoproterenol-stimulated left ventricular contractility in vivo, causing a dampening of myocardial AC activity and, therefore, reduced

functional coupling of β-AR. On the other hand, mice expressing a GRK2 inhibitor express enhanced cardiac contractility in vivo with or without isoproterenol (Koch et al., 1995). The expression of GRK2 mRNA is also significantly increased in the cardiomyopathic Syrian hamster model of congestive heart failure (Urasawa et al., 1996). Accordingly, this enhanced GRK2 expression might provide a negative feedback mechanism to maintain intracellular homeostasis against accelerated stimulation by catecholamines via phosphorylation of β-AR in congestive heart failure. These observations illustrate the potential role of the receptor kinase in enhancing phosphorylation and hence uncoupling of β-AR from the G_s protein, implicating GRKs as critical determinants of the cardiac β_1-AR contractile response; therefore, in the failing heart of patients with idiopathic dilated cardiomyopathy, both the down-regulation of β_1-AR and the uncoupling of β_2-AR are accompanied by an increase in GRK2 mRNA and activity in association with reduced myocardial β-AR responsiveness (Ungerer et al., 1993; Ping et al., 1995). The increase in GRK2-mediated phosphorylation of both β_1- and β_2-ARs in heart failure may contribute to the loss of their responsiveness, leading to impairment of their function through receptor uncoupling (Bouvier et al., 1989; Kaumann et al., 1989; Lohse et al., 1996).

The increase in GRK functional expression might imply that their complementary counterparts, the arrestins, are influenced in a similar fashion in heart failure. Surprisingly, until now, the contrary seemed to be true, at least in heart failure. Thus, in contrast to a slight increase in the GRK3 and a 3-fold increase in the GRK2 mRNAs, the steady-state protein and mRNA and the activities of cardiac β-arrestin-1 and β-arrestin-2 remain unchanged in heart failure (Ungerer et al., 1994). Therefore, in heart failure, the events leading to β-AR desensitization are probably regulated primarily at the level of receptor phosphorylation by the GRKs, without affecting the level of the arrestin function. Besides homologous desensitization mediated by receptor phosphorylation by GRKs at the agonist-receptor-G protein circuit level, theoretically heterologous desensitization might also occur at the level of receptor phosphorylation by PKs. At present, very little information is available regarding possible changes in the PK function and how they may influence β-AR signaling in heart failure. In transfected HEK 293 cells, agonist-induced β_1-AR phosphorylation apparently derives approximately equally from PKA and GRK activity (Freedman et al., 1995). It appears, nevertheless, that the steady-state protein, mRNA, and activity of PKA are not altered in heart failure (Böhm et al., 1994). Together, in heart failure, the down-regulation of β_1-ARs and uncoupling of β_2-ARs are associated with an increased activity and gene expression of GRK2 and $G_{i\alpha}$ protein. In contrast, the AC catalytic subunit and $G_{s\alpha}$ and $G_{\beta\gamma}$ subunits remain unchanged. To date, no defects in the AC or G

proteins have been convincingly demonstrated to influence cardiac function in the presence of an adequately functional β-AR system. On the other hand, alterations in β-AR themselves remain the unequivocally established central trigger for the changes in the level of downstream transduction of the catecholamine-signaling messages; therefore, the search for the underlying cause for changes in the β-AR–signaling pathway in heart failure may have to be directed primarily at factors that regulate the receptor turnover.

B. Left Ventricular Hypertrophy

In general, the manifestation of left ventricular hypertrophy is almost always associated with hypertension, left ventricular pressure, or volume overload diseases. This increase in the left ventricular mass can occur as a pathological consequence of the overload or develop as a compensatory mechanism, possibly to reduce systolic stress on the left ventricle, as in hypertension. Besides heart muscle disease, chronic pressure overload and the associated cardiac hypertrophy are considered to be not only very common causes but also predictors for the development of chronic heart failure (Böhm et al., 1997). However, the cellular markers contributing to the progression from compensated hypertrophy to heart failure have yet to be identified. Understandably, endeavors have also focused on comprehending β-AR functional expression in left ventricular hypertrophy. As in heart failure, β-AR desensitization associated with elevated sympathetic activity has been implicated in the development of overload-related and hypertensive cardiac hypertrophy, as well as progression from hypertrophy to heart failure (Goldstein and Kopin, 1990; Castellano and Böhm, 1997). Early studies in aortic banded dogs showed reduced affinity and increased β-AR density but normal isoproterenol-stimulated AC activity in association with left ventricular hypertrophy (Vatner et al., 1984). More recently, however, myocardial β-AR density was found to be is comparably decreased in both primary and secondary left ventricular hypertrophy in the presence of preserved left ventricular systolic function (Choudhury et al., 1996). It remains to be established whether hypertensive patients who develop heart failure are more prone to β-AR desensitization or whether early intervention to reduce sympathetic activity is more effective in preventing or delaying the transition from compensated hypertrophy to overt failure.

A desensitization of AC stimulation by isoprenaline and depression of its Gpp(NH)p-mediated activity has been demonstrated in association with receptor down-regulation in animal models of volume-overload hypertrophy after circulatory congestion (Böhm et al., 1992a, 1994, 1997; Hammond et al., 1992). The decrease in AC function is apparently not associated with a change in its catalytic subunits and the $G_{s\alpha}$ but is accompanied by an elevation in $G_{i\alpha}$ levels in the absence of β-AR down-regulation (Böhm et al., 1994). Although the underlying

mechanisms for the AC desensitization in animal models of hypertensive cardiac hypertrophy are often different between the heterogeneous models for acquired and genetic hypertension, alterations in G_i protein and β-AR down-regulation have been observed frequently in association with this desensitization. Some evidence suggests that increases in $G_{i\alpha}$ also depress AC in compensated cardiac hypertrophy in monogenic, polygenic, and secondary hypertension (Schnabel and Böhm, 1996). Because cardiac hypertrophy in pressure overload is a strong predictor of cardiac failure, AC desensitization by $G_{i\alpha}$ could therefore be a pathophysiologically relevant mechanism contributing to the progression from compensated cardiac hypertrophy to heart failure (Böhm et al., 1996). To date, there is hardly any documentation of GRK expression in cardiac hypertrophy secondary to hypertension. Pressure overload cardiac hypertrophy in the mouse has been associated with an increase in cytosolic and membrane GRK activity, leading to the suggestion that this is related to neurohumoral activation rather than to the induction of the disease (Choi et al., 1997). In contrast to heart failure, however, the immunoreactive amounts of cytosolic PKC-α, -ψ, and -ζ have been reported to be increased significantly, resulting in stimulation of cAMP-dependent PDE activity in hypertrophic cardiomyopathic hamster hearts (Cai and Lee, 1996). The implications are that signaling systems other than the AR pathway may be sensitized in this disease acting via the PKC stimulation. However, further studies are necessary to reach a general consensus on the potential role of desensitization mechanisms involved in this disease.

C. Left Ventricular Overload Diseases

Although the elevation in catecholamines and attenuation in $β_1$-AR density has been ascribed primarily to heart failure, it is now well established that heart failure per se is not a prerequisite for the manifestation of these phenomena in cardiac disease. Left ventricular overload disorders resulting from a stenosis or tear of the aortic and mitral valves are examples of cardiac diseases in which the severity of the disease depends on factors such as the hemodynamic function rather than the existence of heart failure. In these diseases, therefore, the observed changes can be related in terms of a measurable physiological variable rather the extent of heart failure. In heart valvular patients, although a rise in epinephrine is more closely related to left ventricular pressure overload, a similar but significantly greater rise in norepinephrine is closely associated with left ventricular volume overload (Dzimiri et al., 1996a). Both myocardial $β_1$-AR and lymphocyte $β_2$-ARs are attenuated in patients with left ventricular overload diseases in the absence of severe heart failure (Sylvén et al., 1991; Dzimiri et al., 1996c). The reduction in receptor density is significantly greater in left ventricular volume overload than in pressure overload (Dzimiri et al.,

1996c). It is most evident in the left ventricle and correlates well with the hemodynamic variables, such as the left ventricular end-diastolic and end-systolic pressures or dimensions as well as ejection fractions. This attenuation in receptor density is accompanied by a similar reduction in the mRNAs, which is greater for $β_2$-AR than for $β_1$-AR. This implies that the overload-induced effects are not selective for $β_1$-AR but rather involve both subtypes and often affect the $β_2$-AR more than the $β_1$-AR (Dzimiri et al., 1998a). Moreover, the volume overload emanating from aortic regurgitation seems to reduce the β-AR density more effectively than the overload from mitral regurgitation, depending on the severity of the overload (Dzimiri and Moorji, 1996a). These changes may therefore tell us about the nature of the source and the severity of the overload.

In volume overload patients, not only the AC basal and β-AR-mediated activities but also the sodium fluoride (NaF)-, manganese-, and forskolin-stimulated activities are attenuated in circulating lymphocytes and myocardium (Dzimiri et al., 1998c). This notion finds support in animal models of heart valvular regurgitation and pressure overload showing attenuation in β-AR-coupled AC function in both right and left ventricles (Hammond et al., 1992; Suzuki et al., 1997). The forskolin-dependent activity is significantly attenuated in the presence of Gpp(NH)p, possibly pointing to a sensitization of G_i protein-coupled pathways. These observations suggest that apart from an attenuation of the β-AR-mediated signaling, volume overload may induce heightened activity of pathways that couple to the G_i protein.

Left ventricular volume overload diseases are also associated with a significant increase in the expression of the β-AR-specific GRK2 and GRK3 both at mRNA and protein level in lymphocytes and in the myocardium (Dzimiri et al., 1998a). Interestingly, although GRK5 mRNA is often undetectable in the lymphocytes of the normal healthy blood donors, it is consistently and highly expressed in the patients, suggestive of a de novo expression of this gene as a result of the overload (Dzimiri et al., 1998b). The elevation of the three GRKs in the myocardium appears to be chamber-specific depending on the source of the overload (unpublished observations). Interestingly, these alterations are accompanied with an increase in the expression of lymphocyte β-arrestin-2, but not β-arrestin-1, mRNA. This coexistence of a reduction in $β_2$-AR in conjunction with elevated GRK2 and GRK3 as well as β-arrestin-2 levels vindicates a condition favoring $β_2$-AR desensitization and down-regulation of both myocardial $β_1$-AR and lymphocyte $β_2$-AR. An important difference in heart failure or hypertrophy, however, is the observation of a significant elevation in both the PKC and the cAMP-dependent PKA activities in association with reduced expression of $β_2$-AR in lymphocytes of patients with left ventricular overload disease. A sensitization of PKA indicates that the $β_2$-ARs are also chronically subjected to elevated condition of

heterologous desensitization in left ventricular overload disease. Together with the possible existence of sensitized G_i protein-mediated signaling, this seems to corroborate the existence in humans of a mechanism to switch β_2-AR coupling from the G_s to the G_i under conditions of desensitization to stimulate mitogenesis via the MAPK pathway, as suggested to occur in HEK 293 cells by Daaka et al. (1998).

D. Hypertension

Hypertension is a circulatory disease characterized by sustained elevation of blood pressure. It is often defined as mild (borderline) or severe depending on the blood pressure levels. The disease can be genetic in origin (also termed primary or essential) or may occur as a secondary product of either cardiac diseases such as congenial heart diseases or interactions with environmental factors, such as a high salt diet. As in left ventricular overload disorders, the extent of heart failure is not a primary predictor of the severity of the disease. Only a handful of studies in animals have described β-AR function in environment-mediated hypertension. In salt-sensitive hypertensive Dahl rats, a heterologous desensitization and depressed AC catalytic activity may be caused by an increase of $G_{i\alpha}$ proteins, possibly indicating that heterologous AC desensitization can precede the development of contractile dysfunction in later stages and can occur independently of changes in β-ARs (Böhm et al., 1993). In pulmonary hypertension, the β-AR density apparently correlates directly with the mean pulmonary arterial pressure accompanied with a marked depletion of tissue norepinephrine and neuropeptide Y, as well as decreased AC stimulation by Gpp(NH)p and forskolin (Lopes et al., 1991). In general, pressure-overloaded failing hearts of patients with primary pulmonary hypertension seem to consistently exhibit a decrease in β-AR that is specifically localized to the right ventricles, in association with decreased AC stimulation by $MnCl_2$, pointing to an attenuation in the activity of the AC catalytic subunit (Bristow et al., 1992).

The fact that primary hypertension has a genetic component prompted association studies relating its manifestation with the prevalence of certain polymorphisms in genes coding for proteins such as β-AR receptors and angiotensin-converting enzyme (ACE) known to be involved in cardiac function. Studies addressing the functional expression of β-AR have produced inconsistent results, with some suggesting variations in AR responses as being dependent on population or ethnic variables (Sherwood and Hinderliter, 1993). Some studies found no changes in lymphocyte β-AR density in essential hypertension (Uchiyama et al., 1992; Kahan et al., 1998). However, although hypertension per se may have no direct influence on β-AR signaling, receptor desensitization has been implicated in the pathophysiology of hypertension as well as progression from hypertrophy to heart failure (Goldstein and Kopin, 1990; Castellano and

Böhm, 1997). Thus, a general consensus began to form about a decade ago that most β-AR alterations occur secondary to blood pressure elevation regardless of whether hypertension is genetic and that the mechanisms regulating AR responsiveness on prolonged agonist exposure may be altered in hypertension, thereby contributing to the pathophysiology of the disease (Michel et al., 1992). Accordingly, a hyperadrenergic state might be of some relevance to the pathogenesis of primary hypertension, especially in young adolescents. In one study, the forskolin-induced cAMP production was decreased without a change in β_2-AR and isoprenaline-stimulated AC activity in leukocytes of patients with primary hypertension (Blankesteijn et al., 1993). It was also suggested that the increase in cAMP levels in hypertension is due to an enhancement of the active transport of the cAMP (Mills et al., 1994). An absence of significant alteration in receptor density or AC basal and Gpp(NH)p- and forskolin-stimulated activities, in contrast to a reduction in isoproterenol-stimulated enzyme activity, was also suggested as indicating a reduction in the G_s protein caused by a defect in the protein (Kessler et al., 1989; Yurenev et al., 1992; Yoshikawa et al., 1994). The mechanisms are often different between the heterogeneous models for acquired and genetic hypertension, but alterations in G_i protein and β-AR downregulation have been observed frequently. Interesting, primary hypertension may increase the functional expression of β-AR-specific GRK2 in correlation with a decrease in β-AR-stimulated AC activity (Gros et al., 1997). The finding that GRK2 is increased in both left ventricular overload and hypertension therefore suggests that systemic stress leads to an increase in its functional expression. Furthermore, the fact that in volume overload GRK5 and β-arrestin-2 are elevated in addition to GRK2 clearly points to specific differences in the regulation of GRK function by different cardiac diseases and in heart failure. The underlying mechanism for desensitization is most likely a sympathetic activation in established hypertension rather than genetic alterations of signal transduction proteins (Castellano and Böhm, 1997). Therefore, the changes in β-AR seen in primary hypertension are probably a product of the secondary effects of hypertension rather than of it being the primary cause.

E. Ischemic Heart Diseases

Ischemic heart diseases often occur as a result of an obstruction in the vascular bed, leading to compromised blood circulation. Studies in animal models have produced somewhat inconsistent results with respect to the functional level of β-AR-mediated signaling in ischemic heart disease. Wistar rats with ischemic heart failure induced by coronary artery ligation show no changes in $G_{s\alpha}$ and $G_{i\alpha}$ concentrations as well as the basal and $MnCl_2$-stimulated AC activities (Yamamoto et al., 1994). Myocardial NaF- and forskolin-stimulated AC activities

are significantly decreased, suggesting the presence of myocardial $G_{s\alpha}$ dysfunction that may contribute to the contractile abnormalities in ischemic heart failure (Yamamoto et al., 1994). In a stop-flow rat model of myocardial ischemia, an increase in β-AR density has been reported that is paralleled by an increase in GRK2 mRNA and membrane activity but no alteration in β-arrestin levels (Ungerer et al., 1996). The increased membrane activity is believed to contribute to receptor phosphorylation and inactivation under these conditions. In a rabbit model of ischemic heart disease, Wolff et al. (1994) found that neither the basal nor the isoproterenol-stimulated AC activity in presence of Gpp(NH)p is changed, which is indicative of preservation of β-AR-mediated AC activity in ischemia. In contrast, the Gpp(NH)p-stimulated enzyme activity is depressed, leading to an increased ratio of isoproterenol- to Gpp(NH)p-stimulated AC activity, which is consistent with a more efficient β-AR signaling via less functional G_s protein. These data could not be explained by augmented β-AR density or agonist affinity or by a reduction in G_i protein-mediated inhibition of AC activity. Moreover, both forskolin and Mn^{2+} significantly increase AC activity. Preservation of isoproterenol-mediated AC activity was therefore postulated as a result of enhanced function of its catalytic subunit (Wolff et al., 1994). The observation of enhanced functional responsiveness to β-AR stimulation by isoprenaline, but not to forskolin, in reperfused myocardium in previously ischemic subendocardium also prompted the notion that isoprenaline exerts an action distal to AC in previously ischemic myocardium (Kiuchi et al., 1994). Although β-AR down-regulation has been demonstrated in animal models of the disease (Schmedtje et al., 1996), human ischemic cardiomyopathy per se does not appear to significantly influence β-AR signaling. On the other hand, the failing hearts of patients with ischemic cardiomyopathies exhibit characteristic alterations in β-AR-signaling components in a fashion similar to other cardiac disorders dependent on heart failure, such as dilated cardiomyopathy or hypertension. Comparatively, isoprenaline-induced AC activation is reduced similarly in dilated cardiomyopathy and ischemic cardiomyopathy, whereas the β_1-ARs may be more markedly down-regulated in ischemic cardiomyopathy. Moreover, in both types of cardiomyopathy, GTP-activated AC is significantly diminished, whereas NaF- and Mn^{2+}-mediated activities remain unaltered. Left ventricular positive inotropic responses to β_1- and β_2-AR are significantly and equally reduced in ischemic cardiomyopathy and dilated cardiomyopathy, suggesting that in both diseases, the functional responsiveness of the receptors is similarly reduced (Brodde et al., 1998). In ischemic cardiomyopathy, the $G_{i\alpha2}$ mRNA is significantly increased in left ventricular myocardium, whereas $G_{i\alpha3}$ and $G_{s\alpha}$ mRNA remain unchanged (Feldman et al., 1991; Eschenhagen et al., 1992). However, there are some definable differences in changes associ-

ated with heart failure between idiopathic dilated cardiomyopathy, ischemic heart disease, and hypertension, for example. Thus, unlike in idiopathic dilated cardiomyopathy, which is characterized by down-regulation of mainly myocardial β_1-AR, both β_1- and β_2-AR are attenuated in patients with end-stage ischemic cardiomyopathy or hypertension (Piatak et al., 1991; Brodde et al., 1992; Pitschner et al., 1994). In idiopathic dilated cardiomyopathy, β_1-AR down-regulation is less pronounced than in ischemic dilated cardiomyopathy and slightly more pronounced in primary pulmonary hypertension. Furthermore, depending on the cause of heart failure, abnormalities of the receptor-G protein-AC system result from a reduced number of β_1-ARs, uncoupling of β_1-AR or β_2-ARs, alteration in G protein function, or decrease in the activity of the AC catalytic subunit. This decrease in the catalytic function is most significant in right ventricular preparations from primary pulmonary hypertension and least prominent in ischemic cardiomyopathy, with idiopathic dilated cardiomyopathy occupying an intermediate position. An alteration in the G_i protein may be the basis for β-AR uncoupling in idiopathic dilated cardiomyopathy and ischemic dilated cardiomyopathy, whereas in primary pulmonary hypertension, this phenomenon may result from altered AC function. In summary, numerous desensitization phenomena occur in the failing human heart that are cause or model dependent. These changes might be beneficial because they can partially protect the failing heart from potentially toxic adrenergic stimuli (Bristow and Feldman, 1992).

F. Cardiac Hypoxic Disorders

Hypoxemia is a disorder characterized by the lack of sufficient oxygen supply to the organ as a result of obstructive or adaptive mechanisms to altered functional conditions as in congenital heart disease. It appears to be a general consensus that exposure to chronic hypoxia results in a lower resting heart rate and a blunted cardiovascular responsiveness to β-AR stimulation. Experimental models suggest that left ventricular membrane β_1- and β_2-AR density in newborn lambs is decreased during chronic hypoxia (Bernstein et al., 1992). Left ventricular isoproterenol-stimulated AC activity is decreased, whereas right ventricular enzyme activity is unchanged, suggesting down-regulation of the left ventricular β-AR-AC system during chronic hypoxemia secondary to an intracardiac right-to-left shunt (Bernstein et al., 1990). A decrease in the density of β-AR in chronic hypoxia has been found in rat left ventricle and in human lymphocytes, without modification of the affinity of β-AR for an agonist or antagonist, and a decreased AC activity in the right ventricle. Left ventricular β-AR density is decreased, and a dissociation occurs between increased chronotropic and decreased inotropic responses to chronically elevated sympathetic tone, which is in part secondary to a differential

regulation of β-ARs between the left ventricle and the right atrium (Doshi et al., 1991). In contrast, an increase in β_1 mRNA and receptor expression has also been observed in chronic hypoxia in neonatal rat cardiac myocytes that is apparently not associated with an alteration in AC activity at either the receptor or the postreceptor level and does not affect agonist-induced β-AR down-regulation or desensitization of AC responses (Li et al., 1996).

In contrast to hypoxemia, chronic exposure high-altitude hypoxia appears to consistently lead to a decrease in the β-AR density, possibly as an adaptive mechanism (Richalet et al., 1988; Antezana et al., 1992). Circulating epinephrine is apparent increased significantly, whereas circulating and myocardial norepinephrine is unchanged. Heart rate and chronotropic responses to isoproterenol infusion are decreased in humans after a few days of exposure to high altitudes. This phenomenon has been linked to a desensitization of β-ARs and/or an increase in parasympathetic activity. Basal and isoproterenol-stimulated AC activities are also decreased in membranes prepared from hearts and pulmonary arteries of rats acclimatized to high altitude. The loss of cardiac β-ARs in rats adapted to high altitude might be caused by chronically elevated concentrations of circulating neurally released catecholamines because it can be prevented by the chronic coadministration of a low dose of propranolol (Voelkel et al., 1981; Parer, 1983). In rats acclimatized to hypobaric hypoxia, the β-AR system remains unchanged, but the decreased response to β-AR stimulation limits the efficacy of this system on the mechanisms of systemic O_2 transport and reduces the effect of its blockade on these mechanisms (Clancy et al., 1997). It was also suggested that β-ARs contribute to hypoxemia-induced vasodilatation, despite unaltered epinephrine plasma concentrations (Blauw et al., 1995). The changes in β-AR density may partially explain the hemodynamic adaptation that occurs with chronic hypoxia. Hypoxia is also often associated with cardiac arrhythmias. Moderate hypoxia in normal $[K^+]_o$ is associated with the development of adrenergic-mediated after-depolarizations and triggered activity, whereas the accumulation of $[K^+]_o$ or severe impairment of cellular metabolism is accompanied by an inhibition of adrenergic-mediated after-depolarizations and triggered activity (Priori et al., 1991). During hypoxia, the electrically triggered slow upstroke action potentials in muscles are gradually depressed and catecholamine-induced membrane responses mediated by the β-AR-stimulated slow channel system are enhanced, accelerated by acidosis and reversed by reoxygenation. Changes, not only in catecholamine-β-AR interactions but also intracellular metabolic processes, may be responsible, at least in part, for the enhancement of abnormal automatic activity mediated by the myocardial β-AR-stimulated slow channel system under hypoxic conditions (Hasegawa et al., 1993).

G. Congenital Heart Diseases

Congenital heart diseases encompass several defects in childhood such as tetralogy of Fallot, left-to-right shunts, atrioventricular septal defects, and coarctation of the aorta, which often lead to other circulatory complications such as pulmonary hypertension, cyanosis, or congestive heart failure. In children with varying degrees of congestive heart failure secondary to congenital heart disease, plasma norepinephrine levels are consistently higher than those in patients without heart failure or congenital heart disease (Kozlik et al., 1991a; Kozlik-Feldmann et al., 1993; Dzimiri et al., 1995). There are, however, still a number of uncertainties with regard to the fate of epinephrine levels, with some studies reporting no significant change (Kozlik-Feldmann et al., 1993) and others registering an elevation in its plasma levels (Kozlik et al., 1991a; Dzimiri et al., 1995). It is also not yet clearly discernible in which congenital disorders a change in β-AR signaling might occur without the manifestation of severe heart failure. Left-to-right shunts and the pulmonary stenosis exhibit a significant decrease in lymphocyte β-AR density in the absence of severe heart failure (Dzimiri et al., 1995). The degree of left-to-right shunt flow and pulmonary systolic pressure also correlates directly with plasma norepinephrine levels and inversely with β-AR density (Dzimiri et al., 1995; Wu et al., 1996). A selective β_1-AR down-regulation has also been observed, but in critically ill newborns with congenital aortic valve stenosis or transposition of the great arteries, there is additional significant β_2-AR down-regulation (Kozlik-Feldmann et al., 1993). Patients with tetralogy of Fallot also show attenuated β-AR density (Brodde et al., 1992; Dzimiri et al., 1995), which can be reverted by treatment with propranolol (Kozlik-Feldmann et al., 1993). Furthermore, a follow-up study suggested that in patients with heart failure, there is a significant decrease in plasma norepinephrine and an increase in β-AR density after surgery (Wu et al., 1996). Moreover, in children undergoing valvuloplasty for pulmonary stenosis, the β-AR levels can be restored to almost normal values within a short time after valvuloplasty (Galal et al., 1996). Not only does the reduction in β-AR correlate with the severity of the cardiac disease, but also its reversal similarly correlates with the improvement in the hemodynamics after their surgical correction.

Infants and children with severe acyanotic or cyanotic congenital heart disease exhibit severely reduced β-AR density that correlates with increasing plasma norepinephrine, suggesting an enhanced sympathetic tone as the underlying cause for these changes (Kozlik-Feldmann et al., 1993; Dzimiri et al., 1995). The decrease in myocardial β-AR may be specific for β_1-AR in cyanotic congenital heart disease (Kozlik et al., 1991b). Interestingly, the decrease in β_2-ARs density on mononuclear leukocytes as well as right atrium seems to depend on

the severity of the cyanosis (Kozlik et al., 1991a). A partial decoupling of the β_2-AR to the AC was also suggested to occur in children with severe cyanotic congenital heart disease (Kozlik-Feldmann et al., 1993). A defect at the postreceptor level of AC may also be associated with congenital heart disease due to a decrease in its catalytic subunit and forskolin-stimulated activity in the presence of Mn^{2+}. In contrast, the $G_{i\alpha}$ level is apparently not altered, leading to the conclusion that a defect at the AC catalytic subunit contributes to the decreased effects of cAMP-increasing agents in patients with severe heart failure with congenital heart disease (Reithmann et al., 1997).

H. β-Adrenoceptor Gene Polymorphism in Cardiac Disease

The impact of molecular genetics on the diagnosis, treatment, and prevention of cardiac disorders is culminating at an enormous pace. Already, a number of cardiac diseases, including hypertension and cardiac hypertrophy, are believed to be underlying, at least in part, some genetic etiology. The notion that different cardiomyopathies may also be products of familial genetic defects is becoming more and more vivid, but there is very little evidence to support it. On the other hand, the existence of genetic variations in the β-AR subtypes and their signaling components has stimulated a considerable amount of interest recently, from both a diagnostic and a therapeutic point of view. The realization that both the β_2-AR and β_3-AR play a major role in the regulation of energy expenditure, in part by stimulating lipid mobilization through lipolysis in fat cells, has led to a surge of studies to evaluate the possible role of β-AR genetic polymorphism in lipolytic disorders. At least six different forms of the β_2-AR have been postulated to exist due to genetic polymorphism within the coding block of the receptor gene, some of which have been assigned distinct pharmacological and biochemical phenotypes (Liggett 1995, 1997). Three polymorphic loci within the coding region of the β_2-AR have been recently described for amino acid residues at positions 16, 27, and 164 (Large et al., 1997; Martinez et al., 1997). The Gly[16] has been associated with increased agonist-promoted down-regulation of the β_2-AR compared with Arg[16]. Furthermore, the form of the β_2-AR with Glu[27] has been shown to be resistant to down-regulation compared with Gln[27] but only when coexpressed with Arg[16]. This property has been associated with altered responses of the receptor to therapeutic doses of antiasthmatic agents in children (Martinez et al., 1997). Another study found a marked association of the Gln[27] to Glu[27] polymorphism with obesity and the Arg[16] to Gly[16] polymorphism with altered β_2-AR function but not with obesity (Large et al., 1997). The authors concluded that genetic variability in the human β_2-AR gene could be of major importance for obesity, energy expenditure, and its lipolytic function in adipose tissue. Furthermore, the mutation Cys[116] to

Phe[116] in the third transmembrane domain of the β_2-AR has also been associated with a selective constitutive activation of Na^+/H^+ exchange through a pathway independent of cAMP. These observations point to the existence of multiple and distinct activation states in these receptors (Zuscik et al., 1998). This polymorphism has also been implicated in the pathogenesis of essential hypertension and was significantly associated with variations in blood pressure responses to sodium loading and/or volume depletion in black Americans (Svetkey et al., 1996, 1997). More recently, the Arg[16] to Gly[16] exchange has also been implicated in predisposing individuals to essential hypertension (Timmermann et al., 1998). In contrast to β_2-AR polymorphism, comparatively greater focus has been directed at understanding the role of the β_3-AR in the mechanism that regulates metabolic responses of adipose tissue to stimuli. This mechanism is responsible for lipid mobilization that determines the direction of metabolism and the degree to which adipose tissue can store lipids and release fatty acids in times of need (Lafontan et al., 1997). A Trp[64] to Arg[64] mutation in the human β_3-AR appears to be prevalent in several ethnic groups and is reportedly associated with a series of cardiac and circulatory diseases related to lipid metabolic disorders. These diseases include earlier onset of non-insulin-dependent diabetes mellitus, proliferative diabetic retinopathy, abdominal obesity, weight gain, coronary heart disease, and some features of syndrome X, such as insulin resistance and dyslipidemia (Clement et al., 1995; Sakane et al., 1997; Sipilainen et al., 1997; Mitchell et al., 1998). However, several other investigators failed to establish similar relationships of this mutation with many of these diseases; these include, among others, the development of obesity, non-insulin-dependent diabetes mellitus susceptibility, glucose, and lipid metabolism in the same or other populations (Begin-Heick, 1996; Elbein et al., 1996; Biery et al., 1997; Higashi et al., 1997; Jeyasingam et al., 1997; Nagase et al., 1997; Uekita et al., 1997). Some studies have also implicated all three β-AR subtypes as well the α-ARs in lipolytic disorders, pointing to the ratio of α-AR:β-AR as the regulator of the lipolytic index of adipose depots (Soloveva et al., 1997). Accordingly, β_1-AR might be involved in both the stimulation of lipolysis and the proliferation of brown fat cells in the whole organism, and it is the overall β-AR activity, rather than the particular subtype, that controls these phenomena (Soloveva et al., 1997). As might be expected, a signaling malfunction arising from receptor defects in essential residues is likely to have serious implications for the downward signaling, including complete termination of its propagation. For β-AR signaling in particular, any such disturbance may prevent the synthesis of cAMP, which also plays a major role in the control of the lipolytic machinery by the hormone-sensitive lipases. In some animal models of obesity, such as the ob/ob mouse, the production of cAMP appears to be

abnormal in the adipose tissue. Besides the functional state of the β_3-AR, this abnormal cAMP has been associated with deficient levels of some isoforms of the G proteins and the low receptor expression (Vicario et al., 1998). However, the role of β-AR polymorphisms in cardiac disease must be addressed further to more precisely define its relevance. Besides the β-AR subtypes themselves, factors that may theoretically influence β-AR signaling include deficiencies or mutational changes in the downstream signaling components of its pathway. Although such deficiencies in $G_{s\alpha}$ and AC catalytic units have been associated with some hereditary diseases, currently their impact on the regulation β-AR signaling does not seem to be of significance.

VII. Implications of Receptor Cross-Talk for Signal Transduction in Cardiac Disease

A. Adrenoceptor Signaling and Manifestation of Cardiac Disease

It is now evident that the attenuation in myocardial performance of various origins, such as ischemia, heart valvular lesions, or dilated cardiomyopathy, is often accompanied by a change in the β-AR density and responsiveness to agonists. Thus, although in general it is believed that in heart failure down-regulation is more conspicuous for the β_1 subtype and receptor uncoupling is more eminent for the β_2 subtype (Brodde et al., 1991), it obviously is not the case with cardiac disease-induced changes in receptor expression. Moreover, some previous studies have indicated that the rise in catecholamine levels does not reflect the severity of heart failure (Viquerat et al., 1985), whereas others even observed a reduction in norepinephrine in, for example, patients with congestive heart failure (Regitz et al., 1989a,b). This throws open the question of whether in heart failure these changes are a direct result of increased sympathetic activity or the prevailing disease itself. The fact that the reduction in both the β-AR number and function can be traced back, at least in part, to alterations in the myocardial β-AR mRNA levels suggests that the root for these changes is embedded in the receptor synthesis mechanisms. The changes in the receptor density appear to precede the manifestation of heart failure and to occur at or even before the receptor synthesis step. Thus, heart failure probably constitutes a factor simply segregated coincidentally with the attenuation in β-ARs in severe cardiac disease and therefore may not constitute the underlying cause for the alterations in β-ARs and their downstream signaling components in heart disease. Furthermore, the observation that some of the β-AR signaling regulators, such as their GRKs, are altered in a disease-dependent fashion implies that the underlying basis for the changes in β-AR signaling may be embedded in the disease manifestation. Hardly any effort has been invested to test this notion, yet this might be the key to understanding β-AR regulation in

cardiac disease. It appears indeed that there are several players involved in regulation of β-AR signaling in cardiac disease. For example, in both acute and chronic volume overload in dogs, we observed by differential display technique a persistent increase in the expression of mitochondrial genes involved in energy metabolism within 30 min of the overload. This points to an adjustment of energy utilization as a first line of defense by the heart to protect its function, implying that the attenuation in β-AR is only secondary to these primary changes. It is also thought that in the early stages of heart failure, a number of humoral mechanisms are activated to increase blood pressure as a general mechanism by which the heart mobilizes support to meet its own functional requirements. These compensatory mechanisms involve, among others, the activation of the RAS system, elevated vasopressin levels, and activation of sympathetic nervous system (Dzau et al., 1981; Cohn et al., 1984; Cohn, 1989). Therefore, the alterations in β-AR functional expression probably occur at the end of a chain of events involving an interaction of several signaling pathways. Moreover, β_2-AR down-regulation may occur via other cell type-specific mechanisms in the absence of cAMP elevation and PKA activation (Allen et al., 1989; Bouvier et al., 1989; Danner and Lohse, 1997). The proposition of a postreceptor defect involving the AC catalytic unit strongly suggests the existence of signaling systems capable of triggering alterations in β-AR downstream signaling by interfering directly with the AC function in certain diseases.

B. Receptor Cross-Talk and β-Adrenoceptor Signaling in Cardiac Disease

The role of different cardiac signaling systems in the manifestation of cardiac disease, such as congestive heart failure and cardiac hypertrophy, is still unclear. The majority of studies so far have implicated cross-talk mainly among the AR subtypes and the RAS and ANP systems in cardiac function. This is not surprising because these three receptor systems constitute the three pillars of cardiac circulatory function: β-AR as the main controllers of the contractile apparatus, the RAS as a major player in the control of blood pressure, and the ANP as determinants of circulating volume. Current data suggest, therefore, that an alteration in any one of these pathways is likely to influence the functional expression of the other pathways contributing to cardiac circulatory function. Although the causal relationship between the attenuation in β-AR and elevation in α-AR in cardiac disease points to a cross-regulatory signaling control of these two AR subtypes, there hardly exists any evidence for such a mechanism. At present, the only logical explanation for the increase in α-AR in association with a reduction in β_1-AR density in cardiac disease is the appealing, yet unproved, notion of a trigger mechanism operating as a "switch" to turn on the former in response to malfunction of the latter. It is highly

probable, however, that the cross-talk between the α_1-AR and β_1-AR serves mainly to regulate the positive inotropic machinery in cardiac disease. The α_1-AR system can sustain positive inotropism of the contractile apparatus by stimulating the PLC pathway triggered possibly by an inefficient β_1-AR signaling. The missing link remains, however, the nature of the initiator that turns on this cascade, which is probably embedded in at least one of the signaling circuits. As such, stimulation of receptors by cardiac disease is not restricted to α_1-AR but rather a phenomenon shared by the receptor systems that are presumably dormant physiologically in the heart, such as the RAS and ANP. Besides the elevation in α_1-AR, the down-regulation in β-AR in heart failure and cardiac hypertrophy, for example, is often accompanied by the activation of neurohormones such as angiotensin II or aldosterone (Holmer and Schunkert, 1996). It is not fully understood whether this activation is an adaptive response, a secondary cofactor, or a primary cause of the disease (Bugaisky et al., 1992). In clinical settings, it has also been observed that the administration of ACE inhibitors can increase cardiac and peripheral β_2-AR levels, as well improve prognosis and cardiac function in patients with congestive heart failure (Maisel et al., 1989; Gilbert et al., 1993). Yonemochi et al. (1997) found that in addition to increasing β-AR density, ACE inhibitors also augment the responses to β-AR agonists in cultured neonatal rat myocytes. However, the mechanism by which the AT_1 receptor pathway influences β_1-AR is unclear. It has been suggested that the ACE inhibitors prevent β-AR down-regulation or, alternatively, increase β-AR up-regulation by inhibiting the angiotensin II activity. Initially, it was thought that the ACE inhibitors acted by increasing circulating catecholamine levels (Maisel et al., 1989; Gilbert et al., 1993). This has, however, been refuted by studies showing that β-AR up-regulation induced by ACE inhibitors is not associated with changes in catecholamines (Horn et al., 1988). These observations clearly point to a scenario whereby the inhibition of a potential provider of positive inotropism promotes the activation of a failing β-AR system. Although this seems paradoxical, it may imply that the interactions are regulated at a higher and more complex level that cannot be explained simply by agonist-receptor response relationships. Perhaps the greatest contribution to our current understanding of cross-regulation of cardiac receptors can be attributed to endeavors by a number of researchers at laboratories to explain the mechanisms leading to cardiac hypertrophy. This alteration in heart muscle size and structure develops usually as an adaptive process to reduce wall stress in response to cardiac diseases where pressure is generated (Grossman et al., 1975; Chien et al., 1991) but also provides fertile conditions for the manifestation of cardiac muscle disease and the development of chronic heart failure. Such mechanisms were logically sought in growth receptor signaling, leading to the discovery that

the majority of hypertrophic agonists activate the Ras/ Raf/MAPK pathway. Transfection of ventricular myocytes with components of this pathway has implicated MAPK in the alteration of gene expression observed in the development of hypertrophy (Bogoyevitch and Sugden, 1996). Until recently, it was mainly the RAS and ANP pathways that were believed to contribute to the manifestation of pressure-induced hypertrophic disorders. Interestingly, both receptor systems are involved in cardiac growth during early embryonic development but are drastically down-regulated shortly after birth to negligible levels in adult myocardium, only to be turned on again in cardiac hypertrophy (Appel, 1992; Glennon et al., 1995; Ferrario and Flack, 1996). This finding has lead to the notion that these receptors are turned on to mediate cardiac adaptation to chronic pressure overload by stimulating the Ras/Raf/MAPK pathways. Besides the RAS and ANP pathways, the mitogenic property of AR agonists provides causal evidence for the involvement of this pathway in the development of cardiac hypertrophy, suggesting therefore that this disorder is regulated at a higher level, possibly involving several cardiac signaling pathways.

The fact that virtually all cardiac receptor systems that appear to have no physiological function are capable of exerting positive inotropic and other cardiac-relevant effects suggests that they are potentially required to furnish a supportive role in disease that has yet to be discovered. Cross-talk has been described between the β-AR system and several of these systems in isolated cell systems. However, although cross-talk in cardiac function is a reality, the mechanisms involved remain largely unknown. Currently available data are concordant with the idea that the control of cardiac signal transduction systems converges at certain check points, probably under the humoral control of the heart itself. There are several clues as to the nature of this machinery that should be expected to unfold in the foreseeable future. It might be a matter of time before the clinical relevance of some of these cross-regulatory mechanisms can be exploited for therapeutic purposes. Having said that, we must nevertheless interpret the current data with great caution. To begin with, these studies have been conducted mostly in isolated cell systems. We are all too aware of how drastically the removal of any component from its environment may alter the function of other components or even whole systems. This is particularly true of signal transduction mechanisms, where the absence of one component of a signaling pathway involved in cross-talk with another may even lead to new signaling products. Our knowledge of this subject is still very patchy. The available information so far provides only a taste of an exciting area in cardiac receptor signal transduction, which is gradually unfolding itself. It is likely that some of the mechanisms currently conceived as cross-talk among cardiac receptor systems may

soon be recognized as an integral part of normal cardiac physiological signaling.

VIII. Implications of Altered β-Adrenoceptor Signaling for the Management of Cardiac Diseases

The fact that several components of the β-AR signaling pathway are altered in cardiac disease has rendered them very attractive to exploit their potential as markers for diagnostic and prognostic purposes. Based on the correlation between increased catecholamine levels and attenuated β-AR density with clinical symptoms of heart failure, unsuccessful attempts were made as early as the late 1970s to establish the practicability of using the plasma catecholamine levels as reliable predictors of the events taking place in the myocardium. Although routine analysis of catecholamine levels has found its place in patients with pheochromocytomam for example, it remains difficult to assess the general usefulness of this approach for cardiac disease at present. This is primarily due to the fact that although elevated catecholamine levels may serve as a reliable predictor for increased sympathetic function, the latter is not necessarily a quantitative reflection of the associated changes in β-AR density or functional level. An alternative approach was suggested almost concurrently to use the changes in peripheral β_2-AR density itself as a yardstick for the alterations in the myocardial subpopulations. Brodde et al. (1989) argued that alterations in these receptors do not reflect on the changes in the cardiac β-AR population, mainly because the peripheral lymphocyte β-AR population comprises the β_2-AR subtype, whereas the β_1-AR makes up the majority of the cardiac subpopulations. Although this argument is correct particularly with respect to changes in heart failure, it is now evident that both β_1-AR and β_2-AR are concomitantly reduced in various disease conditions, such as valvular heart diseases (Sylvén et al., 1991; Dzimiri et al., 1996a,c). In a study involving a comparatively large population, we recently established a simple relationship between the reduction in myocardial receptor density and the peripheral β_2-AR using the nonselective β-AR agonist [^{125}I]iodocyanopindolol in patients with left ventricular overload diseases (Dzimiri and Moorji, 1996b). Based on the fact that nonselective agonists bind effectively to both β-AR subtypes, we concluded that this ratio might be informative for prognostic purposes, especially because there is no apparent preferential down-regulation of β_1-AR in these diseases. Besides, even if such a selective action existed, the same parameters should still be valid because this ratio would be affected proportionally under these conditions. Wu et al. (1996) similarly argued that the peripheral β_2-AR levels could be used as an index for assessing the presence and severity of heart failure in infants and children with congestive heart failure. This was based on the findings of a direct correlation between plasma norepinephrine levels with the

degree of left-to-right shunt and pulmonary stenosis and an inverse correlation with β-AR density in children with varying degrees of congestive heart failure. It seems therefore worthwhile to pursue these endeavors to evaluate the applicability of alterations in β-AR for diagnostic and therapeutic purposes. An important difficulty with respect to these efforts, however, is the likelihood that β-AR signaling components change in numbers or function with age, particularly in organs that would lend themselves most conveniently for diagnostic or prognostic purposes. Both human and animal disease models have pointed to age-dependent alterations in the β-AR itself, thereby contributing to its down-regulation as a result of enhanced receptor degradation (Brodde et al., 1995; Wu et al., 1996), AC V and VI (Scarpace et al., 1996), and the G proteins (Feldman et al., 1995; Ferrara et al., 1997). In both settings of chronic heart failure and age, β-AR-mediated effects and all other cAMP-dependent effects are depressed whereas G_i is increased (Brodde et al., 1995). Some of the investigators advocating a diminishing of β-AR signaling with age assume that it is a result of an impaired capacity of the receptors to activate AC, in part as a result of decreased availability of AC catalytic units. On the other hand, however, this dependence of the receptor density or peripheral G protein function on age is still controversial (Barki-Harrington et al., 1996). Moreover, it is not certain whether enhanced receptor degradation indeed contributes to β_1-AR down-regulation in the failing human heart. Apart from the use of spontaneous or disease-induced alterations in the receptors themselves, efforts have also been invested in various other directions, such as the development of antireceptor antibodies as markers for cardiac disease (Jahns et al., 1996; Mijares et al., 1996). However, the antibodies developed so far seem to induce down-regulation and a decline in β-AR responsiveness by interfering with several steps in the cycling of the receptors (Limas et al., 1991). Other modalities for diagnostic strategies include the use of alterations in the ratio of G_s:G_i, based on the argument that only the latter is changed in disease. This has not proved to be useful either. Therefore, the recruitment of alterations in catecholamines and β-AR for diagnostic or prognostic purpose appears to be quite remote, at least for the time being.

In contrast, significantly much more promising benefits have been derived from our knowledge of the β-AR signaling for the purpose of therapeutic management of heart failure, heart muscle disease, and, to some extent, hypertension. The classic approach to counteract detrimental effects of an impaired β-AR system has been to administer β-AR antagonists (β-blockers) as a way of resensitizing the system to improve cardiac function (Swedberg et al., 1980). Great interest continues to grow in evaluating these agents, particularly in the treatment of congestive heart failure and ischemic cardiomyopathies with noticeable success. So far, randomized studies

have shown a good clinical effectiveness of this therapy with, among others, carvedilol, metoprolol, bucindolol, and bisoprolol in combination with cardiac glycosides, diuretics, and ACE inhibitors (Böhm, 1996; Waagstein, 1997). General clinical improvements have also been reported after long-term therapy with a selective β_1-blocker such as celiprolol, which was accompanied by normalization of myocardial β-AR density and improved contractile responses to stimulation by dobutamine (Heilbrunn et al., 1990; Nakamura et al., 1998). However, there appears to be some qualitative and quantitative differences in patient responses to selective and nonselective β-blockers. For example, the administration of a selective β-blocker has been associated with an increase, whereas in contrast, a nonselective β-blocker caused a reduction in cardiac norepinephrine spillover (Newton and Parker, 1996). This observation triggered the notion that nonselective β-AR blockade has favorable inhibitory effects on cardiac sympathetic activity in heart failure. It has also been argued that because both β_1-AR and β_2-AR enhance adrenergic neurotransmission, nonselective β-blockade may inhibit cardiac sympathetic activity more than β_1-selective blockade. Nevertheless, the rationale for choosing between a selective or nonselective β-blocker in the treatment of heart failure remains subjective rather than being based on scientific criteria. The thinking behind β-blocker therapy was the concept of neurohormonal blockade to reduce the stimulation of the contractile apparatus and therefore the load on the failing heart, based on the observation that elevated plasma norepinephrine concentrations closely correlate with the poor prognosis. The reduced cardiac workload associated with such reduced stimulation of the contractile apparatus should increase coronary vasodilatory capacity and myocardial flow reserve as a result of lower myocardial blood flow. However, the validity of these mechanisms has yet to be determined. In a model of congestive heart failure, Yoshikawa et al. (1996) suggested that the late improvement of left ventricular contractile function is preceded by an early reduction of neurohumoral factor levels and may be in part responsible for the efficacy of β-blocker therapy for congestive heart failure. The β-AR antagonists have also been shown to cause functional and hemodynamic improvements in patients with dilated cardiomyopathy (Engelmeier et al., 1985; Waagstein et al., 1989; Gilbert et al., 1990). An improvement in both systolic and diastolic functions in some clinical setups has been attributed to long-term β-AR blockade (Christ et al., 1996; Bristow, 1997; Waagstein, 1997). The up-regulation of myocardial β-AR accompanying the administration of β-blockers has been explained as a response of the myocardial cells to the blocking of the remaining functional receptors. The mechanisms proposed for such a response include a restoration of β-AR signal transduction, an increase in contractile reserve, and protection from toxic catecholamine effects. Moreover, chronic

blockade of stimulatory β-ARs may decrease inhibitory receptors of the adrenergic signal transduction system. This presumably triggers a coordinated cross-regulation of inhibitory receptors and G_i proteins, reducing the effects of inhibitory receptor activation of the heart and contributing to the beneficial effects of β-blocker therapy in heart failure (Borst et al., 1997). This mechanism has been advocated to explain the improved outcome of the β-blocker treatment in some patients (Jaillon, 1987). However, despite the well documented benefits of β-blockade in a variety of cardiovascular conditions, a number of questions still remain open particularly with regard to its usefulness in congestive heart failure and hypertension. The rationale for the use of β-blockers in the treatment hypertension was the assumption that these drugs would reduce elevated blood pressure by counteracting the catecholamine effects, thereby inhibiting sympathetic activity. The β_1-AR selectivity is thought to be important for this therapy (van Zwieten, 1990, 1996); however, other therapeutic modalities involving other signaling pathways seem to have established themselves better that β blockade in the treatment of hypertension.

Some β-AR antagonists, such as bisoprolol, can cause a myocardial chamber-specific down-regulation of $G_{i\alpha2}$ and $G_{s\alpha}$ mRNA and GRK2, on one hand, while triggering an increase in β-AR-dependent stimulation of AC and persistently high-affinity state of β-AR, on the other hand (Ping et al., 1995). As a result of this observation, Ping et al. (1995) suggested that in response to chronic β-AR antagonist administration, the heart may adapt itself in a manner that would be expected to offset reduced agonist stimulation. At present, very little, if anything, is known regarding whether the early administration of β-AR-blocking agents in the patients with minimal or subclinical heart failure may prevent or retard the development of β-AR-coupled AC abnormalities. Also, the use of low-dose treatment with β-blockers has been suggested as way of up-regulating β-AR. This is, however, also not free of risks. Enhanced sympathetic stimulation may contribute to progressive cardiac functional degeneration. It is interesting that all of these mechanisms purport a reversal of events leading to the reduction in β_1-AR, without offering an explanation as to how the sympathetic stimulation may respond, possibly as a reflex mechanism. It is therefore difficult to reconcile these facts with the supposition that β-blocker treatment reduces the effects of sensitized sympathetic function. Nevertheless, β-blocker treatment is still as a promising approach, at least as supportive procedure for standard therapy in the treatment of chronic heart failure.

Besides the use of β-blockers, the budding concept of inverse agonists may have a future role as a therapeutic modality. Inverse agonists are ligands that preferentially stabilize inactive conformations of GPCRs by decreasing the intrinsic ability of a receptor to activate the

cellular G protein population in absence of an agonist (Milligan and Bond, 1997). In a range of systems, sustained treatment with inverse agonists have produced substantially greater up-regulation of receptor levels than antagonists (Lekowitz et al., 1993; Samama et al., 1993). This effect can be magnified by the use of CAM receptors. Furthermore, the use of these receptors may also allow agonists and antagonists to mimic the effect of preventing denaturation of the mutant receptor polypeptide (Milligan and Bond, 1997). These observations have recently led to the notion that CAM receptors may lend themselves as effective therapeutic agents (Milligan et al., 1997). Several occurring CAM GPCRs has already been identified based on the physiology and endocrinology associated with specific familial diseases such as retinitis pigmentosum and thyroid adenoma hyperfunction, among others. These observations have prompted the suggestion that inverse agonist ligands may be more successful than neutral antagonists for the treatment of such patients in a range of clinical conditions in which the elimination of receptor function is believed to be appropriate (Milligan et al., 1997). Some β-AR ligands, such as betaxolol and sotalol, have recently been identified as inverse agonists of the CAM β_2-AR as assessed by their ability to inhibit its induced basal AC activity, which was not observed with equivalent treatment using neutral antagonists (MacEwan and Milligan, 1996). MacEwan and Milligan (1996) further observed that the degree of up-regulation attainable with inverse agonists was greatly enhanced using a CAM β_2-AR. However, although these findings are certainly exciting, at present not much is known about their role in the treatment of cardiovascular diseases. Attempts have also been made to manipulate components of β-AR desensitization mechanisms as a therapeutic strategy for heart failure. For example, some GRK inhibitors have been synthesized with the hope of using them to prevent rapid homologous desensitization of β-AR and reduce their uncoupling. Drazner et al. (1997) reported that β_2-AR gene transfer by recombinant adenoviruses or an inhibitor of GRK2-mediated desensitization may potentiate β-AR signaling. More recently, Rockman et al. (1998) found that overexpression of βARKct, a GRK2 inhibitor, prevents the development of cardiomyopathy in a murine model of heart failure. Similar efforts are being directed at deactivating inducers of disease stimuli by antisense oligonucleotides as a therapeutic model. Because receptor up-regulation may lead to a marked increase in AC activity, atrial tension, and indices of cardiac contractility, efforts have also been invested in evaluating the possibility that genetic modulation of β-AR-signaling cascade can enhance cardiac function. Transgenic mice overexpressing a human β-AR have shown marked improvements in myocardial baseline and left ventricular function, without significant pathological changes (Milano et al., 1995). These findings may have great relevance for the treatment of chronic heart failure and cardiac diseases associated with reduction in β-AR density. These examples present just a few attempts that have been made so far to use our present knowledge of AR signaling for therapeutic purposes. Although not much success can be attributed to these attempts yet, they look promising for future therapeutic management of heart failure and cardiac diseases in general. However, much more basic research will be required before patients will benefit from the clinical results of these findings.

IX. Conclusions

It is now evident that besides being transducers of catecholamine signals, the β-ARs constitute part of a highly complex and tightly regulated cardiac signaling machinery that is geared toward sustaining circulatory function in both healthy and unhealthy individuals. The post-translational regulation of the β-ARs is dominated by their phosphorylation not only by PKs and GRKs but also by the kinases of growth factor receptors with intrinsic tyrosine kinase activity, as well as other signaling pathways. Thus, the emerging realization and appreciation of the fact that cardiac signaling systems are interwoven in an immensely complex machinery add an interesting touch to the level of complexity of this subject. Taking into consideration that hundreds of GPCRs interact with only a handful of G proteins to fulfill all of these functions, the specificity in the fashion by which their coupling mechanisms recognize the different routes of these signaling complexes is fascinating and intriguing. The fact that all of the major downstream components of the GPCR pathways exist in several isoforms, combinations of which can yield different signaling products, point to numerous ways by which these pathways can be regulated. Evolution has evidently provided for various alternatives and conditions by which individual signaling proteins are regulated to permit the choice as to which systems are to be shared with each other and which ones should act as antagonists or independent agents to meet different signaling demands. For the cardiovascular system in particular, the networks contributory to circulatory function require fine tuning not only to maintain homeostasis but, more importantly, also for survival in disease. These mechanisms are just beginning to be unraveled. Great strides have been made in our understanding of β-AR signaling in cardiac function in both normal physiology and disease. These advances are attributable to the success in simulating human disease in both animals and single cells. For example, immunization of rabbit by peptides corresponding to the target sequences for antireceptor autoantibodies in idiopathic dilated cardiomyopathy has been found to induce morphological changes in the heart similar to those found in the human disease (Matsui et al., 1997). To date, a great part of our current understanding of alterations of GPCR signaling in cardiac diseases has also been derived from descriptive studies

comparing healthy and diseased myocardium from different individuals and species. Although this experimental approach provides important phenomenological information, it does not prove any causal relationship between alterations in the signaling components and the onset or progression of the disease. In this regard, in vitro gene transfer into cultured myocytes and transgenic methodologies are promising primarily because the effects of the gene of interest on the whole cell or organism can be traced. We have greatly benefited from animal models of disease. Nevertheless, there are several limitations as to how relevant this information is to human cardiac disease. The greatest hindrance to progress in this direction is the lack of human material, and even in cases where this commodity may be available, is it not possible to use the same sample provider as a control. Moreover, even with the powerful techniques such as differential display to follow sequence of events in alteration in gene expression, it is not possible to use the patients as controls to determine in vivo the predisease status of the gene expression. Putting into consideration the intraindividual and interspecies differences in gene expression, this point becomes more important particularly in relating observations in genetically manipulated animals or cells to the human situations. Apart from that, at present, radiolabeling constitutes part of virtually all currently agents used in signal transduction, such as phosphorylation of receptors and their signaling components. Most studies involving human tissue to investigate AR function in severe heart failure will have received standard treatment including inotropes and diuretics, whereas the donor patients used as controls will not be subjected to such treatment. It is very unlikely that human tissue will become available to exclude the possibility that this treatment might contribute to the observed changes. Future studies have also to address important issues regarding the way by which the different signaling circuits recognize and differentiate the various signals under both physiological and pathophysiological conditions. There are no noninvasive methods of performing these studies in humans yet. Nevertheless, the current progress in the search for research tools that circumvent the need for human material to study signal transduction pathways promises to provide us with answers to some of the questions that until recently appeared insurmountable. Several techniques are emerging as methodologies of the future and will greatly facilitate our understanding of GPCR regulation in living cells, making laborious biochemical fractionation techniques a redundancy. These include recently developed techniques to visualize and monitor receptor trafficking in transfection systems (Kallal et al., 1998) or by fluorescence techniques (Barak et al., 1997; Ferguson and Caron, 1998) promising to be valuable tools with which to study and follow real-time intracellular redistribution and pharmacological regulation of GPCR regulatory proteins. Among others, the

concept of inverse agonism may provide useful information for the understanding of the molecular processes that result in agonist-induced activation of receptors and inverse agonist-induced deactivation by computational mutagenesis and molecular modeling (Milligan et al., 1995; Scheer et al., 1996). Similarly, the use of transgenic and knockout animal models continues to claim an important contribution to our current understanding of the events leading to cardiac disease. Also, yeast may offer a promising alternative vehicle to mammalian cells for the production of ARs and other GPCRs for structural studies (Sizmann et al., 1996). These represent just a few examples of the developments that promise to significantly enhance our knowledge of the functional anatomy of GPCRs in the foreseeable future. As the studies of G protein-mediated signaling progress, they become more and more complex, and our understanding of the diversity of receptors, G proteins, and effectors, as well as cross-talk between different pathways, increases rapidly with it. In particular, with these advances, the already archaic concepts of looking at cardiac signaling pathways as isolated entities will soon disappear as we decipher the regulators of these networks.

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