G-Protein Coupled Receptor Signaling in Myocardium: Not for the Faint of Heart

Catecholamines, endothelin-1 and angiotensin II are among a diverse group of diffusible extracellular signals that regulate pump function of the heart by binding to G-protein coupled receptors (GPCR). When the body demands a temporary boost of power output or if temporary budgeting of resources is required, these signals can adjust heart rate and contractile strength to maintain continuous perfusion of all vascular beds with nutrient- and oxygen-rich blood. Given adequate time in the face of prolonged challenges, activation of GPCRs can also promote “remodeling of the heart” by increasing cell size, organ size, and chamber dimensions, or by varying tissue composition and altering the expression of protein isoforms controlling excitability and contractility. A common feature of heart disease is the state of chronic activation of GPCR signaling systems. Paradoxically, whereas acute activation is beneficial, chronic activation often contributes to further deterioration of cardiac performance. A better understanding of how chronic GPCR activation contributes to the development of heart disease is needed so that it can be translated into better prevention and therapeutic strategies in the clinic.

In this review, we will explore some of the signaling mechanisms employed by the body to regulate the performance of the heart both in the short term, where speed, power, and fuel efficiency are crucial, and in the long term, where the heart attempts to “reinvent” itself to be more competitive in a changing world. Chemical signals targeted to G-protein coupled receptors (GPCRs) regulate a diverse array of cardiac functions such as contractility, response to hypoxia, gene expression, growth, and cell death. We will attempt to weigh the costs and benefits of these regulatory strategies to evaluate their involvement in cardiac pathologies. Therapeutic intervention in heart disease often involves targeting or mimicking GPCR-related signaling mechanisms, but chronic heart disease remains a leading cause of death in the Western world. Clearly, a more complete understanding of cardiovascular signaling from molecules to cells to systems is essential to pave the way for improving the prevention and treatment of a spectrum of complex cardiovascular diseases.

The Neurohumoral Hypothesis

Chemical responses to cardiac stress

The neurohumoral hypothesis is a unifying conceptual framework to account for the heart’s response to stress (60). In this view, when the heart is stressed by high blood pressure, low-flow ischemia, subpar cardiac performance, or other stresses, the body responds by producing endogenous chemical signals. These can be systemic or local and can lead to a variety of beneficial physiological effects: enhanced cardiac contractility to improve cardiac output, vessel dilation to improve blood flow, recruitment of protective mechanisms against oxidative stress, conservation of ATP supplies, and more.

In fact, a bewildering array of local and global chemical messages impinges on the heart at any given time, and many different cell types busily recognize and decode these messages to adjust cardiac performance. Such adjustments increase the probability of survival and improve the quality of life in healthy individuals. Subtle defects in these signaling processes or simply recruiting them into action too often, however, may contribute to a chronic disease state in which the heart can no longer meet expectations, and performance deteriorates progressively. This is the state of heart failure, a relatively common condition in humans and an exceptionally costly one in terms of quality of life and survival (20, 44, 59). We will speculate on how a downward spiral toward heart failure might develop as a result of unbalanced or excessive neurohumoral signaling and explore why certain therapeutic strategies might be more effective than others.

GPCRs in myocardium

Historically, among the most widely studied chemical signaling mechanisms in the heart involve GPCRs. GPCRs represent the largest and most diverse superfamily of receptors in the human genome, and the
largest single class of gene products targeted by therapeutics in clinical use (17). Cardiac GPCRs form the basis for much of classical cardiac physiology and pharmacology and, therefore, are among the most widely studied signaling systems in the heart. These include but are not limited to 1) catecholamines functioning through α-/β-adrenergic receptors, 2) endorphin through ET and ET receptors, 3) angiotensin II through AT and AT receptors, 4) adenosine through A and A receptors, and 5) acetylcholine through muscarinic receptors. With the advent of molecular biology, it is now clear that messenger RNAs for a broad spectrum of GPCRs are expressed in cardiac cells. It has been estimated that ~200 different GPCRs are expressed in various cardiac cell types including endothelial cells, fibroblasts, and myocytes of the atria, ventricles, and coronary vasculature (79). It is quite possible that many (if not all) of these neurohumoral signaling systems play crucial roles, not only in regulating cardiac performance, but also in orchestrating cardiac growth and development and inadvertently contributing to the etiology of heart failure.

To begin to deal with this overwhelming complexity of neurohumoral signaling, we will examine only a few prominent and relatively well understood cardiac signaling systems in detail: α/β-adrenergic receptors, ET endothelin receptors, and AT angiotensin II receptors. Each regulates cardiac performance in the short term but with different goals and outcomes. β-adrenergic receptors mediate the classical fight-or-flight response by translating catecholamine binding into rapid activation of Gαs, adenylyl cyclase, and protein kinase A (PKA). This system also contributes to the systemic cardiovascular response to exercise and to cardiovascular reflex arcs (92). In contrast, ET receptors, AT receptors, and α-adrenergic receptors represent a larger and more diverse group of GPCRs that respond to their respective ligands with an initial activation of Gαq, phospholipase Cβ, and protein kinase C (PKC), which in turn regulate many cardiac functions (14). This dichotomy of Gαq vs. Gα signaling networks provides a glimpse into the complexity of neurohumoral signaling in the heart.

**Ischemic preconditioning**

Research interest in myocardial GPCRs has been catalyzed by the now widely recognized phenomenon of ischemic preconditioning, whereby brief bouts of low flow ischemia protect the heart muscle from subsequent longer and more severe ischemic episodes (66, 97). Autocrine/paracrine release of endogenous ligands for GPCRs (usually Gαq coupled) is now thought to play a central role in cardioprotection by ischemic preconditioning. Indeed, the three Gαq coupled receptor types discussed here each provide cardioprotection following acute activation (16, 25). Moreover, common intracellular mechanisms have emerged including prominent roles for the epsilon isoform of PKC (PKCε) and ATP-regulated potassium channels (KATP, Kir6.2), which in turn protect cardiac tissues against oxidative damage (at least in part) by curbing mitochondrial “overheating” (25). PKCε may also be cardioprotective by reigniting in ATP consumption by the contractile apparatus (63).

Early onset preconditioning (within minutes to hours) substantially reduces ATP utilization by myosin independently of protein synthesis, whereas a delayed phase of preconditioning (1–3 days later) relies on the energetically costly business of invoking gene expression and protein synthesis. In certain cases, GPCR activation may protect the myocardium against necrosis and apoptosis (36). The phenomenon of ischemic preconditioning illustrates the existence of potent chemically mediated protective responses of the heart to stress, which may someday be exploited to treat heart disease in clinical practice (66). It also provides a glimpse into the complexity of neurohumoral signaling in the heart.

**Short-Term Benefits**

**Cellular and molecular mechanisms**

Myocytes (myo = muscle, cyte = cell, Latin) are the individual force-producing cells of the myocardium. A number of cellular processes related to controlling

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**FIGURE 1.** Classical GPCR signaling

Analogous events following activation of various GPCRs. β-adrenergic receptors function through Gαs/adenyl cyclase/PKA, whereas α-adrenergic AT, and ET, signal through Gαq/PLCβ, and PKC. End-effector proteins are typically either distinct or phosphorylated on distinct sites by the actions of Gαq/PKC vs. Gαs/PKA. Norepinephrine also activates β or β-adrenergic receptors with potentially important regulatory roles in the heart (49, 69).
membrane excitability, intracellular Ca\(^{2+}\) levels, and Ca\(^{2+}\) responsiveness of the contractile apparatus can be acutely regulated in each myocyte to influence pump performance. These differ markedly in cardiac and skeletal muscles (FIGURE 2), such that investigation of skeletal muscle fibers and their regulation may be of only limited relevance to understanding cardiac function and dysfunction. Some regulatory and contractile mechanisms are shared by ventricular and atrial cardiac myocytes; for example, both cell types are loaded with GPCRs for neurohumoral control of cardiac excitability and contractility (8). However, ventricular myocytes are the primary force-producing cells and, therefore, largely dictate the heart’s specifications as a muscular pump. Contributions to pump performance provided by the atria are quite modest (perhaps 20% of cardiac output), with the notable exception of the right atrium, which contains a sinoatrial node of cardiac pacemaker cells that drive the electrical rhythm of the heart beat.

**Norepinephrine and PKA**

A classical rendition of a neurohumoral response to alter cardiac contractile performance is the fight-or-flight response mediated by rapid activation of the sympathetic nervous system in preparation for dealing with threatening conditions. The sensation of a pounding heart accompanied by an increase in heart rate is familiar to all who have experienced intense fear or anger. Catecholamines (primarily norepinephrine) bind to \(\beta\)-adrenergic receptors in the sinoatrial node to increase its firing rate and in the ventricular myocardium to enhance contraction strength.

Norepinephrine is packaged into synaptic vesicles of the sympathetic nerve terminals, released onto the heart by electrical activity in these nerves, and released more generally into the blood stream from the sympathetically innervated adrenal medulla. Binding of norepinephrine to \(\beta\)-adrenergic receptors on the heart initiates an intracellular cascade (FIGURE 1) that ultimately targets L-type Ca\(^{2+}\) channels, phospholamban [the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) regulator], and the myofilament regulatory proteins troponin I and myosin-binding protein-C. PKA phosphorylation of L-type Ca\(^{2+}\) channels near the C-terminus of the pore forming \(\alpha\)-subunit increases influx of trigger Ca\(^{2+}\) and contributes to a larger systolic Ca\(^{2+}\) transient (30). Phosphorylation on serines\(^{16/17}\) of phospholamban dissociates it from the SERCA pump and enhances the amount and rate of Ca\(^{2+}\) pumping into the sarcoplasmic reticulum (SR) during diastole (11). The SR becomes “superloaded” with Ca\(^{2+}\) contributing to increased gain of excitation-contraction coupling and a larger Ca\(^{2+}\) transient during systole.

To accommodate the catecholamine-induced increase in heart rate, the systolic phase of the cardiac cycle must be abbreviated to ensure adequate time for ventricular filling during diastole. Therefore, \(\beta\)-stimulation of ventricular myocytes also enhances cardiac twitch kinetics, especially the relaxation phase. This is accomplished by enhancing the Ca\(^{2+}\) transport rate into the SR and by accelerating two potentially rate-limiting processes taking place on the myofilaments: Ca\(^{2+}\) dissociation from troponin-tropomysin and the rate of myosin cross-bridge detachment from actin (FIGURE 3). Phosphorylation of troponin I on serines\(^{23/24}\) stimulates the rate of Ca\(^{2+}\) dissociation from troponin C and (with possible contributions from phosphorylation of myosin-binding protein-C) accelerates rate-limiting steps in the cross-bridge cycle like-

FIGURE 2. Users guide to cardiac performance

Cardiac vs. skeletal muscles: the heart is a muscular organ that alternately contracts and relaxes roughly once every second to pump blood throughout the circulatory system without stopping. It is like an automobile engine that is never turned off: sometimes idling, sometimes cruising, and at other times racing. The walls of the cardiac chambers are comprised of striated muscle cells, not unlike skeletal muscles in their capacity to generate powerful bursts of force by converting ATP’s stored chemical free energy into work without much delay. However, tuning of cardiac pump performance is accomplished quite differently than tuning of skeletal muscles for limb movement or for controlling posture. Skeletal contractions are graded in strength and duration by two phenomena that do not occur in cardiac muscles: motor unit recruitment and tetanus.

Instead, cardiac muscle performance is adjusted at the level of heart rate and contractile strength via myriad neurohumoral signals that regulate 1) pacemaker firing rate, 2) action potential (AP) duration and propagation speed, 3) trigger Ca\(^{2+}\) influx, 4) intracellular Ca\(^{2+}\) release and uptake, 5) myofilament (MF) Ca\(^{2+}\) sensitivity, and 6) performance of the actin-myosin contractile apparatus itself. Regulating these parameters can have a substantial influence on the power and efficiency of cardiac contractions. Energy, work, power, and efficiency: muscles use the energy “stored” in the phosphoanhydride bonds of ATP by a fascinating, precisely orchestrated multi-step transduction process that takes place on the myosin head and involves cyclic interactions with actin (89). The goal of this molecular machinery is to convert as much energy available in ATP as possible into useful work. Energy is the input, work is the output, and both parameters have the same units of measure (J, kcal, ergs, etc.), but some energy is inevitably lost as heat in the transformation, so efficiency (work out/energy in) never reaches 100%. Energy is also consumed in the process of introducing order into macromolecular biological systems (preventing disorder and/or minimizing entropy). Power is defined by the rate of energy conversion, which therefore has units of energy/time or work/time. Power consumption is the rate of energy utilization (\(\text{W}\)), whereas power output is the rate of work performance (\(\text{W}\)), and their ratio is another rendition of efficiency (\(\text{W}\) power output/\(\text{W}\) power consumption). Measurements of heart rate, cardiac work (e.g., pressure-volume relationships), and oxygen consumption provide considerable insight into cardiac muscle energetics, including the high cost of \(\beta\)-adrenergic stimulation (24, 76, 89) and improved efficiency following endothelin stimulation (78).
ly to be ADP dissociation (62, 89). These alterations allow faster force development and shortening in cardiac myocytes during systole and faster force relaxation and re-lengthening during diastole. These are energetically costly adjustments because both the SERCA pumps and myosin motors are cycling faster and consuming more ATP per unit time. Energy expenditure per unit time (i.e., power consumption; FIGURE 2) increases dramatically under these circumstances, but this is the cost of a rapid surge in cardiac power output (26). Such an investment of resources is appropriate to confront imminent danger or, as another example, to prevent precipitous drops in blood pressure as part of the baroreceptor reflex.

**Endothelin, angiotensin II, norepinephrine, and PKC**

Cardiac GPCR signaling initiated by endothelin, angiotensin II, or norepinephrine shares some common properties. Each of these ligands activates a Gq coupled receptor (ET$_1$, AT$_1$, and $\alpha_1$, respectively) located in the vasculature to promote vasoconstriction and also in the myocardium to mediate positive inotropy. Downstream of Gq coupling for each of these receptors involves robust activation of phospholipase C$_{\beta_1}$, hydrolysis of PIP$_2$ to diacylglycerol/IP$_3$, and activation of PKC (FIGURE 1). PKC represents a large gene family with up to twelve isoforms, which target many end effectors in the heart, including L-type Ca$_2^+$ channels (27) and myofilament proteins (91), accounting for the positive inotropic actions (37, 38). PKC and further downstream kinases, such as ERK1/2 are also central to GPCR-mediated regulation of hypertrophic growth (9) and to cardioprotective mechanisms underlying ischemic preconditioning (64).

Not surprisingly, closer inspection of these signaling systems reveals significant differences as well. For example, recent studies of AT$_1$ receptors have revealed a unique ability to cross-talk with receptor tyrosine kinases and MAP kinases via G-protein-independent signaling mechanisms (100, 102). AT$_1$ receptors also can function as a cardiac strain sensor independently of binding angiotensin II (108). Cardiac $\alpha_1$-adrenergic receptors retain normal expression and positive inotropism in the failing heart (56), unlike $\beta_1$, ET$_1$, and AT$_1$ receptors whose expression and function are profoundly altered in failure. Endothelin-1 exerts beneficial regulatory effects on cardiac energetics (see below), which have been difficult to verify in our laboratory for angiotensin II or the $\alpha_1$-adrenergic agonist phenylephrine because of generally less consistent and less robust inotropic responses (at least in rodents). It remains formally possible that smaller animals like rodents have more fully developed endothelin signaling systems, whereas larger animals like dogs, pigs, and humans rely more heavily on angiotensin II signaling for various aspects of cardiovascular regulation (but see Ref. 50).

**Endothelin and contractile efficiency**

Accumulating evidence suggests that endothelin-1 facilitates the cardiac response to chronic stress by enhancing contractile efficiency (48, 78). ATP consumption by the myocardium appears to be spared disproportionately compared with the gain of contractility. This is accomplished in several ways (FIGURE 3). First, although systolic Ca$_{\text{cyt}}$ may be somewhat elevated, it is due to an increase in trigger Ca$_{\text{couch}}$, rather than to an increase in SR Ca$_{\text{couch}}$ load. Thus pumping of Ca$_{\text{out}}$ into the SR between each heart beat is not dramatically enhanced as it is during $\beta$-stimulation. The modest increase in trigger Ca$_{\text{cyt}}$ is cleared through normal “forward mode” operation of the Na$^+$-Ca$_{\text{couch}}$ exchanger. The myofilament regulatory apparatus also appears to be sensitized to Ca$_{\text{couch}}$ such that less systolic Ca$_{\text{couch}}$ is required to initiate contraction and to achieve any given level of twitch force (91). The sensitized regulatory proteins also hold onto Ca$_{\text{couch}}$ longer due to a slower Ca$_{\text{couch}}$ dissociation rate. Finally, the myosin cross-bridge cycle is modified such that the contractile apparatus extracts more chemical energy from each ATP to do work. The principal modification to the cross-bridge cycle appears to be slower cross-bridge detachment at the end of the cycle (62, 63, 89). Importantly, these alterations in reaction rates complement each other and reinforce a prolonged state of attached cross bridges (FIGURE 3). The assumption is that prolonging the attached state of each cross bridge will translate directly into more work output for each ATP hydrolyzed. The costs associated with embarking on this strategy (which can be substantial) are described below.

**Long-Term Costs**

**Chronic challenges**

Given the energetic costs of invoking the fight-or-flight response, sustained $\beta$-adrenergic stimulation is almost certainly not intended to be a status quo condition over the long term. A sustained sympathetic discharge onto cardiac $\beta$-adrenergic receptors may occur during exercise, but for reasons that are not entirely clear, regular intermittent exercise is quite beneficial over the long term (43). In contrast, prolonged chronic overstimulation of ventricular $\beta$-receptors promotes detrimental cardiac remodeling including apoptosis (12, 42, 49).

Considering typical chronic challenges to cardiac performance such as high blood pressure (increased afterload), loss of healthy muscle mass (due to myocardial infarction), ineffective muscle performance due to sarcomeric mutations (hypertrophic cardiomyopathy), and/or a poor oxygen supply to the heart muscle (due to coronary artery blockage), the heart needs to boost its contractile performance to maintain or increase vascular perfusion. Due to limited oxygen supplies and/or chronically increased load under such conditions, it often must accomplish these goals with limited energy resources. In this case,
endothelin-1 would likely be produced locally to encourage efficient cardiac performance. One trade-off or “cost” of employing this kind of regulation by Gq receptors is that relaxation of the ventricles will be somewhat delayed due to slower cross-bridge cycling and slower Ca\(^{2+}\) dissociation from the troponin-tropomyosin regulatory complex. The time available for filling during diastole will also be shortened somewhat in this scenario, but if total cycle time (i.e., reciprocal of heart rate) is not decreased this should have no detrimental effects on cardiac output. A loss of ventricular compliance (enhanced stiffness)
in diastole due to increases in myofilament Ca\(^{2+}\) sensitivity could also provide unwanted resistance to filling, although modest changes should be readily accommodated. In effect, the heart is gambling that there is a “wiggle room” in a trade-off between utilizing ATP more slowly and efficiently vs. maintaining “contractile agility.” Under conditions of limited and controlled endothelin-1 stimulation, the heart appears to benefit by ejecting blood more efficiently.

**Hypertrophy, fetal gene program, and apoptosis**

If stress persists, several long-term strategies can be enlisted by the heart to meet its new circumstances. One is to enlarge the heart muscle mass by hypertrophy (rather than hyperplasia), and another is to adopt a modified gene expression pattern with similarities to a “fetal gene program.” A third is to engage the process of apoptosis, an energy-dependent highly orchestrated program resulting in tidy and selective elimination of individual myocytes. These processes are not necessarily interdependent or tightly coupled, although activation of GPCRs can play central roles in all three. A fourth long-term strategy is to switch its primary fuel source from fatty acids to glucose, although in this case GPCR signaling probably plays only a minor role, if any (1).

Hypertrophy of the heart muscle makes sense in terms of strengthening the pump to deal with a greater long-term work load. The fetal gene program makes some sense in the context of making the heart into a more efficient pump. Two of the big energy-consuming proteins, myosin motors and SERCA pumps, are modified as a result of reexpression of the fetal gene program. The adult α-myosin heavy chain isoform is replaced with the slower and more efficient β-myosin heavy chain isoform. The SERCA pump is downregulated at the gene and protein level again, consistent with sparing ATP supplies. These ATP-conserving measures reinforce the effects of endothelin-1 on cardiac arrhythmias and sudden death (7, 55).

Morphological changes also occur such as loss of the T-tubular compartment, a specialization of adult ventricular myocytes (4). T-tubules represent sites of primary communication between L-type Ca\(^{2+}\) channels and ryanodine receptors during excitation-contraction coupling (10) and sites of primary signaling via GPCRs, receptor tyrosine kinases, and serine/threonine protein kinases (68). Extensive remodeling of T-tubules may account for the altered function of these systems in heart failure.

Norepinephrine operating through β\(_1\)-adrenergic receptors is now recognized to be a potent simulator of apoptosis in ventricular myocytes (74). By contrast, endothelin-1 and α\(_1\)-agonists appear to promote apop-
tosis only under extreme conditions and instead exert a strong anti-apoptotic influence over a range of physiological concentrations (37, 105). More generalities about regulatory mechanisms underlying GPCR-initiated apoptosis will emerge in time and may clarify why in the face of chronic stress the heart resorts to this rather extreme strategy, which ultimately results in irreversible loss of muscle mass. What exactly does the adult heart hope to accomplish by inducing myocyte apoptosis? Is the stressed heart simply "confused" about where it is in its developmental program or is it trapped in a viscous cycle from which it cannot escape?

**Integrating multiple signals**

Our nascent understanding of even these few signaling systems is severely challenged when we next consider the consequences of co-stimulation. What happens during chronic elevation of angiotensin II and/or endothelin-1, when sympathetic reflexes are recruited in response to changes in blood O$_2$-to-CO$_2$ ratios (arterial chemoreceptors), blood pressure (arterial baroreceptors), and wall stress (cardiac afferents) to modulate sympathetic drive? Investigations of interactions between GPCRs, cytokine and growth factor receptors, and other signaling systems in the heart reveal extensive and complex cross talk (19, 28, 54, 90, 96). Even GPCRs as different as β$_1$-adrenergic, angiotensin II, and endothelin receptors may synergize or converge onto similar downstream signaling endpoints during chronic stimulation (19) (**FIGURE 4**). One example of such signal transformation and convergence is illustrated by recruitment of L-type Ca$^{2+}$ channels, CAM kinase II, and calcineurin during long-term stimulation of β$_1$-adrenergic receptors, Goq-coupled receptors, or both (93, 103, 104, 106, 107). Strong chronic activation of these Ca$^{2+}$ signaling pathways, regardless of initial input, often progresses to elevated diastolic Ca$^{2+}$, Ca$^{2+}$ overload, pathological hypertrophy, fetal gene expression, mitochondrial dysfunction, and apoptosis, unmistakable signs of progression toward overt failure (75, 104).

**Systems biology and heart failure**

Another significant challenge to establishing a complete understanding of signaling in the intact organ, even under healthy conditions, is the diversity of cell types that contribute to the ensemble of regulatory cross talk taking place throughout the cardiovascular system. Aside from atrial and ventricular myocytes, appropriate behavior from endothelial cells, vascular smooth muscles and fibroblasts are critical to maintaining a healthy heart. For example, general endothelial cell dysfunction is thought to create sufficient imbalance between two major endothelium-derived signals, endothelin-1 and nitric oxide, to initiate a downward spiral to cardiac failure (6). Excess endothelin-1 would tip the balance toward local and global vasoconstriction and may overdo enhanced myofilament Ca$^{2+}$ sensitivity and depressed cardiac cross-bridge cycling, resulting in excessively compromised relaxation kinetics and progressively more problematic diastolic stiffness. It is also now recognized that when the heart embarks on the long-term strategy of hypertrophy, it must carefully match formation of new blood vessels and endothelium (collectively termed angiogenesis) with increases in myocardial mass. A mismatch between neurohumoral controlled coronary angiogenesis and hypertrophic growth of the myocardium may indeed contribute to early onset cardiac failure (73).

Expression of specific fetal genes can be used as biomarkers for the onset of pathological hypertrophy in the clinic, with BNP currently the serum biomarker of choice (87, 95). Ventricular wall thinning (dilatation) also represents an unmistakable "morphological biomarker" of progression toward failure. The heart may be attempting to enhance cardiac output by increasing chamber volume. However, over the long haul, this strategy is disastrous because as the chamber diameter increases and the walls of the ventricles become thinner, they bear more force [according to the Law of Laplace (83)]. Chronic wall stress subsequently activates further neurohumoral signaling via 1) cardiac afferent reflexes, which further increase sympathetic drive (92), and 2) stretch-induced release of angiotensin II, endothelin-1, and activation of cardiac Goq receptors (70, 108). Such feedback cycles rapidly produce neurohumoral overstimulation and lead to the dreaded downward spiral into failure.

Infiltration of the heart by fibroblasts, macrophages, and other cell types associated with injury or inflammation can also profoundly affect heart function (86). Indeed, endothelin, and angiotensin II represent two of several neurohumoral signals thought to both recruit fibroblasts to injured myocardium and stimulate them to secrete fibrous connective tissue such as collagen (85). This build up of scar tissue has a major detrimental impact on the geometry and mechanical properties of the remodeled heart. Excessive collagenous scar tissue further stiffens the relaxed ventricle and interferes with the diastolic filling phase of the cardiac cycle.

**Heart failure therapy**

Among the most effective clinical treatments for human heart failure are β$_1$-receptor blockers and angiotensin converting enzyme (ACE) inhibitors (20, 44, 59). β-Blockers and ACE inhibitors either alone or in combination consistently improve heart failure symptoms and 5-year mortality rates, albeit only modestly (2). In animal models, β-blockers blunt apoptosis and cardiac remodeling associated with failure, inhibit β$_1$-adrenergic receptor internalization (61), and reduce PKA-dependent Ca$^{2+}$ leak from the sarcoplasmic reticulum (46). ACE inhibitors reduce hypertension, fluid excess, and cardiac hypertrophic remodeling and fibrosis (20). Additional benefit can
sometimes be achieved with blockers of AT₁ or aldosterone receptors (2). Endothelin receptor antagonists have shown promise against heart failure in animal models (53, 71) but thus far have failed to improve morbidity and mortality in clinical trials in humans (41). This is despite the fact that, in many animal models of heart disease including hypertension, ischemia, hypertrophy, infarct, and failure, there is a strong association between disease onset and activation of the endothelin signaling system (3, 21, 32, 52, 77, 80, 84, 88, 98, 99). Given our current level of understanding, can we rationalize why angiotensin II inhibitors aid in the treatment of heart failure in humans whereas endothelin antagonists do not?

Angiotensin II represents part of a complex cascade of signals that influence cardiac function in multifaceted ways. A primary trigger for angiotensin II production is the release of rennin from the kidney (in response to low flow) into the general circulation where it catalyzes the cleavage of angiotensinogen into angiotensin I, which in turn is converted to biologically active angiotensin II by ACE. Low fluid flow through the kidney is corrected by the actions of angiotensin II to increase systemic blood pressure by vasoconstriction, enhance cardiac contractility, and mobilize the mineralocorticoid steroid hormone aldosterone to promote renal Na⁺ and water retention. However, chronic elevation of this signal is detrimental in part because the positive contractile response of the heart to angiotensin II wanes, fibroblasts are recruited to produce scar tissue, growth signaling is activated in myocytes, leading to cardiac hypertrophy, and in vascular tissues, leading to hyperplasia, causing arteries to narrow and stiffen. Inhibition of ACE reduces these chronic effects of angiotensin II stimulation, thereby promoting cardiovascular health (2).

Like catecholamines, angiotensin II is a systemic signaling molecule with strong effects on blood pressure and blood volume, both of which are misregulated in congestive heart failure. Thus blockers of angiotensin II actions should have immediate system-wide impact on blood pressure and cardiac afterload. Angiotensin II is also a powerful chemical inducer of cardiac remodeling including hypertrophy, fetal gene expression, necrosis, and apoptosis (29, 35). Angiotensin II may also promote elevation of catecholamines via actions on central and peripheral nervous control of the cardiovascular system (94). On closer inspection, this may not be the entire story for ACE inhibitors. In mice, ACE inhibition shows beneficial effects on progression of hypertrophy and heart failure even in the absence of AT₁ receptors (in AT₁ knockout mice) (101). ACE inhibition may also reduce circulating aldosterone, which itself can exert direct effects on myocardium to promote cardiac hypertrophy and failure (81). Angiotensin II is thought to upregulate ECE (endothelin converting enzyme) expression, and ACE inhibition has been shown to blunt this upregulation (51). Other neurohormones such as bradykinin may also come into play with inhibitors of ACE because ACE represents a major pathway for inactivation of bradykinin. With ACE inhibitors, bradykinin is elevated and contributes its own positive effects on cardiac remodeling and function. These observations suggest pleiotropic effects of ACE inhibition that are likely to be unique to this regulatory pathway.

Endothelin-1 is an autocrine/paracrine messenger, since circulating levels are virtually undetectable in healthy individuals, and all the requisite machinery for synthesis, secretion, and detection of endothelin-1 is present locally within various cardiovascular tissues (39). Endothelin-1 is synthesized as a 200 amino acid precursor peptide, preproendothelin, and subsequently cleaved to 37 amino acid big endothelin and then to the final 21 amino acid bioactive peptide. The first step involves furin-like metalloproteinases, and the second involves ECE, which has also begun to be targeted therapeutically (5). Transcriptional regulation of preproendothelin mRNA synthesis is a major control point for cardiovascular endothelin-1 production (39). Of the two GPCRs for endothelin, ET₁ receptors predominate in ventricular cardiac myocytes, whereas ET₂ receptors predominate on endothelial cells that line the microvasculature and the cardiac chambers alike (39).

Endothelin’s relatively robust beneficial actions on ventricular contractility and efficiency may not be as well developed for angiotensin II (50) or α₁-adrenergic receptors. Throughout early disease progression then, low tonic levels of endothelin receptor activation may be important for normal myocardial function, survival, and protection against apoptosis (74, 105). However, at end-stage failure, both endothelin-1 and angiotensin II (but not norepinephrine acting on α₁ receptors) can exert substantial negative inotropic actions and further slow already sluggish ventricular relaxation (47, 58, 82). Thus antagonizing both endothelin and angiotensin II signaling should show similar benefits in advanced stages of failure, even in humans (31). Moreover, unlike β-blocker therapy, which does not improve exercise intolerance in patients, endothelin and angiotensin II antagonism either alone or in combination significantly improve exercise intolerance by blunting negative inotropic and lusitropic effects mediated by release of these neurohormones during exercise (13). Thus both similarities and differences exist between endothelin-1 and angiotensin II signaling in the cardiovascular system (13, 31), and reasons for ACE-directed therapies having proven more effective in humans may be due to its pleiotropic systemic roles in blood pressure regulation, cardiac remodeling, and apoptosis.

Concluding Remarks

Cardiac GPCRs as a group play many roles in the life cycle of the heart, such as regulation of contractility,
control of ATP utilization, anticipation of ischemic and hypoxic conditions, induction of cellular hypertrophy, reactivation of a fetal gene program, and initiation of programmed cell death. β-Adrenergic signaling is recruited systemically during exercise, and normal operation of cardiac reflexes, and is rapidly and robustly activated during acute stress. It turbo charges the contractile state of the heart and prepares it for a surge of high power output without concern for energy cost. In contrast, endothelin-1 is called on more locally within the myocardium to deal with chronic stresses like low coronary flow, low oxygen availability, and limited ATP supplies. The endothelin/ET_A receptor system functions to enhance cardiac contractile efficiency by tempering ATP utilization and slowing contractile kinetics.

Therapeutic intervention for heart failure currently involves β-blockers and ACE/AT_1 receptor inhibitors that serve to curtail unwanted prolonged stimulation of β-adrenergic receptors and AT_1 angiotensin II receptors. However, disease progression continues, morbidity and mortality remain high, and better therapeutic strategies are urgently needed. Effective therapeutic targeting of endothelin signaling for heart failure may depend on being able to selectively block its detrimental actions (on fetal gene expression, negative inotropy/lusitropy, and apoptosis) while retaining its beneficial effects on contractility, energy utilization, and survival. Health-promoting effects of red wine on the coronary vasculature have been attributed in large part to its inhibitory actions on endothelin production by the vascular endothelium (15). Beneficial effects of HMG-CoA reductase inhibitors (statins) have been attributed in part to secondary attenuation of endothelin signaling (45–71), and of course mixed ET_1/ET_A receptor antagonists are widely used for treatment of pulmonary arterial hypertension in humans (23, 33). Some investigators have called for more selective ET_A-directed receptor antagonists in patients with heart failure (41). Others have recommended more thorough patient profiling and prescreening to identify those likely to benefit from ET_A-directed therapies. For this, we need to understand much more than we currently do about short- and long-term mechanisms of endothelin signaling and the nature of its interactions with other neurohumoral signaling systems throughout the cardiovascular system.

References


