Clinical trials of beta-adrenergic receptor agonists and cyclic nucleotide phosphodiesterase inhibitors in heart failure have demonstrated a reduction in survival in treated patients despite initial inotropic responses. These findings have led many to infer that activation of the mechanisms through which contractility is increased has deleterious effects on failing myocardium. It should be remembered, however, that these agents act proximately by raising intracellular cyclic adenosine monophosphate (cAMP) content and stimulating protein phosphorylation by cAMP-dependent protein kinase, and that the proteins whose phosphorylation contributes to the inotropic responses may be different from the proteins whose phosphorylation contributes to the reduction in survival. Evidence in support of the latter interpretation is presented, and potential therapeutic approaches through which the phosphorylation of different proteins might be selectively affected are considered. (J Am Coll Cardiol 1999;34:318–24) © 1999 by the American College of Cardiology

Cyclic adenosine monophosphate (cAMP)-mediated signal transduction figures prominently in the pathophysiology of heart failure (for review, see [1]). A decrease in beta1-adrenergic receptor density and increases in beta-adrenergic receptor kinase, G-alpha, and nucleoside diphosphate kinase activity contribute to an impairment in cAMP generation and a reduction in intracellular cAMP content in failing myocardium (2–5). There are several reasons to believe that the consequent decrease in cAMP-dependent protein phosphorylation contributes to the diminished contractility and pathologic dilation characteristic of failing hearts (Fig. 1). Phosphorylation of Ca2+ channels in the sarcolemma and sarcoplasmic reticulum by cAMP-dependent protein kinase increases their open probability, resulting in increases in Ca2+ influx across the plasma membrane and Ca2+ release from intracellular stores during systole (6,7). Phosphorylation of phospholamban, a protein that associates with the Ca2+-transporting adenosine triphosphatase of the sarcoplasmic reticulum, stimulates Ca2+ sequestration by that organelle during diastole; phosphorylation of troponin I reduces the Ca2+ sensitivity of the actin-activated myosin adenosine triphosphatase of the contractile filaments (8,9). A reduction in cAMP content could therefore reduce the amplitude of intracellular Ca2+ transients and the extent of contraction and relaxation in cardiac myocytes. Phosphorylation of cAMP-response element-binding proteins (CREBs) by cAMP-dependent protein kinase activates the transcription of genes containing cAMP response elements (10). Precisely which genes in cardiac myocytes are regulated by CREB phosphorylation is unknown, but transgenic mice expressing a dominant negative (i.e., nonphosphorylatable) CREB develop a dilated cardiomyopathy that very closely resembles the human disease (11).

In view of these actions of cAMP in cardiac myocytes, it would be reasonable to expect agents that increase intracellular cAMP content to have beneficial effects in patients with heart failure. One therapeutic approach based upon this reasoning is the administration of agents that stimulate cAMP formation. Beta-adrenergic receptor agonists such as noradrenaline, adrenaline, dopamine and dobutamine have major inotropic effects in patients with heart failure (12). A trial of intermittent intravenous infusion of dobutamine was halted prematurely, however, because of an increase in mortality in treated patients (13). Xamoterol, a partial beta1-adrenergic receptor agonist, confers hemodynamic and symptomatic benefits in patients with heart failure, but its chronic administration likewise increases mortality in treated patients (14). Another approach is the use of agents that raise cAMP content in cardiac myocytes by inhibiting its breakdown by cyclic nucleotide phosphodiesterases. Enzymes in the PDE3 family of cyclic nucleotide phosphodi-
esterases, which hydrolyze both cAMP and cyclic guanosine monophosphate, constitute the principal high affinity cAMP phosphodiesterase activity in cardiac muscle (15,16). In early clinical trials, PDE3 inhibitors—for example, amrinone, milrinone and enoximone—were shown to improve cardiac index and lower pulmonary capillary pressure in patients with heart failure. Although some of these effects might have been attributable to concomitant vasodilation resulting from increases in cyclic guanosine monophosphate content in vascular smooth muscle myocytes, myocardial contractility, when measured, was clearly increased by PDE3 inhibition (17–21). In long-term clinical trials, however, the hemodynamic improvements seen early in therapy were typically not sustained, and meta-analysis demonstrated an increase in mortality of about 40% after several months of treatment (22–27). The reason for the increase in mortality among patients treated with beta-adrenergic re-

**Abbreviations and Acronyms**
cAMP = cyclic adenosine monophosphate
CREB = cAMP-response element-binding protein

ceptor agonists and PDE3 inhibitors was never established, although in one trial an increase in ventricular arrhythmias in treated patients was noted (24).

In the meantime, other investigators, viewing the abnormalities in cAMP-mediated signal transduction in failing myocardium from a different perspective, pursued a diametrically different approach. Guided by the notion that the high serum catecholamine levels seen in patients with heart failure are toxic and that changes in the level and function of proteins involved cAMP-mediated signal transduction constituting a compensatory response, they hypothesized that treatment with beta-adrenergic receptor antagonists might interrupt the process and thereby improve outcomes. Two decades of research culminated in prospective studies demonstrating that treatment with beta-adrenergic receptor antagonists does indeed increase survival and ultimately improve ventricular function in patients who can tolerate the drugs (28–30).

Taken together, the results of these trials have had a major impact on our current understanding of heart failure and its therapy. They have reinforced the concept that hemodynamic improvement does not ensure long-term therapeutic benefit, and this has contributed to a de-emphasis on hemodynamic improvement as a therapeutic goal. They have also led a large number of physicians to

![Figure 1](image_url)

**Figure 1.** Cyclic adenosine monophosphate (cAMP)-mediated signaling in cardiac myocytes. Cyclic adenosine monophosphate generated by adenylate cyclase activates cAMP-dependent protein kinase (PK-A), some of which is in the cytosol and some of which is bound to intracellular membranes by anchoring proteins (AKAPs). Proteins phosphorylated by cAMP-dependent protein kinase include L-type and ryanodine (Ry)-sensitive Ca$^{2+}$ channels in the sarcolemma and sarcoplasmic reticulum, respectively; phospholamban (PL), which interacts with the Ca$^{2+}$-transporting adenosine triphosphatase (SERCA2) of the sarcoplasmic reticulum; troponin (Tn) I, complexed with troponin C, troponin T, tropomyosin (TM) and actin in the thin filaments, and transcription factors such as cAMP-response element-binding protein (CREB), whose phosphorylation is dependent upon the translocation of cAMP-dependent protein kinase from the cytosol to the nucleus. ATP = adenosine triphosphate.
conclude that agents that increase contractility have adverse effects on survival in patients with heart failure.

**SHIFTING THE FOCUS FROM INOTROPY TO cAMP**

When examined carefully, the latter conclusion presents serious problems. Cyclic adenosine monophosphate has many important actions in cardiac myocytes apart from those involved in inotropic responses. The regulation of gene transcription by CREB phosphorylation and its importance in maintaining normal myocardial function and structure were noted earlier (11). Phosphorylation by cAMP-dependent protein kinase also alters the activity of glycogen synthase and phosphorylase kinase so as to stimulate glycogen hydrolysis; its phosphorylation of beta-adrenergic receptors and R subunits of cAMP-dependent protein kinase serves an autoregulatory function for these pathways (31–33). In view of these diverse actions, the basis upon which the increased mortality in patients treated with beta-adrenergic receptor agonists and PDE3 inhibitors can be ascribed specifically to the activation of molecular mechanisms involved in their inotropic effects must be questioned. There are, in fact, several reasons for believing the increased mortality is not linked mechanistically at the molecular level to increased contractility. For one, hemodynamic responses to beta-adrenergic receptor agonists and PDE3 inhibitors are immediate, whereas increases in mortality require months to demonstrate. Furthermore, withdrawal of PDE3 inhibitors after chronic administration is not accompanied by any diminution in contractility (22). This observation is not surprising, given that the inotropic efficacy of PDE3 inhibitors correlates inversely with the severity of the cardiomyopathy and that the severity of the cardiomyopathy progresses with time (34). But the fact that survival is adversely affected despite a loss of inotropic efficacy makes it difficult to conclude that the increased mortality in patients treated with beta-adrenergic receptor agonists and PDE3 inhibitors is inextricably tied to the increase in contractility these agents elicit. This difficulty is heightened by the fact that digoxin, an inotropic agent whose mechanism of action does not involve cAMP-mediated signal transduction, does not increase cardiovascular mortality in patients with heart failure (35).

For all these reasons, it may be preferable to regard the adverse effects of beta-adrenergic receptor agonists and PDE3 inhibitors in patients with heart failure as a function of the increase in intracellular cAMP content they generate rather than as a consequence of the mechanisms involved in inotropic responses (Fig. 2). From this perspective, increased mortality would be viewed as the net result of the multiple effects of a nonselective increase in the phosphorylation of literally dozens of substrates of cAMP-dependent protein kinase involved in diverse intracellular functions in cardiac myocytes. This interpretation has two important corollaries. The first is that although the overall balance of the effects of raising intracellular cAMP content in cardiac myocytes is harmful, it is possible that some of the individual effects are beneficial. It may be, for example, that inotropic responses are desirable but are outweighed by an increase in ventricular arrhythmias. The second is that the reduction in survival in patients treated with beta-adrenergic receptor agonists and PDE3 inhibitors might be attributable to the phosphorylation of a subset of protein substrates that either is separate from or only partially overlaps with the subset of protein substrates to which potentially desirable effects on contractility and gene transcription are attributable (Fig. 3). Increases in the phosphorylation of sarcolemmal Ca$^{2+}$ channels, troponin I and CREB, for example, might augment contractility and slow or reverse the pro-
gression of myocardial dilation without adversely affecting mortality, whereas increases in the phosphorylation of phospholamban, sarcoplasmic reticulum Ca\(^{2+}\) channels, glycogen synthase and phosphorylase kinase might reduce survival by increasing ventricular ectopic activity and depleting myocardial energy reserves. If this is the case, interventions that could increase the phosphorylation of “beneficial” substrates without increasing the phosphorylation of “harmful” ones might allow inotropic and other benefits to be achieved without the concomitant increase in mortality seen with nonselective increases in cAMP-stimulated protein phosphorylation.

In the absence of a clearly identified mechanism to which the increased mortality that accompanies increases in intracellular cAMP content can be ascribed, the possibility that separate substrates of cAMP-dependent protein kinase contribute to beneficial and harmful responses is a matter of speculation. But the difficulties noted earlier in attributing the increase in mortality in patients treated with beta-adrenergic receptor agonists and PDE3 inhibitors to mechanisms involved in inotropic responses certainly makes this speculation plausible. Also, increases in our understanding of cAMP-mediated signal transduction in cardiac myocytes now offer several opportunities for selectively increasing the phosphorylation of specific substrates of cAMP-dependent protein kinase, at least one of which may be involved in a recently reported therapeutic approach.

**NEW THERAPEUTIC DIRECTIONS**

One opportunity for selectively modulating the phosphorylation of different substrates of cAMP-dependent protein kinase relates to the fact that the cAMP content in different intracellular compartments of mammalian cardiac myocytes can be differentially regulated. Some of this differential regulation takes place at the receptor level. Occupancy of beta\(_1\)-adrenergic receptors by their agonists results in an increase in both cytosolic and membrane-bound cAMP content; the same is true for beta\(_2\)-adrenergic receptors, but in the latter case the increase in membrane-bound cAMP content is significantly lower than is seen when beta\(_1\)-adrenergic receptors are occupied, and when prostaglandin E1 receptors (which, like beta-adrenergic receptors, are coupled by G-alpha\(_S\)) to adenylate cyclase) are occupied by their agonists, the increase in cAMP content is restricted to the cytosol (36–39). Exactly how compartmental cAMP levels are selectively regulated at the receptor level is unclear—coupling of beta\(_1\)- and beta\(_2\)-adrenergic receptors to different classes of G proteins may be involved (40)—but the result is a differential activation of cAMP-dependent protein kinase in functional compartments of cardiac myocytes and a consequent phosphorylation of different substrates. Increases in the amplitude of intracellular Ca\(^{2+}\) transients in response to beta\(_2\)-adrenergic receptor agonists correlate with changes in membrane-bound cAMP content, and are accompanied by increases in phospholamban phosphorylation and the rate of relaxation; occupancy of beta\(_2\)-adrenergic receptors by their agonists, in contrast, results in an increase in the amplitude of intracellular Ca\(^{2+}\) transients that does not correlate with changes in cAMP content and occurs without increases in phospholamban phosphorylation or relaxation rates (38,41,42). In fact, the stimulation of Ca\(^{2+}\) influx through dihydropyridine-sensitive channels by beta\(_2\)-adrenergic receptor agonists, although dependent on cAMP-dependent protein kinase activity, occurs without any detectable change in total intracellular cAMP content (43,44). The latter observation is evidence that changes in intracellular cAMP content in response to receptor occupancy can be localized with exquisite precision.

This compartmental regulation of cAMP-dependent protein phosphorylation may be important in heart failure for several reasons. The selective down-regulation of beta\(_2\)-adrenergic receptors in failing myocardium may cause a selective decrease in membrane-associated cAMP content (2,45). Thus, the pattern of proteins phosphorylated in failing myocardium in response to beta-adrenergic receptor agonists may differ significantly from what is seen in normal tissue. Compartmentation may also be relevant to the recently reported use of the combination of metoprolol and enoximone in patients with advanced contractile failure (46). Selective beta\(_1\)-adrenergic receptor antagonism in conjunction with nonselective PDE3 inhibition might produce what would amount to a selective potentiation of beta\(_2\)-stimulated responses, with a resulting pattern of protein phosphorylation that would differ from that obtained with nonselective beta-adrenergic receptor stimulation or nonselective PDE3 inhibition alone. For reasons noted above, this might lead to an increase in contractility without an increase in Ca\(^{2+}\) accumulation by the sarcoplasmic reticulum; if the latter is arrhythmogenic, this might prove beneficial. Compartmentally selective pharmacologic manipulation of cAMP-mediated signal transduction might also be achieved through selective PDE3 inhibition. PDE3 activity is found both in the cytosol and associated with intracellular membranes in cardiac myocytes, and there is evidence that cytosolic and membrane-bound forms of PDE3 are separate molecular species (47). Selective inhibition of the specific compartmental isozyme could lead to a selective increase in compartmental cAMP content and kinase activity. Determining whether the differences between cytosolic and membrane-associated forms of PDE3 at the molecular level are sufficient to make selective inhibition of the membrane-associated isozyme feasible is a central issue.

A second opportunity for selectively increasing the phosphorylation of different substrates of cAMP-dependent protein kinase involves the different isoforms of the enzyme, of which two classes have been described (for review, see [1]). The two classes, designated type I and type II, differ with respect to intracellular localization, activation and regulation, and cyclic nucleotide analogs that preferentially activate one or the other class of isoform have been
identified (48–50). The use of these analogs has demonstrated that the two isoforms serve different functions. In T lymphocytes, for example, proliferation is inhibited by activation of the type I kinase but not by activation of type II kinase; lipolytic effects in adipocytes are attributable to activation of RII-associated C subunits (51–53). Selective activation of type I or type II isoforms, both of which are present in cardiac myocytes (54), may offer a means of selectively altering the phosphorylation of the substrates of cAMP-dependent protein kinase. Whether the two isoforms phosphorylate different substrates in cardiac myocytes—and, if so, whether selective activation of the appropriate isoforms can be used to separate beneficial and harmful effects—will be an important area for future experiments.

The possibilities for selectively increasing the phosphorylation of targeted substrates discussed rely upon selective stimulation of cAMP-dependent protein kinase activity. Another possibility involves the inhibition of the protein phosphatases that dephosphorylate these substrates (for review, see [55,56]). For many years it was believed that protein phosphatases are relatively few in number and relatively nonspecific with respect to substrates, so that opportunities for selectivity seemed unlikely. More recently, though, the number of different phosphatases that have been identified has increased dramatically, and it has become clear that noncatalytic subunits of some of these phosphatases can confer exquisite substrate specificity, through either substrate recognition or intracellular targeting. If the substrates of cAMP-dependent protein kinase are dephosphorylated by different protein phosphatases, it may be possible to selectively increase the phosphorylation of substrates contributing to the beneficial effects of increases in cAMP content by selectively inhibiting the protein phosphatases to which they are coupled (Fig. 4).

There are several reasons to be optimistic about phosphatase inhibition as a therapeutic approach in heart failure. First, protein phosphatases have very different patterns of tissue expression, with some being expressed abundantly and somewhat selectively in myocardium (57). Cardiac-specific phosphatase inhibition may be feasible. Furthermore, phosphatase inhibition has been shown to result in major inotropic effects that, unlike those of PDE3 inhibition, are not diminished in magnitude in failing myocardium (58). Also, more recently, studies in animals have pointed to a role for one phosphatase, calcineurin, in the pathogenesis of cardiomyopathies. Calcineurin inhibitors prevent the development of myocardial hypertrophy in animals predisposed to this condition because of abnormal expression of contractile proteins; animals overexpressing calcineurin develop myocardial hypertrophy (59,60). Phosphatase inhibition thus offers the prospect of not merely improving the outcome in patients with cardiomyopathies but of actually preventing or reversing the underlying myocardial pathology.

CONCLUSIONS

The recognition that beta-adrenergic receptor agonists and PDE3 inhibitors have adverse effects on survival in patients with heart failure has profoundly influenced thinking about this syndrome and its treatment. What remains unclear, however, is whether this reduction in survival is mechanistically linked to the inotropic actions of these drugs or results from the activation of other cAMP-mediated processes in cardiac myocytes. A shift in focus from inotropy to cAMP-mediated signaling and a consideration of the molecular mechanisms involved in this signaling opens the door to several new therapeutic strategies through which these mechanisms might be modulated selectively, possibly allowing the hemodynamic and other potentially beneficial effects of increases in intracellular cAMP content to be achieved without the concomitant reduction in survival seen with currently available agents.

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