Cardiac Hypertrophy
Mechanical, Neural, and Endocrine Dependence

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Cardiac hypertrophy usually follows an increase in work load that is imposed on the heart. Usually, greater work is due to increased afterload in patients with hypertension or after myocardial infarction in which the remaining muscle must assume the work of the segment that was lost as a result of myocardial cell death and scar formation. In some instances, increased pressure or volume work of the left ventricle is associated with increased release of neurotransmitters or plasma concentrations of hormones that may also have direct effects on cardiac myocyte growth, including activation of the sympathetic nervous system, pheochromocytoma, hyperthyroidism, and high renin hypertension. In these cases, is hypertrophy of cardiac myocytes due to a direct effect of the neurotransmitter or hormone receptor binding, or is hypertrophy due to increased work load imposed on the heart? Does pressure-overload hypertrophy depend on receptor binding to generate the intracellular signals required to transduce increased work into cell growth? In addition to the classic neural and hormonal pathways for signal transduction, is there a third pathway originating within the cell itself due to stretching or other types of deformation that can generate intracellular signals, such as increased intracellular Na⁺, pH, cytosolic free Ca²⁺, cyclic AMP (cAMP), inositol phosphates, and diacylglycerol concentrations without reliance on receptors for neurotransmitters or hormones? How do the intracellular signals that are generated increase rates of protein synthesis, decrease rates of protein degradation, or both to result in growth of the heart? These questions will be addressed in dealing with the mechanisms that translate greater heart work to cardiac hypertrophy. This last question was discussed in recent reviews.¹⁻³

Mechanical Factors in Cardiac Hypertrophy

In Vivo Studies

Increased work of the heart is usually associated with accelerated growth and cardiac hypertrophy. Pressure-overload hypertrophy in either human or animal models often is associated with areas of focal necrosis (for reviews, see References 1 and 3). These findings raise the question as to whether the heart grows in response to the pressure load or to compensate for loss of myocardial mass. Volume overload usually results in rates of hypertrophy that are not sufficiently fast for studies of the mechanism of either accelerated rates of protein synthesis or decreased protein degradation. Rapid rates of growth that are not associated with myocardial cell death occur in the neonatal pig heart, in which the left ventricle grows threefold or more rapidly than the right ventricle during the first 10 days of life.⁴ Injection of replacement doses of thyroid hormone (T₃) into hormone-deficient animals and swimming exercise of female rats also lead to rapid growth without myocardial cell death.⁵ All of these in vivo conditions, however, are associated with changes in the release of neurotransmitters or plasma concentrations of hormones. In this situation, a rigorous assessment of the direct effects of mechanical parameters such as increased active or passive wall tension on growth is impossible.

In Vitro Studies

The complexity of the in vivo environment has caused virtually all studies of the effects of mechanical factors on generation of intracellular signals and cell growth to be carried out in vitro. Deformation of the tissue or cell by stretching or swelling has been identified as the mechanical parameter most closely linked to these events. Acceleration of protein synthesis by stretch of quiescent papillary muscle was first observed by Peterson and Lesch.⁶ Stimulation of the muscle to contract did not increase the rate of protein synthesis in stretched muscle; rather, myocardial protein synthesis increased in proportion to total muscle tension.⁷ Takala⁷ and Kira et al⁸ found that elevation of aortic pressure in hearts arrested by potassium or tetrodotoxin accelerated protein synthesis. In the experiments of Kira et al,⁸ increased aortic pressure did not change oxygen consumption, substrate use, or energy availability, and intraventricular pressure and heart rate were zero. Smith and Sugden⁹ found that increased left atrial filling pressure increased the rate of left atrial protein synthesis in the perfused working rat heart. Increased aortic pressure also decreased the rate of protein degrada-
tion and accelerated rates of ribosome formation in isolated rat hearts. An increase in intraventricular pressure from 0 to 25 mm Hg also increased the rate of protein synthesis in tetrodotoxin-arrested hearts. These findings support the hypothesis that stretch of the ventricular wall sets in motion a signal transduction pathway that leads to enhanced efficiency of protein synthesis via accelerated initiation, elongation and termination of peptide chains on preexisting ribosomes, and faster formation of new ribosomes and other components of the protein synthetic pathway.

In contrast to quiescent cells, contracting neonatal rat myocytes hypertrophied during 3 days in culture due to accelerated rates of protein and RNA synthesis. In these studies, growth was not correlated to any specific index of contractility, such as rate or amplitude of contraction. In adult feline cardiocytes, contracting cells had a larger cardiocyte surface area than nonbeating cells after 2 weeks in culture. When these feline myocytes were plated onto a deformable, laminin-coated substrate, linear deformation that increased myocyte length by approximately 10% increased the rate of incorporation of [3H]uridine into nuclear RNA and of [3H]phenylalanine into cytoplasmic protein. These data indicate that an increase in load is a sufficient stimulus for induction of accelerated RNA and protein synthesis in the adult mammalian cardiac muscle cell. A similar result was obtained earlier in skeletal muscle in which stretching of adherent myotubes on a filter disc accelerated macromolecular synthesis. Recently, a new method to impose a load on isolated myocytes was reported that uses viscous incubation media. This method of loading affected both the rate and the extent of sarcomere shortening. Studies of viscous loading on myocyte growth have not been reported.

**Proposed Mechanism of Action**

Mechanical stretch and cell deformation generate a wide range of intracellular signals that may modify rates of RNA and protein synthesis and degradation (Table 1). In this section, generation of these signals is reviewed; the effects of the signals on macromolecular synthesis and degradation is discussed later in the article. Muscle cell membranes as well as many other tissues contain mechanotransducer ion channels that are either activated or inactivated by stretch (for a review, see Reference 21). Most stretch-activated ion channels appear to be rather nonselective. The material experiencing these mechanical forces may be more than the bilayer alone—probably a combination of bilayer plus membranous cytoskeletal network and extracellular matrix.

In regard to intracellular ions, Na⁺ uptake was increased in quiescent or contracting ferret papillary muscle as load on the muscle was increased. The addition of streptomycin, a cationic blocker of mechanotransducer ion channels, had no effect on protein synthesis in slack muscles that were stimulated but inhibited the faster rate of protein synthesis observed in contracting muscles developing tension. To provide further support for the role of Na⁺, inotropic agents were chosen that increased development of muscle tension but either enhanced (monensin or veratridine) or inhibited (amiloride) sodium entry. Protein synthesis varied directly with sodium influx despite the positive inotropic effect observed with each of these agents. This study of Kent et al. identified deformation-dependent sodium influx as an early signal in the transduction of load into growth. In isolated, perfused rat hearts, however, addition of streptomycin or dihydrostreptomycin did not block the effect of elevated aortic pressure to accelerate protein synthesis. However, each drug decreased heart rate 20–30% compared with the value obtained at the end of 10 minutes of preliminary perfusion. These studies in the rat did not provide support for the hypothesis that sodium influx was a link between stretch of the ventricular wall and an accelerated rate of protein synthesis.

Stretch-activated ion channels that are permeable to Ca²⁺ have been reported in endothelial cells and oocytes. Similarly, hyposmotic swelling of S49 lymphoma cells increased intracellular calcium concentration within 1 minute of osmolarity reduction. In the isolated, perfused rat heart, elevation of aortic pressure from 60 to 120 mm Hg increased the initial rate of ⁴⁵Ca²⁺ uptake and the steady-state level that was achieved. Although elevation of perfusate Ca²⁺ from 0.5 to 2.9 and 5.0 mM doubled oxygen consumption rates of protein synthesis and ribosome formation were unchanged. These studies indicated that stretch of the ventricular wall increased intracellular calcium but failed to link cytosolic calcium to macromolecular synthesis.

Alkalization of the cell interior may represent a signal for cell growth. Recently, Schwartz et al. reported that intracellular pH (pHi) was increased in well-spread 3T3 cells in serum on tissue culture plastic compared with round cells on a nonadhesive surface. Addition of ethylisopropyl amiloride decreased pH, and eliminated the difference between round and spread cells. Na⁺-H⁺ exchange appeared to have a primary role in determining steady-state pH, and was responsible for the lower pH level in

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**Table 1. Possible Effector Mechanisms and Intracellular Signals That May Be Generated by Stretch and Contribute to Cardiac Hypertrophy**

<table>
<thead>
<tr>
<th>Component</th>
<th>Intracellular signal</th>
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<tr>
<td>Stretch-sensitive channels</td>
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<tr>
<td>Activated Cation channels</td>
<td>Na⁺, K⁺</td>
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<tr>
<td>Ca²⁺ channels</td>
<td>Ca²⁺</td>
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<tr>
<td>Inactivated K⁺ channels</td>
<td>K⁺</td>
</tr>
<tr>
<td>Adenyl cyclase</td>
<td>cAMP</td>
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<tr>
<td>Phospholipase C</td>
<td>Inositol phosphates</td>
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<td>Na⁺-H⁺ exchange</td>
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round cells. Bicarbonate-dependent regulatory mechanisms were also inhibited in round cells. These studies suggested that cell deformation could modify rates of macromolecular synthesis by modifying pH.

Increased intracellular content of cAMP has been suggested to play a role in regulating cardiac hypertrophy resulting from a variety of physiological and pharmacological stimuli. Schriever et al. observed an increase in adenyl cyclase activity within 10 minutes of initiation of acute hemodynamic overload in the perfused guinea pig heart, but the content of cAMP did not change. Singh reported that mechanical stretch of the isolated frog ventricle in vitro elicted a rapid rise in cAMP content. Studies from this laboratory showed that elevation of aortic pressure from 60 to 120 mm Hg in either beating or tetrodotoxin-arrested hearts accelerated rates of protein synthesis and ribosome formation and increased cAMP content and cAMP-dependent protein kinase activity. Exposure of hearts to methacholine, a muscarinic-cholinergic agonist, for 5 minutes before elevation of perfusion pressure blocked the pressure-induced increases in cAMP content, cAMP-dependent protein kinase activity, and rates of protein synthesis and ribosome formation. These studies indicated that the effects of increased aortic pressure to accelerate protein synthesis and ribosome formation appeared to involve a cAMP-dependent mechanism that was independent of changes in contractile activity but could be blocked with a muscarinic-cholinergic agonist. Hyposmotic swelling of avian erythrocytes and S49 lymphoma cells increased intracellular cAMP, indicating that cellular deformation directly stimulated cAMP accumulation. Experiments involving cyc mutants of S49 cells, lacking the G,-guanine nucleotide binding protein, and 2',5'-dideoxyadenosine indicated that increased adenyl cyclase activity with swelling was not mediated by G. Disruption of the actin membrane skeleton with cytochalasin B resulted in a significant accumulation of cAMP that was not increased further by swelling. These studies concluded that the S49-adenyl cyclase responded directly to mechanical forces transmitted through the actin membrane skeleton.

Myocardial stretch also increased the contents of inositol monophosphate, inositol biphosphate, and inositol triphosphate in isolated, perfused rat hearts. The right atrium and right ventricle were dilated with fluid-filled latex balloons. Perfusion with propanolol, prazosin, or atropine did not alter the inositol phosphate response to dilatation, indicating that it was not due to release of norepinephrine or acetylcholine. Increased contents of inositol phosphates presumably are due to activation of phospholipase C, but activity of the enzyme has not been directly measured. Similarly, increased tissue content of diacylglycerol would be expected but has not been reported. As is discussed later, activation of protein kinase C leads to cardiac cell growth and could result from increased intracellular concentrations of calcium and diacylglycerol.

Myocardial stretch also generates an intranuclear signal in the form of increased expression of a proto-oncogene. Whether this event is due to deformation of the nucleus itself or whether it depends on generation of an intracellular signal of the types discussed above has not been determined. Increased expression of proto-oncogenes was observed earlier in cardiac hypertrophy, including that induced by pressure overload in vivo. In cultured rat myocytes, accumulation of c-fos messenger RNA (mRNA) was detected as early as 15 minutes after stretching of the cells by 10% and reached a maximal level by 30 minutes. Stretching appeared to regulate the c-fos gene at the transcriptional level because transcription of a transfected chimeric gene containing the upstream sequences and promoter of the fos gene and the chloramphenicol acetyltransferase coding sequence was markedly increased. This experiment also showed that the stretch-responsive element was located in the 5'-flanking DNA of the c-fos gene.

Overall, cellular stretch modifies the intracellular contents of signaling compounds, including Na+, Ca++, cAMP, inositol phosphates, and H+. These changes occur without the participation of humoral factors. Signal transduction by stretch or other types of deformation represents a third pathway that complements transduction of neural and humoral signals. A major challenge is to determine which of these intracellular signals are important for the regulation of RNA and protein synthesis and to determine the molecular mechanism of the regulatory event.

Neural Control of Cardiac Hypertrophy

As was discussed, changes in hemodynamic loading appear to be an important stimulus for cardiac growth. The question is whether trophic factors are also directly involved. A dissociation between elevated arterial pressure and increased myocardial mass has been demonstrated in hypertensive cardiac hypertrophy in animals and humans. This dissociation suggests the existence of stimuli other than blood pressure that are responsible for the development and regression of cardiac hypertrophy. In some instances, a permissive effect of neurotransmitters or hormones for the action of a mechanical stimulus on growth of cardiac muscle cells may be involved, whereas in other circumstances, the neurotransmitter may exert a direct affect on growth. During the past several decades, many neural and hormonal stimuli have been implicated in cardiac muscle growth, including but not limited to α- and β-adrenergic agonists, thyroxine, angiotensin II, glucocorticoids, insulin, growth hormone, and glucagon. In vivo and in vitro data pertaining to adrenergic stimuli, thyroxine, and angiotensin II, all of which have been suggested to directly modulate cardiac muscle growth, are reviewed, and possible mechanisms of action are discussed. The other factors will not be discussed further because there is no substantial evidence that they act directly to initiate cardiac hypertrophy fol-
lowing a hemodynamic load. Glucocorticoids, for example, appear to play only a permissive role in pressure-induced hypertrophy.30

**α₁-Adrenergic–Mediated Cardiac Hypertrophy**

*In vivo studies.* Adrenoceptor activation could be a primary effector initiating and maintaining cardiac hypertrophy as a result of increased cardiac sympathetic nerve activity and elevated levels of circulating catecholamines. α-Adrenergic receptors are present in cardiac myocytes, and effects of nonhydrolyzable analogs of GTP on agonist binding suggest that the α₁-adrenergic receptor is coupled via a guanine nucleotide binding protein (G protein).41 α₁-Adrenergic receptor stimulation produces positive chronotropic and inotropic effects that may, in part, be related to phosphatidylinositol turnover42 and Ca²⁺ entry.43 cAMP levels decrease due to activation of cAMP phosphodiesterase activity.44 Norepinephrine infusion into dogs resulted in significant myocardial hypertrophy, and the changes in ventricular mass were suggested to be independent of changes in arterial blood pressure, intracardiac pressures, myocardial shortening rate or velocity of shortening, or cardiac work.45,46 It was recently shown in the rat that norepinephrine or norfenephrine (α₁-adrenergic agonist) increased left ventricular weight–to–body weight ratios and was accompanied by an increase in total RNA and the RNA-to-DNA ratio.47 Prazosin (α₁-adrenoceptor blocker) and metoprolol (in an amount that blocked both β₁- and β₂-adrenoceptors) each partially antagonized the increase in left ventricular weight; when administered simultaneously, they prevented the norepinephrine-induced increase in left ventricular mass. Changes in functional parameters caused by norepinephrine were reversed with verapamil; however, this treatment did not prevent the development of cardiac hypertrophy. The data suggest that cardiac hypertrophy that develops in the rat in response to norepinephrine was directly mediated by stimulation of myocardial α- and β-adrenoceptors and was not secondary to hemodynamic changes.

It has been inferred that pressure-overload cardiac hypertrophy may be partially secondary to concurrent sympathetic stimulation (for a review, see Reference 48). However, Cooper et al48 reported two sets of negative data that refute the hypothesis that adrenergic stimuli are important in mediating the cardiac hypertrophy during pressure overload. Using an in vivo model of ventricular hypertrophy in the cat, the right ventricle was loaded by pulmonary artery banding while a constituent papillary muscle was unloaded. When differential loading was imposed on two segments of the same ventricle, the unloaded papillary muscle atrophied while the remainder of the right ventricle hypertrophied.46 To investigate sympathetic input as a contributing factor to the hypertrophy, the experiment was repeated after epicardial denervation. The loaded ventricular muscle hypertrophied to the same degree in the denervated heart. When β- or α-adrenoceptor blockade was produced before and maintained during the pressure overload, the hypertrophic response was not impaired compared with control cats whose right ventricles were pressure overloaded.48 These data indicate that adrenergic activation is not necessary and does not modify the cardiac hypertrophic response to an increased hemodynamic load. Although the adrenergic system has been suggested to play an important role in regression of myocardial hypertrophy in spontaneously hypertensive rats (SHR),49 central and peripheral sympathectomy did not prevent the development of cardiac hypertrophy but did prevent the development of hypertension.50–52 Overall, these studies suggest that cardiac hypertrophy in SHR may develop as a genetic cardiovascular abnormality that does not require catecholamines or systemic hypertension for its expression. Similarly, the available data do not indicate that α₁-adrenergic stimuli are important in mediating cardiac hypertrophy due to pressure overload.

*In vitro studies.* Although adrenergic agonists appeared to cause growth of the heart in vivo without dependence on increased contractility or afterload, a direct test of norepinephrine’s growth potential was examined in cardiac myocytes and perfused hearts.53–56 Many of these studies involved neonatal cardiac myocytes in cell culture.53–55 Neonatal cells may still divide, but hypertrophic effects of α₁-adrenergic agonists were clearly demonstrated. Recently, accelerated rates of protein synthesis that appeared to be mediated by the α₁-adrenoceptor were observed in cardiac myocytes and perfused hearts from adult rats.56 In cultured neonatal myocytes, the growth effect was characterized by increased rates of protein synthesis, myocyte surface area, and protein content. DNA synthesis was unaffected by α₁-adrenergic stimuli. The hypertrophic response to α₁-adrenergic stimuli also involved selective upregulation of the myosin light-chain gene and of the early developmental contractile protein isoforms, skeletal α-actin, and β-myosin heavy-chain genes.57–59 α-Adrenoceptor stimulation was also accompanied by a twofold to threefold increase in total transcriptional activity, which was dependent on the concentration and duration of the exposure to the agonist and displayed α₁-adrenergic receptor specificity.57 In cardiac myocytes and perfused hearts from adult rats, increased rates of protein synthesis dependent on α₁-adrenergic stimulation were due to faster rates of translation of preexisting mRNA.56 In summary, there are substantial data to indicate that α₁-adrenergic stimuli can mediate cardiomyocyte growth in vitro. Whether this effect can be shown to occur in vivo with physiological concentrations of α₁-adrenergic agonists remains to be determined.

Proposed mechanisms of action. The intracellular transducers involved in α₁-adrenoceptor–mediated growth and gene expression in the heart are unknown. Potential mediators of the α-adrenergic signal transduction include inositol triphosphates and
diacylglycerol (Figure 1). \(\alpha_1\)-Adrenergic–stimulated decreases in intracellular levels of cAMP due to an increase in cAMP phosphodiesterase activity\(^{44}\) are not likely to be coupled to cardiomyocyte growth. Therefore, the role of this second messenger is not discussed further as it pertains to \(\alpha_1\)-adrenergic stimulation. The \(\alpha_1\)-adrenergic stimulation of phospholipase C results in selective cleavage of the plasma membrane lipid phosphatidylinositol-4,5-biphosphate.\(^{60}\) This cleavage generates two biologically active intracellular messengers—1,2-diacylglycerol (DG) and inositol-1,4,5-triphosphate (Ins-1,4,5-IP\(_3\)). DG activates the membrane-bound, phospholipid-dependent, Ca\(^{2+}\)-dependent protein kinase C, and in skeletal muscle Ins-1,4,5-IP\(_3\) can release Ca\(^{2+}\) from stores in the sarcoplasmic reticulum.\(^{60}\) Whether Ins-1,4,5-IP\(_3\) or other water-soluble inositol phosphates are important in the regulation of cell Ca\(^{2+}\) in cardiomyocytes is not known.

\(\alpha_1\)-Adrenoceptor–stimulated increases in DG and activation of protein kinase C provide a starting point for determining the growth effects of these stimuli in cardiomyocytes. The tumor promoters phorbol myristate acetate and 12-0-tetradecanophorbol-13-acetate are potent activators of protein kinase C that produce effects similar to those of \(\alpha_1\)-adrenergic stimuli (i.e., increased rates of protein synthesis, cardiomyocyte growth, and upregulation of skeletal \(\alpha\)-actin and \(\beta\)-myosin heavy-chain genes) when administered to cardiomyocytes in culture.\(^{61,62}\) Activation of protein kinase C could lead to phosphorylation of a transcription factor that binds to a regulatory region of a sensitive gene such as skeletal \(\alpha\)-actin or to another protein bound to a regulatory region, thereby activating RNA polymerase II.

The activities of several nuclear enhancer–binding proteins (AP-1 and AP-2) were shown to be increased by phorbol esters and, in the case of AP-2, by cAMP-dependent protein kinase A as well. AP-1 is the transcription factor responsible for basal expression of many genes and mediates transcriptional induction in response to phorbol ester.\(^{53,64}\) Thus, AP-1 is at the receiving end of a complex pathway responsible for transmitting the effects of these tumor promoters from the cell membrane to the transcriptional machinery. Treatment of HeLa cells with phorbol esters and agents that elevate cAMP increased activity of the enhancer element AP-2.\(^{65,66}\) Therefore, the latter transcription factor (AP-2) mediated the effects of two distinct signaling transduction systems that have been implicated in control of cell growth. At the present time, neither AP-1 nor AP-2 has been demonstrated in heart muscle, although both appear to be ubiquitous in their distribution. To further complicate this scenario, at least two distinct regions of the transforming growth factor–\(\beta_1\) (TGF-\(\beta_1\)) gene are responsive to autoregulation and activation by phorbol ester.\(^{67}\) TGF-\(\beta_1\) is important in the regulation of cellular growth and differentiation in many cell types. TGF-\(\beta_1\) responsiveness is conferred by the transcription factor AP-1, which appears to be involved in the second promoter–derived transcription of the TGF-\(\beta_1\) gene. The phorbol ester–responsive element serves as a binding site for the AP-1 complex that mediates the induction of transcription by phorbol ester. The AP-1 complex has been shown to result from dimeric association of AP-1 and the cellular oncogenes Jun and Fos.\(^{67}\) To this end, it has recently been shown that TGF-\(\beta_1\) enhances the expression of two genes encoding serum- and phorbol ester–regulated transcription fac-
tors, the Jun B gene, and the c-jun proto-oncogenes, respectively. Although the α₁-adrenergic receptor has been reported to couple to stimulation of c-myc expression in neonatal cardiomyocytes, the role of this or any oncogene as a putative inducer of cardiac cell growth is unknown.

There is evidence that an increase in pH is a necessary signal for the initiation of growth and development in many cell types. Regulation of pH is in large part controlled by Na⁺-H⁺ exchange. Activation of Na⁺-H⁺ exchange can be brought about by an increase in cytosolic-free [Ca²⁺] and formation of Ca²⁺-calmodulin complexes. In addition, phorbol esters can activate Na⁺-H⁺ exchange without any change in [Ca²⁺], suggesting that an increase in cytosolic-free Ca²⁺ is not essential for activation of Na⁺-H⁺ exchange. There are several lines of evidence indicating that activation of Na⁺-H⁺ exchange by external stimuli is mediated by protein kinase C. First, biologically active phorbol esters, which directly activate protein kinase C, stimulate Na⁺-H⁺ exchange and increase pH in many cell types. Second, the addition of synthetic diacylglycerol can mimic phorbol esters in increasing pH. Whether growth factors can use additional pathways to activate the Na⁺-H⁺ exchange or protein kinase C is the final common regulator of Na⁺-H⁺ exchange is not known. As noted above, a change in cell shape can also increase pH, possibly by activating Na⁺-H⁺ exchange.

Overall, adrenergic agonists stimulate heart growth in vivo, and this effect appears to involve α- and β-receptor-mediated components. However, adrenergic stimuli do not appear to be important for induction of pressure-overload hypertrophy. In vitro, α₁-agonists stimulate cellular hypertrophy, and the signaling pathway appears to involve phospholipase C, diacylglycerol, protein kinase C, and activation of transcription and translation. An increase in pH may be an important component of the growth response to α₁-adrenergic stimulation.

β-Adrenergic–Mediated Cardiac Hypertrophy

In vivo studies. The difficulty of establishing that β-adrenergic agonists directly stimulate cardiac growth in vivo independent of hemodynamic changes is greater than with α-agonists. Stimulation of myocardial β-adrenergic receptors markedly increases cardiac contractility, cAMP accumulation, glycogenolysis, and heart rate. As discussed above, infusion of norepinephrine into dogs induced cardiac hypertrophy in doses that did not cause hemodynamic changes. In rats, evaluation of the mechanism of isoproterenol-induced hypertrophy is further complicated by necrosis of cardiac myocytes. Loss of cardiac muscle after isoproterenol administration raises the additional possibility that the hypertrophy is compensatory to increased workload. Isoproterenol-induced cardiomegaly in rats was a dose- and time-dependent event, but so was myocardial necrosis. A more recent study showed that isoproterenol produced myocyte necrosis, even when administered in low doses and with chronic infusion to avoid transient high catecholamine levels. In the presence of myocardial necrosis, the gain in heart weight also was an unreliable index of hypertrophy because growth of the remaining myocytes was obscured by loss of necrotic tissue. After a single high dose and multiple low doses of isoproterenol, myocyte volume increased by 27% and 92%, respectively, whereas heart weight increased by 15% and 56%, respectively. Although cardiac hypertrophy clearly follows in vivo infusions of norepinephrine and isoproterenol, direct stimulation of the anabolic pathway by β-receptor occupancy has not been established because of the difficulty in controlling hemodynamic and metabolic effects and myocardial necrosis.

In vitro studies. Many of the problems that plague in vivo studies of the effects of β-adrenergic agonists on cardiac growth also occur in vitro. Early studies of the effects of isoproterenol, epinephrine, and norepinephrine on the rate of protein synthesis in the perfused rat heart showed that all of these agonists increased the rate when measured during 60 minutes of perfusion, whereas only epinephrine resulted in higher rates of protein synthesis after 90 minutes. A decrease in ATP levels in hearts exposed to catecholamines for 90 minutes may have obscured the effects on protein synthesis because β-stimulation increased the rate of synthesis and ATP depletion decreased it. Similar difficulties in studying catecholamine effects have been encountered in recent studies. In both instances, catecholamines decreased ATP but increased creatine phosphate contents. In the study by Chua et al., isoproterenol decreased rates of protein degradation and content of ATP. In tetrodotoxin-arrested hearts, however, rates of protein degradation and ATP content were unaffected by addition of isoproterenol, but cAMP levels were still increased. These studies indicate that the perfused rat heart is a difficult preparation in which to study direct effects of β-receptor occupancy because of accompanying changes in contractility, heart rate, and ATP content. To circumvent these difficulties and to demonstrate that increased cAMP content was associated with faster rates of protein synthesis, glucagon, forskolin, and the cAMP phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine were used to increase cAMP levels. All three of these agents increased rates of protein synthesis during the second hour of perfusion in beating and tetrodotoxin-arrested hearts in which ATP depletion did not occur.

In cultured neonatal cardiac myocytes, chronic exposure to isoproterenol resulted in cell hypertrophy, but the effect was not as large as with α₁-adrenergic stimulation. Addition of the β-agonist also induced beating in the sparsely plated cultures. In other studies in confluent cultures in which cells beat spontaneously, McDermott and Morgan found that myocytes hypertrophied compared with K⁺-depolarized myocytes that were quiescent, suggesting that mechanical activity could have accounted for the
β-agonist–stimulated growth. Recently, a preliminary report indicated that additions of cAMP analogues, forskolin (an activator of adenyl cyclase), or cholera toxin (an inhibitor of the cAMP phosphodiesterase) resulted in hypertrophy of cardiac myocytes.75 Overall, studies in perfused hearts and cultured neonatal cardiac myocytes indicate that increased cAMP content is associated with cellular growth.

Proposed mechanisms of action. The strongest mechanistic link of β-adrenoceptor stimulation with cardiac cell metabolism is the early increase in cAMP content that precedes other events such as cell growth. As discussed in the first portion of this review on mechanical factors in cardiac hypertrophy, there are data to support a role for stretch-induced activation of adenyl cyclase and cAMP-dependent protein kinase in the regulation of protein synthesis and ribosome formation in isolated heart preparations. Similarly, agents that increase cAMP without depleting ATP accelerate protein synthesis and ribosome formation. Although no studies have been carried out in cardiac myocytes, many cAMP-regulated genes have been isolated and are expressed in tissues that are responsive to hormones or regulatory factors (for a recent review, see Reference 76). In cAMP-regulated genes, transcription factors appear to bind to cAMP-regulatory elements and the AP-2 sites (described above) in a cAMP-independent manner. cAMP-inducible enhancer elements may be regulated by rapid modifications of proteins already bound to specific DNA elements. These proteins presumably contain DNA binding and transcriptional activation domains. Modification of the binding protein by increased intracellular levels of cAMP could alter transcription as follows. The binding of transcription factors such as RNA polymerase II is increased; interactions increase between the cAMP-responsive element(s) binding proteins and AP-2 and other transcription factors (i.e., the TATA binding factor and polymerase II), and cAMP-induced phosphorylation of the transcriptional regulatory domains in the binding proteins leads to interactions with another non-DNA binding protein, resulting in the increased rate of transcription of a given gene.76 Although regulation of cell growth in eukaryotes via induction of gene expression by cAMP is clearly established, the molecular mechanisms involved in the transduction of increased cAMP content to acceleration of ribosome formation and synthesis of specific proteins in the mammalian heart are not understood. Recently, convergence of the distinct cellular transduction pathways involving cAMP-dependent protein kinase A and phorbol ester–stimulated protein kinase C was shown to involve common as well as distinct nucleotide sequences in the 5′-flanking regions of a chloramphenicol acetyltransferase fusion gene and DNA binding proteins.77 Both protein kinase A and C activated transcription by the phorbol ester–responsive element, but activation of both kinases was synergistic.77 Interaction and convergence of the signaling pathways involving protein kinases A and C provide a sensitive mechanism for control of gene expression.

In regard to the possibility that protein kinase C may participate in the signal transduction pathway from the β-receptor, it has recently been reported that β-adrenergic stimulation of isolated, perfused hearts was associated with changes in basal phosphatidylinositol turnover that may be partially mediated by inhibition of the enzymatic activity of phospholipase C.76 Stimulation of β-adrenoceptors by isoproterenol resulted in increased labeling of phosphatidylinositols as well as a decline in the contents and radioactivity of water-soluble inositol triphosphates, including Ins-1,4,5-IP₃, in perfused guinea pig hearts. These changes in phosphatidylinositol turnover were blocked by the β-receptor antagonist propranolol. Isoproterenol-stimulated increases in the labeling of phosphatidylinositols may be related to increased cAMP content because formation of phosphatidylinositols was stimulated by cAMP-dependent protein kinase, (for a review, see Reference 78). However, in this study,78 increased labeling of phospholipids did not correlate with increased phosphorylation of phospholamban and troponin I or with cAMP levels. Thus, the effects of β-adrenoceptor activation on the phosphatidylinositol cycle is opposite to that of α₁-receptor activation. These findings appear to exclude a role for protein kinase C in the growth effects of β-adrenergic stimuli on cardiac myocytes.

Endocrine Control of Cardiac Hypertrophy

Thyroid Hormone

In vivo studies. Mechanisms of cardiac hypertrophy produced by T₄ administration include a direct effect of the hormone on the heart, indirect effects related to stimulation of the adrenergic nervous system, or altered left ventricular loading conditions. One of the early studies demonstrated that thyroxine administered to mice resulted in cardiac hypertrophy.79 Circulatory changes associated with thyrotoxicosis were greatly reduced by concomitant reserpine treatment but did not prevent the cardiac hypertrophy. After synthetic l-thyroxine administration, the rate of cardiac protein synthesis appeared to be increased before any definite circulatory changes appeared.79 In a more recent study, β-adrenoceptor blockade with propranolol blunted the increase in heart rate but had no effect on left ventricular systolic pressure, left ventricular dP/dt, or development of cardiac hypertrophy induced by thyroid hormone (T₄).80 Alteration of cardiac load with captopril decreased left ventricular systolic and diastolic pressures, but heart rate, dP/dt, and left ventricle–to–body weight were unchanged compared with treatment with T₄.80 In the studies of Zierhut and Zimmer,81 triiodothyronine (T₃) induced hemodynamic and metabolic alterations and resulted in development of cardiac hypertrophy in the rat. After 18 hours of administration, T₃ increased heart rate and left ventricular dP/dt max. At 48–72 hours, there were increases in cardiac output.
and RNA concentration, and cardiac hypertrophy developed. β-Receptor blockade reduced left ventricular functional parameters but had no effect on the T3-induced increases in RNA or on the development of cardiac hypertrophy. α-Receptor blockade also had no effect on the T3-induced cardiac hypertrophy. These data indicate that α- and β-receptors are not involved in the development of cardiac hypertrophy in this model and that exogenous thyroid hormone may directly mediate increased protein synthesis and the development of cardiac hypertrophy. In contrast to the study of Zierhut and Zimmer and other studies, Klein found that T3-induced cardiac hypertrophy could be blocked by propranolol. Klein’s study supported the conclusion that the hypertrophic response to excess thyroid hormone administration was mediated indirectly.

In support of an indirect effect of thyroid hormone on heart growth, Klein and Hong obtained data in a heterotopically transplanted rat heart model suggesting that thyroxine-induced cardiac hypertrophy is mediated by changes in cardiac work. The nonworking, denervated, vascu larly perfused transplanted heart could be maintained for prolonged periods of time in vivo and permitted dissociation of the direct effects of thyroid hormone on the myocardium from those mediated indirectly by increased cardiac work. Thyroid hormone caused a marked increase in heart weight and protein content of the in situ working heart but in contrast did not increase heart weight, left ventricular weight, total heart protein, or left ventricular protein in the heterotopically transplanted, nonworking heart. However, excess thyroid hormone did reestablish the myosin isozyme distribution in the heterotopic heart from 61% to 95% V1 and increased heart rate. These results support the concept that thyroxine-induced cardiac hypertrophy is mediated by changes in cardiac output and work, although there appear to be direct effects on regulation of myosin gene expression and heart rate.

Thyroid hormone has been shown to affect the developmental expression of the ventricular isomyosins V1, V2, and V3. During the latter part of gestation in rats, V3 accounted for 80–90% of fetal ventricular myosin. After birth, a transition began between V3 and V1; by 3 weeks of age, there was 100% V1. Increased serum levels of endogenous T3 and T4 correlated with the maximal expression of V1 during the same period. The relative amount of V1 was also increased in fetal ventricles by administration of T3 and T4. Thus, endogenous thyroid hormone induced the synthesis of ventricular heavy-chain α, which dimerized to form the V1 isomyosin. Similarly, rapid induction of the β-mysin heavy-chain mRNA, which paralleled the increase in left ventricular weight in response to aortic coarctation, was completely reversed by thyroxine administration. Formation of α-mysin heavy-chain mRNA and protein was induced despite progression of the left ventricular hypertrophy.

Hypertrophy of the heart in thyroid hormone-treated animals is due to faster rates of protein synthesis. When measured in isolated, perfused hearts, the rate of protein degradation was either unchanged or slightly decreased. When calculated from measurements of heart growth and protein synthesis in vivo, rates of protein degradation were increased in the hearts of T3-treated rabbits. As discussed earlier, however, these calculations overestimate rates of proteolysis. Faster rates of protein synthesis depended to a large extent on increased capacity as reflected in higher contents of recombinant RNA (rRNA) and mRNA.

In vitro studies. In vitro effects of thyroid hormone are usually delayed several hours in onset, a situation that has restricted studies to organ cultured hearts and cardiac myocytes. With mouse hearts in organ culture, T3 increased the rate of protein synthesis, although no changes in contractility were observed. In cultured fetal cat cardiomyocytes, thyroid hormone caused a shift in the content of myosin isozyme from V3 to V1, an accumulation of α-mysin heavy-chain mRNA, and inhibition of expression of β-mysin heavy-chain mRNA. In cultured chick cardiomyocytes, the addition of T3 increased the fractional rates of protein synthesis and cell growth by 10–16% and 20–40%, respectively. These studies demonstrate that thyroid hormone directly controls gene expression and cellular growth in cardiac myocytes. In unpublished studies by Peffer and Morgan, addition of T3 increased rates of protein synthesis in contracting neonatal rat myocytes by only 10–15%. The control beating cells, however, were growing much more rapidly than the quiescent myocytes. A larger effect of T3 on rates of protein synthesis in K+-depolarized noncontracting myocytes—approximately 35%—was observed. In contrast to the in vivo studies presented above in which there is division as to whether the cardiac effects of thyroid hormones are directly or indirectly mediated, the effect in vitro is clear but may be relevant only to conditions of high circulating levels of hormone.

Proposed mechanisms of action. Thyroid hormone could act through changes in tissue contents of intracellular signaling compounds, such as cAMP or Ca2+, or directly on gene transcription via nuclear thyroid hormone receptors. Zimmer and Peffer found that thyroid hormones increased cAMP content of the heart within 12 hours of T3 administration and that cAMP remained elevated for 48 hours. The cAMP increase occurred before the maximal enhancement of adenine nucleotide and protein synthesis. β-Blockade prevented the hemodynamic effects of T3 but did not prevent cardiac hypertrophy. The possible mechanisms by which increased cAMP content could contribute to cardiac hypertrophy were reviewed in the section on β-adrenoceptors. As discussed, there are no substantial data to indicate a primary involvement of increased Ca2+ availability in mediating the cardiac cell growth response.
Thyroid hormone regulates transcription of contractile protein genes in cardiac myocytes97 and probably also regulates cardiac muscle cell growth in the same way. Thyroid hormone receptors are part of a family of ligand-dependent transcriptional factors that include steroid hormone and retinoic acid receptors.98 Members of this family activate transcription by the binding of the hormone-receptor complex to specific DNA sequences on target genes.97,98 In cardiac tissue, thyroid hormone can act as either a positive (α-myosin heavy-chain) or negative (β-myosin heavy-chain) regulator of transcription. DNA sequences in the myosin heavy-chain gene have been identified in gene transfer experiments that appear to be responsible for regulation by thyroid hormone.97 Thyroid hormone-responsive elements in the 5′-flanking hormone subunit genes bind not only the thyroid hormone receptor but also in vitro synthesized Hc-erb-Aβ, a T3 receptor.99 A recently identified 65-kDa nuclear protein may be an essential component in the response of a given gene to thyroid hormone.99 Whether thyroid hormone mediates overall cardiac growth at the level of gene transcription has not been demonstrated conclusively.

Angiotensin II

In vivo studies. There are both indirect and direct actions of angiotensin II on cardiac tissue. The indirect cardiovascular effects of angiotensin II include central nervous system actions, such as stimulation of thirst and increased sympathetic outflow; stimulation of aldosterone synthesis and release; decreased renal excretion of Na⁺; maintenance of vascular tone; and increased heart rate secondary to enhanced sympathetic activity. The indirect cardiac actions of angiotensin II were recently reviewed and are not discussed further.100 The direct cardiac actions of angiotensin II that are mediated by membrane receptors and are coupled to effector responses by guanine nucleotide binding proteins include stimulation of cardiac contractility101–103; acceleration of protein synthesis that results in cardiac hypertrophy104,105; and activation of a membrane phospholipase with resultant increases in Ins-1,4,5-IP₃, diacylglycerol, and protein kinase C activity (References 106 and 107 and unpublished data).

The classic example of involvement of the renin-angiotensin system in cardiac hypertrophy is experimentally produced renovascular hypertension. The increased blood pressure and left ventricular mass that occurred in this model were prevented by unclipping the kidney or by inhibiting angiotensin converting enzyme, both of which decreased production of angiotensin II.108 However, in this model, the possible direct hypertrophic effects of angiotensin II were not separated from indirect effects mediated through increases in blood pressure and vascular resistance. In a genetic model, SHR, neither the cardiac hypertrophy that developed nor the regression once present was affected by normalization of blood pressure with sympatholytic agents or vasodilators.109–111 In contrast, administration of an angiotensin converting enzyme inhibitor not only prevented the cardiac hypertrophy but also caused regression of established left ventricular hypertrophy.109–111 Chronic infusion of angiotensin II into rats increased left ventricular mass, a response that occurred even when the pressor activity of the peptide was blocked.104 Using a similar model but one in which a subpressor dose of angiotensin II was chronically (1–2 weeks) infused into rats, we found increased left ventricular-to-body weight ratios in the animals receiving angiotensin II, even when there were no differences in mean arterial pressure between sham-operated controls and experimental animals (unpublished data). These data indicate that the pressure or load against which the heart functions is not the only factor regulating cardiac growth. The data also indicate that angiotensin II is an in vivo stimulus for cardiac hypertrophy. Using a model of "pressure-overload" cardiac hypertrophy (i.e., abdominal aorta constriction), it was recently shown that treatment of the animals with enalapril maleate, an angiotensin converting enzyme inhibitor, completely prevented the increased left ventricular mass associated with coarctation.112 Cardiac afterload was the same in both groups of animals in that carotid artery pressures were not different in conscious, awake aortic-constricted rats receiving enalapril and those not receiving enalapril.112 These data indicate that the cardiac hypertrophy that occurs in this model of pressure overload may be related to increased activity of the renin-angiotensin system. In all of the studies cited above, the effects of angiotensin II to stimulate cardiac hypertrophy could have been mediated by direct or indirect (or both) actions of the peptide.

In vitro studies. In cultured embryonic chick myocytes, angiotensin II induced cellular hypertrophy that was associated with an increased rate of protein synthesis.105 The stimulatory effects of angiotensin II on cardiomyocyte growth were blocked by a specific angiotensin receptor antagonist105; the responses were not blocked by α- or β-adrenoceptor antagonists.105 The angiotensin II–stimulated rates of protein synthesis and cardiomyocyte growth were not secondary to an angiotensin II–induced increase in chronotropic activity, because the hypertrophic response to angiotensin II was the same in nonbeating potassium chloride–depolarized cells.113 These data indicate that angiotensin II is a direct receptor–mediated stimulus of cardiomyocyte growth. We have recently confirmed these findings using neonatal rat cardiomyocytes in culture, in which angiotensin II appears to be an even more potent stimulus of cardiomyocyte growth than in the avian heart (unpublished data). The combined in vivo and in vitro data suggest that the renin-angiotensin system may be integrally involved in the process of cardiac muscle growth and likely contributes to certain pathological forms of cardiac hypertrophy.
### Proposed mechanisms of action

The hypertrophic action of angiotensin II could be mediated by circulating or locally produced hormone. The precursor genes for the renin-angiotensin system, angiotensinogen, and renin mRNA have been detected by Northern and dot-blot hybridization analyses and S1 nuclease mapping in all four cardiac chambers.\(^{112,114-116}\) Cellular localization of these genes by in situ hybridization has not been reported. The genes appear to be expressed in cardiac tissue in that the translatable products renin, angiotensin I, and angiotensin II have all been detected (Reference 117 and unpublished data). Although the constituents for an intracardiac renin-angiotensin system are present, the question remains as to whether there are physiologically relevant paracrine or autocrine functions of the peptide in the heart. Upregulation of left ventricular angiotensinogen mRNA has been described in association with pressure-overload cardiac hypertrophy, suggesting that a localized renin-angiotensin system may be activated in this experimental model of hypertension.\(^{112}\)

Radiolabeled angiotensin II perfused into isolated rat hearts localized to the perinuclear region of myocytes.\(^{118}\) In addition, rat liver nuclei exposed to angiotensin II had increased chromatin solubility putatively from the action of endogenous nucleases.\(^{119}\) These data suggest that angiotensin II produces structural changes in chromatin. Nuclear localization of angiotensin II may be similar to T4 (discussed above) in that angiotensin II receptor internalization could promote interaction with regulatory sites on DNA.

Angiotensin II receptor–mediated increases in diacylglycerol and resultant translocation of protein kinase C could also contribute to the effects of this peptide on cardiomyocyte growth. As discussed above, phorbol ester–induced increases in protein kinase C activity were associated with cardiomyocyte growth.\(^{61,62,113}\) In cultured vascular smooth muscle cells, angiotensin II–stimulated translocation of protein kinase C was associated with angiotensin II–induced phosphorylation of nuclear lamins.\(^{120}\) Phosphorylation of nuclear proteins may be one of the steps by which the protein kinase C signaling pathway regulates angiotensin II–induced nuclear events. Alternatively, the angiotensin–stimulated increase in protein kinase C activity could also lead to intracellular alkalization, which, as discussed above, may be related to initiation of cellular growth. Angiotensin appears to be important for regulation of cardiac growth and may prove particularly interesting if some of the effector responses to this peptide in cardiac tissue are mediated by local production of the hormone. The molecular and biochemical mechanisms of the angiotensin II–induced hypertrophic response in the heart remain to be elucidated.

### Conclusion

Cardiac hypertrophy involves a sequence of events, including initiating signals, coupling mechanisms, and regulation of gene expression (Figure 2). Initiating signals include cell deformation, neurotransmitters, and hormones. As a result of these signals, membrane ion channels and enzymes are activated that lead to increased intracellular contents of Na\(^+\), H\(^+\), cAMP, inositol phosphates, and diacylglycerol and to greater activity of protein kinases A and C. Regulation of gene expression involves DNA binding proteins, such as transcription factors, and regulatory elements in the 5′-flanking regions of the genes, such as the cAMP response element. Enhanced gene expression includes sequences coding for contractile proteins, atriopeptin, angiotensinogen, ribosomal

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<tr>
<th>Initiating Signals</th>
<th>Regulation of Gene Expression</th>
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<tbody>
<tr>
<td>- stretch of ventricular wall</td>
<td>- AP-1 transcription factor</td>
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<tr>
<td>- α(_1) and β-adrenergic agonists</td>
<td>- AP-2 transcription factor</td>
</tr>
<tr>
<td>- thyroid hormone</td>
<td>- cAMP response (CRE) - elements</td>
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<tr>
<td>- renin-angiotensin system (All)</td>
<td>- contractile protein genes (β myosin light chain, α-skeletal actin)</td>
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### Physiological Sequelae

- adaptive hypertrophy
- altered contractility
- decompensated hypertrophy

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\(^{All}\)AP-1 and AP-2, nuclear enhancer–binding proteins; cAMP, cyclic AMP; rDNA, ribosomal DNA.
RNA, and oncogenes. The physiological sequelae are adaptive growth and altered contractility, due in part to transcription of different contractile protein isoforms. The mechanisms of decomposition of the hypertrophied heart leading to mechanical failure could involve impaired function of any of these steps in this complex pathway.

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