Pathological Hypertrophy and Cardiac Interstitium
Fibrosis and Renin-Angiotensin-Aldosterone System

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Left ventricular hypertrophy (LVH) is the major risk factor associated with myocardial failure. An explanation for why a presumptive adaptation such as LVH would prove pathological has been elusive. Insights into the impairment in contractility of the hypertrophied myocardium have been sought in the biochemistry of cardiac myocyte contraction. Equally compelling is a consideration of abnormalities in myocardial structure that impair organ contractile function while preserving myocyte contractility. For example, in the LVH that accompanies hypertension, the extracellular space is frequently the site of an abnormal accumulation of fibrillar collagen. This reactive and progressive interstitial and perivascular fibrosis accounts for abnormal myocardial stiffness and ultimately ventricular dysfunction and is likely a result of cardiac fibroblast growth and enhanced collagen synthesis. The disproportionate involvement of this mononuclear cell, however, is not a uniform accompaniment to myocyte hypertrophy and LVH, suggesting that the growth of myocyte and nonmyocyte cells is independent of each other. This has now been demonstrated in vivo studies of experimental hypertension in which the abnormal fibrous tissue response was found in the hypertensive, hypertrophied left ventricle as well as in the normotensive, nonhypertrophied right ventricle. These findings further suggest that a circulating substance that gained access to the common coronary circulation of the ventricles was involved. This hypothesis has been tested in various animal models in which plasma concentrations of angiotensin II and aldosterone were varied. Based on morphometric and morphological findings, it can be concluded that arterial hypertension (i.e., an elevation in coronary perfusion pressure) together with elevated circulating aldosterone are associated with cardiac fibroblast involvement and the resultant heterogeneity in tissue structure. Nonmyocyte cells of the cardiac interstitium represent an important determinant of pathological LVH. The mechanisms that invoke short- (e.g., collagen metabolism) and long-term (e.g., mitosis) responses of cardiac fibroblasts require further investigation and integration of in vitro with in vivo studies. The stage is set, however, to prevent pathological LVH resulting from myocardial fibrosis as well as to reverse it. (Circulation 1991;83:1849–1865)

Heart failure is a major health problem. In the United States, where myocardial failure (i.e., circulatory failure resulting from valvular heart disease) is the most common cause of heart failure, a history of systemic hypertension is obtained in a majority of symptomatic patients; coronary artery disease with previous myocardial infarction is present in nearly half, whereas 10% have idiopathic cardiomyopathy. In both men and women, left ventricular hypertrophy (LVH) is the major risk factor associated with adverse cardiovascular events, including the appearance of myocardial failure. Why should LVH predispose this normally efficient biologic pump to failure? Does the hypertrophied myocardium simply give out? Is LVH an inadequate compensatory response, or is the hypertrophic process intrinsically faulty? These questions have been the subject of extensive debate and research for more than 50 years. A substantial portion of this research, particularly that conducted since 1970, has been focused on the hypertrophied cardiac myocyte and potential biochemical abnormalities that may accompany its growth and would explain an impairment in contractility of the hypertrophied myocardium. In 1973, Arnold Katz noted, “We are now in a position to search for the causes of reduced contractility in the hemodynamically stressed human
heart and to define such alterations in terms of specific changes in the interactions of the heart's contractile proteins and in the processes of excitation-contraction coupling..." Several recent reviews should be consulted for the many contributions that this line of inquiry brought forward in subsequent years. At present, however, the biochemical basis of pathological hypertrophy remains uncertain.

Another, perhaps broader perspective has sought answers to these questions by addressing the structure of the hypertrophied myocardium. In his 1940 Harvey Lecture, Joseph Wearn wrote, "The frequent finding at necropsy of an hypertrophied heart that has failed is familiar to all. Other than the hypertrophy, the muscle of these hearts often shows no abnormalities. Why, then, should an enlarged muscle without demonstrable abnormality fail? Hypertrophy is frequently spoken of as being compensatory. On the other hand, it is also considered to be one of the most dependable signs of heart damage." In extending Wearn's logic, we might ask what other than muscle cell enlargement is abnormal about the structure of the hypertrophied myocardium that accompanies chronic hypertension and that, according to the 30-year prospective Framingham experience, so frequently predisposes to symptomatic failure? In other words, what could impair the contractility of the myocardium while preserving that of its cardiac myocytes?

Important clues reside in the structural remodeling of the cardiac interstitium or extracellular space of the myocardium, where an abnormal accumulation of fibrillar collagen can occur. This is also true for the adventitia of intramyocardial coronary arteries, where medial thickening is also frequently seen. The interstitial and perivascular fibrosis is a reactive process, morphologically distinct from the replacement fibrosis that follows myocyte necrosis. Fibrosis, however, is not a constant feature of the hypertrophic process that accompanies every elevation in myocyte loading, mediated by, for example, an increase in the systolic pressure or diastolic volume of the ventricular chamber. In his textbook Human Pathology, Howard Karsner wrote, "Diffuse (myocardial) fibrosis may be part of widespread fibrosis of organs such as... atrophies of the aged. It also occurs in hypertrophy of the heart, especially when caused by hypertension. Grossly the myocardium is firm, and on tangential section there are fine parallel lines of retraction which give the coarsely striated appearance of tough beef. Microscopically, narrow bands of collagenous connective tissue course between the fiber cells of the muscle."

From a morphological point of view, the following could be deduced from this structural remodeling of the hypertrophied myocardium in hypertension: 1) the alteration in collagen architecture is based on the growth of nonmyocyte cells (i.e., cardiac fibroblasts) that contain the genetic code responsible for initiating the synthesis of fibrillar collagens, whereas the growth of cardiac myocytes accounts for LVH, expressed as either an increment in myocardial mass or wall thickness; and 2) myocardial hypertrophy need not be a homogenous process. Instead, a heterogeneity in myocardial structure can occur if myocyte and fibroblast growth and the trophic factors responsible for their respective growth are independent of each other. What are these trophic factors, and why should there be a reactive fibrosis of the cardiac interstitium in the LVH that accompanies hypertension and not other conditions? These questions are of central importance given that the reactive interstitial fibrosis and "tough beef" appearance of the myocardium are associated with abnormalities in its stiffness and pumping function, whereas the perivascular fibrosis and medial thickening of coronary resistance vessels probably impair their vasodilator reserve. Of equal importance, what can be done about it? This report addresses these questions as they pertain to the reactive myocardial fibrosis that is seen in hypertension and that represents only one expression of what has been termed "interstitial heart disease."

**Myocyte and Nonmyocyte Cells: General Principles**

**Cell Population of Myocardium**

The myocardium comprises many different cells (see Figure 1). Cardiac myocytes (cardiocytes), the largest of these cells, occupy 75% of its structural space; cardiocytes, however, constitute only one third of the cell population. All remaining cells, by definition, are found in the cardiac interstitium. They include 1) endothelial cells, which form the ubiquitous lining of the coronary and lymphatic vasculature and endocardium and are now known to influence the vasomotor reactivity of blood-containing vessels; 2) vascular smooth muscle cells, which are found in epicardial and intramyocardial coronary arteries and arterioles and, like endothelial cells, influence the reactivity and vasodilatory capacities of these vessels; 3) cardiac fibroblasts, which have the responsibility to both produce and degrade the structural proteins collagen and elastin in the interstitium; and 4) macrophages and mast cells, which are defenders against invasion by foreign proteins.

Unlike endothelial and vascular smooth muscle cells, which are highly specialized, fixed anatomic elements of the vascular compartment, cardiac fibroblasts are multipotential cells that are free to move within the extracellular space. Fibroblasts, not cardiac myocytes, contain the messenger RNA for type I and type III collagens, the major fibrillar collagens of the myocardium that constitute its normal structural protein network. These collagens are also involved in the interstitial and perivascular fibrosis of the myocardium, which is discussed later in this report, and the replacement scarring that follows cell death. Like endothelial and vascular smooth muscle cells, fibroblasts are capable of reentering the cell cycle and can therefore undergo
mitosis or hyperplastic growth. On the other hand, adult cardiocytes are thought to be terminally differentiated and therefore do not proliferate. This view, based on biochemical studies conducted over short periods of myocardial growth, was recently questioned by Anversa et al., whose morphometric findings in long-standing hypertension indicate that myocyte nuclei hyperplasia can occur.

**Myocyte and Nonmyocyte Cell Growth**

Cardiocyte growth is expressed as an increase in cell breadth and/or length, a morphometric measure of cellular hypertrophy. Cardiocyte growth creates an increment in the mass and thickness of the myocardium, criteria used to identify organ hypertrophy; therefore, LVH is based on the growth of the cardiocytes. Cardiocyte growth is the common denominator to the LVH seen in all disease states and in exercise training. On the other hand, it should not be assumed that LVH is accompanied by nonmyocyte cell growth irrespective of its etiologic basis.

Nonmyocyte cell growth is expressed as a structural remodeling of the interstitium. In the case of endothelial and vascular smooth muscle cells, respective intimal and medial thickenings of the coronary microvasculature follow. Either of these events could reduce the luminal area of these vessels and compromise their vasomotor reactivity. The accumulation of fibrillar collagen is indicative of fibroblast growth and increased myocardial collagen synthesis, relative to its degradation. Save for the caveat that collagen synthesis per given cell may increase without fibroblast proliferation, one could predict that nonmyocyte cell growth had occurred from the microscopic finding of fibrosis in the hypertrophied myocardium.

**Myocyte and Nonmyocyte Cell Growth Are Independent of Each Other**

Based on the heterogeneity in myocardial structure found in postmortem hearts, it has been suggested that myocyte and nonmyocyte cells grow independently of each other. Recent in vivo studies have confirmed this. Hence, any one of the following growth responses to cardiovascular disease could occur: 1) nonmyocyte cell growth without myocyte hypertrophy, 2) myocyte hypertrophy without nonmyocyte cell growth, and 3) concomitant equal or disproportionate growth of these cells. Coronary vasculitis and radiation-induced myocardial fibrosis are examples of the growth and remodeling of nonmyocyte cells that occur without myocyte growth. This independence of nonmyocyte and myocyte growth is explored further as the interstitial and perivascular fibrosis that occurs in the nonhypertrophied right ventricle in systemic hypertension is considered. The forms of hypertrophy seen in association with exercise training, arteriovenous fistulas, chronic anemia, or administration of thyroxine or growth hormones are examples of myocyte growth that occurs without the disproportionate involvement of fibroblasts and in which myocardial collagen concentration remains normal. In various forms of hypertension, congenital and acquired aortic valvular stenosis, and coartation of the aorta, LVH is accompanied by a reactive fibrosis and increase in collagen concentration, representing...
examples of myocyte hypertrophy and disproportionate nonmyocyte cell growth.

In examining the structure of the hypertrophied left ventricle in hypertension, it is difficult to identify the trophic factors responsible for myocyte and nonmyocyte cell growth. This problem is partially resolved by considering that the right and left ventricles are arranged in series by their anatomic communication as provided by the pulmonary circulation and in parallel by their common coronary circulation. In the absence of pulmonary venous hypertension resulting from left heart failure, the right ventricle represents a negative internal control relative to the role of hemodynamic factors such as elevated ventricular systolic and/or diastolic pressures. Conversely, a circulating substance that may be involved in mediating nonmyocyte cell growth independent of ventricular pressure would make the right ventricle a positive internal control in that it also would demonstrate a structural remodeling independent of hemodynamic factors.

Pathological Hypertrophy Defined

From a morphological standpoint, the hypertrophic remodeling of the myocardium is either a homogenous or a heterogeneous process, based on whether there is a proportionate or disproportionate growth of nonmyocyte cells, respectively.20 When tissue homogeneity is preserved, the proportionality of muscular, vascular, and interstitial compartments is maintained and hypertrophy is adaptive. This is the type of hypertrophy that occurs in response to isotonic or isometric exercise training, chronic anemia, or arteriovenous fistulas. The adaptive nature of the hypertrophy, with preserved myocardial structure, is further evidenced by the uneventful regression in hypertrophy and restoration in ventricular chamber size that occur when the overload terminates or is corrected.42 In contrast, a heterogeneity in myocardial structure, based on disproportionate nonmyocyte cell growth and loss of intercompartmental proportionality, will cause pathological hypertrophy. In the case of adaptive hypertrophy, myocardial collagen concentration remains unchanged from normal even though collagen content (concentration multiplied by ventricular weight) is increased. A disproportionate response in collagen would lead to pathological hypertrophy. This could take the form of an inadequate increment in collagen in the presence of cardiac myocyte growth. In this case, collagen concentration would decline. Alternatively, a disproportionate rise in collagen concentration (and content) in hypertrophy leads to myocardial fibrosis and structural remodeling of the hypertrophied myocardium.

This concept of adaptive and pathological hypertrophy, which is based on the proportions of cell growth and corresponding proportionality among myocyte, vascular, and collagen compartments, is not time dependent. For example, the initial structural remodeling of the myocardium may be pathological; alternatively, an adaptive hypertrophy can become pathological when disproportionate nonmyocyte cell growth occurs at any point in time, thereby creating a heterogeneity in tissue structure.

Hence, trophic factors that promote disproportionate nonmyocyte cell growth or collagen gene expression, for example, and thereby lead to abnormal structure represent a determinant of pathological hypertrophy with myocardial failure. Therefore, it is essential to view the hypertrophic process as more than the mere growth of cardiocytes; the entire cell population of the myocardium as well as various proteins and enzymes and the expression of corresponding genes must be considered potentially recruitable into the growth process.

Trophic Factors

Circulating hormones (e.g., growth hormone and norepinephrine) are thought to regulate cardiocyte growth.36,43,44 Recent evidence obtained in cultured cardiocytes suggests that mechanical conditions (e.g., stretch) will also contribute to the growth of these cells.45,46 Whether stretch would also promote the growth of cardiac fibroblasts or enhance their synthesis of collagen in a manner analogous to stress-mediated collagen deposition in bone is uncertain. Mechanical factors do not appear to account for the disproportionate accumulation of collagen that occurs with LVH in some conditions and not in others despite comparable elevations in wall stress resulting from ventricular pressure or volume overload. As noted, collagen concentration remains normal in the hypertrophied myocardium seen with arteriovenous fistulas, chronic anemia, or thyroxine or growth hormone administration. This is also true for an atrial septal defect47 and when arterial hypertension is created by banding the abdominal aorta below the renal arteries.48 Furthermore, type I and type III collagen gene expression and collagen synthesis are temporally dissociated from the onset of myocyte growth.49–51 This topic is addressed further; nevertheless, trophic factors that mediate myocyte and nonmyocyte (i.e., cardiac fibroblast) cell growth in the myocardium can be independent of each another.

Structural Remodeling of Cardiac Interstitium in Hypertension and Its Functional Consequences

Myocardial Fibrosis in Hypertension

At birth, the concentrations of collagen in the right and left ventricles are equal.37 With the regression in right ventricular myocyte size seen during the neonatal period as well as the increment in left ventricular mass, collagen concentration is higher (30%) in the adult right ventricle; collagen normally occupies 2–4% of the adult left ventricle.37,48 The collagen concentration of the myocardium can be obtained from a determination of its collagen-specific amino acid, hydroxyproline, or the morphometric assessment of connective tissue in specially stained specimens. Various histochemical techniques have been used for this purpose, including nonspecific tri-
appears in intermuscular spaces previously devoid of collagen; 2) a perivascular fibrosis, or accumulation of collagen within the adventitia of intramyocardial coronary arteries and arterioles; 3) a replacement (or reparative) fibrosis, which represents microscopic scarring that follows myocyte necrosis; and 4) a plexiform fibrosis or swirling arrangement of collagen fibers that is frequently seen in association with muscle fiber disarray.

**Interstitial and Perivascular Fibrosis**

The fibrillar nature and sequence of the fibrous tissue response have been examined in various experimental models of arterial hypertension in the nonhuman primate\(^{22,60,65}\) and rat.\(^{66-72}\) Findings for the two species were essentially similar and therefore are not distinguished in this article. In brief, the following was observed. First, within the first several weeks after the induction of renovascular hypertension, an interstitial edema of the myocardium was present. Thereafter, the edema resolved, followed by the accumulation of fibrillar collagen—first within the adventitia of intramural vessels and subsequently within the interstitial space surrounding muscle bundles. Second, this interstitial and perivascular fibrosis was progressive in nature. After 8 weeks of unilateral renal hypertension, a twofold increase in CVF was present; after 12 weeks, fibrous tissue increased to threefold its normal value (see Figure 3). Reparative scarring was now evident as well, particularly in the endomyocardium. Capasso et al\(^{73}\) have shown that after 32 weeks of renovascular hypertension, collagen occupied 18% of the left ventricle, a sixfold increase, whereas within the endomyocardium, 25% of its space was now fibrous tissue. Anversa calculated that the left ventricle and septum contain nearly 20 million cardiocytes and that for every millimeter cubed of scar tissue that appears, approximately 75,000–100,000 myocytes were lost (personal communication). At 32 weeks of renovascular hypertension, there was more than 75 mm\(^3\) of fibrous tissue in the left ventricle. This would either equal the unlikely loss of 7.5 million myocytes or indicate that a reactive accumulation of collagen was a major part of the fibrous tissue response.

**Functional Significance of Myocardial Fibrosis**

The stress–strain relation of the myocardium has been used to gauge the functional impact of collagen accumulation on systolic and diastolic myocardial stiffness.\(^{66-70}\) Diastolic stiffness is increased, particularly at larger strains (or filling volumes), at both 8 and 12 weeks of renovascular hypertension, whereas the force-generating capacity of the myocardium is enhanced, thereby preserving systolic function (e.g., ejection fraction). At 32 weeks, diastolic stiffness is abnormal at normal strains, accounting for diastolic dysfunction at rest with elevated left ventricular filling pressure. Systolic dysfunction with chamber dilatation and reduced ejection fraction now appears.\(^{73}\) We have therefore
concluded that the accumulation of fibrillar collagen is a major determinant of impaired stiffness and pump dysfunction and that its progressive accumulation accounts for a spectrum of ventricular dysfunction that first appears during diastole and subsequently involves systole.

Additional evidence in support of fibrillar collagen representing the major elastic element responsible...
for myocardial stiffness has been presented elsewhere\textsuperscript{74} and is not recounted.

**Trophic Factors and Myocardial Fibrosis in Hypertension**

Because LVH is not always associated with an increase in CVF, despite comparable pressure or volume overload, nonhemodynamic factors such as circulating hormones may represent growth stimuli to cardiac fibroblasts. The importance of various hemodynamic factors and circulating hormones on the fibrous tissue response of the rat right and left ventricles was examined in many different models of experimental hypertension\textsuperscript{48,75,76} (summarized in Table 1). Morphometric evaluation of interstitial CVF by videodensitometry was used to quantitate the reactive fibrous tissue response seen in coronal sections of the myocardium prepared with the collagen-specific stain Sirius red F3BA. Microscopic scars, differentiated from the interstitial and perivascular fibrosis on the basis of morphological presentation,\textsuperscript{60,64,70} were excluded from the analysis. Pulsatile and mean carotid artery systolic pressures were determined at the time of death, and the presence of LVH was established from the ratio of left to right ventricular weights. Right ventricular weights were not found to be different among the various experimental groups and their respective controls. Plasma concentrations of angiotensin II and aldosterone were determined by radioimmunoassay.\textsuperscript{48}

**Hemodynamic Factors**

Comparable levels of systemic hypertension and LVH were observed with unilateral renal ischemia (renovascular hypertension) and infrarenal aortic banding (nonrenovascular hypertension) and in uninephrectomized animals receiving enhanced dietary sodium plus the mineralocorticoid aldosterone. Control animals included a group with previous uninephrectomy receiving a high sodium diet in which aldosterone administration was withheld. A diverse profile for plasma angiotensin II and aldosterone (outlined in Table 1) was purposefully created with these models to dissociate the importance of elevations in ventricular systolic pressure from circulating angiotensin II and aldosterone.

In the hypertrophied left ventricle that accompanied renovascular hypertension or hyperaldosteronism (i.e., uninephrectomized animals receiving aldosterone plus enhanced dietary sodium), CVF was significantly increased. CVF was also significantly increased in the normotensive, nonhypertrophied right ventricle in these two experimental groups. CVF was not increased in either ventricle in the nonrenovascular model of hypertension with LVH or the normotensive control group with uninephrectomy-enhanced dietary sodium in which aldosterone was not received but LVH accompanied the circulatory overload. Thus, despite comparable elevations in left ventricular systolic pressure and increments in left ventricular mass
Figure 3. Photomicrographs of normal and hypertensive rat left ventricles. Picrosirius technique in direct light (×40). Panel A: Control animal. Panel B: After 12 weeks of unilateral renal ischemia, interstitial and perivascular fibrosis is evident.
in each of the models of experimental hypertension, the fibrous tissue response was only associated with an elevation in plasma angiotensin II and/or aldosterone. The relative importance of angiotensin II and aldosterone to myocardial fibrosis is discussed. Hypertension alone did not evoke the growth of cardiac fibroblasts and/or an increase in collagen synthesis. Hence, a heterogeneity in tissue structure accompanied the LVH in these various forms of hypertension. These findings support the view that ventricular loading is the primary determinant of myocyte growth, whereas nonmyocyte growth can occur in the absence of myocyte growth, as was the case with myocardial fibrosis appearing in the normotensive, nonhypertrophied right ventricle in renovascular hypertension and hyperaldosteronism.

To examine the role of elevations in ventricular filling pressure in mediating the fibrous tissue response, the myocardia of dogs with comparably elevated left ventricular filling pressure, secondary to either rapid ventricular pacing or an abdominal aorta-to-inferior vena caval fistula, for approximately 4 weeks or longer were examined. Interstitial and perivascular fibrosis and elevation in left ventricular CVF were found only in the group with impaired cardiac output resulting from rapid pacing; CVF remained normal in the fistula group, in which cardiac output was elevated. In contrast to the circulatory failure and high cardiac output that accompany the fistula, sustained elevations in plasma angiotensin II and aldosterone are seen in the low cardiac output model of myocardial failure resulting from rapid pacing. Thus, current evidence does not favor a role for an elevation in diastolic wall stress in mediating the fibrous tissue response of the interstitium. Conversely, elevations in circulating angiotensin II and/or aldosterone appear to be involved.

Conclusions similar to those drawn for ventricular systolic and diastolic pressures in mediating myocardial fibrosis hold true for the contribution of arterial hypertension alone, representing an elevation in coronary perfusion pressure (see Table 1). Arterial hypertension associated with infrarenal aortic banding is accompanied by LVH, but interstitial CVF remains normal in the right and left ventricles. Thus, it appears that elevations in coronary perfusion pressure must be accompanied by elevated plasma angiotensin II and/or aldosterone to mediate the fibrous tissue response and elevation in CVF, as is the case in unilateral renal ischemia and hyperaldosteronism. These findings also indicate that myocyte growth is not governed by coronary perfusion pressure. Right ventricular hypertrophy was not seen in any of the hypertension models. These findings further suggest that the adaptive LVH seen with either isometric or isotonic exercise training is related to myocyte growth associated with the respective pressure or volume loading of the left ventricle, whereas disproportionate cardiac fibroblast growth is not involved and is probably related to the absence of elevated circulating angiotensin II or aldosterone. On the other hand, exercise training together with the use of anabolic steroids, lead to pathological LVH and a structural remodeling of the interstitium.

Angiotensin II and Aldosterone

Various pharmacological probes were used to identify the association between elevations in plasma angiotensin II and/or aldosterone and the reactive myocardial fibrosis (see Table 1). When rats with unilateral renal ischemia were pretreated with oral captopril to prevent the expected increase in plasma angiotensin II and aldosterone and then continued on this angiotensin converting enzyme inhibitor for 8 weeks, neither the expected increase in CVF nor the appearance of arterial hypertension and LVH was observed. These findings did not identify the relative importance of angiotensin II and aldosterone to the fibrous tissue response, but they did support the importance of these hormones. The aldosterone antagonist spironolactone, administered orally before the induction of renovascular hypertension or hyperaldosteronism, was examined next to determine if it would prevent the fibrous tissue response and clarify whether aldosterone or angiotensin II was responsible for cardiac fibroblast proliferation and enhanced

**TABLE 1. Interstitial and Perivascular Fibrosis of Rat Left and Right Ventricles in Various Experimental Models With and Without Systemic Hypertension and Hypertrophy**

<table>
<thead>
<tr>
<th>Model</th>
<th>Fibrosis</th>
<th>HT</th>
<th>LVH</th>
<th>Angiotensin II</th>
<th>Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>↑</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>→</td>
<td>→</td>
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<tr>
<td>Aldosterone/1K/high Na⁺</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>1K/high Na⁺</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>RHT+captopril</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>RHT+S (low)</td>
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<td>+</td>
<td>+</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Aldosterone/1K/high Na⁺</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Aldosterone/1K/high Na⁺</td>
<td>–</td>
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RHT, renovascular hypertension resulting from unilateral renal ischemia; IRB, infrarenal band; 1K, uninephrectomy; high Na⁺, enhanced dietary sodium; captopril, angiotensin converting enzyme inhibition; S, spironolactone in variable dosage, low, small dose that did not prevent hypertension or LVH in RHT or hyperaldosteronism and a larger dose (high) that did; fibrosis, increased interstitial and perivascular collagen volume fraction of right and left ventricles; HT, hypertension; LVH, left ventricular hypertrophy.
collagen synthesis. Two different doses of spironolactone were used in hyperaldosteronism: a small dose that did not prevent arterial hypertension or LVH and a larger dose that did. In our model of unilateral renal ischemia, only the small dose of spironolactone was used because the chance of preventing hypertension was modest. We found that either dose of spironolactone prevented the appearance of myocardial fibrosis in either ventricle in hyperaldosteronism. The reactive fibrosis of the myocardium was also not seen in renovascular hypertension or hyperaldosteronism when animals received spironolactone. Thus, irrespective of the presence of LVH, the perivascular and interstitial fibrosis of the myocardium seen in arterial hypertension is in some manner related to elevations in plasma aldosterone.

Experimental evidence from other studies suggest a role for circulating angiotensin II in contributing to a wound-healing response in the myocardium, including structural abnormalities of endothelial cells and consequent alterations in coronary vascular permeability. These morphological studies demonstrated that the permeability of intramyocardial coronary arteries was abnormal in the rat within 4 weeks of unilateral renal ischemia; this could be reproduced after a 4-hour intravenous infusion of angiotensin II that increased arterial pressure to hypertensive levels. Studies of cardiac lymph in the dog with renovascular hypertension for 4 weeks confirmed the presence of abnormal coronary permeability. These findings of abnormal coronary permeability in renovascular hypertension do not distinguish between the importance of angiotensin II and aldosterone in altering permeability because the plasma concentration of each hormone is elevated in response to renal ischemia. The administration of angiotensin II does not clarify the issue because plasma aldosterone increases quickly in response to this peptide hormone.

A cardiotoxic effect of angiotensin II must also be considered responsible for raising myocardial collagen concentration through the appearance of a reparative fibrosis, or scarring. Previous studies have demonstrated a nonhypertensive intraperitoneal dose of angiotensin II to be associated with the antinymosin antibody labeling of cardiocytes, indicative of abnormal sarcolemmal permeability and impending myocyte necrosis. The degree of reparative scarring and, by inference, the extent of necrosis, however, were modest and involved only a small portion of the myocardium (unpublished data). Thus, elevations in circulating angiotensin II may contribute to the fibrous tissue response by inducing myocyte necrosis, thereby setting into motion a wound-healing-like response. Furthermore, a direct role for angiotensin II-mediated fibroblast proliferation and enhanced myocardial collagen synthesis cannot be presently excluded since it was reported that angiotensin II has mitogenic properties in cultured 3T3 cells, a nontumorigenic murine fibroblast cell line.

Myocardial Fibrosis: Theoretical Models of Regulatory Mechanisms

Morphological studies of the fibrous tissue response seen in vivo in the rat right and left ventricles in various experimental models of arterial hypertension suggest that an elevation in arterial pressure and circulating aldosterone are required to promote a reactive myocardial fibrosis; the role of plasma angiotensin II is less clear. Responsible pathogenetic mechanisms must now be sought. It will be necessary to conduct in vitro studies in cultured adult cardiac fibroblasts, in which their short- and long-term responses to peptide and steroid hormones can be directly examined, as well as additional in vivo studies to further identify responsible mechanisms. Theoretical models of the regulatory mechanisms that may explain the potential contributions of the peptide hormone angiotensin II and the steroid hormone aldosterone to cardiac fibroblast growth and collagen synthesis are presented below. They should be considered in the context of the molecular biology of myocyte growth.

Hormone–Fibroblast Interaction

A specialized cell can quickly respond (e.g., within seconds or minutes) to a given stimulus. Contraction, secretion, movement, and altered metabolism are examples of such short-term cellular responses to hormones. Long-term responses require hours to days and include cell division.

The influence of various hormones on short- and long-term cellular responses has been studied extensively in cultured Swiss 3T3 cells, a murine fibroblast cell line. Of course, these cells are not cardiac fibroblasts, and the following discussion must be viewed as supposition. Nevertheless, the response and mechanisms responsible for the behavior of 3T3 cells provide valuable insights for the in vitro study of cultured cardiac fibroblasts. Several reports have proven invaluable in developing these theoretical models.

Figure 4 depicts the generation of angiotensin II that can occur either within circulating or tissue (i.e., cardiac interstitium) compartments and its interaction with a cell surface receptor of the fibroblast. A series of intracellular events that follow, which are depicted and described in the figure legend, are likely to involve inositol triphosphate and protein kinase C pathways and may be responsible for mitosis and short-term responses, such as collagen synthesis and cell motility, in this mesenchymal cell. In response to angiotensin II, zona glomerulosa cells of the adrenal gland synthesize aldosterone. This is an example of an epithelial cell’s short-term response to a hormone.

Circulating aldosterone may bind to a cell surface receptor of the fibroblast; however, it is more likely that its primary mode of action will be mediated through a cytosolic receptor. The interaction of circulating aldosterone with the corticoid receptor of fibroblasts and its possible stimulation of collagen
FIGURE 4. Schematic representation of potential interaction between peptide hormone angiotensin II and cardiac fibroblasts. Beginning with angiotensinogen-renin-angiotensin cascade that occurs in either plasma or interstitium, renin's cleavage of angiotensinogen yields decapeptide angiotensin I. Messenger RNA for angiotensinogen has been observed in periatrial and periaortic brown adiopocytes and fibroblasts of periaortic connective tissue of adventitia. Tissue-generated angiotensinogen may not contribute substantively to circulating pool, but it could play an important role in expressing paracrine effects of angiotensin II on cardiac fibroblasts. Angiotensin converting enzyme (ACE), located in endothelial cells of coronary vasculature and interstitium, cleaves a dipeptide from angiotensin I to form octapeptide angiotensin II. Angiotensin II attaches to ligand-binding domain of a membrane receptor of fibroblast. Two different angiotensin II receptors (type A and B) may exist, and each may promote a different outcome when activated. Signal transduction or second-messenger pathway that couples angiotensin II to type A receptor activation with intracellular events in other cells include a G protein, representing cell membrane's effector domain and driven by the conversion of GTP to GDP and activation of phospholipase C. Phospholipase C converts membrane phospholipids for their participation in second-messenger pathways. In this case, phosphatidylinositol biphosphate (PIP₂) is converted to inositol triphosphate (IP₃) and diacylglycerol (DAG). IP₃ diffuses through cytosol, subsequently binding to endoplasmic reticulum at specific binding sites. Binding of three IP₃ molecules is considered necessary to open calcium channels in endoplasmic reticulum and release calcium from this intracellular calcium storage site. Additional messenger pathways that regulate a variety of short-term cellular responses may also be involved by calcium. In vascular smooth muscle cells, for example, increase in cytosolic calcium leads to calmodulin-mediated phosphorylation of myosin light-chain kinase and cell contraction. In cardiac myocytes, calcium-calmodulin kinase II activation leads to phosphorylation of phospholamban and their subsequent contraction. Whether such Ca²⁺-mediated messenger pathways modulate cardiac fibroblast movement or collagen synthesis is unknown. DAG remains within cell's lipid membrane to activate protein kinase C (PKC). In zona glomerulosa cells of adrenal, DAG-mediated rise in PKC leads to biosynthesis of aldosterone. Role of PKC in cardiac fibroblasts remains unknown. It is likely that these messenger pathways play an important role in short-term responses of cardiac fibroblasts (see Figure 6).
 syntheses through DNA binding and collagen gene expression are depicted in Figure 5. A discussion of intracellular events that probably lead to enhanced protein synthesis is presented in the figure legend.

In Figure 6, the interrelation between cardiac fibroblasts and various circulating or tissue-generated hormonal substances present in the tissue fluid of the extracellular space is schematically represented. Short- and long-term responses of the cell are suggested and discussed in the figure legend.

**Hormone–Blood Vessel Interaction**

The contribution of arterial hypertension together with elevations in circulating and/or tissue peptide and steroid hormones on short- and long-term responses of cardiac fibroblasts will not be easily repro-
FIGURE 6. Schematic of protein kinase C representing a signal transduction pathway for mitogenesis that is used by various trophic factors, either hormonal (e.g., vasopressin, angiotensin II) or nonhormonal (e.g., platelet-derived growth factor) in nature. Protein kinase C activates an amiloride-sensitive Na+/H+ antiporter, which serves to increase both intracellular Na+ and pH by extrusion of H+ from the cell. This translocation of Na+ across cell membrane is coupled to Na+ efflux, mediated by digitalis-sensitive Na+,K+-ATPase. Na+,K+-ATPase activation displaces three Na+ ions out of the cell and brings two K+ ions into the cell. This serves to restore electrochemical gradient across the cell membrane. This movement of monovalent cations is an early response of cells that have left their quiescent G0 state to reenter cell cycle. Protein kinase C may directly influence nucleus and expression of proto-oncogenes c-fos and c-myc. Hormones, such as aldosterone, that enhance cellular ion fluxes in epithelial cells may also serve to stimulate Na+ entry and Na+,K+-ATPase activity in non-epithelial cells and thereby augment DNA synthesis in fibroblasts. This would probably require the presence of important cofactors, such as insulin, in what has been described as the competence-progression model for fibroblasts. It is now recognized that gene-specific modulation of Na+,K+-ATPase is dependent on the nature of the hormonal stimulus, is specific for various isoforms of the Na+,K+ exchanger, and is tissue specific. Furthermore, through its stimulation of protein synthesis, aldosterone leads to insertion of new Na+ channels and Na+,K+-ATPase molecules into epithelial cell membranes. If this were to occur in cardiac fibroblasts, it would augment their mitogenic potential. Potential interrelation between angiotensin II and another angiotensin II receptor (type B) may resemble...
an α1-receptor in its activation of adenylate cyclase and cyclic AMP (cAMP) similar to that seen with other peptide hormones such as norepinephrine (NE) and vasopressin (AVP). Cellular levels of cAMP may also represent a growth-promoting signal in cultured fibroblasts, particularly as they relate to prostaglandin E, neuropeptides, and platelet-derived growth factor-mediated increments in cAMP. Activated cell surface receptor, coupled to adenylate cyclase, increases intracellular cAMP, which in turn binds to regulatory subunits of a cAMP-dependent protein kinase to free its catalytic subunits. cAMP-dependent pathway of mitogenesis provides a mechanism for fibroblast growth that may be independent of protein kinase C pathway or may be synergistic to it. α1-Receptor pathway is opposed by G proteins that inhibit adenylate cyclase (Gi) and are activated by agonists of the α2-receptor.

duced in tissue culture. Despite the theoretical attractiveness of growing fibroblasts on stretchable membranes to simulate mechanical loading, animal models, such as those described, can be used to advantage and are likely to have more meaningful pathophysiological significance. Furthermore, the sequence to the progressive myocardial fibrosis, which begins as an accumulation of fibrillar collagen within the adventitia of intramyocardial coronary arteries and then extends into intramuscular spaces between bundles of cardiac myocytes, resembling the response of the kidney to antiglomerular basement membrane IgG, strongly implicates an abnormality in the permeability of these intramural vessels. The abnormality in coronary vascular permeability may include the entrance of growth factors (e.g., platelet-derived growth factor) or the activation of in-residence growth factors (e.g., transforming growth factor β) within the interstitium that, in turn, lead to fibroblast proliferation and enhanced collagen synthesis. It is also possible that blood-borne protease inhibitors enter the interstitial space to retard collagen degradation. Any of these pathophysiological scenarios could be reproduced in culture if the proper sequence to the modification in tissue fluid were reproduced. This would be a logical goal of combining in vivo with in vitro studies and might first involve sampling interstitial fluid and then determining the fibroblast’s response in collagen metabolism (synthesis and degradation) and growth. Based on the outcome of these in vitro studies, biochemical analysis of the tissue fluid for the substances responsible for promoting collagen gene expression, altering collagen metabolism, or enhancing fibroblast proliferation could be undertaken. The results of this line of investigation would probably make it possible to design complementary in vivo experiments that address the mechanism responsible for abnormal permeability of these intramural vessels and the prevention of the subsequent fibrous tissue response.

Such studies typify the importance associated with integrating the resources of the molecular and cellular biology laboratories into studies of abnormal myocardial structure and physiology. They further underscore the potential for developing clinically relevant therapeutic strategies and suggest that myocardial failure resulting from fibroblast growth and interstitial fibrosis will be largely preventable and perhaps even correctable. Such a scenario would be a welcomed outcome to the creative potential of the academic process and lead to more remedial forms of therapy for the management of the patient with heart failure by the turn of this century.

**Myocardial Fibrosis and Management Strategies**

The fibrous tissue response of the cardiac interstitium is responsible for the abnormal stiffness of the myocardium and accounts for ventricular dysfunction in either the diastolic or the systolic phase of the cardiac cycle or both. Prevention of the reactive myocardial fibrosis would therefore be a logical goal. In patients having LVH and myocardial fibrosis with ventricular dysfunction, regression in myocardial structure and restoration in tissue distensibility are additional necessary objectives.

**Prevention of Fibrosis**

The prevention of arterial hypertension and elevation in circulating angiotensin II and aldosterone are logical targets for the prevention of myocardial fibrosis. Our initial studies with spironolactone or captopril pretreatment in the rat with either unilateral renal ischemia or hyperaldosteronism in which it was possible to attenuate the fibrous tissue response suggest that these agents have cardioprotective properties. This approach appears to interrupt the process at the outset of the hormone-mediated response that leads to fibroblast activation. This concept will need to be systematically examined. One could also argue that the fibrous tissue response could be interrupted within the cardiac fibroblast at any point beginning with the signal-transduction pathway and continuing beyond. Given the potential importance of calcium as an intracellular signal in mediating angiotensin II–mediated cardiac fibroblast collagen synthesis, calcium channel blockers may prove to have cardioprotective properties. This too needs to be examined. A third possibility is based on the premise that the angiotensin II–mediated response is related to its interaction with adrenergic neurons and cyclic AMP–mediated pathways. Thus, sympatholytic agents may prevent fibrosis.

**Regression of Fibrosis**

Removal of the interstitial and perivascular fibrosis will prove equally challenging but represents a means by which myocardial failure resulting from collagen accumulation could be reversed. Agents that restore myocardial structure to normal and thereby alleviate abnormalities in myocardial stiffness would have cardio reparative properties. The premise of this approach is based on either inhibiting the stimulus to fibrous tissue formation while retaining the myocardium’s proteolytic enzyme system for continued gradual collagen degradation or actively invoking pro-
teolytic digestion for more rapid degradation. Presently, little is known about the regulation of the active or inactive forms of myocardial collagenase. In other tissues, this neutral metalloproteinase is known to be influenced by pH, calcium concentration, and temperature, whereas the propensity of the fibrillar collagen to proteolytic digestion favors type III collagen. Laurent underscored the fact that it is no longer tenable to view collagen as an inert protein and that its turnover may be more rapid than previously appreciated. He estimates that in the heart collagen degradation may be as high as 5% per day and that a major portion of this proteolysis may occur within lysosomes and/or Golgi apparatus of fibroblasts.

Twelve weeks of lisinopril treatment in 14-week-old spontaneously hypertensive rats (SHR) with established LVH, myocardial fibrosis, and abnormal diastolic stiffness removed excess fibrous tissue accumulation and restored stiffness to values seen in normotensive genetic controls while at the same time regressing myocardial mass. The manner in which this occurred requires systematic evaluation. However, the results are encouraging. Shorter (6-week) treatment trials with captopril in either 8-week-old SHR or adult rats with 6-week unilateral renal ischemia were found to regress LVH but not reduce collagen concentration. In longer (45-week) treatment trials, the calcium channel blocker verapamil did not reduce collagen concentration or collagen synthesis in 10-week-old SHR despite the reduction in LVH associated with its normalization of arterial pressure. On the other hand, minoxidil, a non-specific antihypertensive agent, increased collagen concentration in similarly treated animals. These findings indicate that myocyte size declines more rapidly than the invasion of collagen, a stable protein having a half-life of 80–120 days in the myocardium, and that not all agents are associated with an inversion in collagen mass. Motz and Strauer recently reported that 20 weeks of treatment with another calcium channel blocker, nifedipine, restored collagen concentration in 20-week-old SHR to values seen in age-matched genetic controls. Certain pharmacological agents may therefore prove to have a greater influence on myocardial collagen concentrations because of their specific mechanism of action, enhanced uptake, or tissue specificity. This will prove a worthy line of investigation. The opportunity is at hand to determine which antihypertensive agents have antifibrotic effects and are cardiovascular tissue specific. In so doing, such agents may have the potential to restore myocardial structure and function to normal and eliminate pathological hypertrophy as a major determinant of myocardial failure.

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