Heart Failure Reviews, 7, 71–88, 2002

Studies of Prevention, Treatment and Mechanisms of Heart Failure in the Aging Spontaneously Hypertensive Rat

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Abstract. **The spontaneously hypertensive rat (SHR) is an animal model of genetic hypertension which develops heart failure with aging, similar to man. The consistent pattern of a long period of stable hypertrophy followed by a transition to failure provides a useful model to study mechanisms of heart failure with aging and test treatments at differing phases of the disease process. The transition from compensated hypertrophy to failure is accompanied by changes in cardiac function which are associated with altered active and passive mechanical properties of myocardial tissue; these events define the physiologic basis for cardiac decompensation. In examining the mechanism for myocardial tissue dysfunction, studies have demonstrated a central role for neurohormonal activation, and specifically the renin-angiotensin-aldosterone system. Pharmacologic attenuation of this system at differing points in the course of the process suggests that prevention but not reversal of myocardial tissue dysfunction is possible. The roles of the extracellular matrix, apoptosis, intracellular calcium, beta-adrenergic stimulation, microtubules, and oxygen supply-demand relationships in ultimately mediating myocardial tissue dysfunction are reviewed. Studies suggest that while considerable progress has been made in understanding and treating the transition to failure, our current state of knowledge is limited in scope and we are not yet able to define specific mechanisms responsible for tissue dysfunction. It will be necessary to integrate information on the roles of newly discovered, and as yet undiscovered, genes and pathways to provide a clearer understanding of maladaptive remodeling seen with heart failure. Understanding the mechanism for tissue dysfunction is likely to result in more effective treatments for the prevention and reversal of heart failure with aging. It is anticipated that the SHR model will assist us in reaching these important goals.**

Key Words. **aging, hypertrophy, heart failure, heart failure prevention, myocardial function, fibrosis, energetics, intracellular calcium, apoptosis, microtubules, angiotensin II**

Introduction

Age is a leading risk factor for the development of heart failure in humans. The incidence of heart failure increases more than 5-fold during the 7th and 8th decades of life [1]. For Americans over the age of 65, heart failure is the most common hospital discharge diagnosis and the most expensive Medicare item. Despite advances in the treatment of heart failure, pharmacologic therapies for heart failure remain ameliorative rather than reparative [2]. Considering the projected increase in the population of aged individuals in the 21st century, studies of the mechanisms that contribute to heart failure in the aging human population are of great interest and importance. Aging in healthy humans and experimental animals is associated with changes to the myocardium that reduce the reserve capacity of the heart to respond to stress [3,4]. These factors and others contribute, in a poorly understood manner, to the progression from stable hypertrophy to ventricular dysfunction and heart failure in an aging population. The decompensated, failing heart is characterized by impaired functional performance, despite compensatory hypertrophy. Manifestations of decompensated function include ventricular dilation, depressed fractional shortening, and diminished ejection fraction. Interstitial fibrosis is a hallmark of heart failure associated with left ventricular hypertrophy [5–7] as well as normal aging [4,8,9]. Fibrosis increases the stiffness of the myocardium, contributing to diastolic dysfunction, and aggravating heart failure [10].

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While studies in man have defined the effects of aging and hypertension on morbidity and mortality, much further work is needed before we understand the fundamental basis for pathological events. The heterogeneity of human heart failure and the limited ability to carry out carefully controlled studies of mechanisms of heart failure render studies of humans as inadequate for solving the problems of heart failure in a timely manner. The difficulty in obtaining mechanistic information from studies in humans provides a strong rationale to study animal models of heart failure associated with aging. The spontaneously hypertensive rat (SHR) develops hypertension and cardiac hypertrophy during the first quartile of life. Stable, compensated hypertrophy is present for most of its subsequent lifespan. Male SHRs ultimately develop cardiac dysfunction and heart failure between 18–24 months of age [11,12]. Thus, the SHR may be a useful model to study the transition from compensated hypertrophy to decompensated heart failure in the context of aging. This review will focus on studies of the aging SHR which demonstrate a transition to heart failure.

The Aging SHR Model of Heart Failure

The SHR was originally introduced by Okamoto and Aoki [13] as a model of genetic hypertension. It has been pointed out that the progression from hypertrophy to impaired cardiac function in the SHR is similar to the clinical course of patients with hypertension [14]. Persistent hypertension develops in the SHR after approximately 2 months of age [15,16]. Following a relatively long period of stable hypertension and compensated hypertrophy, at approximately 18 months of age, animals begin to develop evidence of impaired function [11]. Clinically, these rats are observed to develop tachypnea and labored respirations. After the age of 18 months, mortality rate is increased; on pathologic evaluation, cardiac hypertrophy is found in virtually all SHR. If animals with tachypnea and labored respirations are studied, all were found to have one or more of the pathologic features suggesting heart failure; e.g. effusions, left atrial thrombi and right ventricular hypertrophy [17]. Findings in these animals are consistent with observations of cardiac insufficiency in late stages of pressure overload in the SHR, associated with impaired contractility, cardiac chamber dilatation and fibrosis [18]. Echocardiographic studies and cardiac catheterization were performed in a subgroup of animals including those later demonstrated to have pathological features of failure (SHR-F) on post mortem examination. In comparison to age matched non-failing SHR (SHR-NF) and non-hypertensive Wistar-Kyoto rats (WKY), LV systolic and diastolic chamber dimensions and filling pressures were increased [12]. In a group of 63 SHR, 54 (85%) died or developed heart failure by 24 months of age. 37 (59%) had pathologic evidence of heart failure at a mean age of 19 ± 2 months [19]. Thus, over 50% of SHR develop heart failure over the course of their lifespan.

Myocardial Function

The relation between the syndrome of heart failure and mechanical dysfunction of the heart as a muscle, deduced from catheterization laboratory or hemodynamic studies in humans, is not straightforward. Studies in experimental animals may likewise be difficult to interpret. To more clearly define the physiologic state of SHR hearts under study we have characterized the SHR model of the transition to heart failure using an integrated approach which includes echocardiography, cardiac catheterization, isolated perfused heart studies, isolated papillary muscle studies and pathological evaluation [12]. Increased chamber dimensions, elevated filling pressures, impaired pump function, and pathological findings document clinical heart failure. Isolated papillary muscle studies provide a direct measure of the mechanical properties of the myocardium as a tissue.

Heart failure ultimately results from an impairment of the pump function of the heart. This is easily understood in the heart with impaired muscle function. However, it is important to realize that failure may occur in the heart in which myocardial function is normal but an excessive load is imposed—load induced heart failure. A sudden obstruction to flow in a normal heart—for example—from a pulmonary embolus, which impedes right ventricular outflow, may result in acute right ventricular failure with intrinsically normal right ventricular muscle function. A common cause of abrupt LV failure with normal muscle function is sudden or paroxysmal hypertension. Myocardial infarction will likewise suddenly augment the load on adjacent normal myocardium. It has been suggested that the transition to failure in the SHR may reflect an increased load due to a pulsatile contribution to total load, secondary to changes in conduit vessel function [20]. In an analogous manner, a reduced ability to develop hypertrophy might result in a relative increase in load and heart failure in otherwise normally functioning myocardium. The work of Gerdes et al. [21] studying the spontaneously hypertensive heart failure rat (SHHF) supports this possibility. LV myocyte cross-sectional area was found to reach

a maximum by 2–3 months of age. Systolic wall stress continued to rise and cell length was increased by 12 months of age while myocyte cross-sectional area failed to increase. Interestingly, when the hypertrophied RV was studied from SHHF with heart failure, RV myocyte length and width increased proportionately [21]. These studies suggest that transverse growth of the LV myocyte is impaired in the SHHF.

The findings of studies examining contractile properties from isolated muscle preparations from the SHR are reviewed below (see also Table 1). While studies of isolated muscle preparations have their own set of limitations which include the need to carry out studies at low temperatures and low stimulation rates, findings are considered to reflect intrinsic properties of myocardial tissue. Additional studies of myocyte function help sort out the role of tissue components (myocytes, extracellular matrix) as contributing to cardiac dysfunction and heart failure. Studies of isolated papillary or trabecular muscle preparations include a determination of the active and passive properties of myocardium in a controlled environment under fixed experimental conditions; i.e., temperature, nutrients, including dissolved gasses, stimulation rate, and load. Parameters measured include active contraction parameters; e.g. active tension (AT), maximum or mean rate of isometric tension development (dT/dt) and maximum shortening velocity measured at minimum load. Temporal parameters of contraction include electromechanical delay time (EMD), time to peak tension (TPT), and the time from peak tension to 50 % tension decline ($RT_{1/2}$), which is used as an index of relaxation. The passive properties or stiffness (k) of the muscle preparation are derived from measurements of force-length determinations during passive stretches of the preparation. k_{cs} is stiffness determined from a central undamaged segment of the papillary muscle preparation [22].

Using this approach, it has been found that active properties of isolated papillary muscle preparations from the SHR at 6, 12 and 18 months are enhanced in comparison to normotensive controls (Fig. 1). AT and dT/dt were found to be approximately 25 % increased in SHR from 6–18 months of age. Enhanced calcium transients [23] and contractile state have been reported in SHR myocytes [24,25]. Shorofsky et al. [26] have recently reported that calcium sparks linked to calcium entry through L-type Ca^{2+} channels and release of Ca^{2+} from SR are increased in 6 month old non-failing SHR in association with an enhanced contractile state. In the 18 month SHR an increase in TPT was observed [27]; otherwise myocardial performance was not impaired until pathological manifestations of heart failure

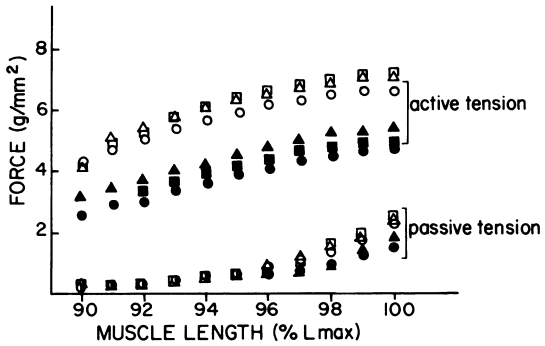

Fig. 1. *Passive and active force (tension)-length relationships obtained from isolated papillary muscle preparations from 6, 12 and 18 month old (circles, squares and triangles, respectively) spontaneously hypertensive (open symbols) and normotensive rats (closed symbols). Active tension is increased in the SHR at all ages studied.* L_{max} *is muscle length at which peak active tension is developed (adapted from [102]).*

(including effusions, atrial thrombi and right ventricular hypertrophy) are present [12]. In the SHR-F, in comparison to age-matched SHR-NF and WKY, a depression of active muscle properties is observed; active tension (Fig. 2, left), dT/dt and maximum shortening velocity are depressed. It is interesting that if corrected for the histological constituents of the myocardium, which are altered as a result of myocyte loss and fibrosis, the active properties of remaining myocardium may not be impaired. That is, if calculations of muscle active tension are corrected for fractional myocyte cross-sectional area, myocardial active tension is not different from control [28]. This suggests that depressed active tension developed by whole papillary muscles might be explained by a shift in the composition of the muscle preparation; an increased portion is made up by non-contracting material including extracellular matrix components, and that the function of remaining myocytes is relatively unimpaired. Findings are consonant with studies of myocytes; Emanuel et al. [29] found no differences in myocyte properties from failing and non-failing SHR hearts and concluded that myocyte apoptosis and remodeling of the extracellular space were more likely responsible for the development of heart failure. On the other hand, a study of right ventricular pressure overload in cats demonstrated impaired myocyte contractile dysfunction [30]. Differences may relate to the ventricle (left vs. right), species studied, or stage of hypertrophy/failure; therefore, the critical issue of the basis for impaired cardiac function observed in heart failure associated with chronic pressure overload remains unresolved.

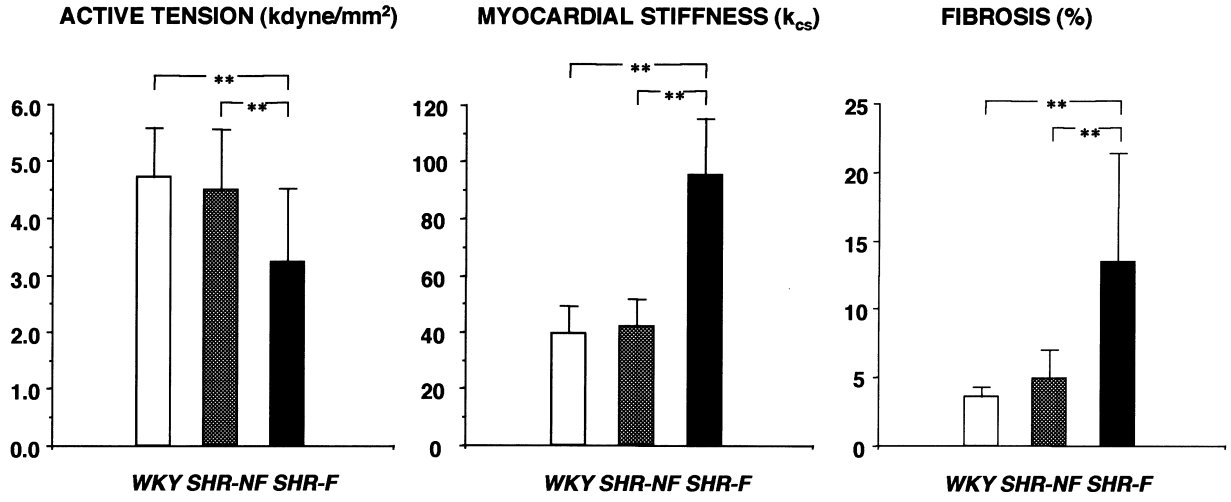

Fig. 2. *Active tension, myocardial stiffness (k_{cs}; central segment stiffness) and fibrosis of papillary muscles from WKY (n = 12), SHR-NF; (n = 8) and SHR-F (n = 7). Active tension is depressed while k_{cs} and fibrosis are increased in preparations from the SHR-F relative to age-matched WKY and SHR-NF. Stiffness is determined from central segments of papillary muscle preparations (adapted from [28]). ** P < 0.01.*

Associated changes in temporal parameters of contraction include a prolonged TPT which is seen in both SHR-NF and SHR-F. A prolonged EM delay time and an abbreviation of the half-relaxation time are observed only in the SHR-F. Studies of the passive properties of the myocardium demonstrate an increase in muscle stiffness (k_{cs}) in the SHR-F, which is associated with fibrosis determined from histological studies and collagen determinations (Fig. 2; center, right) [28].

A consistent finding that has been observed in studies of failing (but not hypertrophied non-failing) SHR myocardium is an abbreviated $RT_{1/2}$ [17,19,28,31]. A similar abbreviation of $RT_{1/2}$ is found in preparations from failing hearts from the aortic banded WKY (unpublished observations); thus, this is not a strain specific finding. Traditional thought has been that relaxation is impaired in cardiac hypertrophy, and further impaired in failure. Impaired relaxation has been correlated with molecular data suggesting impairment of calcium handling, and decreases in the transcripts of components of the cardiac relaxing system have been reported in studies of cardiac hypertrophy and failure.

The finding of an abbreviated $RT_{1/2}$ in the SHR-F does not appear consistent with impaired relaxation, and suggests that biochemical and molecular findings may not relate directly to physiologic relaxation in heart failure in the SHR. Studies of myocardial relaxation in the intact heart must take into account the multiple factors that affect cardiac mechanics. Certainly, an analysis of the time course of in vivo force or pressure tracings alone is inadequate. Myocardial performance, including both contraction and relaxation, are considerably affected by loading conditions. A discussion of the complexities of the mechanics of contraction and relaxation is beyond the scope of this review. However, an understanding of these events is integral to understanding the relation between mechanical function and heart failure. A number of mechanical and neurohormonal factors are well recognized to alter the time course of mechanical activity. Simply increasing afterload will prolong contraction and relaxation. The finding of "impaired relaxation" based on an examination of the time course of pressure or stress recordings after myocardial infarction, for example, may simply reflect the relative increase in load borne by surviving myocardium, which must generate a more isometric and prolonged contraction—giving the appearance of "impaired relaxation". A similar analysis of pressure or force traces in cardiac hypertrophy and failure may yield a similar erroneous conclusion of "impaired relaxation". Efforts to develop load independent measures of relaxation have spawned a number of indices. Using tau, a load independent measure of the time course of force decline [32] and a number of indices to study relaxation in the failing SHR heart, no evidence for mechanical impairment of relaxation studying isolated muscle preparations has been found (unpublished observations).

While mechanical studies of myocardial relaxation provide empirical information, findings appear to be of limited value in furthering

Table 1. *Hypertrophy indices and physiologic data from WKY, SHR-NF and SHR-F*

	BP mm Hg	BW g	LV/BW mg/g	RV/BW mg/g	AT g/mm^2	dT/dt $g/mm^2/s$	EMD ms	TPT ms	$RT_{1/2}$ ms	$V_{0.5}$ lengths/s	k_{cs}	OHP mg/g	Fibrosis %
WKY	125 ± 5*+	563 ± 74*+	1.84 ± 0.17*+	0.54 ± 0.06*+	6.17 ± 14.7+	68.8 ± 14.7+	32 ± 5+	157 ± 14+	218 ± 30+	2.15 ± 0.48+	40 ± 10+	1.05 ± 0.16+	8.0 ± 2.8+
SHR-NF	200 ± 12	400 ± 22	3.49 ± 0.21	0.65 ± 0.07#	7.17 ± 0.94#	65.5 ± 15.6#	32 ± 4#	174 ± 14	208 ± 19#	1.60 ± 0.3#	42 ± 10#	1.24 ± 0.24#	9.5 ± 4.2#
SHR-F	191 ± 11	369 ± 50	3.65 ± 0.35	1.14 ± 0.17	3.58 ± 1.75	31.8 ± 16.2	48 ± 10	180 ± 11	161 ± 29	0.95 ± 0.38	96 ± 20	1.83 ± 0.66	20.1 ± 10.5

WKY indicates Wistar-Kyoto; SHR, spontaneously hypertensive rats; NF, non-heart failure; F, heart failure; BP, systolic blood pressure; BW, body weight; LV/BW, left ventricle to body weight ratio; RV/BW, right ventricle to body weight ratio. Papillary muscle data are: AT, active tension; dT/dt, peak positive derivative of tension; EMD, electromechanical delay time; TPT, time from onset of contraction to peak tension; $RT_{1/2}$, time from peak tension to 50% relaxation; $V_{0.5}$, shortening velocity determined from quick release to 0.5 g/mm^2 at 100 ms (normalized by muscle length at L_{max}); k_{cs}, myocardial stiffness constant; OHP, hydroxyproline; Fibrosis, fractional area of connective tissue. Values are means ± S.D. of 8–10 rats/group.

*$P < 0.01$ WKY vs. SHR-NF.

+$P < 0.01$ WKY vs. SHR-F.

our understanding of cardiac relaxation in heart failure. Myocardial relaxation is a complex [33] multicomponent process. For example, the time course of calcium release must overlap with that of calcium uptake; this interaction will affect relaxation dynamics. In addition to calcium sequestering mechanisms, the lifetime of cross-bridge attachment may also affect relaxation. In the case of ATP depletion, (hypoxia or ischemia), crossbridges may bind with high affinity at very low calcium concentrations [34]. Thus, rigor bond formation may alter the diastolic properties of myocardium largely independent of $[Ca^{2+}]_i$. With respect to calcium handling, it is unclear how the multiple components of the cardiac calcium handling system interact to modulate the contraction-relaxation cycle and how disease might effect the individual components as well as their integrated activities. Thus, mechanical relaxation represents the sum or net of a complex multicomponent process and the relation of a change in a relaxation index such as $RT_{½}$ or tau to a specific finding such as impaired SR calcium uptake may be difficult to define.

Prevention and Treatment of Heart Failure

Considering the well documented clinical/pathological outcome of long-term studies, the SHR is a useful model to study therapies aimed at prevention and treatment of heart failure. Several treatment studies were carried out to examine mechanisms of heart failure as well as to provide pilot data for potential clinical studies. Studies designed to evaluate prevention of failure involved administering therapy to SHR and control animals at 12 and 18 months which is before the mean age of failure (19 ± 2 months) and to study animals at 24 months, an age where there are normally few surviving untreated SHR. One group of clinically non-failing SHR was treated at 21 months of age which is beyond the mean age of failure for the SHR group as a whole; treatment was carried out to 24 months. In an attempt to reverse failure, treatment was initiated in animals with evidence of heart failure. Echocardiograms were performed on a subgroup of these animals [12]; LV dilation and reduced ejection fraction were documented. Treatments studied in our laboratory to date, in an attempt to prevent/reverse failure in the SHR, included the angiotensin converting enzyme inhibitor, captopril [19], colchicine, which lyses microtubules, suggested to modulate cardiac cytoskeletal mediated impairment of function [35], L-arginine, a substrate for NO synthesis, thought to improve coronary flow and attenuate cardiac fibrosis [36] and losartan, an AT_1 receptor blocker (Lakatta et al, in preparation).

Outcome parameters included mortality, pathological data including measures of hypertrophy and failure, isolated muscle function, changes in gene expression and changes in calcium handling. In brief, these studies demonstrated no protective effect of colchicine (1 mg/L drinking water) administered from age 13–24 months [35] or L-arginine (16 g/L drinking water) administered from 18–24 months [36]. Captopril (2 g/L in drinking water) administered to SHRs prior to failure beginning at 12, 18 and 21 months until 24 months, totally prevented the clinical and pathological manifestations of failure. Although a few captopril treated animals died before 24 months of age, none was found to have pathological evidence of failure at autopsy. Studies of the myocardium from captopril treated 24 month old SHR revealed that in comparison to SHR-F, depression of papillary muscle active properties (Fig. 3), myocardial fibrosis and increased stiffness did not take place [19]. These findings are generally consonant with those reported by Tan et al. [37] using lisinopril. In addition, changes in gene expression associated with the transition to failure [38] did not occur, and prolongation of the intracellular calcium transient associated with hypertrophy and failure was prevented [39]. In contrast to untreated SHR, positive inotropic responses of papillary muscles to isoproterenol were present in SHRs treated with captopril [19].

It has been theorized that the beneficial effects of captopril may be due to angiotensin converting enzyme (ACE) inhibition as well as non-ACE effects of captopril. In a recent study comparing captopril with losartan, a specific angiotensin II receptor (AT1) blocker, protection appeared to be identical to that observed with captopril (Lakatta et al., in preparation). Losartan has also been shown to attenuate the electrophysiological alterations seen in the old SHR [40]. Therefore, it appears unnecessary to invoke a role for non-ACE effects of captopril, at least with respect to the parameters studied. These data are consistent with the well recognized concept that angiotensin II (Ang II) plays a critical role in the transition to heart failure.

Interestingly, if captopril is administered to clinically non-failing SHR at 21 months of age (which is beyond the mean age that failure develops), significant protection is observed. The slight decline in performance parameters observed in animals in which treatment was initiated at progressively older ages may reflect the presence of early failure (not detected at the time treatment was initiated) in some of the animals prior to treatment.

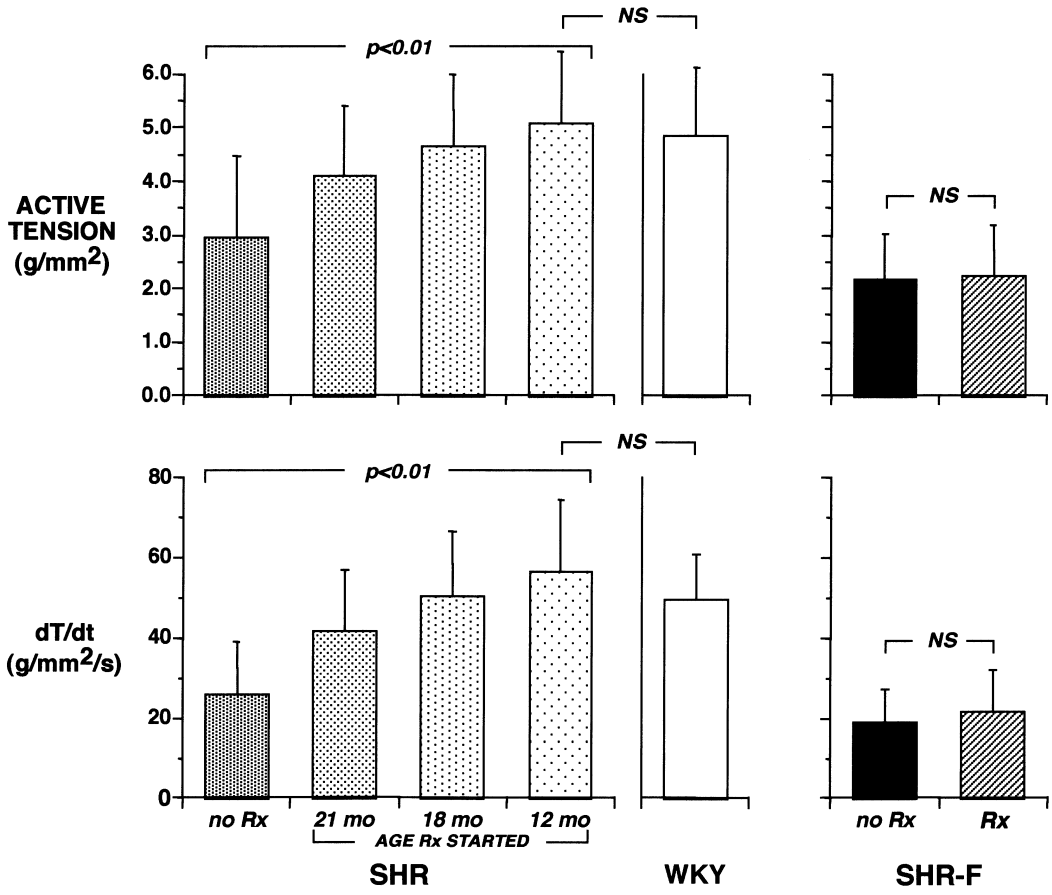

Fig. 3. *The effect of no treatment (n = 24) and captopril treatment (2 g/L drinking water) on active tension and dT/dt developed by papillary muscle preparations from SHR. Captopril was begun at 12 (n = 6), 18 (n = 12) and 21 (n = 11) months of age and administered to 24 months. Data from 24 month untreated WKY (n = 10) are shown. Captopril was also administered to a group of SHR-F (n = 6; mean age 21) months until 24 months and data are compared with untreated SHR-F (n = 8). 6 of 12 SHR treated with captopril after failure survived to 24 months and study. The earlier captopril is administered to SHR the greater the performance of papillary muscles studied at 24 months. If captopril is administered after failure there is no difference in the performance of preparations from captopril treated and untreated SHR-F (adapted from [19]).*

The overall data suggest that ACE inhibition (or Ang II blockade), before the advent of failure prevents clinical and pathological manifestations of heart failure in the SHR, consistent with the landmark study in the SHR by Pfeffer and coworkers [41]. Moreover, of paramount interest and clinical importance, impairment of intrinsic myocardial tissue function does not occur. Additionally, increased myocardial fibrosis, changes in the intracellular calcium transient, impaired inotropy to catecholamines and changes in molecular markers associated with failure are not observed. Swynghedauw et al. [42] reported converting enzyme inhibitor administration to middle-aged SHRs reduced hypertension, LVH, ventricular fibrosis, and ventricular irritability, and normalized heart rate variability. These data support the concept of a primary role for neurohormonal stimulation; specifically angiotensin II in the transition to overt failure, and secondary roles for parameters measured in this study including fibrosis, altered calcium handling and changes in expressions of genes encoding components of these systems. On the other hand, while these events may occur secondary to the action of Ang II, their effects may be of primary importance in contributing to the heart failure phenotype. Therefore, blocking specific secondary events may have a therapeutic role. It is conceivable that not all of the actions of Ang II in heart failure are detrimental and that inhibiting specific components of Ang II actions may be of greater benefit than total inhibition of Ang II production or blocking all of its effects.

When captopril was administered to SHR after clinical and echocardiographic manifestations of

failure [19], the clinical course appeared to stabilize; often there was a decrease in respiratory distress and approximately 50% of treated animals survived to 24 months at which time they were studied. Findings are in general agreement with Secchi et al. [43], who reported a lower mortality rate in trandolapril and enalapril treated SHRs with congestive heart failure. Although clinical manifestations improved, and there was a decrease in LVH, echocardiographic parameters, impaired papillary muscle function and fibrosis were not different from untreated SHR-F. Therefore, it appears that ACE inhibition does not reverse the effects of failure on the functional properties of the myocardium, at least within the time frame of this study. Stabilization of the clinical course of these animals may be related to improved myocardial loading conditions and/or to arresting the deleterious effects of Ang II. In any case, ACE inhibitor therapy administered to the failing SHR for a 2–4 month period, fails to reverse pathological features of the myocardium, improve papillary muscle function, or reduce myocardial fibrosis and stiffness. Sen [44] has pointed out that hypertrophy may regress with treatment but myocardium of the post-hypertrophic heart no longer has the same composition as it did prior to the development of hypertrophy; the same may be true for failure. Connective tissue remodeling of the heart is thought to contribute to impaired performance [10]. Reversal of fibrosis, preservation of viable myocardium and regeneration of contractile elements are among goals that merit consideration in the treatment of the chronically failing heart.

The studies cited above demonstrate that the SHR serves as a useful model for testing treatments which may prevent or reverse heart failure. Studies of papillary muscle function are of particular interest because the effects of a treatment on intrinsic myocardial tissue properties may be defined. Additional studies of myocyte preparations will help to define whether treatment acts on myocytes or other constituents of the myocardium such as the extracellular matrix. Isolated muscle studies utilizing the SHR model might then be useful for the assessment of other classes of treatments. Carvedilol, a non-selective vasodilating beta blocker with antioxidant activity administered to stroke prone SHR for 18 weeks is reported to reduce histopathologic coronary artery hypertrophy, myofiber degeneration, myocardial inflammation, microinfarction and fibrosis [45]. A matrix metalloprotein inhibitor has been observed to reduce collagen volume fraction and preserve LV function in the SHHF [46]. Iwanaga et al. [47] have determined that cardiac endothelin-1 plays an important role in the transition from hypertrophy to failure in salt-sensitive hypertensive rats and that bosentan, an endothelin receptor antagonist, attenuated impairment of fractional shortening as determined by *in vivo* transthoracic echocardiography. Although Pinto et al [48] have pointed out that response to treatment may differ between rat models of hypertension, studies of heart failure prevention and reversal with differing classes of agents in the SHR failure model are of interest.

Studies of Possible Mechanisms Contributing to Heart Failure

Extracellular Matrix

Interstitial fibrosis is a hallmark of heart failure associated with cardiac hypertrophy [5–7]. Fibrosis is also observed in the "normal" aging heart [4,8,9,49]. Fibrosis increases the stiffness of the myocardium leading to diastolic dysfunction and contributing to heart failure [50]. The transition to failure in the SHR is accompanied by a marked increase in the levels of mRNA encoding extracellular matrix components (for review of matrix gene expression in the aging SHR model see Boluyt and Bing [51]). These include fibronectin, collagen Type I, collagen Type III [38] and osteopontin [52]. Decorin and elastin have been found to be decreased and the ratios of gelatinase A and elastase to tissue inhibitor of metalloproteinase-4 increased in the failing SHR heart [53]. In situ hybridization studies suggest interstitial as well as perivascular localization of α_1(I) collagen mRNA in failing SHR hearts (Fig. 4 [54]). Fibrosis and stiffness were studied in the SHR with the ACE inhibitor lisinopril, at doses that allowed dissociation of hypertrophy and fibrosis [55]. These studies report that lisinopril could cause regression of fibrosis and normalize myocardial stiffness independent of LV hypertrophy. In our experience, changes in myocardial fibrosis and stiffness in hypertrophied hearts are small prior to the advent of heart failure. It is also important to distinguish between prevention and regression of fibrosis. In studies by Brooks and coworkers [19] using high dose captopril (2 g/L drinking water) administered to failing SHRs, partial regression of LVH was found without a change in fibrosis or stiffness, or improvement of myocardial function. It is clear however, that if captopril is administered prior to heart failure, increases in the expression of genes encoding extracellular matrix components, fibrosis and stiffness are attenuated, and heart failure is prevented.

Collagen alignment and type are factors that contribute to force transmission. It has been shown that collagen increases in SHR myocardium after 40 weeks of age, and that an increase

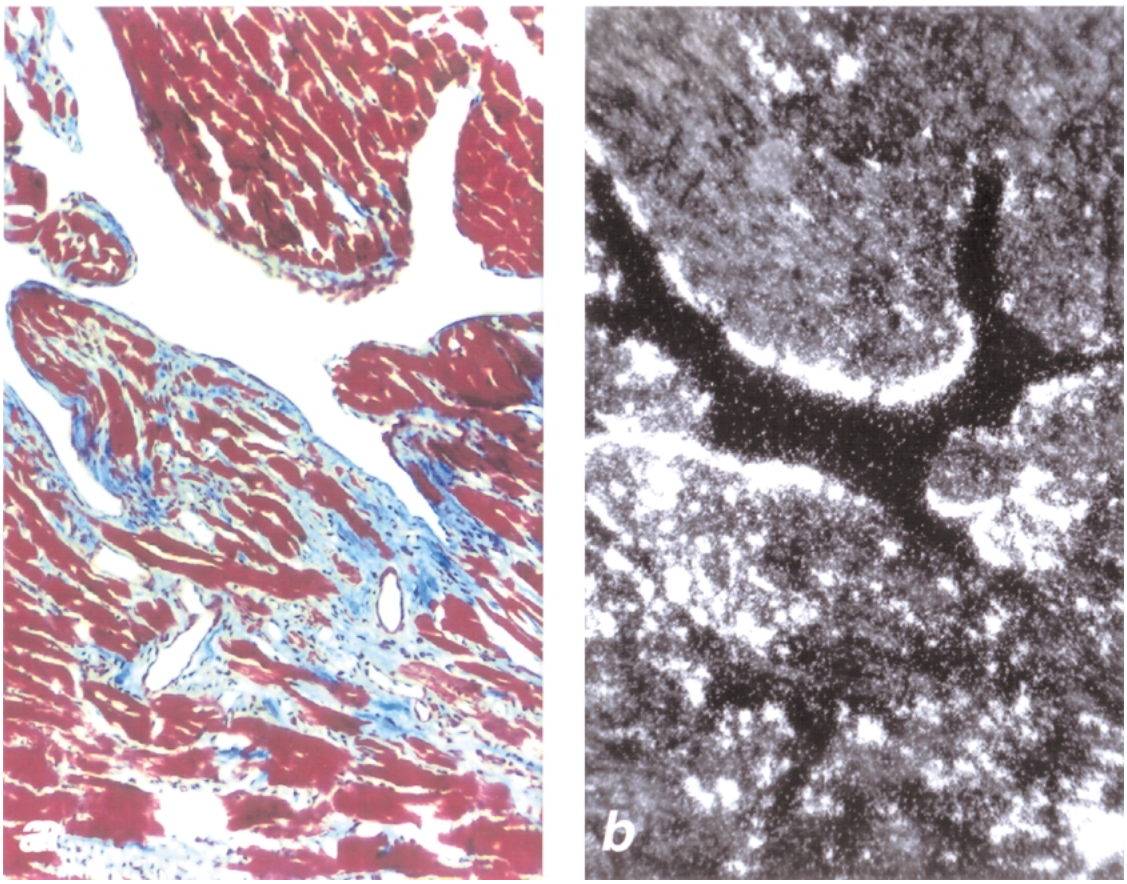

Fig. 4. *Connective tissue deposition in the SHR-F. a) Masson's trichrome section demonstrating extensive perivascular and interstitial fibrosis. b) In situ hybridization demonstrates extensive subendocardial as well as focal intramyocardial α_1(I) collagen mRNA expression (adapted from [54]).*

in type III collagen is observed. After 65 weeks of age, there is a change in the type I to III ratio in the SHR. It is thought that changes in myocardial stiffness may be related to these changes in collagen composition [56]. Norton et al. [57], studying 44 week old rats, have suggested that increased myocardial stiffness in the SHR (non-failing) is due to enhanced collagen cross-linking. In the failing SHR heart, the preferential expression of alternatively spliced fibronectin transcripts containing EIIIA and EIIIB segments is consistent with a wound healing response [38]. Scar tissue involved in wound healing may have different effects on force transmission than fibrils laid down to support stress. For example, as the heart heals from injury after infarction, some of the mechanical properties of the scar have been shown to change [58].

Identification of upstream instigators of extracellular matrix proliferation could provide targets for therapies to prevent possible fibrosis mediated deterioration of cardiac function associated with heart failure. There is evidence that transforming growth factor β_1 (TGF-β_1) plays an important role in many aspects of cardiac remodeling including the upregulation of ECM genes. In the SHR-F hearts that exhibited markedly increased levels of fibronectin and collagen gene expression, a small but significant increase in TGF-β_1 was found [38]. The upregulation of TGF-β_1 observed in the failing heart suggests that it may direct accumulation of extracellular matrix, as it does in wound repair [59]. Treatment of SHR with captopril for 2–4 months after failure was identified results in a significant reduction of TGF-β_1 and reverses some of the ECM changes in gene expression, but as described, does not significantly reverse myocardial fibrosis or papillary muscle stiffness [19,60]. On the other hand, it has been recently reported that selective inhibition of the TGF-β_1 family of cytokines using a soluble type II receptor reduces fibrosis and collagen formation in the adventitial layer of balloon injured rat carotid arteries [61],

suggesting TGF-β_1 may be of importance in mediating the connective tissue response in some tissues, but its role in modulating of cardiac fibrosis in the SHR has not yet been clearly defined.

Apoptosis

In addition to fibrosis, hypertrophy and cell loss are recognized to accompany heart failure (e.g. [62]). In recent years considerable attention has focused on the role of apoptosis. Apoptosis was initially hypothesized to be a mechanism for the transition to heart failure because of several features; these include 1) the presence of single myocyte loss or dropout 2) the relationship between $[Ca^{2+}]_i$ and apoptosis in a variety of tissues and abnormalities of intracellular calcium handling in heart failure and 3) the expression of growth factors in cardiac hypertrophy and the relationship between trophic factors in differing tissues, which may under some conditions lead to growth or hypertrophy, and in others to apoptosis [63]. The possibility that apoptosis may contribute to the progression from hypertrophy to heart failure in patients with hypertensive disease has been reviewed [64]. To test the hypothesis that the reduction in functional myocyte mass is due to apoptosis in the failing SHR heart, apoptotic cells were quantified in cross sections of myocardium from WKY and non-failing and failing SHR hearts by the nick end labeling technique [65]. An approximately fourfold increase in the index of apoptotic myocytes was found in LV myocardium from the non-failing SHR compared with age matched WKY; a further 5-fold increase in apoptotic myocytes was found in failing SHR hearts compared to non-failing SHR (Fig. 5 [65]). Ikeda et al. [66] also reported an increase in non-cardiomyocyte apoptosis in 20 month SHRs with LV decompensation. Kajstura et al. [67] demonstrated that angiotensin II induces apoptosis in adult rat ventricular myocytes; these findings are consonant with the effects of ACE inhibitor therapy of the failing SHR heart where captopril reduced the number of apoptotic cells to values similar to those in the non-failing SHR heart [65]. Apoptosis is a relatively rapid process in which cell surface blebbing, shrinkage and phagocytosis occur rapidly. If we estimate an average time period for the process of 6 hrs, calculations in the failing SHR heart suggest approximately 50 % of myocytes may be lost over a 10 month period, consistent with the relatively long period of time required for the development of heart failure in the SHR. As discussed earlier, Emanuel et al. [29] found no differences in myocyte contractile properties from failing and non-failing SHR hearts and concluded that myocyte apoptosis

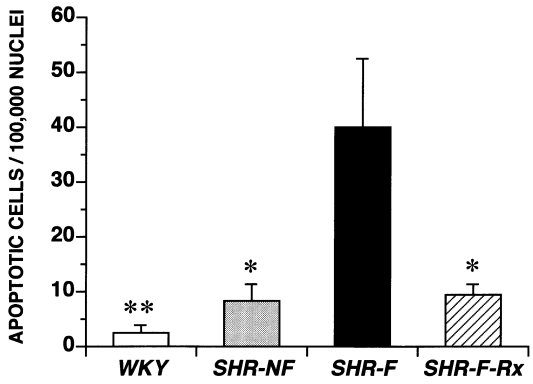

Fig. 5. *Number of apoptotic cells/100,000 nuclei in sections of hearts from age matched (18–24 months) WKY, SHR-NF and SHR-F and SHR-F treated (Rx) with captopril (2 g/L drinking water for 2–4 months after the onset of failure). * $P < 0.05$, SHR-F vs. SHR-NF and SHR-F-Rx; ** $P < 0.01$ SHR-F vs. WKY (adapted from [65]).*

and remodeling of the extracellular space were more likely responsible for the development of heart failure. Since the dysfunction seen in failing and aging hearts are similar in a number of respects [68], apoptosis may contribute to heart failure seen in the elderly population. While the roles of apoptosis and necrosis in myocardium remains controversial, it appears likely that myocyte loss contributes to the dysfunction of myocardium in heart failure. Possibly, with the loss of a critical mass of myocytes, the renin-angiotensin-aldosterone system (RAAS) is activated and the "vicious cycle" culminating in overt heart failure is initiated. A further understanding of the control of the myocardial cell cycle may conceivably lead to methods which will maintain or regenerate myocytes and possibly stabilize or reverse failure.

Calcium Handling

The time course of intracellular calcium concentration ($[Ca^{2+}]_i$) reflects the integrated activities of complex intracellular calcium handling systems in the heart. Intracellular calcium handling events have been well summarized in a recent review of contractile dysfunction in heart failure [69]. In the present review, the role of calcium handling in the SHR during the transition to failure is summarized.

Using the bioluminescent indicator aequorin, intracellular calcium transients were recorded from left ventricular papillary muscle preparations from age-matched WKY, SHR-NF, and SHR-F [31]. In addition, responsiveness to calcium and β-adrenergic stimulation with isoproterenol were studied. Despite baseline depression of active tension and dT/dt in SHR-F

myocardium, no major differences in resting and peak intracellular calcium concentration $[Ca^{2+}]_i$ were found (Fig. 6). Thus, depression of the active properties of muscle preparations from failing SHR myocardium is not explained by a corresponding depression of the intracellular calcium transient. Zaugg et al. [70], studying 8–10 month SHR and WKY, found no differences in $[Ca^{2+}]_i$ handling parameters.

Additional studies were carried out to evaluate mechanical responsiveness of hypertrophied and failing SHR myocardium to inotropic stimulation. Addition of calcium resulted in proportional increases in peak $[Ca^{2+}]_i$ and mechanical function; AT and dT/dt (inotropy) in myocardium from WKY, hypertrophied and failing SHR. However, in studies of increasing heart rate using the isolated buffer perfused preparation [71], it was found that in failing SHR hearts, in contrast to SHR-NF and WKY, the amplitude of the calcium transient, peak pressure and dP/dt declined at increasing heart rates. These responses to changes in stimulation rate are similar to findings of studies of human dilated cardiomyopathy [72].

Isoproterenol resulted in an increase in the calcium transient and inotropy in WKY preparations. However, despite an increase in the $[Ca^{2+}]_i$ there is little change in dT/dt and a fall in AT after isoproterenol in both SHR groups (Fig. 7). Findings of impaired inotropy are consistent with well recognized reduced catecholamine responsiveness observed with heart failure. As has recently been reviewed [73] impaired responsiveness may be due to desensitization of the β-adrenergic receptor in response to catecholamine excess coincident with failure, modulation of G proteins and/or adenyl cyclase activity. Bohm et al. [74] studying the SHR have reported an increase in inhibitory G proteins in the SHR without failure which is thought to regulate adenyl cyclase activity and contractile force.

Experimental findings suggest that impaired inotropy to β-adrenergic stimulation in the SHR may not involve the β-receptor, G-proteins, or adenyl cyclase signaling. The peak of the intracellular calcium transient is augmented in response to isoproterenol and the lusitropic response appears intact at the same time the inotropic response is impaired (Fig. 7 [31,75]).

Overall, findings suggest that impaired inotropy in SHR myocardium reflects a "downstream mechanism" acting in the presence of apparently adequate cAMP production. It is not yet clear that the specific phenomenon described for SHR myocardium is applicable to other models of hypertrophy and failure.

Studies of skinned muscle preparations indicate that calcium sensitivity of left ventricular preparations from the SHR is similar to WKY in both hypertrophy and failure [76]. In the SHR-NF relative to the WKY, maximum calcium-activated force (F_{max}) is increased. In the SHR-F, calcium-activated force is depressed compared with SHR-NF. Interestingly, preparations from hypertrophied right ventricles from the SHR-F demonstrate increased maximum calcium activated force in comparison to WKY and the non-hypertrophied RV from SHR-NF. Thus, F_{max} is observed to be increased in hypertrophied myocardium from both RV and LV. The increased F_{max} may be related to a change in cross-bridge kinetics, an increase in relative force generation per attached cross bridge or an increase in contractile density per unit cross-sectional area of the muscle preparation. The mechanism for the decline in F_{max} in LV preparations from failing myocardium is unknown but may be related to

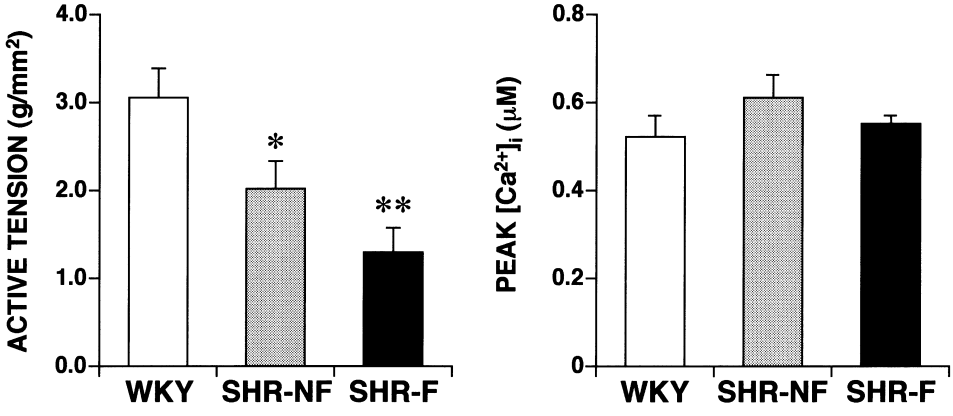

Fig. 6. *Peak active tension and peak $[Ca^{2+}]_i$ recorded simultaneously from isolated papillary muscle preparations from age matched (18–24 month) WKY (n = 6), SHR-NF (n = 8) and SHR-F (n = 4). Peak active tension is depressed in the SHR groups relative to WKY; particularly, the SHR-F. Peak $[Ca^{2+}]_i$ does not differ among groups *P < 0.05; **P < 0.01 vs. WKY (adapted from [31]).*

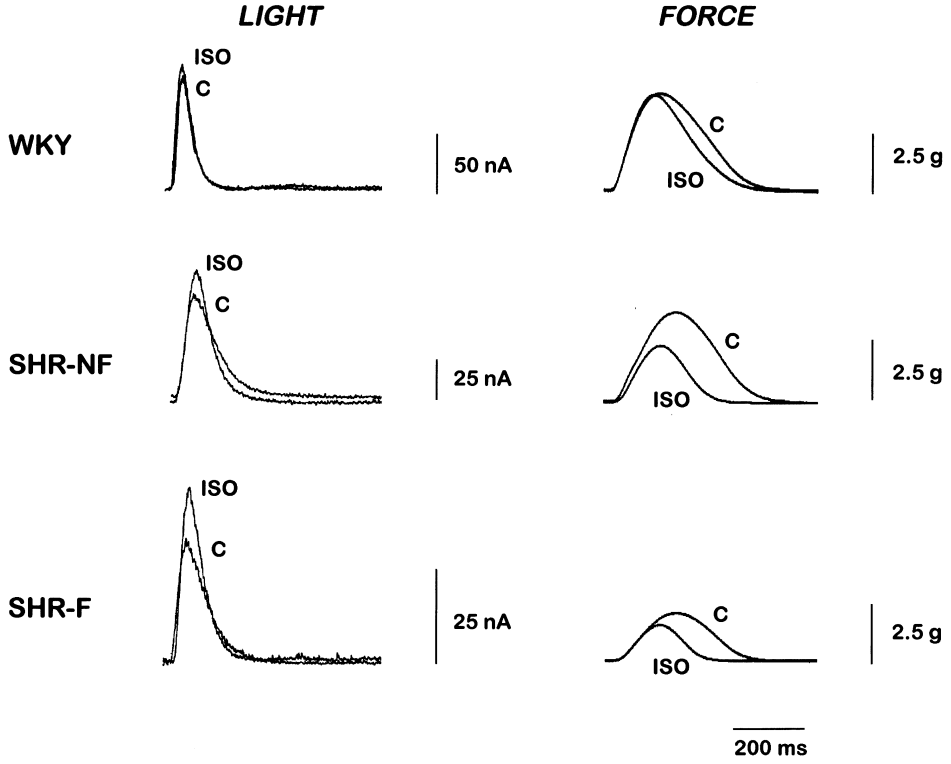

Fig. 7. Aequorin light signals and isometric mechanical activity recorded simultaneously from isolated papillary muscle preparations obtained from aged (18–24 months) WKY, SHR-NF and SHR-F. It can be seen that in the control state (C), the calcium transient is prolonged in both SHR preparations relative to WKY. Isoproterenol 10^{-6}M (ISO) increases peak light in all groups. Peak isometric tension falls considerably in both SHR groups while little decline is seen in WKY. Temperature 30°C, stimulation rate 20/min (adapted from [31]).

extracellular factors such as a decrease in contractile unit density, cell loss, possibly due to apoptosis, and to an increase in collagen [28].

In summary, depressed function of LV papillary muscles from the failing SHR is not explained by a corresponding decline in the amplitude of the calcium transient. A prolonged calcium transient is observed in both hypertrophied and failing myocardium from the SHR. Perfused heart studies suggest that at the stage of heart failure, impaired calcium cycling in association with increased heart rates may contribute to systolic and diastolic dysfunction. While myofilament calcium responsiveness appears normal and papillary muscles from failing myocardium remain normally responsive to calcium mediated inotropic stimulation, inotropy in response to β-adrenergic stimulation is impaired in both SHR-NF and SHR-F despite appropriate augmentation of the calcium transient and of lusitropy. It is presently unclear whether the specific mechanism(s) responsible for impaired inotropy to catecholamine stimulation is necessarily related to the cause(s) of failure in the SHR.

Energetics

One of the consequences of ventricular pressure overload is the requirement for more energy to meet the demands of an increased workload. Rakusan et al. [77] (1992) have shown that LV pressure overload hypertrophy in adult human myocardium is associated with failure of capillary angiogenesis. Medial hypertrophy of small coronary vessels associated with increased wall thickness were considered to explain impaired coronary flow and decreased coronary reserve in hypertensive LVH [78]. Engelmann et al. [79] reported a decrease in the LV capillary density in the SHR at 6 months. A number of investigators have reported decreases in CrP levels in the aging SHR heart [80–82]. Bittl and Ingwall [83] reported a 50% decrease in Cr kinase activity in the 18 month SHR relative to age-matched WKY. O'Donnell et al. [84] observed that a decline in PCr and creatine signals the transition to failure in the SHHF. Thus, among possibilities, decreased microvascular blood flow might impair oxygen delivery, decrease the pool of high energy intermediates and performance of the hypertrophied

heart, contributing to heart failure. To examine the role of oxygen delivery on impaired function seen in the failing SHR heart, the relation between function and oxygen consumption was studied in 18–24 month control (WKY), hypertrophied, and failing SHR hearts [85]. Utilizing the isolated perfused isovolumic (balloon in LV) heart preparation, it was found that at constant intraventricular volume, systolic pressure development was greatest in the SHR-NF and lowest in the SHR-F group. Correcting for wall thickness, midwall stress development was greatest in the WKY and lowest in the SHR-F heart, consistent with impairment of function observed in papillary muscle studies from aged and failing SHR hearts. Increasing perfusion pressure did not reverse depression of systolic stress in the SHR-F. Myocardial oxygen consumption per gram LV was lowest in the SHR-F group. Correcting for stress development, however, MVO_2 was lowest in the WKY and greatest in the SHR-F. Thus, the oxygen cost of stress development was greatest in the SHR-F (Fig. 8). An increase in the oxygen cost of stress development has also been observed in papillary muscle preparations from the pulmonary artery banded cat [86], which was found to be due to nonphosphorylating mitochondrial respiration [87]. Increased "economy of contraction" based on myothermal data from hypertrophied papillary muscles [88,89] is thought related to

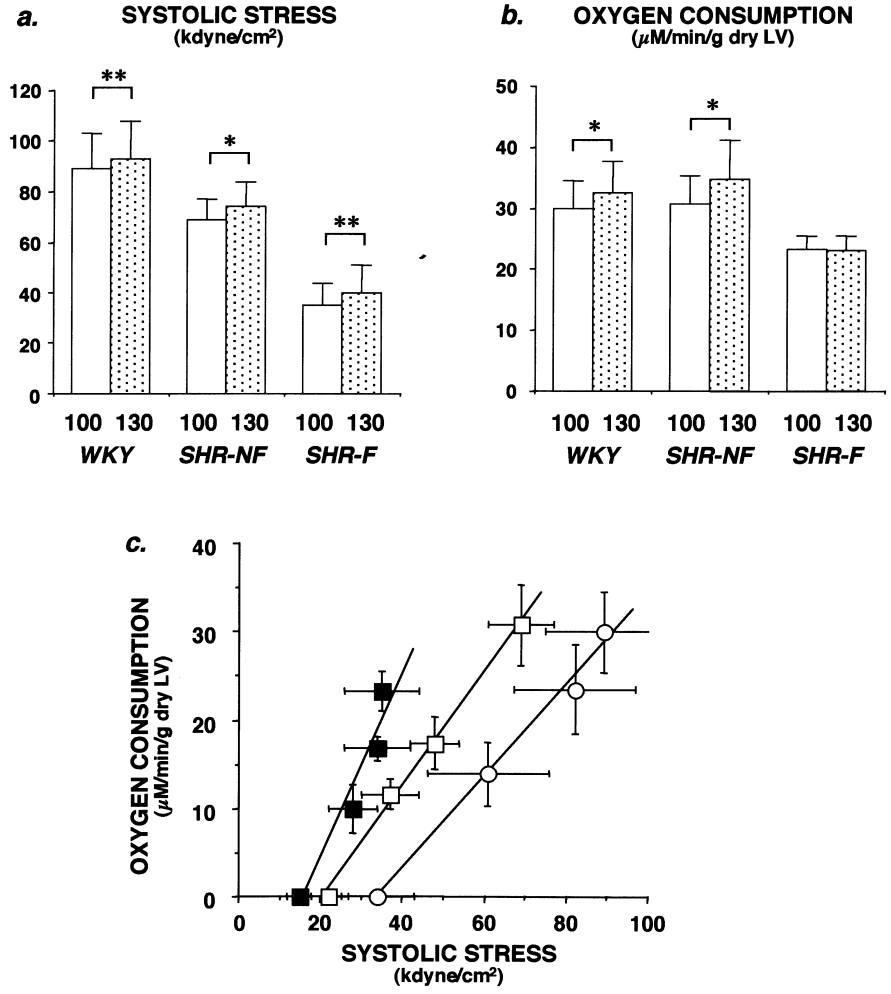

Fig. 8. *Systolic Stress (a) and myocardial consumption (b) determined from isolated (balloon in ventricle) buffer perfused heart preparations. Age matched (18–24 months) WKY (n = 13), SHR-NF (n = 8) and SHR-F (n = 8) are compared. Systolic stress and myocardial oxygen consumption are decreased in the SHR-F group relative to WKY and SHR-NF. Increasing perfusion pressure from 100 to 130 mmHg fails to restore parameters in the SHR-F group to values seen in non-failing hearts. The relation between myocardial consumption and systolic stress (c) is shifted to the left in the SHR-F (solid squares) and SHR-NF (open squares) groups demonstrating an increased oxygen cost of stress development in the SHR groups relative to age-matched WKY (open circles); particularly the SHR-F. (*P < 0.05; **P < 0.01 respectively; perfusion pressure 130 vs. 100 mmHg) (adapted from [85]).*

an adaptive shift in myosin isozymes [90]. Thus, improved energetic efficiency based on myothermal data appears to be offset by decreased efficiency based on oxygen consumption data; the latter may be a factor contributing to the observed decrease in PCr.

In perfused hearts, no lactate was produced by any of the groups in the baseline state [85]. Lactate was produced, however, during graded hypoxia; reducing perfusate PO2 from 95 to 50, 25 and 0 (%) resulted in a progressive fall in systolic stress and MVO2 while lactate was observed to increase progressively in all groups. Since isolated heart preparations from the SHR-F produces lactate during hypoxia but not in the baseline state, these data suggest that the baseline impairment of function observed in the SHR-F, which is not associated with lactate production, is not due to hypoxia—or that hypoxia/ischemia are present without lactate production ('hibernating myocardium' [91]).

Tolerance of isolated muscle preparations to hypoxia was studied comparing 6 and 18 month SHR and age matched WKY [92]. Papillary muscles from 18 month SHR had the lowest CrP and adenylate energy charge which may explain increased contracture and depressed tension development when carbohydrate is limited (5.5 mM glucose) after protracted (60 min) hypoxia. Although performance of hypoxic papillary muscles is improved in the presence of high glucose (20 mM glucose [93]), performance of perfused hearts during hypoxia is not associated with augmented lactate production (see earlier). These data suggest that while ischemia or hypoxia does not appear to be responsible for impaired baseline stress development in the failing SHR, hypertrophied and failing myocardium appear to have impaired tolerance to prolonged hypoxia. Findings may be relevant to cardiac preservation where it has been noted that hypertrophied hearts exhibit decreased tolerance to prolonged arrest [94].

Microtubular Polymerization

Based on the studies of Tsutsui et al. [95,96], who studied right ventricular preparations from cats, it was suggested that microtubular polymerization associated with hypertrophy might act as an internal load impairing the performance of cardiac muscle and contributing to heart failure. Lysing the microtubules with colchicine was observed to improve the performance of cardiac myocytes from hypertrophied hearts. Accordingly, in an attempt to prevent heart failure in the SHR, colchicine (1 mg/L) was added to the drinking water of 12 month old SHR and WKY until 24 months of age, or the appearance of clinical manifestations of heart failure [35]. No differences in the clinico-pathological manifestations of heart failure were found between colchicine treated and untreated SHR. Likewise, the mechanical performance of papillary muscle preparations from these animals was not improved. The direct effects of colchicine were also studied in papillary muscle preparations from control, hypertrophied, and failing SHR hearts [97]. Colchicine at concentrations from 10^{-4}–10^{-6} M failed to augment the performance of preparations from hypertrophied and failing SHR hearts. Thus, studies utilizing colchicine fail to suggest a role for microtubular polymerization in the depressed function found in the failing SHR heart. As pointed out by Tsutsui et al. [98], differences between their findings [95,96] and those of others may reflect variables such as the animal species, the ventricle studied, and the stage of hypertrophy/failure.

Summary and Future Directions

The SHR has been found to be a useful model in which to study the combined effects of aging and hypertension on the heart. A valuable aspect of studies in the SHR is that long term studies over the course of its lifespan are feasible from a practical perspective. While there are a number of points for questioning the SHR as a suitable model of human hypertension, the clinical course of animals and response to treatment appears similar in a number of aspects to those observed in humans. The effectiveness of ACE inhibitors and angiotensin receptor blockers in both the SHR and humans support the SHR as a model for pilot studies of potential treatments. However, it remains to be determined which conclusions derived from this model regarding specific treatments and the mechanism of heart failure are applicable to humans.

Studies have revealed that the SHR develops heart failure between 18–24 months of age. Failure is associated with recognized clinical, pathological, and hemodynamic findings, as well as myocardial fibrosis and impairment of intrinsic myocardial function. Virtually all manifestations of failure and associated changes in gene expression can be prevented by inhibition of production or blocking the effects of Ang II, a component of the neurohormonal system well recognized to be activated in association with heart failure. Findings are consistent with the concept that the renin angiotensin aldosterone system (RAAS) has evolved to support the circulation in a young, generally healthy population without cardiovascular dysfunction but is maladaptive in the aged, failing heart. Findings in the SHR demonstrate that treatment with ACE inhibitor or AT_1 blocker

prior to heart failure is able to prevent not only failure but intrinsic myocardial tissue dysfunction; treatment after failure demonstrates clinical improvement, as seen in humans, but impairment of intrinsic myocardial function is not reversed. Thus, a practical goal of studies in animals such as the SHR is to improve impaired myocardial function or prevent dysfunction.

Some of the possible mechanisms directly contributing to failure in the SHR have been briefly reviewed. To further understand the basis for heart failure, a common approach in recent years has been to measure changes in transcripts encoding proteins thought to play a role in heart failure. In addition to studying early changes in traditional genes, comparison of gene expression using the techniques of subtractive hybridization [99] and differential display [52,100,101] suggests a role for previously unsuspected genes and pathways which may be related to the genesis of heart failure. Consistent with this approach, a number of laboratories are comparing arrays of transcripts using gene chip technology. It should be pointed out that unlike subtractive hybridization and differential display, only changes in selected transcripts will be found using this technique, and that possibly important unsuspected genes will not be discovered or evaluated. Despite this limitation, the potential of this approach to identify novel mechanistic contributors to heart failure is excellent.

While Ang II plays a major role in the transition to overt heart failure in the SHR, specific mechanisms by which Ang II and other neurohormones remodel the myocardium remain under study. A further question of major interest and importance, not yet well studied in the SHR model, is the role of factor(s) which precede and lead to neurohumoral activation. It is not clear whether the mechanisms responsible for overt failure are the same as those leading to the point where the neurohormonal system is activated. To examine this question, strategies will have to be developed to study the compensated hypertrophied heart for mechanisms which trigger neurohormonal activation initiating the vicious cycle culminating in overt heart failure. Animals models with a predictable clinico-pathologic course such as the SHR will provide an important resource for advancing our understanding of the genesis of heart failure in the aging hypertrophied heart.

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