The Myocardial β-Adrenergic System in Spontaneously Hypertensive Heart Failure (SHHF) Rats

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Abstract—Responsiveness to β-adrenergic stimulation is reduced in the failing human myocardium. This results principally from reduced β-adrenergic receptor (βAR) density, elevated β-adrenergic receptor kinase 1 (βARK1) levels, and functional uncoupling of remaining receptors. The temporal nature of changes in the human myocardial β-adrenergic system relative to onset of symptomatic heart failure (HF) has been difficult to discern. A relatively new model of HF, the spontaneously hypertensive heart failure (SHHF) rat spontaneously and reproducibly develops left ventricular hypertrophy (LVH) and progresses to HF, thus enabling longitudinal studies to examine the cellular and molecular bases for hypertension-induced cardiac hypertrophy and subsequent HF. The purpose of this study was to examine age-dependent changes in the βAR system in this model. Lean male SHHF rats at 3, 7, 14, and 20 months were compared with age-matched Sprague-Dawley (SD) control rats ([C]; 4 animals/group). At all ages the SHHF rats had elevated blood pressures and left ventricular end-diastolic pressure relative to the SD control rats (P<0.05). Compared with age-matched SD control rats, LVH was evident by 3 months in SHHF rats; 20-month-old SHHF rats had significantly greater LVH compared with the other SHHF rat groups. β-adrenergic responsiveness (maximal heart rate to isoproterenol) was reduced only in 20-month-old SHHF rats. βARK1 protein levels and activity were elevated at 14 months (162±10% and 195±20% C, respectively), and βARK1 protein remained elevated at 20 months (140±14% C). In contrast, G protein–coupled receptor kinase 5, a second receptor kinase in the heart, remained unchanged at all ages. βAR density did not change with age in the SD control rats and was similar in the SHHF rats until 20 months of age when the receptor number was reduced (30±1%). These data indicate that cardiac dysfunction is coincident with reduced βAR density. Importantly, cardiac dysfunction was preceded by elevated βARK1 levels and activity, thus suggesting that βARK1 may be a precipitating factor in the transition from hypertension-induced compensatory cardiac hypertrophy to HF. Furthermore, these results indicate that the SHHF rat is a powerful model for use in examination of the mechanisms involved in alterations of β-adrenergic signaling that occur in human HF. (Hypertension. 1999;33[part II]:402-407.)

Key Words: hypertension, experimental ▪ hypertrophy, cardiac ▪ heart failure ▪ receptors, adrenergic, beta ▪ kinase ▪ adenylyl cyclase ▪ rats, inbred SHR

Hypertension and hypertension-induced cardiac hypertrophy are recognized risk factors for the development of heart failure (HF). The fundamental molecular mechanisms involved in the initial compensatory hypertrophic response to elevated systemic pressures and subsequent progression to HF remain largely undefined. It has been proposed that desensitization of the β-adrenergic signaling system contributes to the progression from hypertrophy to HF.1 Indeed, patients with chronic HF demonstrate reduced cardiac β-adrenergic receptor (βAR)–mediated responsiveness in the face of high circulating catecholamine levels.2–6 Evaluation of ventricular samples from patients with near-end or end-stage HF indicates that in HF there is reduced βAR density,6–9 elevated levels and activity of β-adrenergic receptor kinase 1 (βARK1), which functionally uncouples βARs,10–13 alterations in G proteins,14–17 and decreased adenylyl cyclase (AC) activity.18–20 Difficulty in obtaining serial ventricular samples from patients with HF and variability in changes in β-adrenergic signaling in different contrived models of heart failure render it difficult to distinguish whether such changes are causally related or merely correlative with progression to symptomatic HF.

A relatively new genetic model of hypertension-induced heart failure, the spontaneously hypertensive heart failure (SHHF/Mcc-α+α−) rat, is now commercially available. Originally derived from a cross between spontaneously hyperten-
sive rats (SHR) and Koletsky obese rats, and then bred to SHR at the NIH (SHR-N), the colony had been maintained by McCune (Mcc designation) at the Ohio State University. These rats exhibit early-onset hypertension, and all animals develop HF. While in HF the SHHF rats exhibit numerous symptoms and biochemical changes that parallel documented changes in patients with hypertension, cardiomyopathy, and HF. Evaluation of SHHF animals at different ages should facilitate elucidation of mechanisms temporally and causally involved in the progression from stable compensatory myocardial hypertrophy to HF. Thus, the purpose of this study was to functionally and biochemically evaluate components of the βAR signaling cascade over a wide range of ages in lean males from this unique genetic model of HF.

Methods

Assessment of Hemodynamics and Heart Weight in the SHHF and Sprague-Dawley Rats

Lean male SHHF (n=16; Genetic Models, Inc, Indianapolis, IN) rats and Sprague-Dawley (SD; n=16; Charles River Laboratories, Raleigh, NC) rats were housed in an accredited laboratory animal facility, and all procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (US Department of Health, Education, and Welfare, Department of Health and Human Services, NIH Publication 85–23). All procedures were approved by the Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals.

In all animals, anesthesia was induced with isoflurane (4% in O2), and a catheter was placed in the femoral vein for the administration of drugs. Isoflurane was discontinued, and anesthesia was maintained with pentobarbital (35 mg/kg, IV; with 5 mg/kg supplemental doses as needed). The right common carotid artery was exposed, and a micro-tipped pressure transducer (Millar Instruments) was inserted retrogradely for recording arterial blood pressure. The transducer was approved by the Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals.

Total AR density was determined using myocardial sarcolemmal membranes. Portions of the LV (membrane fraction) were resuspended in 50 mmol/L HEPES (pH 7.3) and 5 mmol/L EDTA, 5 mmol/L EGTA, 10 mg/mL leupeptin, 20 mg/mL aprotinin, and 1 mmol/L phenylmethylsulfonyl fluoride). Nuclei and tissue were separated by centrifugation at 800g for 15 minutes. The crude supernatant was then centrifuged at 20,000g for 15 minutes. Protein concentrations were determined using Bradford reagent (Pierce) on the supernatant (cytosolic fraction). Sedimented proteins (membrane fraction) were resuspended in 50 mmol/L HEPES (pH 7.3) and 5 mmol/L MgCl2. βAR binding was determined by incubating 25 μg of sarcolemmal membranes with a saturating concentration of [3H]cyanopindolol and 20 mmol/L alprenolol to define nonspecific binding.

Western Blot Analysis of βARK1 Protein Levels

Immunoblotting of βARK1 was performed on 500 μg of protein from the left ventricular cytosolic fraction after immunoprecipitation with a monoclonal βARK1/βARK2 antibody as described previously. The βARK1 protein was visualized with a monoclonal antibody raised against an epitope within the carboxyl terminus of βARK1 and chemiluminescent detection of anti-mouse IgG conjugated with horseradish peroxidase (Renaissance; Amersham). To detect G protein–coupled receptor kinase 5 (GRK5), 30 μg of protein from the LV+S membrane fraction was loaded on a 12% Tris-glycine gel. GRK5 was visualized with a monoclonal antibody to the carboxy-terminus and chemiluminescent detection of anti-mouse IgG.

Evaluation of βARK1 Activity

βARK1 activity was determined in LV+S cytosolic fractions with rhodospin-enriched rod outer segment membranes as an in vitro substrate and [γ-32P]ATP as described previously. [32P] Incorporation into rhodopsin was quantified by using a Molecular Dynamics PhosphorImager.

Determination of AC Activity

AC activity was determined on 20 μg of left ventricular sarcolemmal membranes in triplicate. Membranes were incubated for 15 minutes at 37°C with [α-32P]ATP under basal conditions or after stimulation with 10-5 mol/L isoproterenol to stimulate βARs or 10 mmol/L sodium fluoride (NaF) to activate all G proteins. cAMP was quantified as described previously.

Results

Assessment of Hemodynamic Parameters and Cardiac Mass

The SHHF rats were significantly hypertensive relative to the SD control rats as early as 3 months of age (mean arterial pressure, 193.3±4.0 and 111.3±4.6 mm Hg, for SHHF and SD rats, respectively; mean±SEM; n=4 per strain). As shown in Figure 1, mean arterial, systolic, and diastolic pressures remained stable and elevated in SHHF rats until 20 months of age when mean and systolic pressures fell significantly. Body weights were significantly greater in SD control rats at all ages; although SD rat and SHHF rat body weights increased between 3 and 7 months, body weight did not change in the SD or SHHF rats between 7 and 20 months (SD: 562±13.92 versus 575±14.45 g; SHHF: 435±8.42 versus 437.5±2.5 g, 20 months versus 7 months). LV mass...
was increased as early as 3 months in the SHHF rats and increased more with age (Figure 2), reflecting hypertension-induced cardiac hypertrophy and suggesting that these animals become hypertensive much earlier than 3 months. As shown in Figure 3A, the maximum rate of contraction \((dP/dT)\) is significantly depressed at 20 months, and this is preceded by a reduced lusitropy \((-dP/dT)\). Relative to age-matched SD rats, LVEDP was elevated in SHHF rats at 3 months \((14.2\pm2.8\text{ mm Hg, mean}\pm\text{SEM, n}=4/\text{group})\) and progressively increased with age (Figure 3B). Cardiac responsiveness to infused isoproterenol was diminished in 20-month-old SHHF rats as demonstrated by a significantly depressed chronotropic response (Figure 4). Isoproterenol decreased blood pressure by \(35.2\pm1.99\text{ mm Hg}\) in all SHHF rats with no difference among the 4 age groups.

**Myocardial βAR Signaling Properties**

Biochemical and molecular biological approaches were used to determine whether abnormalities in βAR signaling contributed to the depressed cardiac function in older SHHF rats and to examine the chronology of any noted changes in βAR signaling relative to the observed onset of cardiac dysfunction in this genetic model of heart failure. βAR density for control SD rats was \(34.6\pm3.4\text{ fmol/mg protein}\) and was unchanged with age. βAR density progressively declined in SHHF rats, and at 20 months of age was significantly attenuated (Figure 5). βAR affinity was unchanged with age or between control and SHHF rat groups.

We assessed AC activity to examine the signaling properties of the βARs in control versus SHHF rats. Basal AC was not different between SD and SHHF rats (data not shown), and there was no difference with age or between control SD \((137.3\pm17.3\text{ pmol}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}, \text{n}=16)\) and SHHF \((133.1\pm18.1\text{ pmol}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}, \text{n}=16)\) rats for NaF-stimulated AC activity. However, isoproterenol-stimulated AC activity was significantly depressed at 14 (24%) and 20 (36%) months in the SHHF rats (Figure 6). Therefore, SHHF rats concomitantly exhibit age-dependent decreased βAR density and βAR-mediated signaling.

**Expression and Activity of βARK1**

β-adrenergic receptor kinase (BARK1 or G-protein–coupled receptor kinase 2 [GRK2]) is a prototypical member of a

![Figure 2](image1.png)

**Figure 2.** Left ventricular weights from SHHF rats and age-matched SD control rats. After removing the atria and right free wall, the weight of the left ventricular free wall and ventricular septum (LV+S) was obtained as an index of LVH. The data were normalized to body weight and are expressed as the ratio of LV+S per 100 g body weight. The SD rats showed no evidence of LVH at any age. Relative to the age-matched SD control rats, the SHHF rats had significantly greater LV+S mass at all ages, with the most pronounced difference at 20 months. *P<0.05, 2-tailed t-test. Comparisons among the SHHF rat groups indicated significantly increased LVH at 20 months (†P<0.05; ANOVA and Bonferroni test).

![Figure 3](image2.png)

**Figure 3.** Left ventricular (LV) hemodynamic parameters in anesthetized rats (SHHF). A. The rates of contraction (+dP/dT) and relaxation (–dP/dT) were significantly depressed in the older age groups. B. Elevations in LVEDP were linearly correlated with increasing age, n=4 animals per group. *P<0.05 (ANOVA and Bonferroni test).

![Figure 4](image3.png)

**Figure 4.** β-Adrenoceptor reactivity in anesthetized SHHF rats. The maximum chronotropic response elicited by isoproterenol (10 μg/kg, IV). Results from 4 age groups (3, 6, 14, and 20 months) were compared (n=4 animals per group). *P<0.05 (ANOVA and Bonferroni test).
family of at least 6 serine/threonine kinases known as the GRKs that specifically phosphorylate agonist-occupied G protein–coupled receptors leading to desensitization.\textsuperscript{12,13,28} βARK1 is a critical in vivo modulator of βAR-mediated myocardial function.\textsuperscript{28} Human HF is associated with alterations in βAR signaling, including downregulation and desensitization of βARs.\textsuperscript{6–13} To test the hypothesis that βARK1 is involved in the decreased βAR number (Figure 5) and depressed signaling (Figure 6) in SHHF rats, expression and activity of the myocardial GRKs βARK1 and GRK5 were examined. Similar to what we have observed before, neither βARK1 expression nor activity changed with age in the control rats.\textsuperscript{31} However, in the SHHF rats, βARK1 expression progressively increased with age and was significantly greater than that in control rats at both 14 (63±10%, n=4, \(P<0.0008\)) and 20 (40±14%, n=4, \(P=0.0289\)) months (Figure 7A).

Enzymatic activity of soluble βARK1 on the G protein–coupled receptor substrate rhodopsin (see “Methods”) was measured in cytosolic extracts. βARK1 activity was significantly increased in SHHF rats by 14 months (95±36%, n=4, \(P=0.038\); Figure 7B). Unlike βARK1, there were no changes with age or between control and SHHF rat groups in membrane-bound GRK5 expression (data not shown).

### Discussion

It is well established that in human heart failure compromised cardiac function is associated with alterations in the β-adrenergic signaling cascade including reduced myocardial βAR density, increased βARK1, and decreased AC activity.\textsuperscript{6–13,18–20} The purpose of the present study was to characterize in vivo cardiac adrenergic responsiveness with increasing age in a unique genetic model of spontaneous hypertension-induced myocardial hypertrophy and HF (the SHHF/Mcc-fα\textsuperscript{2} rat) and to examine components of the βAR signaling cascade in the same animals. SHHF rats were studied at 3, 7, 14, and 20 months. Relative to age-matched SD control rats, SHHF rats had significantly elevated blood pressure, elevated LVEDP, and LVH as early as 3 months of age. Compared with age-matched SD rats, resting blood pressure in SHHF rats increased by 14 months (95±36%, n=4, \(P=0.038\); Figure 7B). Unlike βARK1, there were no changes with age or between control and SHHF rat groups in membrane-bound GRK5 expression (data not shown).
pressure was elevated in all SHHF rat groups; compared with 6-month-old SHHF rats, systolic and mean arterial pressures were significantly decreased in 20-month-old SHHF rats. This was coincident with depressed cardiac contractility ([+] dP/dT) and was preceded by depressed lusitropy [(−) dP/dT]. Cardiac sensitivity to isoproterenol was significantly attenuated in 20-month-old SHHF rats. This was coincident with reduced βAR density and was preceded by elevated βARK levels and activity. Reduced AC activity at 14 and 20 months was consistent with elevated βARK at these ages.

The onset of overt HF in the SHHF rats varies with the phenotype of the rat, ranging from 10 to 13 months (obese males) to approximately 24 months in lean females. The lean males used in this study reportedly exhibit symptoms of overt HF at 14 to 18 months. Documented symptoms and biochemical changes that occur in SHHF rats and that parallel human HF include dyspnea, cyanosis, orthopnea, subcutaneous edema, ascites, hepatomegaly and congestion, pleural effusion, lethargy, piloerection, increased urinary excretion, cachexia, weight loss, reversal of contractile proteins (myosin and actin) to fetal forms, elevated atrial natriuretic peptide, elevated plasma renin activity, elevated circulating norepinephrine, and reduced enzymes involved in fatty acid oxidation. We monitored our animals closely via daily visual examination. The 20-month-old lean males used in this study were included on the basis of their exhibition of initial overt symptoms of HF, specifically increased respiratory rate and effort. It was somewhat surprising that they did not exhibit overt HF until 20 months of age, but it is possible this study was performed on homozygote animals because they tend to live longer than the lean males that are heterozygote for the corpulent gene.

The fundamental molecular mechanisms that contribute to progression in humans from compensatory hypertension-induced cardiac hypertrophy to cardiac dysfunction are poorly understood, but it has been proposed that altered βAR signaling plays a role. Consistent with data from left ventricular samples of patients with HF, the present study demonstrates that SHHF rats with compromised cardiac function have reduced βAR density. Importantly, the in vivo functional changes and the reduction in βARs observed in the SHHF rats are preceded by an increase in the level and activity of βARK1. Given that the 20-month-old SHHF rats had just begun to show overt symptoms of HF, elevation of βARK1 preceded symptomatic HF by at least 6 months. Thus, these data suggest that alterations in βARK1 may represent a key trigger to the initiation of HF and support the hypothesis that βARK1 is a good target for development of a therapeutic agent for treatment of HF.

The present study is the first to examine the in vivo myocardial response of SHHF rats to direct β-adrenergic stimulation, and it is the first to document definitive changes in components of the β-adrenergic system in the intact myocardium of this model. Gomez et al. reported that myocytes isolated from 17- to 18-month-old lean male SHHF rats in overt HF and from hypertrophic hearts of hypertensive Dahl salt-sensitive rats had diminished sarcoplasmic reticulum calcium release in response to electrical depolarization. Interestingly, βAR stimulation via isoproterenol overcame this deficit in the myocytes from Dahl salt-sensitive rats but produced virtually no response in the myocytes from the animals in HF, perhaps, the authors speculate, because of downregulation of the cardiac βARs. The data presented herein from 20-month-old lean males demonstrating initial symptoms of overt HF support their conclusion. Consistent with our results in intact myocardium, SHHF myocytes from 6-month-old obese female animals retain their full complement of βARs relative to myocytes from 4-month-old SD rats. However, reduced isoproterenol-stimulated cAMP production by the cells suggested a decrease in AC activity by 6 months of age. Exciting preliminary data from our laboratories confirm that, compared with age-matched SD rats, basal cAMP production and isoproterenol-stimulated cAMP production are significantly attenuated in myocytes isolated from 20-month-old lean males and demonstrate that this can be reversed to near normal by adenosinergic-mediated expression of a βARK inhibitor, the βARKct, a peptide encoding the carboxy-terminus of βARK1.

To summarize, lean male SHHF rats at the initial stages of overt HF have reduced chronotropic response to β-adrenergic stimulation. This in vivo manifestation of cardiac dysfunction is coincident with reduced βAR density and, importantly, preceded by elevated βARK1 levels and activity. Thus, the data are concordant with the hypothesis that elevated βARK1 may be an important precipitating factor in the transition from hypertension-induced compensatory hypertrophy to HF. Furthermore, these results provide additional data that strengthen the relevance of the SHHF rat model to the study of human HF.

References
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