SHHF/Mcc-cp Rat: Model of Obesity, Non-insulin-dependent Diabetes, and Congestive Heart Failure

Sylvia A. McCune, Peter B. Baker, and Harold F. Stills, Jr.

Introduction

It is well established that obesity and diabetes contribute to increased cardiovascular complications in humans. Many feel that the increased prevalence of heart disease in obese or diabetic patients can be attributed to increased atherosclerotic lesions caused by hyperglycemia, hyperlipoproteinemia, hypertension, obesity, and hyperinsulinemia. However, more recent data indicate there may be a specific diabetic cardiomyopathy not associated with coronary artery disease that may explain some of the increased mortality and morbidity seen in diabetics (Crepaldi and Nosandini, 1988; Fein and Sonnenblick, 1985).

Knowledge of the heart and cardiovascular system in genetic animal models of obesity and diabetes is limited (Chobanian et al., 1982; McCune, 1989). Various combinations of risk factors may cause different heart problems, with obesity and non-insulin-dependent diabetes mellitus (NIDDM) combined with hypertension probably causing the most deleterious effects on the heart. The use of animal models with obesity, diabetes, or both conditions, combined with other risk factors, may clarify what each component contributes to the cardiovascular disease. This report summarizes some of the current information on the cardiovascular and diabetic complications found in the SHHF/Mcc-cp rat model, which exhibits obesity, NIDDM, and congestive heart failure (CHF).

Origin and Genetics of the Colony

The breeding stock we used to found our colony was transferred to Sylvia McCune in 1983 from J. E. Miller of G. D. Searle (Rubin et al., 1984). Originally designated as the congenic strain SHR/N-c/?, the animals were developed by backcrossing “Koletsky obese” rats (i.e., rats heterozygous for the cp gene) (Koletsky, 1975) to SHR/N (spontaneously hypertensive) rats. We obtained the rats after the seventh backcross. The only other SHR/N-cp rats currently available are from the fourteenth backcross (Michaelis et al., 1986) and may have divergence in as many as 20 percent of their genes from our substrain, now designated SHHF/Mcc-cp.

This colony exhibits the following traits: hypertension (100 percent), obesity (25 percent), and CHF (100 percent in recent sibships). Obesity is expressed as an autosomal recessive (cp/cp) trait, and hypertension is multifactorial (Yen et al., 1974). The CHF trait has been maintained through at least 15 generations. The colony is closed, but it has not been completely brother x sister mated. The incidence of CHF has increased with inbreeding, and the onset of CHF seems to be occurring at an earlier age. The mode of inheritance of the CHF is probably multifactorial, much like hypertension.

Most of the lean animals that died of CHF were heterozygous for the obesity trait (+/cp). It is possible that the presence of the cp gene on this genetic background is required for the expression of the CHF or at least for the earlier age of onset of CHF. We have been successful in getting our obese male rats to breed (McCune et al., 1989); therefore, all of their lean offspring will carry the cp gene. A homozygote lean (+/+) line is also being bred, and their offspring will be compared for incidence of CHF with the heterozygote lean parents.

Characteristics of the Colony

NIDDM and Obesity

This colony shows a sexual dimorphism in the expression of overt NIDDM (Hoversland et al., 1988). The obese male exhibits fasting hyperglycemia, polyuria, glucosuria, hyperinsulinemia, proteinuria, hypertriglyceridemia, hypercholesterolemia, and abnormal glucose tolerance (Figure 1; Table 1). The obese female does not manifest fasting hyperglycemia but is hyperinsulinemic and has some glucose intolerance to an oral load (Figure 1; Table 1).

Most of the animal models of diabetes and obesity have insulin resistance and hyperinsulinemia. Some feel
TABLE 1 Characteristics of Eight-Month-Old Lean and Obese SHHF Rats

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lean</td>
<td>Obese</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>404 ± 6</td>
<td>673 ± 15</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>95 ± 7</td>
<td>176 ± 17</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>51 ± 8</td>
<td>408 ± 32</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>97 ± 13</td>
<td>702 ± 110</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>81 ± 9</td>
<td>148 ± 7</td>
</tr>
</tbody>
</table>

NOTE: All values are mean ± SE, N = 6 rats per group. The plasma samples were collected after the animals had been fasted overnight.

CHF in our rats (Dunn et al., 1988), an observation also reported in humans and animals with CHF (Burnett et al., 1986; Edwards et al., 1986). Levels of plasma aldosterone, renin, and norepinephrine follow similar patterns to those observed in humans with CHF.

Cardiac Studies  Compared with Wistar Furth (WF) and Sprague Dawley rats, the hearts of SHHF rats in CHF are enlarged with dilation of all four chambers (Figure 2). From records on several hundred colony rats, the heart weight range for CHF rats was 1.7-4.5 g for males and

Congestive Heart Failure

Symptoms  Both lean and obese male and female SHHF can develop overt CHF (McCune et al., 1988; Rubin et al., 1984). These rats develop many of the clinical signs of CHF: generalized and subcutaneous edema, hydrothorax, ascites, dyspnea, cyanosis, enlarged hearts, left atrial thrombosis, and hyperemia of the lungs, liver, and kidneys. These rats are hypertensive, but blood pressure falls to normal levels with the onset of severe CHF. Increased levels of plasma atrial natriuretic factor (ANF) seem to be positively correlated with the severity of the

FIGURE 1 Oral glucose tolerance curves for four-month-old obese and lean SHHF rats. □ = lean male; ■ = obese male; ○ = lean female; ● = obese female.

that hyperinsulinemia is an independent risk factor for both heart disease and hypertension and that with increased insulin resistance cardiovascular complications become more severe (Reaven and Hoffman, 1987; Ruderman et al., 1984). The SHHF obese rats are very insulin resistant (Table 1), and both the obese male and obese female die of CHF at an earlier age than do their lean littermates. The male rat has a lower hepatic clearance rate and slower degradation rate for insulin than does the female (Hennes et al., 1988), and the male dies earlier of CHF than does the female. It is possible that hyperinsulinemia causes more deleterious effects depending on adiposity and gender.

Congestive Heart Failure

Symptoms  Both lean and obese male and female SHHF can develop overt CHF (McCune et al., 1988; Rubin et al., 1984). These rats develop many of the clinical signs of CHF: generalized and subcutaneous edema, hydrothorax, ascites, dyspnea, cyanosis, enlarged hearts, left atrial thrombosis, and hyperemia of the lungs, liver, and kidneys. These rats are hypertensive, but blood pressure falls to normal levels with the onset of severe CHF. Increased levels of plasma atrial natriuretic factor (ANF) seem to be positively correlated with the severity of the

CHF in our rats (Dunn et al., 1988), an observation also reported in humans and animals with CHF (Burnett et al., 1986; Edwards et al., 1986). Levels of plasma aldosterone, renin, and norepinephrine follow similar patterns to those observed in humans with CHF.

Cardiac Studies  Compared with Wistar Furth (WF) and Sprague Dawley rats, the hearts of SHHF rats in CHF are enlarged with dilation of all four chambers (Figure 2). From records on several hundred colony rats, the heart weight range for CHF rats was 1.7-4.5 g for males and

Congestive Heart Failure

Symptoms  Both lean and obese male and female SHHF can develop overt CHF (McCune et al., 1988; Rubin et al., 1984). These rats develop many of the clinical signs of CHF: generalized and subcutaneous edema, hydrothorax, ascites, dyspnea, cyanosis, enlarged hearts, left atrial thrombosis, and hyperemia of the lungs, liver, and kidneys. These rats are hypertensive, but blood pressure falls to normal levels with the onset of severe CHF. Increased levels of plasma atrial natriuretic factor (ANF) seem to be positively correlated with the severity of the

CHF in our rats (Dunn et al., 1988), an observation also reported in humans and animals with CHF (Burnett et al., 1986; Edwards et al., 1986). Levels of plasma aldosterone, renin, and norepinephrine follow similar patterns to those observed in humans with CHF.

Cardiac Studies  Compared with Wistar Furth (WF) and Sprague Dawley rats, the hearts of SHHF rats in CHF are enlarged with dilation of all four chambers (Figure 2). From records on several hundred colony rats, the heart weight range for CHF rats was 1.7-4.5 g for males and

Congestive Heart Failure

Symptoms  Both lean and obese male and female SHHF can develop overt CHF (McCune et al., 1988; Rubin et al., 1984). These rats develop many of the clinical signs of CHF: generalized and subcutaneous edema, hydrothorax, ascites, dyspnea, cyanosis, enlarged hearts, left atrial thrombosis, and hyperemia of the lungs, liver, and kidneys. These rats are hypertensive, but blood pressure falls to normal levels with the onset of severe CHF. Increased levels of plasma atrial natriuretic factor (ANF) seem to be positively correlated with the severity of the

CHF in our rats (Dunn et al., 1988), an observation also reported in humans and animals with CHF (Burnett et al., 1986; Edwards et al., 1986). Levels of plasma aldosterone, renin, and norepinephrine follow similar patterns to those observed in humans with CHF.

Cardiac Studies  Compared with Wistar Furth (WF) and Sprague Dawley rats, the hearts of SHHF rats in CHF are enlarged with dilation of all four chambers (Figure 2). From records on several hundred colony rats, the heart weight range for CHF rats was 1.7-4.5 g for males and
1.5-3.0 g for females. For WF rats, a range of 1.03-1.24 g was found in males and 0.85-1.05 g for females. Progression of cardiac structural alterations has been quantitatively characterized grossly and by light microscopy. In SHHF rats at 6 and 10 months of age, the left ventricular free wall (LVFW) and interventricular septum (IVS) were significantly heavier, and the LVFW was thicker compared to WF controls. The rats in overt CHF had biventricular hypertrophy with increased weights and thickness of the LVFW, IVS, and the right ventricular free wall (RVFW) as seen in Figure 2. In many of the SHHF rats with CHF, thrombi were observed in the left atrium, right atrium, superior vena cava, and left ventricle. No thrombi were found in control rats.

Histologic studies revealed increased myocyte diameter (Figure 3) corresponding to increased ventricular weights and thicknesses. Myocyte diameters were larger in the SHHF rats at 6 and 10 months of age and in all three ventricular walls in CHF rats. The area fraction of fibrous tissue, consisting of replacement and interstitial fibrosis, was increased in all three ventricular walls in CHF rats and tended to be most pronounced in the inner one-third of the LVFW. These morphologic changes probably reflect early compensatory LV hypertrophy at 6-10 months of age. This is followed by LV failure with elevated right heart pressures and RVFW hypertrophy, finally leading to biventricular CHF. This progression is similar to that of hypertensive heart disease in humans. Other similarities between the SHHF model and human hypertensive-diabetic cardiomyopathy (Factor et al., 1980) and another rat model of hypertension and diabetes (Factor et al., 1983) include increased heart weight and prominent interstitial fibrosis. Pathologic alterations in SHHF corresponding to human dilated cardiomyopathy include four-chamber dilation, intracardiac thrombi, myocyte hypertrophy, and interstitial fibrosis. (Unverferth et al., 1986)

FIGURE 3 Light microscopy of left ventricular myocardium (hematoxylin and eosin, 520x). Top. WF control. Scant fibrous connective tissue is found between the myocytes. Bottom. SHHF. The myocytes are severely hypertrophied with a prominent increasing myocyte diameter.

Preliminary ultrastructural observations on the LVFW revealed prominent degenerative changes in CHF rats compared to controls (Figure 4) similar to those described in...
Renal lesions on selected animals have been evaluated by both light microscopy and immunofluorescent microscopy. Preliminary data indicate a general increase in glomerular cellularity and mesangial matrix with increasing age in both lean and obese SHHF rats (Figure 5). The glomerular lesions are more pronounced in males than females. The glomerular cellularity and mesangial matrix in both male and female SHHF rats is greater than that in either Wistar or SHR rats from one year of age until death. The primary glomerular lesions in these animals have been classified as a diffuse intercapillary sclerosis, with nodular intercapillary sclerosis only rarely observed. The lesions observed to date in these animals are consistent with diabetic nephropathy and are similar to those reported for the SHR/N-cp rat (Michaelis et al., 1986).

Other Lesions There are several prominent lesions in SHHF rats that die of CHF. Pulmonary hyperemia and edema are commonly seen. Vascular lesions—consisting of a macroscopic periarteritis nodosa involving primarily the mesenteric vessels but also often involving the adrenal, renal, and testicular arteries—are present in approxi-

mately 10 percent of aged rats dying of CHF. Adrenal cortical hemorrhage and necrosis are seen in over 90 percent of rats dying of CHF. Pancreatic lesions are limited to increased islet cell size with beta cell hyperplasia (Figure 6). No abnormalities were noted in the exocrine pancreas.

Future Studies and Appropriate Uses

Diabetes is one of the four major risk factors for heart disease. Cardiovascular disease develops early in diabe-
tes, and about 75 percent of diabetic deaths are due to cardiovascular disease. Patients with type II diabetes (insulin resistant) have a four- to five-fold greater risk of developing CHF; therefore, the high incidence of CHF in this colony may be related to insulin resistance. Many of the lean animals that died of CHF were proven heterozygotes for the corpulent (cp) gene, and some preliminary results show that the rats that carry the cp gene are more insulin resistant than normal rats. More detailed studies in the colony will help correlate insulin resistance with the age of onset and severity of CHF.

The development of a new genetic animal model for naturally occurring CHF offers great potential for understanding the causes and finding treatments for the condition in humans. The fact that hypertension is an important risk factor for CHF in humans makes this rat model a good one to study, particularly because the changes that occur in the rat are so similar to those that occur in the human condition. The advantage of rat models of CHF is that they can be compared with normal rats about which there is extensive information. If we could find some parameter that seems to be associated with increased risk of CHF in these animals, it might indeed be a marker that could be used in humans. An important area of further study is to alter metabolic control of diabetes or obesity in the various animal models and record how it affects heart lesions.

human dilated cardiomyopathy (Baker, 1985). No abnor-
malities were noted in the capillaries, including the basement membranes, and only rare inflammatory cells were found. Fibrosis as well as prominent inflammatory cell infiltration has been observed in the ventricular myocardium of the normotensive LA/N-cp rat, although these animals do not develop CHF.

The obese male Jcr:LA-cp develops different myocardial lesions from those seen in SHHF rats (Russell et al., 1986), and in the obese male Zucker rat no evidence of myocardial lesions was found (Amy et al., 1988). These findings suggest that on some genetic backgrounds, obesity or diabetes may be a significant independent predi-
tor of cardiovascular disease, which may explain the heterogeneity seen in the obese human population when it comes to cardiovascular lesions.

Renal

Renal section from a SHHF rat showing diffuse intercapillary sclerosis with glomerular hypercellularity (Jones methenamine silver, 400x).

Pancreatic islet from obese SHHF rat. Islet cell hyperplasia, primarily beta cells, is evident (hematoxylin and eosin, 100x).
Some of the experiments planned or in progress will examine the relationship between the level of insulin resistance and CHF; metabolism and calcium transport in isolated myocytes; metabolism using nuclear magnetic resonance imaging; the effect of treatment on hemodynamics; cardiac protein changes in CHF; and the correlation of heart size with the level of ANF, whether the level of ANF is predictive of the age at which overt CHF appears, and the role of ANF in both renal and heart function.

References


Jcr:LA-corpulent Rat: A Strain with Spontaneous Vascular and Myocardial Disease

J. C. Russell and D. G. Koeslag

Introduction

Following isolation of the mutant corpulent (cp) gene (Koletsky, 1973), rats containing it were initially bred into two standard strains: the LA/N and the SHR/N (Hansen, 1983). The progeny were then backcrossed more than 12 times to the parent strains to yield two congenic strains: LA/N-cp and SHR/N-cp.

The fully backcrossed, congenic strains of rats incorpo