The Role of Stressor Intensity in Influencing the Course of Heart Disease in Cardiomyopathic Hamsters (44013)

QIANG CHANG,*·†·‡ BENJAMIN H. NATELSON,*·†·[1] JOHN E. OTTENWELLER,*·† AND ROBERT S. CONWAY§

Neurobehavioral Unit (127A), VA Medical Center, East Orange, New Jersey 07018–1095; Department of Neuroscience,† and Graduate School of Biomedical Sciences,‡ University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103-2714; and Department of Surgery,§ University of Medicine and Dentistry of New Jersey, Robert W. Johnson Medical School, New Brunswick, New Jersey 08903*

Abstract. **Our earlier work showed that stress had progressively more serious consequences in a hamster model of congestive heart failure as the magnitude of heart failure worsened. Based on that study, we hypothesized that the intensity of the stressor used might play an important part in determining this outcome as well as in influencing coronary reactivity to arginine vasopressin (AVP). Cardiomyopathic (2.5, 6.5, and 10 months) hamsters (CMHs) were stressed with a 2-hr period of supine immobilization for five consecutive days. Stressor intensity was increased by exposing the hamsters to progressively longer periods at 4°C: the low stress group was never put in the cold; the moderate stress group was exposed to cold for 1 hr, and the high stress group for 2 hr. CMHs were anesthetized and sacrificed 5 days after stress, and their hearts were perfused using a modified Langendorff system. Maximum $\pm dP/dt$, developed pressure, ventricular relaxation time, (T), and coronary vascular resistance (CVR) were recorded, and CVR was also measured following coronary infusion of AVP. Stressor intensity had no effect on cardiac mechanics in 2.5-month CMHs. In 6.5-month CMHs, only the high-intensity stressor impaired ventricular mechanics (decreased maximum $\pm dP/dt$ and developed pressure, increased T; $P < 0.05$), while low and moderate stress produced no effects. In 10-month CMHs, stress at all intensities exacerbated ventricular dysfunction (decreased maximum $\pm dP/dt$ and developed pressure; $P < 0.05$). These results support our first hypothesis that stressor intensity interacts multiplicatively with severity of the underlying disease to influence the course of heart failure. However, our second hypothesis was not supported, because stress—regardless of intensity—affected reactivity of the coronary vasculature to AVP only in 2.5-month CMHs. A further test of the relation of stressor intensity and coronary vascular reactivity requires study of additional groups of CMHs during the period of their disease characterized by coronary vasospasm.** [P.S.E.B.M. 1996, Vol 212]

The cardiomyopathic hamster (CMH) inherits its disease as an autosomal dominant trait characterized by a necrotic phase in which myocardial myocytolysis occurs at 1–3 months of age (1). Spasm of coronary microvessel (2), myocardial hypoperfusion (3), and coronary hyperreactivity to arginine vasopressin (AVP) (4) were found in the CMH during this necrotic phase, suggesting a global coronary vasculopathy. Thereafter, active necrosis declines and some healing occurs from 3 to 6 months of age before the process of congestive heart failure (CHF) begins—a process that kills the animal at about 13 months of age, approximately half the life span of healthy hamsters (5).

Because of our interests in the role of stress in the pathogenesis of serious heart disease, we used the natural history of inherited heart disease in hamsters as a sliding scale of cardiac vulnerability on which to superimpose stressors of different intensity. In earlier

[1] To whom requests for reprints should be addressed at Neurobehavioral Unit (127A), VA Medical Center, 385 Tremont Avenue, East Orange, NJ 07018-1095.

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work (6), we stressed both healthy hamsters and CMHs at 4, 6, and 10 months of age. In healthy hamsters at all three ages, stress produced no significant effects. Stress did not alter disease in 4-month CMHs whose hearts had not yet enlarged. In contrast, stress precipitated overt heart failure in 6-month CMHs that were beginning to undergo compensatory cardiac enlargement. When 10-month CMHs in overt heart failure were stressed, stress dramatically exacerbated their failure and half of these animals succumbed to stress. These data led us to suggest that in the hamster model of gradually worsening heart failure, the pathological outcomes of stress were a multiplicative function of organ vulnerability. However, the role of stressor intensity was not evaluated in this psychosomatic equation.

In another series of experiments, we found that 2- to 3-month CMHs, which we expected to be less vulnerable than 4- and 6-month CMHs, succumbed to stress using a more intense stressor (7, 8). Additionally, different stressor intensities produced different survival curves in 2.5- and 5-month CMHs; younger hamsters, in their lesion-forming period, were at an increased risk from stress (9). These data suggested that stressor intensity may play an important role in pathological outcomes of hamster heart disease following stress. Therefore, we arrived at the following hypotheses: first, stressor intensity would interact multiplicatively with cardiac vulnerability to alter the rate of progression of CHF; and second, stressor intensity would alter the *in vitro* reactivity of the coronary vasculature to AVP. AVP was chosen because it is an endogenous vasoconstrictor that does not produce inotropic or chronotropic responses at low doses (10).

Materials and Methods

The study was reviewed and approved by our institutional animal care and use committee, and conforms with the *USPHS Guide for the Care and Use of Laboratory Animals* and the *U.S. Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals*. Cardiomyopathic hamsters (CHF 146 strain), obtained from Canadian Hybrid Farms (Nova Scotia, Canada), were housed individually in shoe-box cages with freely available food and water for at least 2 weeks prior to the start of the experiment. Animals were delivered at four different ages, thus allowing us to run a cohort every week for four consecutive weeks. Experiments were performed when hamsters were 2.5, 6.5, and 10 months old. At these ages, CMHs are at the following stages of disease: active vasculopathy, compensated heart failure, and overt CHF (1). Healthy hamsters were not used in these studies because of the lack of a stress effect noted in our earlier work (6, 7).

Hamsters were randomly assigned to one of four groups: nonstress, and low, moderate, and high intensity stress groups. For each age group, two animals were randomly assigned to the nonstress groups, two to the low stress groups, and three each to the moderate and high stress groups. Thus, 30 hamsters were used in each cohort ($n = 10$ in each age). The total number of hamsters in all four cohorts was 120 ($n = 8$ in nonstress and low stress groups; $n = 12$ in moderate and high stress groups for all three ages).

The stressor was a 2-hr period of supine immobilization for five consecutive days. To increase stressor intensity, we exposed groups of hamsters to different durations of 4°C cold. The low stress group was kept at room temperature for the entire 2 hr; the moderate stress group was in room temperature for 1 hr and in cold for 1 hr; and the high stress group was kept in the cold for 2 hr. The high intensity stressor was the one which had produced equal mortality outcomes for 2.5- and 5-months CMHs in our previous work (9). Hamsters were immobilized by extending their four limbs with string loops taped to the corners of a small board and then covering their bodies with a cloth flap to limit trunk movement. Nonstressed, control hamsters were moved to other shoe-box cages with no food or water during the 2-hr period of stress.

Five days after stress, surviving hamsters were deeply anesthetized with 50 mg/kg body wt of sodium pentobarbital (ip) and then anticoagulated with 200 units of heparin (iv). The chest was opened and the heart was rapidly removed with the aortic root intact. The tricuspid, mitral, and pulmonary valves were rendered incompetent to provide a nonworking preparation. The aorta was mounted on a glass cannula tip that allowed for retrograde perfusion of the coronary arteries with a roller pump. Krebs-Henseleit buffer containing NaCl 117 m*M*, KCl 4.7 m*M*, $CaCl_2$ 2.7 m*M*, $MgSO_4 \cdot 7H_2O$ 1.2 m*M*, KH_2PO_4 1.2 m*M*, $NaHCO_3$ 21 m*M*, glucose 11.2 m*M*, EDTA 0.2 m*M*, and pyruvate 2.0 m*M*, and pyruvate 2.0 m*M* was perfused at a pressure of 80 mm Hg at 37°C. The perfusion medium was gassed with a 95% O_2/5% CO_2 mixture.

Coronary perfusion pressure was measured in line with a transducer-tip catheter. A Millar catheter fitted with an adjustable water-filled balloon at the tip was inserted into the left ventricle via the mitral valve and the heart was paced at 240 beats/min (1 ~ 3 volt, SD5 Stimulator; Grass Instrument Co., Quincy, MA). The volume of the balloon within the left ventricle was adjusted to provide a left ventricular end-diastolic pressure of 10 mm Hg. After a 15-min equilibration period, baseline left ventricular pressure measurements were recorded on a chart recorder (Model RPS 7C; Grass Instrument).

The analog signal was simultaneously digitized at 300 samples/sec using a DAS-16 A/D card (Metrabyte,

Taunton, MA) and an IBM computer. The maximal positive first derivative of the left ventricular pressure trace ($+dP/dt$) as well as developed pressure (left ventricular systolic-diastolic pressure) were determined as indices of systolic function. The maximum $-dP/dt$ and the time constant of isovolumic pressure decay, T, were used as indices of diastolic function (11). After these measurements were made, the balloon catheter was withdrawn from the left ventricle, and the AV node was crushed to produce long diastoles.

The perfusion of the coronary arteries was switched to a syringe pump at the same pressure (80 mm Hg) using the same perfusion solution to provide a more stable pressure trace. The perfusion pressure was fairly flat during the latter stage of these extended diastoles. Constant flow perfusion was chosen to avoid potential confounding effects of vasoactive species that can be released by vasoconstriction-induced ischaemia under constant pressure perfusion. Coronary vascular resistance was calculated as the quotient: coronary perfusion pressure/syringe pump flow rate. After resting coronary resistance was determined at 80 mm Hg perfusion pressure, we began an infusion of AVP at 0.2 pressor units/min. This dose was chosen empirically as an infusion rate that would produce a modest increase of perfusion pressure in a normal hamster heart. Coronary vascular resistance (CVR) was calculated for AVP infusion once the perfusion pressure stabilized at its new elevated level. The increase in CVR after AVP infusion (CVRavp − CVR-resting) served as an index of coronary vascular responsiveness to vasoconstrictor infusion.

Maximum $+dP/dt$, developed pressure, maximum $-dP/dt$, ventricular relaxation time (T), resting CVR, and AVP-induced CVR increases were analyzed with 3 × 4 (age × stress intensity, analysis of variance (ANOVA) $n = 7$ for nonstressed hamsters at 2.5, 6.5, and 10 months of age and for low and moderate stress groups at 2.5 months of age, $n = 8$ for the rest of the groups). *A priori* comparisons among the groups were performed using Dunn's tests (12). All values are reported as means ± SEM. Differences were considered significant at $P < 0.05$.

Results

Of all 120 CMHs, 14 animals succumbed to stress. Seven of the 2.5-month CMHs (one in low, four in moderate, and two in high stress groups), two of the 6.5-month CMHs (two in moderate stress group), and five of the 10-month CMHs (three in moderate and two in high stress groups) died after stress. A chi-square test showed no significant differences in mortality among the three ages.

The overall 3 × 4 ANOVA of developed pressure showed a significant age effect ($F [2, 17] = 91.1$; $P < 0.01$) and stress intensity effect ($F [3, 58] = 3.32$; $P < 0.05$; Fig. 1). Developed pressure was significantly higher in the 2.5-month nonstressed group than in nonstressed CMHs at 6.5 ($t_D = 3.75$; $P < 0.01$) and 10 months of age ($t_D = 6.2$; $P < 0.01$). There was no

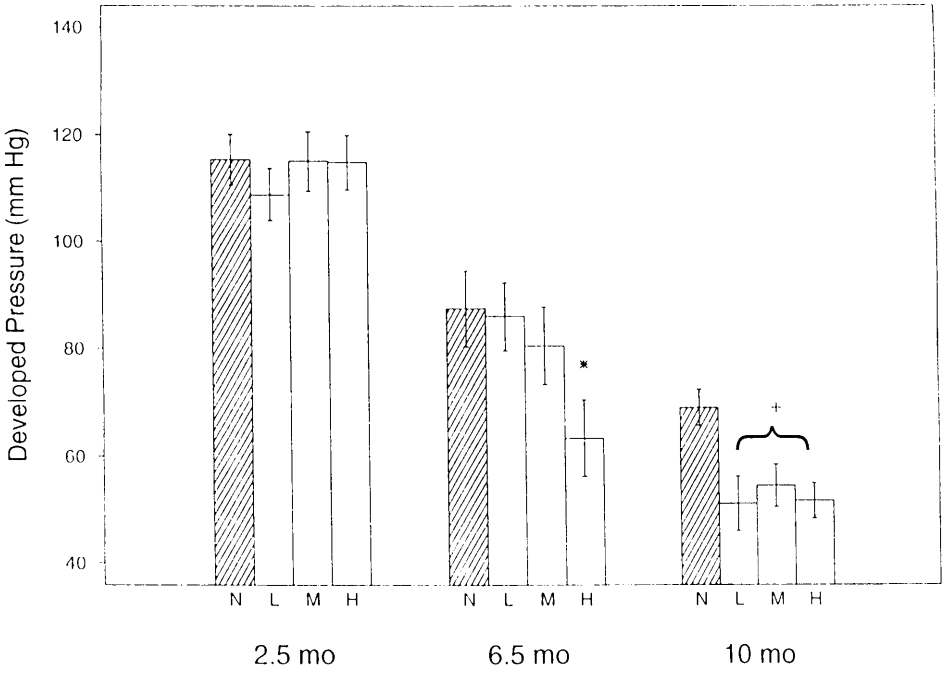

Figure 1. Developed pressure in nonstress (N), low (L), moderate (M), and high (H) stress groups of 2.5-, 6.5-, and 10-month CMHs (mean ± SEM). Stress at all intensities produced no effects in 2.5-month CMHs. Low and moderate stress intensity produced no effects, but the high-intensity stressor impaired developed pressure in 6.5-month-old hamsters. Stress of all three intensities impaired developed pressure in 10-month CMHs to the same degree. $^*P < 0.05$, compared with nonstress animals in 6.5-month group; $^+P < 0.05$, compared with nonstress animals in 10-month group.

significant difference between nonstressed 6.5- and 10-month CMHs in developed pressure. Stress produced a decrease of developed pressure in 6.5- and 10-month CMHs but not in 2.5-month CMHs. Dunn's tests also showed that in 6.5-month CMHs only the high stressor intensity reduced developed pressure compared to the nonstress group (t_D = 3.28; $P < 0.05$). However, in 10-month CMHs, low stress produced the same magnitude of decrease in developed pressure as moderate and high stress (t_D = 2.82; $P < 0.05$).

The results for maximum $+dP/dt$, another index of systolic ventricular function, were the same as those for developed pressure (data not shown). A monotonic decrease in maximum $+dP/dt$ occurred as nonstress CMHs aged: 2604 ± 100 (SEM), 2381 ± 184, 1695 ± 156 (mm Hg/sec) for 2.5-, 6.5-, and 10-month CMHs respectively. Stress was ineffective in influencing this variable for the 2.5-month group. For the 6.5-month group, only the most intense stressor was effective at reducing maximum $+dP/dt$ when compared to values in the nonstress group. For the 10-month group, even the low-intensity stressor was effective.

The overall 3 × 4 ANOVA of maximum $-dP/dt$ showed a significant age effect (F [2, 17] = 10.13; $P < 0.01$) and stress effect (F [3, 56] = 2.79; $P < 0.05$; Fig. 2). No significant differences for nonstressed CMHs at three ages were seen. In 2.5-month CMHs, stress at all three intensities produced no significant effect. In 6.5-month CMHs, low and moderate stress produced no effects, but high stress reduced maximum $-dP/dt$ (t_D = 3.24; $P < 0.05$). In 10-month CMHs, stress at all three intensities decreased $+dP/dt$ to the same degree (t_D = 2.70; $P < 0.05$). Similarly, a 3 × 4 ANOVA of the time constant of isovolumic pressure decay, T, showed an age effect (F [2, 14] = 14.14; $P < 0.01$) and an age × stress interaction (F [6, 52] = 2.84; $P < 0.05$; Fig. 3). There were no significant differences among nonstressed CMHs at three ages. Stress produced no effects in 2.5- and 10-month CMHs. In 6.5-month CMHs, only high stress increased the ventricular relaxation time to the levels seen in the stressed 10-month CMH (t_D = 2.9; $P < 0.05$).

The overall 3 × 4 ANOVA of resting CVR showed that 2.5-month CMHs uniformly had greater CVRs than either 6.5- or 10-month CMHs (F [2, 55] = 52.77; $P < 0.01$; Fig. 4). Stress at all three intensities produced no effects on resting CVR at any of the three ages. Dunn's tests showed that resting CVR in all the 2.5-month groups were significantly higher than those groups at 6.5 (t_p = 5.32; $P < 0.01$) and 10 months of age (t_p = 9.45; $P < 0.01$). In addition, resting CVR was higher in nonstressed 2.5-month group than in nonstressed 6.5- (t_D = 3.60; $P < 0.05$) and 10- (t_D = 4.31; $P < 0.01$) month groups. There were no significant differences in resting CVR between 6.5- and 10-month CMHs.

AVP produced coronary vasoconstriction in every heart under every condition. To evaluate the reactivity of the coronary vasculature to AVP, we compared the responses in all groups by calculating the absolute

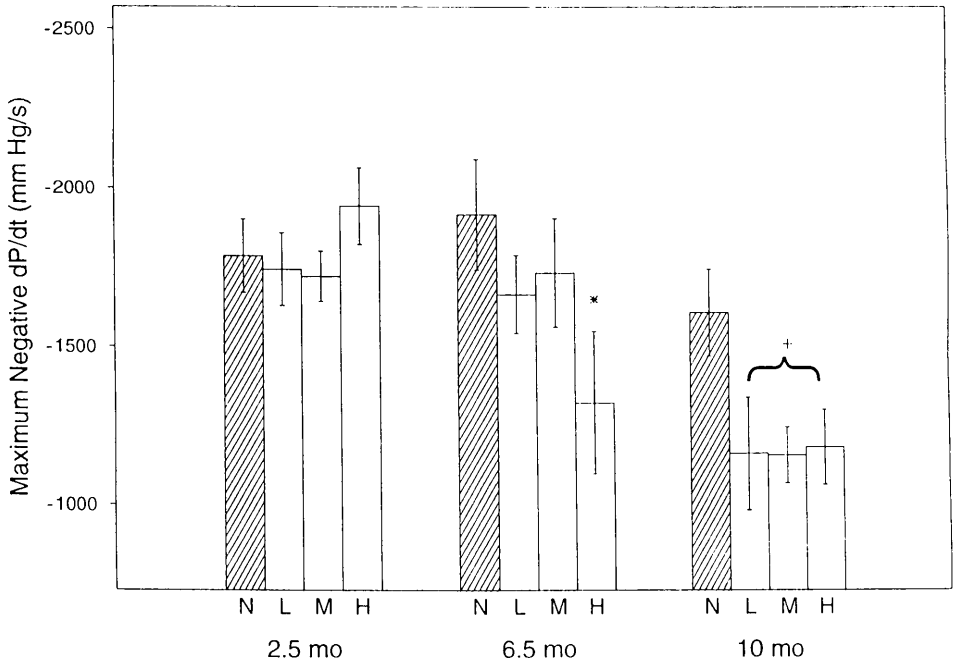

Figure 2. Maximum $-dP/dt$ in nonstress (N), low (L), moderate (M), and high (H) stress groups of 2.5-, 6.5-, and 10-month CMHs. Stress at all intensities produced no effects in 2.5-month CMHs. In 6.5-month CMHs, while low and moderate stress intensity produced no effects, the high stressor intensity impaired maximum $-dP/dt$. Low stressor intensity reduced maximum $-dP/dt$ in 10-month CMHs to the same degree as moderate- and high-intensity stress. $^*P < 0.05$, compared with nonstress animals in 6.5-month group; $^+P < 0.05$, compared with nonstress 10-month CMHs.

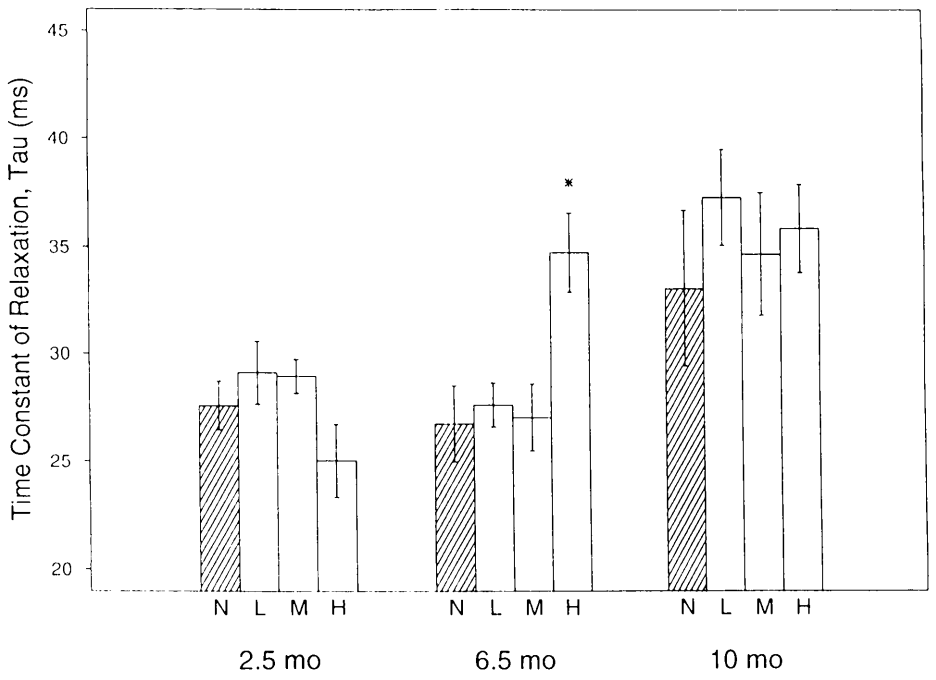

Figure 3. T in nonstress, low, moderate, and high stress groups of 2.5-, 6.5-, and 10-month CMHs. Stress at all intensities produced no effects in 2.5- and 10-month CMHs. In 6.5-month CMHs, when low and moderate stress intensity produced no effects, the high stressor intensity impaired T. $^{*}P < 0.05$, compared with nonstress 6.5-month CMHs.

change in CVR with AVP infusion for each heart (Fig. 5). The overall 3 × 4 ANOVA showed a significant age effect (F [2, 55] = 30.19; $P < 0.01$) and one of stress intensity (F [3, 55] = 3.37; $P < 0.05$). The age effect indicated that AVP produced a greater response in 2.5-month CMHs than either 6.5- or 10-month CMHs. Stress of all three intensities produced significant increases in CVR only in 2.5-month CMHs ($t_D = 3.14$; $P < 0.05$), and no significant effects of stress were found in 6.5- and 10-month CMHs.

Discussion

Our first hypothesis was that stressor intensity would interact multiplicatively with cardiac vulnera-

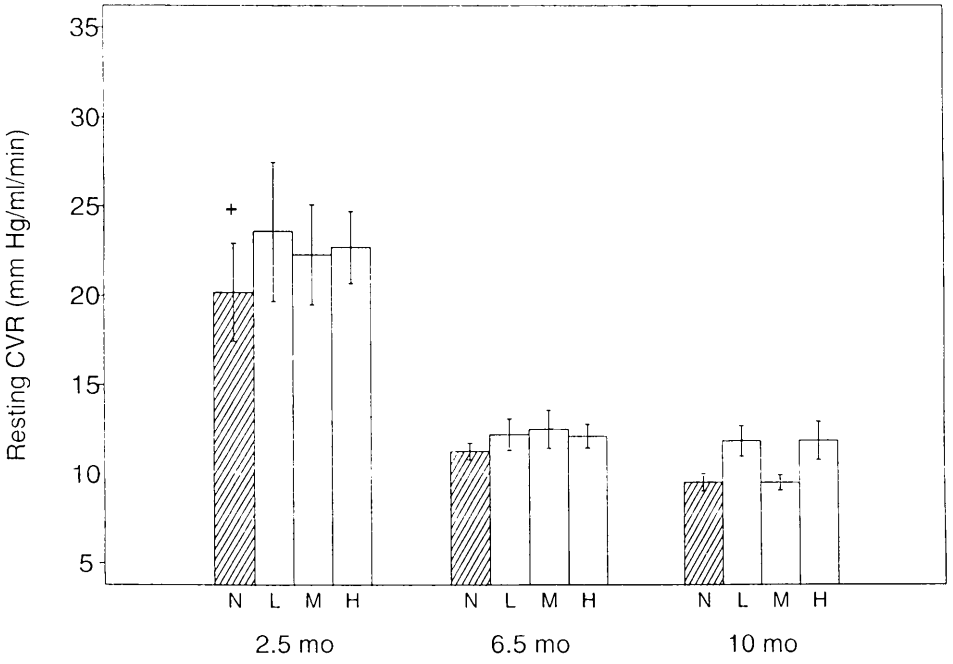

Figure 4. Resting CVR in nonstress (N), low (L), moderate (M), and high (H) stress groups of 2.5-, 6.5-, and 10-month CMHs. Resting CVR in nonstressed 2.5-month CMHs was greater than in nonstress 6.5- and 10-month groups. Stress produced no effects in resting CVR at three ages. $^{+}P < 0.05$ compared with nonstressed 6.5- and 10-month CMHs.

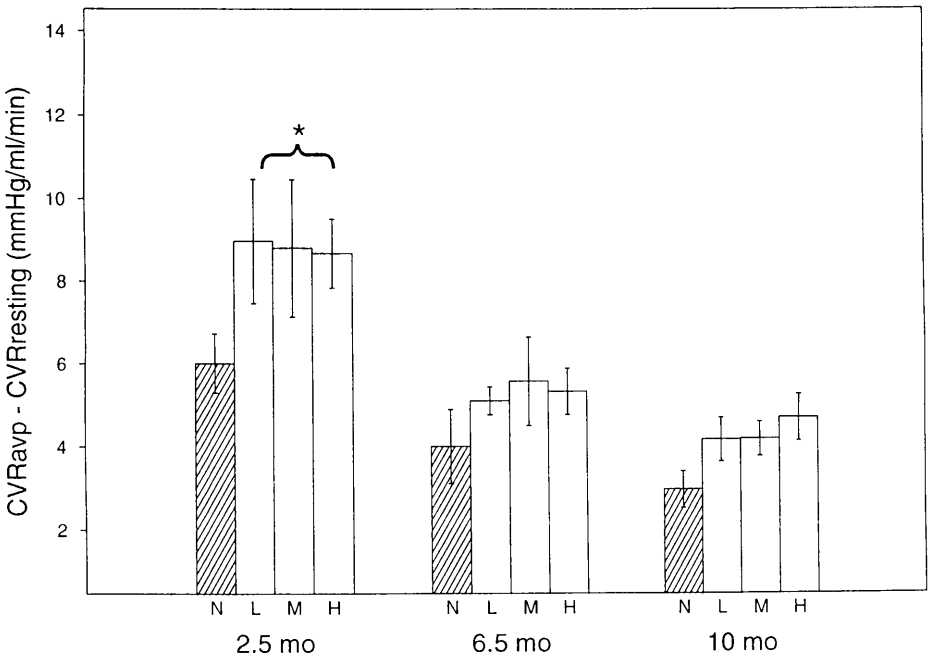

Figure 5. CVR increase after AVP infusion (CVRavp – CVRresting) with nonstress (N), low (L), moderate (M), and high (H) stress groups of 2.5-, 6.5-, and 10-month CMHs. Stress at all intensities produced no effects in 6.5- and 10-month CMHs. In 2.5-month CMHs, low stress intensity produced a coronary hyperreactivity of the same magnitude as moderate and high stress intensity. $^*P < 0.05$ compared with nonstressed 2.5-month CMHs.

bility to alter the rate of progression of CHF. The data supported this hypothesis. Regardless of stressor intensity, stress did not exacerbate heart failure in 2.5-month CMH in the necrotic stage of their disease. A more intense stressor than the one used here might have been able to affect mechanics in these young hamsters, but that would still be consistent with our hypothesis. In 6.5-month CMHs with compensated heart failure, low and moderate stress did not change cardiac mechanics, but high-intensity stress produced a further decrement in ventricular function indicative of increased CHF (Fig. 1–3). Finally in 10-month CMHs, in the early stage of cardiac decompensation, even low-intensity stress produced the same magnitude of deterioration in ventricular function as more intense stress.

Diastolic dysfunction, an important manifestation of CHF (13) was not found in nonstressed 6.5- and 10-month CMHs (Fig. 2 and 3), despite their having depressed systolic function in comparison with nonstressed 2.5-month CMHs. These data suggested that ventricular function in 6.5- and 10-month nonstressed CMHs might be only mildly impaired and that the degree of impairment in the nonstressed 10-month hamster was greater than in the 6.5-month hamster. When the high-intensity stressor was administered to 6.5-month CMHs, the depressed systolic function was further reduced and diastolic function became impaired. In 10-month CMHs, low stress intensity magnified the depressed systolic function and impaired diastolic function (maximum $-dP/dt$) to the same degree as moderate and high stress intensity. The fact that the milder CHF was exacerbated by high stress intensity in 6.5-month CMHs and by lower stress intensity in 10-month CMHs supports our interpretation that cardiac mechanics in 10-month CMHs were compromised more than those in 6.5-month CMHs. Thus, 10-month CMHs were more vulnerable to stress than 6.5-month CMHs, a fact we have noted previously using autopsy data (6). These data also suggested that impaired diastolic function has an important role in the transition form compensated ventricular hypertrophy to uncompensated heart failure (14).

Our second hypothesis was that different stressor intensities might alter the *in vitro* reactivity of the coronary vasculature to AVP. The data did not support this hypothesis. Even at the lowest intensity, stress produced the same sensitization to AVP as more intense stressors. However, in contrast to the effects seen with cardiac mechanics, the effect here was not a multiplicative one. Stress was only effective in sensitizing the coronary microvasculature in 2.5-month CMHs—animals in the vasospastic phase of their disease. In these animals, low stressor intensity produced coronary hyperreactivity to challenge with AVP to the same degree as moderate- and high-intensity stressors (Fig. 5). The sensitivity of stress in these animals is strikingly similar to that of 10-month CMHs. For those animals, low-intensity stress also had the same deleterious effect on cardiac mechanics as more intense stress. Despite different pathophysiological mechanisms, the similarity of the results seems to suggest

that in the face of marked cardiac vulnerability—despite its cause—the effect of stress may be all or nothing. However, we have recently completed another experiment in which we stressed 2.5-month CMHs with even milder intensity stressors than those used here. Since milder stressors did not elicit coronary vascular hyperreactivity, a multiplicative relation between stressor intensity and underlying vasculopathy remains possible. Further research is needed to address this issue.

The data in Figure 4 indicate that resting CVR in 2.5-month CMHs is significantly higher than that in 6.5- and 10-month CMHs. Higher resting coronary vascular resistance suggests an increased coronary vascular tone in 2.5-, but not 6.5-, or 10-month CMHs. Since vascular resistance is a function of small vessel diameter, two explanations for this finding are possible: either all the vessels in the lesion forming period are mildly constricted or some vessels are normal while others are more markedly constricted. Several lines of evidence point to the latter possibility and suggest that microvascular spasm occurs in the coronary circulation of the CMH. Factor *et al.* (2) demonstrated small vessel reperfusion injury and marked arteriolar constrictions in silicone rubber casts of the coronary bed. Myocardial hypoperfusion zones were also observed before the development of necrosis, and the size of these zones closely approximated that of the myocytolitic zones (3). Therefore, the physiological findings in the present study are in agreement with pathological data, which indicate that microinfarcts in the necrotic CMH heart reflect a vasculopathy in resistance arterioles (15).

Our earlier work showed that CMHs in the lesion-forming period of their diseases are more susceptible to the lethal effects of stress than older CMHs (9). Using stressors of moderate and high intensities, we found different survival curves in younger and older CMHs. Therefore we hypothesized that stress might trigger different pathophysiological mechanisms: coronary vasospasm in 2.5-month CMHs and CHF in older CMHs. The current study supports that hypothesis. The coronary vasculature was hyperreactive to AVP in stressed 2.5-, but not 6.5- and 10-month CMHs. In contrast, decreased cardiac mechanics following stress were found in 6.5- and 10-month CMHs, but not 2.5-month hamsters. The lethal effects of stress in younger and older CMHs may occur because of the activation of different pathophysiologic processes.

The fact that the disease progresses from coronary vasospasm to CHF in CMHs may have direct clinical relevance. Syndrome X patients have typical exertional angina, but normal coronary angiograms. Epstein and Cannon (16) suggest that the prearteriolar vessels, too small to be seen by angiography, undergo abnormal vasoconstriction and produce anginal symptoms in these patients. A study of dilated cardiomyopathic patients showed that a subgroup of them with a history of angina pain had increased CVRs when challenged with ergonovine (17). Patients without angina showed no such increase in CVR. Cannon *et al.* speculated that some dilated cardiomyopathic patients may suffer from "a primary vasoconstrictor disorder of the coronary microvasculature," and that patients with otherwise healthy hearts may progress from angina to dilated cardiomyopathy. This was supported by studies showing that a subgroup of Syndrome X patients eventually developed heart failure in 4-year follow-up studies (18, 19), perhaps *via* mechanisms similar to those operating in the CMH.

Since our experimental design employed a relatively brief period of stress in the hamsters' lives, our data do not answer the question of long-term effects of stress or the question of how stress initiates disease. However, our results focus on the much less studied interaction between stressor intensity and cardiac vulnerability as well as how stressor intensity relates to the pathological effects of stress.

We must emphasize that our conclusions are based on work with an animal model of heart disease and using a laboratory stressor, immobilization. If our results can be extended to humans with heart disease, we think the parallel would be best for those with the sort of progressive heart disease manifested by the hamsters studied here and undergoing some acute stressful event. If, as we believe, similar relationships between stress effects and organ vulnerability hold for that group of humans, physicians probably should be progressively more concerned about stress and stress management as patients with known heart disease undergo progression of their disease. Thus, the present study is clearly of interest to physicians trying to contend with stress-related problems in their practice.

The Animal Research Facility of the Veterans Administration Medical Center, East Orange, NJ, is an approved facility of the American Association for the Accreditation of Laboratory Animal Care. This research was submitted in partial fulfillment of the requirements for the PhD degree at UMDNJ-GSBS, by Q. C.

This paper is dedicated to the memory of Dr. Robert S. Conway, who died unexpectedly in April 1995.

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