ABSTRACT

Stress is known clinically and experimentally to contribute to the development or exacerbation of cardiovascular dysfunction. In an attempt to construct an animal model of stress-induced cardiovascular dysfunction and to understand its mechanisms, the effects of cold-immobilization stress and its cardiovascular consequences were investigated in cardiomyopathic Syrian hamsters (BIO 14.6) and age-matched healthy control hamsters. Repeated exposure (5 days) to cold-immobilization in the supine position induced no detectable ill effects in the healthy control hamsters but had a lethal effect in the cardiomyopathic hamsters: more than half of the animals died suddenly during or after the stress sessions. Autopsy study of these animals showed significant increases in the weights of the heart, adrenal, liver and kidney and in the serum levels of alkaline phosphatase, urea nitrogen, creatinine and glucose in the cardiomyopathic hamsters subjected to the stress. Propranolol (0.1–10 mg/kg i.p.) administered just before each cold-immobilization for 5 consecutive days dose-dependently and significantly prevented the lethal effects of the stress. Furthermore, it was demonstrated that the drug significantly reduced the increase in the weights of the heart, adrenal, liver and kidney observed in the stressed cardiomyopathic hamsters, whereas phentolamine (0.1–10 mg/kg) and atropine (0.1–10 mg/kg) did not prevent the stress-induced sudden death. This series of acute experiments using single exposure of this stress revealed that the stress evoked severe arrhythmia in some of the cardiomyopathic hamsters and increased the levels of circulating catecholamines in both healthy and cardiomyopathic hamsters. These results taken together suggest that stress accelerates the cardiovascular dysfunction in cardiomyopathic hamsters and provide the first evidence that excitation of the sympathetic nerves, in which \( \beta \)-adrenoceptors appear to be involved, but not the parasympathetic nerves, has an important role in the etiology of stress-induced cardiac sudden death of cardiomyopathic hamsters.

A growing body of evidence suggests that behavioral stress has a part in the precipitation of life-threatening cardiovascular dysfunction. Indeed, stress has been known clinically and experimentally to contribute to the development or exacerbation of cardiovascular dysfunction, and it is identified as a risk factor of hypertension, cardiac dysrhythmia, or even sudden cardiac death (Eliot, 1987; Galosy et al., 1981). Among such cardiovascular disorders, sudden cardiac death is the leading mode of death in adults in the industrial world (Manolio and Furberg, 1994). Although the precise mechanisms of cardiac sudden death are not yet fully understood, increasing evidence has indicated that sudden death resulting from ventricular fibrillation may be triggered by behavioral and neural factors (Engel, 1971; Kamarck and Jennings, 1991; Lown, 1987). For instance, several physiological precursors of sudden death are promoted by psychological stress, especially in persons with coronary heart disease (Kamarck and Jennings, 1991). In experimental animals, various stressors that can augment sympathetic neural traffic to the heart reportedly lower the vulnerable period threshold for ventricular fibrillation, resulting in sudden death in dogs or pigs (Kolman et al., 1976; Parker et al., 1987).

On the other hand, the cardiomyopathic Syrian hamster is known to develop a genetically determined cardiomyopathy, with progressive development of congestive heart failure, resembling human congestive cardiomyopathies (Bajusz et al., 1966; Bajusz and Lossnitzer, 1968; Strobeck et al., 1979). The cardiomyopathy is characterized by multifocal myocardial necrosis that begins at 40 to 50 days of age and causes premature death from congestive heart failure or arrhythmia, usually within 1 year. Although the pathogenesis of the disease is imperfectly understood, a body of evidence has suggested there are defects in the myocytes that are susceptible to the effect of stressful stimuli such as transient ischemia (Lossnitzer et al., 1975). It has recently been reported that cold-restraint stress had lethal consequences in cardiomyopathic hamsters (Tapp et al., 1989a, 1989b). These findings taken together prompted us to construct an animal model of stress-induced sudden death in this animal.
model of cardiac sudden death associated with stress by investigating the effect of cold-immobilization stress and its cardiovascular consequences in cardiomyopathic hamsters. The purpose of this study was to characterize the sudden death of these animals and to clarify the role of the autonomic nervous systems in the genesis of the sudden death.

Materials and Methods

Animals. The animals used were 2-month-old BIO 14.6 cardiomyopathic hamsters and age-matched F1B healthy hamsters purchased from Canadian Hybrid Farms (Nova Scotia, Canada). The hamsters were individually housed in plastic cages and given unlimited access to Purina mouse chow and tap water. Both groups were placed in a temperature-controlled environment (22 ± 1°C) under a 12-hr light/dark schedule with lights off at 3 p.m. and were allowed to adapt to these conditions for 4 weeks before the experiment. All animals procedures were carried out as approved by the Animal Care and Use Committee at Fujisawa Pharmaceutical Co. Ltd.

Stress procedures. Hamsters of each group were further subdivided into stress and nonstress groups. The stress protocol was carried out on 5 consecutive days and began at 3 p.m. The stressed hamsters including the healthy controls were subjected to daily 1-hr periods of supine immobilization at 4°C. They were immobilized by extending their four limbs and taping them onto the corners of a small board; they were then left in a refrigerator for 1 hr. The nonstressed healthy and cardiomyopathic hamsters were not immobilized and were just left in their housing cages outside a refrigerator for 1 hr. Immediately before and after each stress session as well as the next morning after each session, the hamsters were checked to measure their body weights and to see if they were still alive. Thereafter, we checked the hamsters twice daily until 7 days after the last stress session.

Autopsy. Hamsters were autopsied after either being found dead or after decapitation on the final day of the experiment (day 12). In the animals that died from sudden death, 10 hours was the maximum time that was allowed to elapse between death and autopsy. Autopsy consisted of removal of the organs and absorption of pleural and peritoneal fluid on a preweighed gauze pad. Organs and BCF were then weighed quickly. In a experiment, organs were dried at 90°C for 6 hr, and their dry weights were determined. The surviving animals were killed at the end of the experiment, 7 days after the last stress session, and autopsied as above, including the collection of blood. Trunk blood was collected into tubes and centrifuged, and the serum was separated. Serum concentrations of CPK, GOT, GPT, ALP, urea nitrogen, creatinine, total cholesterol, total bilirubin, triglycerides and glucose were measured using an autobiochemical analyzer (TBA-20R; Toshiba, Tokyo, Japan). Corticosterone levels in ALP, urea nitrogen, creatinine, total cholesterol, total bilirubin, triglycerides, plasma NE and E levels were investigated in separate series of acute experiments. Blood of the hamsters was obtained from the hamsters without stress 20 min after pentobarbital anesthesia (40 mg/kg i.p.) served as base-line data. Four or five healthy hamsters and cardiomyopathic hamsters were used in each group of different time points. NE and E in the plasma were immediately extracted by the method of Hallman et al. (1978) and assayed electrochemically by HPLC as described by Watson (1981). The analytical conditions were as follows: HPLC pump, model EP-10 (Eicom, Kyoto, Japan); electrochemical detector, model ECD-100 (Eicom); HPLC column, CA-5ODS, 4.6 × 150 mm (Eicom); mobile phase, 0.1 M sodium phosphate buffer solution (pH 6.0) with 10% (v/v) methanol and 277 µM 1-octanesulfonate (Nacalai Tesque, Kyoto, Japan) and 10 µM disodium EDTA (Nacalai Tesque); flow rate, 1 ml/min.

ECG telemetry. To examine the acute effect of the cold-immobilization stress on electrocardiographic responses, telemetry ECG was recorded before and after the stress session for 60 min using the Cardiotel telemetry system (Data Sciences, St. Paul, MN) in a separate series of acute experiments. Two healthy hamsters and two cardiomyopathic hamsters were used in the experiment. Briefly, the animals were anesthetized with pentobarbital (40 mg/kg i.p.), and dorsal celiotomy was performed. Each transmitter (model TA11CTA-F40) consisted of a small body (volume, 4 ml; weight, 9 g; 15 × 9 × 25 mm) with two coiled wires protruding from one end of the cylinder. The body of the transmitter was placed in the dorsal cavity. Subsequently, the two recording leads were pulled along two subcutaneous tunnels toward the left and right clavicular regions, where the tips of the leads were sutured to the pectoral muscles. The receiver (model CTR85-SA) measured 350 × 220 × 30 mm and was placed outside the refrigerator during the recording sessions. Before the beginning of immobilization stress, a 10-sec ECG recording was performed every 20 min while the animal was alone and undisturbed in its home cage (base-line condition). The recording was not performed during the stress session for 60 min, but immediately after the animals were taken out of the refrigerator, ECGs were recorded again, and the recordings continued until either the animals died or 3 days after conclusion of the stress sessions. The ECG was monitored on a cathode-ray tube, registered on paper and fed into a dedicated instrument that automatically performed amplification, sampling (500 Hz) and analog-to-digital conversion of the signal. The digitized data were stored on IBM-PC for offline processing and analyzed by using a software package.

Drugs. The drugs used here were dl-propranolol hydrochloride, phenolamine hydrochloride and atropine hydrochloride (Sigma Chemical, St. Louis, MO). All drugs were prepared just before the tests. They were dissolved in physiological saline and given intraperitoneally in a volume of 2 ml/kg just before the immobilization stress for 5 consecutive days.

Statistical analysis. All results were expressed as mean ± S.E.M. Statistical significance of differences was calculated using Student's t test (two-tailed) for the changes in organ weights and serum parameters. Mortality results were analyzed by Fisher's exact probability test. Cumulative surviving percents of hamsters during the course of the experiments were analyzed using a generalized Wilcoxon test.

Results

Effects of cold-immobilization stress on cardiomyopathic hamsters. Figure 1 shows the mortality. No healthy hamsters with or without stress or nonstressed cardiomyo-

![Fig. 1. Percentage of surviving cardiomyopathic and age-matched control healthy hamsters in stress treatment. **P < .05, statistically significant compared with nonstressed cardiomyopathic hamsters (by Fisher's exact probability test). Numbers in parentheses indicate the number of animals in each group.](image-url)
pathic hamsters died during the course of the experiment. In contrast, 8 of 13 stressed cardiomyopathic hamsters died; there was a statistically significant difference between the mortality of the two groups (P < .01 by Fisher’s exact probability test). As shown in figure 2, which represents the cumulative surviving percent, 5 animals among the stressed cardiomyopathic hamsters died during the 5 days of stress sessions, and 3 animals died after the stress termination. The difference between the two groups was statistically significant (P < .01 by generalized Wilcoxon’s test).

Table 1 summarizes the daily changes in the body weight of the animals during the experiment. Cold-immobilization had a minimal effect on the body weights of the healthy control hamsters, with a statistically significant (P < .05 by Student’s t test) reduction only on days 6 and 8; thereafter, the body weights recovered. In the cardiomyopathic hamsters, however, the stress significantly (P < .01) decreased body weight, and the mean body weight loss persisted during the 1 week of observation after the termination of the stress sessions.

**Effects of cold-immobilization stress on the organ weights of cardiomyopathic hamsters.** The effects of cold-immobilization stress on the organ wet weights of the cardiomyopathic hamsters were evaluated, and the results are summarized in figure 3. Seven days after the conclusion of the stress sessions, BCF was increased in both the control and cardiomyopathic hamsters, although the change was not statistically significant. Also, the wet weights of the adrenal, kidney, liver and heart were significantly (P < .05 by Student’s t test) increased in the stressed cardiomyopathic hamsters compared with the nonstressed cardiomyopathic animals, whereas the spleen was not affected by the stress (fig. 3). The stress produced no detectable changes in the healthy animals except that it significantly but slightly increased the weights of the heart and liver. To clarify the nature of the increase in the organ weights, the dry weights of the organs were determined. Table 2 shows that the stress significantly increased the dry weight of the adrenal, kidney, liver and heart in a comparable magnitude with the measurement of wet weight of each organ, suggesting the presence of hypertrophy or remodeling of each organ.

The pooled data from our previous experiments were reanalyzed to clarify the time course changes in the organ weights of the stressed cardiomyopathic hamsters without any drug dosing, and the results were summarized in figure 3.

**Fig. 2.** Cumulative survival curves of unstressed and stressed cardiomyopathic hamsters. P value was calculated by generalized Wilcoxon test.
4. All data were extracted and arranged from the stressed cardiomyopathic hamsters (96 animals) that died from sudden death on each time point during the experimental period. As shown in figure 4, BCF weight was slightly increased during the stress sessions and then returned toward the levels of the nonstressed animals when the stress sessions were stopped. On the other hand, the stress quickly and progressively elevated the weight of the adrenal, and the increases were sustained even after the termination of the stress. A similar time course change was observed in the weights of the kidneys. Spleen weight increased transiently, followed by a marked reduction caused by the stress, whereas the heart and liver increased in their weight at a slow onset starting from day 3 or 4, and the increases persisted after the conclusion of the stress sessions.

**Effects of cold-immobilization stress on the serum parameters in cardiomyopathic hamsters.** Changes in serum parameters were determined from the blood of surviving hamsters on day 12. The data obtained are summarized in figure 5. Serum levels of CPK in the nonstressed cardiomyopathic hamsters were found to be significantly higher than in the nonstressed healthy control hamsters, but cold-immobilization stress hardly affected the levels in either group. Similar results were obtained in GPT (fig. 5) and GOT (data not shown). On the other hand, the stress markedly elevated serum levels of urea nitrogen and creatinine in the stressed cardiomyopathic hamsters, but produced no changes in these parameters in the healthy hamsters. Similarly, significant increases were observed in the serum levels of alkaline phosphatase and total protein in the stressed cardiomyopathic hamsters (data not shown). Blood glucose, a sensitive indicator of stress response (De Boer et al., 1990), was also markedly elevated only in the stressed cardiomyopathic hamsters. Serum levels of total bilirubin,
calcium and triglycerides were minimally affected by the stress (data not shown), and the stress tended to increase the serum corticosterone level in the cardiomyopathic hamsters, although the change was not statistically significant.

**Changes in plasma catecholamine levels.** As shown in figure 6, single exposure to cold-immobilization stress increased the plasma NE and E levels significantly in healthy control hamsters. Plasma NE level in the stressed healthy hamsters peaked immediately after the beginning of stress and declined gradually during the 1-hr stress period to recover to the base-line levels. Plasma E level in the stressed hamsters reached to the plateau at 15 min, and this increase was sustained throughout the stress period. However, there were no significant changes in the time course of these catecholamine levels between the cardiomyopathic and control healthy hamsters.

**ECG telemetry.** The acute effect of the cold-immobilization stress on electrocardiographic responses was investigated by telemetry system in two healthy and two cardiomyopathic hamsters before and after the stress. As shown in figure 7, the nonstressed healthy hamsters showed no significant changes in their sinus rhythm after cold-immobilization stress (row A, prestress; row B, poststress), whereas the cardiomyopathic hamsters showed a regular sinus rhythm with shorter RR interval compared with the healthy controls before the stress, as seen in row C of figure 7. However, the animal that received immobilization stress for 1 hr a day showed significant arrhythmia; there was obvious heart rate slowing and an occasional atrial fibrillation (row D). As shown in row E of figure 7, one of two animals showed an abnormal auriculo-ventricular block before its death.

**Pharmacological analysis on the stress-induced sudden death in cardiomyopathic hamsters.** Propranolol, a beta blocker, was evaluated for its effect on the sudden death of stressed cardiomyopathic hamsters in an attempt to elucidate the role of the sympathetic nerves. Figure 8 shows the mortality results. No healthy hamsters with or without stress and no nonstressed cardiomyopathic hamsters died during the course of the experiment. In contrast, 5 of 6 stressed cardiomyopathic hamsters died. Administration of propranolol (0.1–10 mg/kg i.p.) dose-dependently reduced the mortality seen in the stressed cardiomyopathic hamsters with statistically significant changes in the mortality for the group dosed with 10 mg/kg propranolol compared with the saline-treated stressed cardiomyopathic hamsters (P < .05 by Fisher’s exact probability test). As shown in figure 9, no cardiomyopathic animals receiving 10 mg/kg propranolol died during the experiment.

Six days after the completion of the stress sessions, BCF and organ weights were measured; the results are presented.

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**Fig. 4.** Time course changes in hamster organ weights and BCF weights caused by cold-immobilization stress. Each symbol and bar represent the mean ± S.E.M. Broken line in each graph represents the level of nonstressed cardiomyopathic hamsters that were killed and autopsied on day 12. Numbers in parentheses indicate the number of animals in each group that died on each day.
in figure 10. The heart, adrenal and kidney weights were significantly \((P < .05)\) increased in the stressed cardiomyopathic hamsters compared with the nonstressed cardiomyopathic animals. However, the stress caused no detectable changes in healthy animals. The administration of propranolol significantly and dose-dependently prevented the increase of weight of any organ in the stressed cardiomyopathic hamsters, with statistically significant \((P < .05)\) difference in the group dosed with 10 mg/kg of propranolol (fig. 10). BCF was minimally affected by the drug (data not shown).

Phentolamine, a nonselective \(\alpha\) adrenoceptor blocker, was evaluated for its effect on the sudden death of the stressed cardiomyopathic hamsters. Figure 11 shows the mortality results. No healthy hamsters with or without stress and no nonstressed cardiomyopathic hamsters died during the course of the experiment. In contrast, 5 of 6 of the stressed cardiomyopathic hamsters died, and administration of phentolamine (0.1–10 mg/kg i.p.) hardly affected the mortality seen in the stressed cardiomyopathic hamsters.

When atropine, an antimuscarinic, was investigated for its action in this model, atropine (0.1–10 mg/kg i.p.) minimally affected the sudden death seen in the stressed cardiomyopathic hamsters, as shown in figure 12. Although doses of <1 mg/kg atropine tended to accelerate the lethal effect of the stress, in turn the larger dose (10 mg/kg) showed rather a tendency to delay death in the cardiomyopathic hamsters.

**Discussion**

The first important finding of the present study is that cold-immobilization stress exerted a lethal effect in cardiomyopathic Syrian hamsters but not in the control hamsters, suggesting that the stress aggravated the cardiovascular dysfunction seen in cardiomyopathic hamsters with a covert
heart disease. The evidence is principally in line with the findings of Ottenweller et al. (1987). Extending their findings, however, the present study for the first time adds evidence of the possible cause of death. The fact that only the animals with covert heart disease died suggests that stress has serious and even lethal consequences in cardiovascular function. Other, more direct evidence is derived from the present telemetry recording data, which showed the presence of severe arrhythmia in some of the cardiomyopathic hamsters after cold-immobilization stress. Acute heart failure and/or lethal arrhythmia might be responsible for the death of the stressed cardiomyopathic hamsters because heart and adrenal weights were markedly increased in the animals. Given the fact that cardiomyopathic hamsters are known to develop hypertrophy of the heart spontaneously (Bajusz et al., 1966; Bajusz and Lossnitzer, 1968; Strobeck et al., 1979), the stress could accelerate the cardiac hypertrophy in cardiomyopathic hamsters as revealed by the dry organ weights. It was also supported by the result that stress elevated BCF in the animals, an index that is known to reflect the cardiovascular age of each animal (Ottenweller et al., 1987, 1988), suggesting the presence of fluid retention in the body cavity commonly seen in animals with congestive heart failure. The absence of an additional increase in the activity of CPK, one of the reliable measures of cardiac myolysis (Van Der Veen and Willebrands, 1966), in stressed cardiomyopathic hamsters may be explained by the fact that the enzyme activity in the nonstressed cardiomyopathic animals was already much higher and more saturated than that in the healthy ones.

In the present study, stress produced a marked increase in the kidney weight and the serum levels of ALP, urea nitrogen and creatinine in the cardiomyopathic hamsters, indicating a
Fig. 8. Effects of propranolol (0.1–10 mg/kg) on sudden death caused by immobilization stress in cardiomyopathic hamsters. Each value represents the percentage of surviving hamsters. *P < .05, statistically significant compared with saline-treated stressed cardiomyopathic hamsters (by Fisher’s exact probability test). Number in parentheses indicates the number of animals in each group. Propranolol was administered intraperitoneally just before the stress for 5 consecutive days. C; nonstress control group, S; stress group.

Fig. 9. Survival curves of unstressed and stressed cardiomyopathic hamsters receiving propranolol treatment. Propranolol was administered intraperitoneally just before the stress for 5 consecutive days in the first week.

reduction in glomerular filtration rate possibly related to a decrease in myocardial performance indicative of sudden ventricular dysfunction. These changes were suggestive of the involvement of acute failure in the kidney as well as in the heart for the cause of sudden death in the stressed cardiomyopathic hamsters. This view is compatible with earlier findings by others that certain forms of stress can produce abnormalities in the kidney (Altland and Highman, 1961; Knocker, 1955). Increased activity of the sympathetic nerves is one of the most important factors responsible for the increased afterload in cardiac dysfunction. Efferent sympathetic activity is known to be distributed in a nonuniform way, with significant increases to the heart and kidney, but normal activity to some other organs, such as the liver or lung (Esler et al., 1985). Increased renal sympathetic activity is known to contribute significantly to altered renal hemodynamics, sodium and water retention and modulation of the actions of other vasoactive hormones. For example, renal blood flow is reduced in animals with heart failure (Millard et al., 1972). In humans, the sympathetic outflow to the kidney is significantly increased in patients with heart failure (Hasking et al., 1986; Zelis and Flaim, 1982). Our present results coincide with this evidence and suggest an important role of activated sympathetic drive in renal failure that could have resulted in the sudden deaths of the stressed cardiomyopathic hamsters. Multiple organ failure might further be involved in the mechanism underlying the vulnerability of the cardiomyopathic hamsters to stress because the liver weight also was increased in the cardiomyopathic animals.

Another important finding of the present study is that a beta blocker, propranolol, dose-dependently prevented the lethal effect of the stress in the cardiomyopathic hamsters. Taken together with the fact that the drug significantly re-
ischemia in the stressed cardiomyopathic hamsters. The potentially lethal arrhythmia and resulted in myocardial levels of catecholamines might have caused or exacerbated mechanisms of cardiac sudden death, increases in the circulating catecholamine levels are major factors involved in the mechanical effects of catecholamines released from the adrenal medulla are generally supportive of the actions of the rest of the sympathetic nerves, which depend mainly on the release of NE. In the present study, plasma E concentration increased more slowly after the onset of stress than NE, and the increase was more prolonged than that of NE. The changes in the time course of plasma NE and E are in accordance with well known differences in the actions of E released from the adrenal and NE released from sympathetic postganglionic nerve terminals. Given the evidence that E is usually the predominant amine released from the adrenal and it has a much greater affinity for beta adrenoceptors than NE, circulating E could be involved in the sudden death of stressed cardiomyopathic hamsters. Interestingly, however, there were no significant changes in the time course of NE levels between the cardiomyopathic and healthy control hamsters. This finding suggests that circulating NE is an important factor but not a sole mechanism of sudden death of stressed cardiomyopathic hamsters. More detailed studies using cardiomyopathic hamsters with adrenalectomy or sympathetic denervation would be required to better address the qualitative difference of the contributions of sympathetic nerve discharge and circulating E to the sudden death of cardiomyopathic hamsters by stress.

The present finding that phentolamine failed to prevent the sudden death of the animals subjected to cold-immobilization stress would suggest that alpha adrenergic receptors had little or no part in the stress-induced cardiac sudden death. This finding may rule out the possibility of the involvement of increased resistance of the peripheral vasculatures in the etiology of the stress-induced death in cardiomyopathic hamsters. It has been well documented that both divisions of the autonomic nervous system are usually tonically active and the inhibitory effects of the tonic vagal activity on the heart oppose the facilitatory influences of the tonic sympathetic activity. Thus, these antagonistic interactions of the autonomic nervous systems are supposed to be involved in the cardiac dysfunction that leads to the sudden death observed in stressed cardiomyopathic hamsters (Verrier and Hagestad, 1983). However, the fact that atropine failed to prevent the lethal effects of cold-immobilization stress or to affect the increase of weights of the heart, liver, adrenal and kidney caused by the stress, these results suggest that sympathetic beta adrenoceptors play an important role in the stress-induced cardiac sudden death of the cardiomyopathic hamsters. An activation of the sympathetic nerves triggered by stress might participate in the sudden death as a consequence of increased incidence of heart failure and/or cardiac arrhythmia. Furthermore, it is possible that the protective action of propranolol against sudden death might involve not only the inhibitory action of propranolol on the reduced tone of sympathetic nerve discharge that was excited by stress but also the direct antiarrhythmic and anti-ischemic effects of the drug.

The sympathetic nervous system and circulating catecholamine levels are major factors involved in the mechanisms of cardiac sudden death, increases in the circulating levels of catecholamines might have caused or exacerbated potentially lethal arrhythmia and resulted in myocardial ischemia in the stressed cardiomyopathic hamsters. The present results determining plasma NE levels strongly suggest the acceleration of peripheral sympathetic activity after the stress. Catecholamines released from the adrenal medulla are generally supportive of the actions of the rest of the sympathetic nerves, which depend mainly on the release of NE. In the present study, plasma E concentration increased more slowly after the onset of stress than NE, and the increase was more prolonged than that of NE. The changes in the time course of plasma NE and E are in accordance with well known differences in the actions of E released from the adrenal and NE released from sympathetic postganglionic nerve terminals. Given the evidence that E is usually the predominant amine released from the adrenal and it has a much greater affinity for beta adrenoceptors than NE, circulating E could be involved in the sudden death of stressed cardiomyopathic hamsters. Interestingly, however, there were no significant changes in the time course of NE levels between the cardiomyopathic and healthy control hamsters. This finding suggests that circulating NE is an important factor but not a sole mechanism of sudden death of stressed cardiomyopathic hamsters. More detailed studies using cardiomyopathic hamsters with adrenalectomy or sympathetic denervation would be required to better address the qualitative difference of the contributions of sympathetic nerve discharge and circulating E to the sudden death of cardiomyopathic hamsters by stress.

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In light of the validity of the present experimental paradigm as an animal model of stress-associated cardiac sudden death, the present results so far are in good accord with experimental and clinical findings that have shown beneficial effects of the blockade of beta adrenoceptors on cardiac
sudden death (Beta-blocker Heart Attack Trial Research Group, 1982; Parker et al., 1987; Widerhorn and Rahimtoo, 1994). There is considerable clinical evidence of the beneficial effects of beta blockers without intrinsic sympathomimetic activity, such as propranolol, timolol or metoprolol, on cardiac sudden death (Beta-blocker Heart Attack Trial Research Group, 1982; Widerhorn and Rahimtoo, 1994). In dogs, Verrier (1986, 1987) found that beta blockade by propranolol or tolamolol but not atropine protected against the reduction of the cardiac vulnerable threshold of ventricular fibrillation evoked by psychological stress. Our present findings are also in agreement with previous results by others suggesting the important role of the sympathetic nervous system in the genesis of inherited cardiomyopathy of the hamster. For example, the course of the disease can be delayed by sympathetectomy (Anderson et al., 1982) or pharmacological blockade of the sympathetic nervous system (Jasmin et al., 1979). As well, experimental cardiomyopathy similar to that seen in cardiomyopathic hamsters has been induced after the administration of isoproterenol or NE in various animals (Bloom and Cancilla, 1969; Rona, 1985). In addition, a positive correlation has been found between levels of myocardial NE and the degree of cardiomyopathy in hamsters (Angelakos et al., 1973).

It is tempting to further speculate that beta adrenoceptors in the brain might be involved in the response. The blockade of the central beta receptors reportedly improved the stress-related increase in cardiac vulnerability to ventricular fibrillation in studies with pigs (Parker et al., 1990). There also is evidence that there are changes in the brain of cardiomyopathic hamsters that might contribute to the disease development; that is, in the lateral parabrachial nucleus of the hamsters, catecholaminergic innervation is very dense in comparison to that in healthy control hamsters (Allen et al., 1995). Although future studies will be needed to address the detailed mechanisms by which propranolol prevented the sudden death in stressed cardiomyopathic hamsters, it would be fascinating to further clarify the central mechanisms by which stress information was conveyed from the brain to the sympathetic nerves (Galosy et al., 1981; Skinner, 1988).

In conclusion, the present study demonstrated that cold-immobilization stress can have lethal consequences in cardiomyopathic hamsters and may provide a novel animal model of stress-induced cardiac sudden death. We propose that the stress produces lethal changes in the heart and/or kidney as a consequence of excitation of the sympathetic drive and results in sudden death, although further studies will be needed to clarify the detailed mechanisms by which heart and/or renal failure occurred in our stressed cardiomyopathic hamsters. Furthermore, the present results provided the first experimental evidence that propranolol can hinder the lethal consequences in cardiovascular function produced by stress and imply a role of sympathetic beta adrenoceptors in the stress-induced sudden death of cardiomyopathic hamsters. Taken together, this is compelling experimental evidence regarding cardiac sudden death in terms of its mechanisms and suggests therapeutic implications.

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