Pharmacology Biochemistry and Behavior, Vol. 66, No. 4, pp. 707–712, 2000

0091-3057/00/$–see front matter

PII S0091-3057(00)00287-2

A Dopamine $D_{1/5}$ Receptor Antagonist, SCH23390, Prevents Stress-Induced Sudden Death in Cardiomyopathic Hamsters

HIROYUKI ARAKAWA, HIROSHI KODAMA, ISAMU YAMAGUCHI AND NOBUYA MATSUOKA

Basic Research Group, Tsukuba Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., Tsukuba, Ibaraki, Japan

Received 10 September 1999; Revised 20 January 2000; Accepted 25 January 2000

ARAKAWA, H., H. KODAMA, I. YAMAGUCHI AND N. MATSUOKA. *A dopamine $D_{1/5}$ receptor antagonist, SCH23390, prevents stress-induced sudden death in cardiomyopathic hamsters.* PHARMACOL BIOCHEM BEHAV **66**(4) 707–712, 2000.—Stress is known to have an impact on the development of life-threatening cardiovascular dysfunction. We have previously demonstrated that repeated exposure to cold-immobilization stress had lethal effects on cardiomyopathic Syrian hamsters (BIO 14.6), and that stress-induced sudden death was prevented by daily treatment with propranolol, suggesting an important role of sympathetic nerves in the etiology of stress-induced cardiac sudden death. In an attempt to clarify further the mechanisms of the sudden death, in the present study we investigated the effects of $D_{1/5}$ receptor blockade by SCH23390 on the sudden death of cardiomyopathic hamsters. In accordance with our previous results, repeated exposure for 5 days to cold-immobilization stress induced a lethal effect in the cardiomyopathic hamsters but not in control healthy hamsters. SCH23390 (0.1–10 mg/kg, IP), administered just before the exposure 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 adrenal and kidneys observed in the stressed-cardiomyopathic hamsters. On the other hand, specific D_2 antagonist haloperidol (0.1–10 mg/kg) failed to prevent the stress-induced sudden death and minimally affected the increase in organ weights. Correctively, these results suggest that $D_{1/5}$ receptors had an important role in the etiology of stress-induced cardiac sudden death of the cardiomyopathic hamsters, and provide the first experimental evidence of the potential therapeutic values of $D_{1/5}$ antagonists against cardiac sudden death associated with stress. © 2000 Elsevier Science Inc.

Stress Cardiomyopathic hamsters Sudden death Cardiac sudden death Heart failure Kidney failure $D_{1/5}$ receptors Sympathetic nerves SCH23390 Haloperidol

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 (8,11). Among such cardiovascular disorders, sudden cardiac death is becoming a leading mode of death in adults in the industrially developed world (17). Although the precise mechanisms of cardiac sudden death is still not fully understood, increasing evidence has indicated that sudden death resulting from ventricular fibrillation may be triggered by behavioral and neural factors (9,13,16). For instance, several physiological precursors of sudden death are promoted by psychological stress, especially in persons with coronary heart disease (13). 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 (14,21).

The cardiomyopathic Syrian hamster (BIO 14.6) is known to develop a genetically determined cardiomyopathy, with progressive development of congestive heart failure, resembling human congestive cardiomyopathies (2,3,25). We have recently demonstrated that a certain form of stress can accelerate the death of cardiomyopathic Syrian hamsters (18). Furthermore, we have shown that the sudden death of these hamsters was prevented by propranolol but not by atropine (18), suggesting an important role of the sympathetic nerves in the etiology of stress-induced cardiac sudden death of cardiomyopathic hamsters. These results have prompted us to explore neural mechanisms that could convey stress information that

Requests for reprints should be addressed to Nobuya Matsuoka, Department of Neuroscience, Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan.

leads to the excitation of the sympathetic nerves, and result in the sudden death of the cardiomyopathic hamsters.

On the other hand, dopamine in the brain is known to function as an important neural signal in the cascade of central response to stress (23). Indeed, the mesocortical and the mesolimbic dopaminergic systems are well known to be activated by various forms of stress (4,7,23,27). Moreover, recent evidence has suggested that central D_1-like ($D_{1/5}$) receptors among the diverse DA receptor subtypes may participate in the cascade of stress responses (5,19,22). From these findings, we hypothesized that an activation of the brain $D_{1/5}$ receptor could be etiologically involved in the disorders associated with stress.

Therefore, the aim of the present study was to clarify the role of dopamine receptor subtypes in stress-induced sudden death. The effects of $D_{1/5}$ receptor blockade by a highly selective blocker SCH23390 on sudden death were investigated using cardiomyopathic Syrian hamsters, and compared the results with those of haloperidol, a specific D_2-like receptor antagonist.

METHOD

Animals

The animals used here were 2-month-old BIO 14.6 cardiomyopathic hamsters and age-matched F1B healthy hamsters purchased from Canadian Hybrid Farms (Nova Scotia, Canada). Hamsters were housed individually in plastic cages in a temperature-controlled environment (22 ± 1°C) under a 12 L:12 D schedule, with lights off at 1500 h and given unlimited access to Purina mouse chow and tap water. The animals were accliminated 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

All experiments were performed according to a method described previously (18). Briefly, 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 1500 h. The stressed hamsters, including the healthy controls, were subjected daily to 1-h periods of supine immobilization at 4°C, during which they were immobilized by extending their four limbs and taping them to the corners of a small board and left in a refrigerator. The nonstressed healthy and cardiomyopathic hamsters were not immobilized, and just left in their housing cages outside a refrigerator for 1 h. Immediately before and after each stress session as well as the next morning after each session, the hamsters were checked for body weight 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 succumbed to sudden death, the maximum time allowed to elapse between death and autopsy was 10 h. Autopsy consisted of removal of the organs, and organs were then weighed quickly. The surviving animals were sacrificed at the end of the experiment 7 days after the last stress session, and autopsied as above.

Drugs

The drugs used here were SCH23390 hydrochloride [(+)-8-chloro-7-hydroxy-3-hydroxy-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-bezazepine HCl, Research Biochemicals Incorporated, Natik, MA) and haloperidol hydrochloride (Sigma Chemical Co., St. Louis, MO). SCH23390 was dissolved in physiological saline, and haloperidol was suspended in 0.5% methylcellulose. Both drugs were prepared just before the tests and given intraperitoneally in a volume of 2 ml/kg just prior to the immobilization stress for consecutive 5 days.

Statistical Analysis

All results were expressed as mean ± SEM. Statistical significance of differences in the changes in organ weights was calculated using one-way analysis of variance followed by Dunnett's multiple comparison post hoc test for dosed multiple groups, and was calculated by unpaired Student's *t*-test for two groups comparison between nonstressed and stressed animals. Mortality results were analyzed by Fisher's exact probability test. Cumulative surviving percents of hamsters during the course of the experiments were analyzed using generalized Wilcoxon test.

RESULTS

Effects of SCH23390 on Sudden Death Produced by Cold-Immobilization Stress to Cardiomyopathic Hamsters

No healthy hamsters with or without stress, or nonstressed cardiomyopathic hamsters succumbed during the course of the experiment (Fig. 1A). In contrast, six out of seven

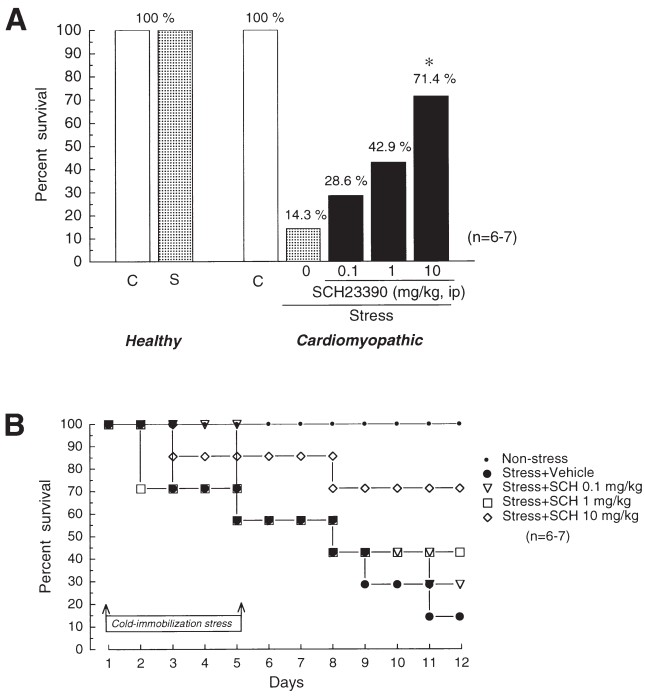

FIG. 1. (A) Effects of SCH23390 (0.1–10 mg/kg) on sudden death caused by immobilization stress in cardiomyopathic hamsters. Each value represents the percent of surviving hamsters. *$p < 0.05$; statistically significant compared to saline-treated stressed cardiomyopathic hamsters (by Fisher's exact probability test). Number in parentheses indicates the number of animals in each group. (B) Survival curves of unstressed and stressed cardiomyopathic hamsters receiving SCH23390 treatment. SCH23390 was administered IP just prior to the stress for 5 consecutive days in the first week. Saline was administered to the vehicle control group.

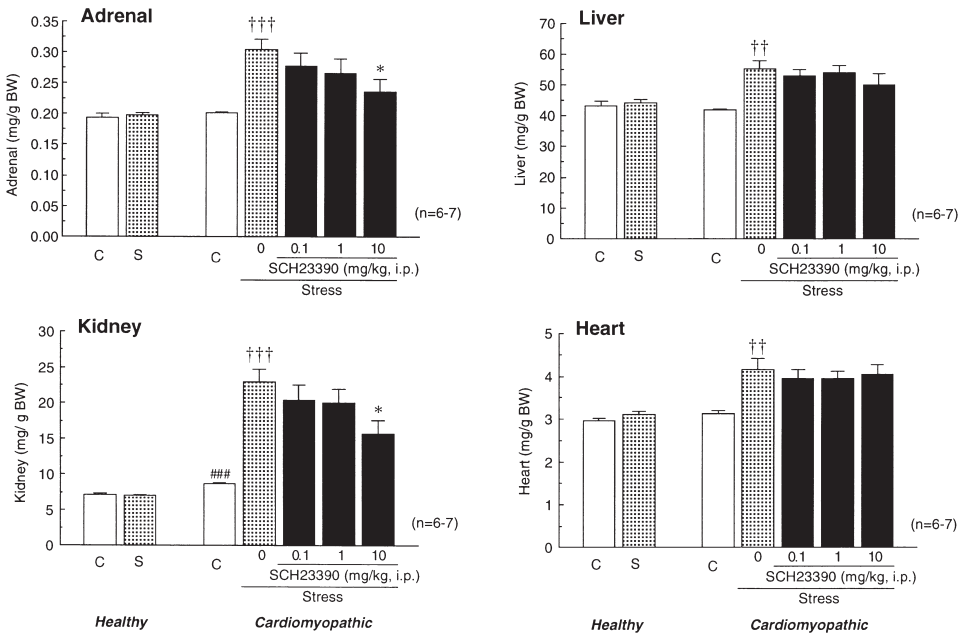

FIG. 2. Effects of SCH23390 on organ weights in stressed cardiomyopathic hamsters. Each column and bar represents the mean ± SEM. *$p < 0.05$; significantly different compared with the values of saline-treated stressed cardiomyopathic hamsters (by one-way analysis of variance followed by Dunnett's multiple comparison post hoc test). ++$p < 0.01$, +++$p < 0.001$; significantly different from the value of each corresponding non-stressed group (by unpaired Student's *t*-test). ###$p < 0.001$; significantly different from nonstressed healthy animals (by unpaired Student's *t*-test). Saline was administered to the vehicle control group.

stressed cardiomyopathic hamsters died, and there was a statistically significant difference between the mortality of the stressed and nonstressed groups of cardiomyopathic hamsters ($p < 0.01$ by Fisher's exact probability test). As shown in Fig. 1B, which represents the cumulative surviving percent of hamsters, three animals among the stressed-cardiomyopathic hamsters succumbed during the 5 days of stress sessions, and three animals died after the stress termination. The difference between the stressed and nonstressed groups of cardiomyopathic hamsters was statistically significant ($p < 0.01$ by a generalized Wilcoxon test).

SCH23390, a selective antagonist of $D_{1/5}$ receptors, was evaluated for its effect on the sudden death of stressed cardiomyopathic hamsters in an attempt to elucidate the role of $D_{1/5}$ receptors. Daily administration of SCH23390 (0.1–10 mg/kg, IP) during the stress period dose-dependently reduced the mortality seen in the stressed-cardiomyopathic hamsters with statistically significant changes in the mortality of the group dosed with 10 mg/kg SCH23390 compared to saline-treated stressed cardiomyopathic hamsters ($p < 0.05$ by Fisher's exact probability test).

Six days after the completion of the stress sessions, organ weights were measured, and the results were presented in Fig. 2. The heart, adrenal, and kidney weights were significantly ($p < 0.05$) increased in the stressed cardiomyopathic hamsters compared with the nonstressed cardiomyopathic animals; however, the stress caused no detectable changes in healthy animals.

Administration of SCH23390 significantly and dose-dependently prevented the increase in weights of the adrenal and kidney in the stressed-cardiomyopathic hamsters, with statistically significant ($p < 0.05$ by one-way analysis of variance followed by Dunnett's multiple comparison test) difference in the group dosed with 10 mg/kg of SCH23390 (Fig. 2). The drug minimally affected the weights of the liver and heart.

Effects of haloperidol on Sudden Death Produced by Cold-Immobilization Stress to Cardiomyopathic Hamsters

Haloperidol, a selective antagonist of DA receptors of D_2-like (D_2, D_3, D_4) families, was evaluated for its effect on the sudden death of the stressed cardiomyopathic hamsters. Figure 3 shows the mortality results. No healthy hamsters with or without stress, and no nonstressed cardiomyopathic hamsters succumbed during the course of the experiment. In contrast, five out of six of the stressed cardiomyopathic hamsters died, and the administration of haloperidol (0.1–10 mg/kg, IP) hardly affected the mortality seen in the stressed-cardiomyopathic hamsters.

When the effect of haloperidol was evaluated on the organ weights, the drug failed to affect the increases in adrenal and kidney weights of the stressed-cardiomyopathic hamsters (Fig. 4). The drug was also without effects on the weights of the liver and heart.

DISCUSSION

In accordance with our previous study (18) and earlier studies by Natelson et al. (20,26), the present study has shown that cold-immobilization stress exerted a lethal effect in cardiomyopathic Syrian hamsters but not in control healthy hamsters, suggesting that the stress had serious and even lethal

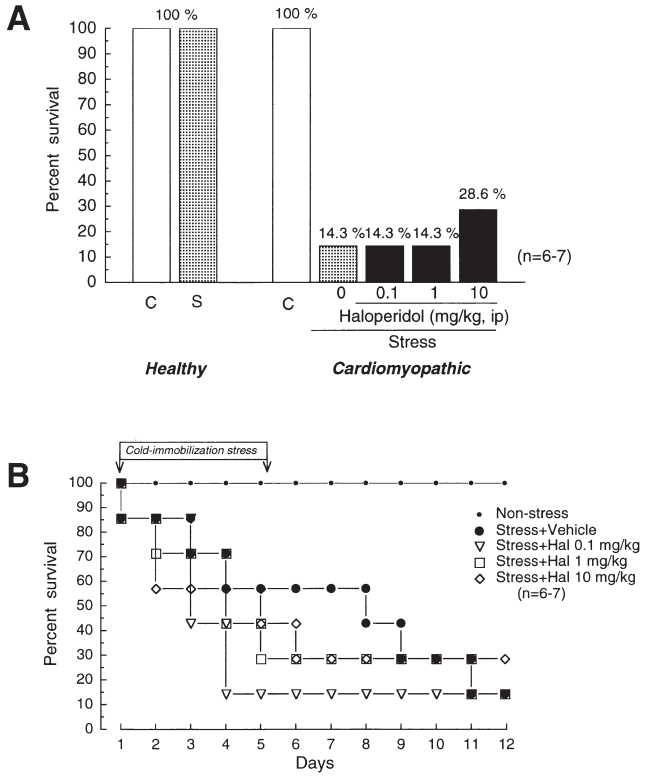

FIG. 3. (A) Effects of haloperidol (0.1–10 mg/kg) on sudden death caused by immobilization stress in cardiomyopathic hamsters. Each value represents the percent of surviving hamsters. Number in parentheses indicates the number of animals in each group. (B) Survival curves of unstressed and stressed cardiomyopathic hamsters receiving haloperidol treatment. Haloperidol was administered IP just prior to the stress for 5 consecutive days in the first week. Methyl-cellulose (0.5%) was administered to the vehicle control group.

consequences in cardiovascular dysfunction seen in cardiomyopathic hamsters with a covert heart disease (2,3,25). Our previous study with telemetry ECG recording demonstrated that severe arrhythmia was observed before sudden death in stressed-cardiomyopathic hamsters (18), implying that acute heart failure and/or lethal arrhythmia might be responsible for the death of the stressed cardiomyopathic hamsters. Supporting this view, heart and adrenal weights were markedly increased in the animals, as confirmed in the present study. Given the fact that the stress produced a marked increase in kidney weight and serum levels of alkaline phosphatase, urea nitrogen, and creatinine in the cardiomyopathic hamsters, the reduction in glomerular filtration rate was possibly related to a decrease in myocardial performance indicative of sudden ventricular dysfunction.

The major finding of the present study is that the dopamine $D_{1/5}$ receptor antagonist SCH23390 significantly and dose dependently prevented the sudden death induced by cold-immobilization stress in cardiomyopathic hamsters, whereas the D_2 receptor antagonist haloperidol failed to prevent the stress-induced sudden death. Our group previously demonstrated the specificity of SCH23390 and haloperidol on the subtypes of DA receptors in terms of their binding affinities in vitro; K_i values for inhibition of [^{3}H]SCH23390 to D_1-like receptors of SCH23390 and haloperidol were 0.56 and 165 nM, respectively, and those for [^{3}H]N-0437 binding to D_2-like receptors of SCH23390 and haloperidol were 35 and 2.1 nM, respectively (19). The dosages of SCH23390 employed here are also known to be specific for D_1-like receptors in vivo as well in terms of its occupancy of brain D_1 receptors labeled with [^{3}H]SCH23390 but inactive on D_2-like receptors labeled by [^{3}H]raclopride, and those of haloperidol are conversely highly specific for D_2-like receptors in vivo (1). These results taken together clearly demonstrate an involvement of $D_{1/5}$ receptors but not of D_2-like receptors in sudden death of stressed-cardiomyopathic hamsters.

We have previously proposed that an activation of the sympathetic nerves triggered by stress participates in sudden death as a consequence of increased incidence of heart failure and/or cardiac arrhythmia (18). Although the present study does not solely depict the precise mechanisms of $D_{1/5}$ receptors in participating in the stress-induced sudden death, one explanation is that central $D_{1/5}$ receptors could play a role in the mechanism by which stress information was conveyed from the brain to the sympathetic nerves (11,24). There has been accumulating evidence suggesting that the mesolimbic and mesocortical dopamine projections are strongly activated by various forms of stress (4,7,23,27). Giardino et al. (12) have shown that after chronic restraint stress dopamine D_1 receptors decreased in the nucleus accumbens, whereas dopamine D_2 receptors were not modified in any investigated area. Another example is that Fadda et al. demonstrated a pivotal role of limbic D_1 receptors in the generation of arousal and insomnia related to sleep deprivation induced stress (6,10). Bueno et al. recently found that SCH23390 administered intracerebroventricularly (ICV) but not sulpiride reduced the colonic motility stimulated by foot shock stress in rats, suggesting an involvement of central $D_{1/5}$ receptors in the response (5). Therefore, taken together, it would be conceivable to assume that the activation of central $D_{1/5}$ receptors could have contributed to the etiological cause of sudden death of stressed-cardiomyopathic hamsters in the present study.

The present studies, however, cannot rule out the possible involvement of the blockade of peripheral dopamine D_1-like receptors located at the sympathetic nerve or vascuratures in the heart, kidney, and adrenal in the preventive effect of SCH23390 on sudden death. For example, in the kidney, $D_{1/5}$ receptors are known to be located at the luminal and basolateral membranes of the proximal tubules rather than at the sympathetic nerve terminals innervating the renal blood vessels (15). Activation of the D_1-like receptors by means of dopamine or D_1 agonist like fenoldopam reportedly produces natoriuresis and diuresis. Future studies will be required to address the detailed mechanisms by which the blockade of kidney D_1-like receptors by SCH23390 resulted in the prevention of the sudden death in stressed-cardiomyopathic hamsters.

Our previous results determining plasma levels of NE (norepinephrine) and E (epinephrine) in this stress model strongly suggested the acceleration of peripheral sympathetic activity following the stress. Given the evidence that E is usually the predominant amine released from the adrenal and it has a much greater affinity for β-adrenoceptors than NE, circulating E could be involved in the sudden death of stressed-cardiomyopathic hamsters. In the present study, SCH23390 reduced the increase in the adrenal weight produced by stress, indicating the drug could effectively attenuate sympathetic nerve discharge. To address this hypothesis, detailed studies measuring plasma NE and E in stressed-cardiomyopathic hamsters with or without SCH23390 treatment are now in progress in our laboratory.

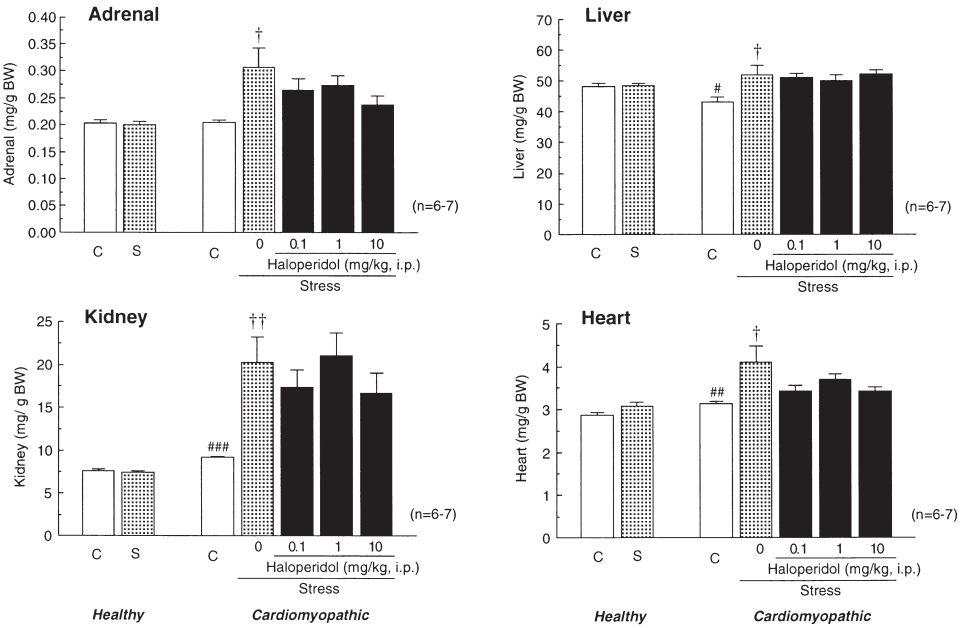

FIG. 4. Effects of haloperidol on organ weights in stressed cardiomyopathic hamsters. Each column and bar represents the mean ± SEM. $+p < 0.05$, $++ p < 0.01$; significantly different from the value of each corresponding nonstressed group (by unpaired Student's *t*-test). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$; significantly different from nonstressed healthy animals (by unpaired Student's *t*-test). Methyl-cellulose (0.5%) was administered to the vehicle control group.

In conclusion, the present studies have shown that the blockade of $D_{1/5}$ receptors by SCH23390 prevented the sudden death of cardiomyopathic hamsters induced by coldimmobilization stress, and suggest that $D_{1/5}$ receptors have an important role in the etiology of stress-induced cardiac sudden death of cardiomyopathic hamsters. The findings provide the first experimental evidence of potential therapeutic values of $D_{1/5}$ antagonists against cardiac sudden death associated with stress.

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