Effects of Some \( \beta \)-Adrenoceptor Antagonists on Orthostatic Hypotension in Repeatedly Cold- (SART-) Stressed Rats

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Received September 5, 2006; accepted November 15, 2006; published online November 17, 2006

Rats stressed by specific alternation of rhythm in temperature (SART) show various symptoms of disautonomia, increased pulse rates, continuous hypotension, and severe orthostatic hypotension (OH) when they are subjected to postural change. The OH symptoms are improved by muscarinic \( M_3 \)-receptor blockers. In the present study, effects of \( \beta \)-adrenoceptor blocking agents on OH in SART-stressed rats were investigated. Anesthetized rats were restrained on a board in the supine position, and direct blood pressure and ECG were measured automatically using Fluclet® Jr.2. Postural change was performed by raising the rat’s head up to a 60° angle for 4 min. Unstressed rats treated with hexamethonium showed large decrease in blood pressure, small reflex from the bottom of pressure and decreased tachycardia reflex, whereas isoproterenol showed little changes. In SART-stressed rats, isoproterenol alleviated the decrease in blood pressure in postural change, brought large reflex from the bottom of pressure and increased tachycardia reflex, whereas hexamethonium had little changes. Propranolol and atenolol induced the similar changes as those seen by hexamethonium. ICI-118,551, a selective \( \beta_1 \)-adrenoceptor antagonist showed large reflex from the bottom of pressure and increased tachycardia reflex in stressed rats, whereas little changes in unstressed rats. In conclusion, it was suggested that the hypotension in OH manifestation time of rats reflects the state of peripheral blood vessels, and \( \beta_1 \)-adrenoceptors played a role in compensatory tachycardia reflex and \( \beta_2 \)-adrenoceptors in blood pressure reflex. The circulatory regulation in SART-stressed rats seems to be poorly functioning in nervous reflex in postural changes.

Key words SART stress; orthostatic hypotension; \( \beta \)-adrenoceptor; autonomic nervous system; head-up tilt

Hypotension has not been recognized as a disease and has been neglected till now, because the symptoms are quiet and rarely life-threatening unlike those of hypertension. However, many people suffer from hypotension, and these people also suffer from orthostatic hypotension (OH). While the role played by stress in hypertension is often talked about, there are nearly no reports on the relationship between hypotension and stress and that between OH and stress just like there are nearly no reports on hypotension itself. We have been studying the relationship.1,2) We receive various kinds of stress in our everyday life. Among many stressors, those we talk about daily seem to be psychological stresses arising from jobs or personal relationships. The majority of these stressors are anger and hostility, as well as suppression and anxiety. Hypotensive patients often manifest symptoms when they have these psychological factors.3) Hypotension also occurs when patients are under excessive environmental stress, such as cold and heat. Many cases are thought to be induced not by organic injury but by the breakdown of circulatory regulation due to the dysfunction of the hypothalamo-autonomic nervous system.4) Various tests often find no abnormalities, when the breakdown of circulatory regulation due to autonomic dysfunction occurs in patients with normal circulatory functions. Head-up tilt test (HUT)5) is one method often used to induce OH in humans for the purpose of testing OH of undetermined origin and neurally mediated syncope (NMS). However, details of HUT vary by researchers, such as degree of HUT or the positive criteria of OH and NMS. The dose of isoproterenol, which is often used in HUT in order to increase the induction rate of NMS, also varies by researchers.6—11)

In the present study, the role played by the autonomic nervous system, especially \( \beta \)-adrenoceptors in OH was investigated by performing HUT to SART (specific alteration of rhythm in temperature)12)-stressed rats with various symptoms of autonomic imbalance13) and hypotension.1) There are many people who complain of poor physical condition at a season with severe temperature changes or at a change of seasons, such as early spring or the beginning of autumn. SART-stressed animals are created in simulation of such a state by rearing in an environment where temperature changes rapidly, intermittently and repeatedly between room temperature and low temperature.12,14) The animals are used widely in research as an autonomic imbalance model animal of systemic parasympathetic hypertonia and sympathetic hypotonia13) based on results of, the Aschner’s test, Mecholyl test and GSR (galvanic skin response) test.

SART-stressed rats have been reported to have increased heart rate,13) abnormal electrocardiogram (ECG),1) continuous hypotension1) and other circulatory changes.16,17) SART-stressed rats develop OH easily when subjected to HUT.1) It has also been reported that these symptoms are improved by muscarinic \( M_3 \)-receptor antagonists.18,19)

Effects of hexamethonium, an autonomic ganglion blocker, isoproterenol, a \( \beta \)-adrenoceptor stimulant and \( \beta \)-adrenoceptor antagonists on OH induced by HUT were studied using the above-described SART-stressed rats. Results of the study were obtained in mean blood pressure measured automatically using an electrocardiogram and blood pressure-waveform recognition frequency change analysis software (Fluclet®).

MATERIALS AND METHODS

Experimental Animals Male Wistar rats (Japan SLC Inc., Hamamatsu) aged 8—10 weeks and weighing 250—300 g at the start of the study, were used in accordance with ethical procedures following the guidelines for the care and

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use of laboratory animals issued by the Japanese government and the Japanese Pharmacological Society. The animals were housed in groups of three in a wire-net cage (38×25×17 cm) placed in a temperature- and light-controlled room (24±1 °C, with a 12-h light–dark cycle, lights on at 8:00 am, off at 8:00 pm) with free access to a standard diet (MF, Oriental Yeast, Tokyo) and tap water ad libitum.

Procedure for SART Stress Loading  According to the method by Hata et al.,1,4,6 three rats per group were alternately transferred to two cages, one of which was placed in a room at 24 °C and the other in a room at −3 °C every hour from 9:00 am to 4:00 pm and kept in a room at the low temperature from 4:00 pm to 9:00 am the next morning. Rats were allowed to eat and drink freely in both cages. This procedure was repeated for 6—8 d. On the morning of experiments, in order to eliminate direct effects of low temperature, the stressed rats were kept at room temperature (24 °C) for at least 30 min before the experiments.

Drugs Used  Drugs used were hexamethonium (Wako Pure Chemical Industries, Ltd., Osaka), a nonselective β-adrenoceptor stimulant isoproterenol (Sigma, St. Louis, MO, U.S.A.), a nonselective β-adrenoceptor antagonist propranolol, a selective β1-adrenoceptor antagonist atenolol, and a selective β2-adrenoceptor antagonist ICI-118,551 (all by Wako Pure Chemical Industries, Ltd., Osaka). Urethane (Sigma, St. Louis, MO, U.S.A.), heparin (Shimizu Pharmaceutical Co., Osaka), and ascorbic acid (Wako Pure Chemical Industries, Ltd., Osaka) were used. The above drugs were dissolved in 0.9% physiological saline at use, and concentrations were prepared so that doses were 0.1 ml/100 g when administered to rats. Isoproterenol was dissolved with ascorbic acid for prevention of oxidation, and injected continuously after standing, and turned to increase shortly thereafter, and recorded for 2 min before HUT was initiated.

Measurement of Blood Pressure and Heart Rate  Heart rate did not increase after standing and continued to decrease though blood pressure turned to increase, it took a little longer time, and the recovery was less than unstressed rats. Although blood pressure increased after standing, it took a little longer time, and the recovery was less than unstressed rats did. Heart rate in unstressed rats increased after standing and returned to that before standing after returning to the horizontal position. In SART-stressed rats, heart rate did not increase but decreased after standing and continued to decrease for a while without recovering after returning to the horizontal position. The increase due to compensatory tachycardia reflex to decreases in blood pressure was not observed.

Table 1 shows mean blood pressure and heart rate at rest and during stress. In unstressed rats, blood pressure decreased maximally immediately after standing, and recovered to the horizontal position within −10 mmHg. In SART-stressed rats, blood pressure decreased maximally immediately after standing.

RESULTS  Experimental data are expressed as mean±S.E.M. Significant difference from control rats were tested in Student’s t-test between 2 groups and one-way analysis of variance (ANOVA) or Tukey’s test among 3 groups or more. p values less than 0.05 (p<0.05) were considered to indicate significant difference.

Effects of SART Stress on Blood Pressure and Heart Rate at the Time of HUT  Time-related changes in blood pressure and heart rate in HUT are shown in Fig. 1. In unstressed rats, blood pressure decreased maximally immediately after standing, and returned to the horizontal position within −10 mmHg. In SART-stressed rats, blood pressure decreased maximally immediately after standing. The decrease was less than that in unstressed rats. Although blood pressure increased after standing, it took a little longer time, and the recovery was less than unstressed rats did. Heart rate in unstressed rats increased after standing and returned to that before standing after returning to the horizontal position. In SART-stressed rats, heart rate did not increase but decreased after standing and continued to decrease for a while without recovering after returning to the horizontal position. The increase due to compensatory tachycardia reflex to decreases in blood pressure was not observed.

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Observation confirmed that data in Fig. 1 and Table 1 based on mean blood pressure and heart rate that were mea-
Effects of hexamethonium and isoproterenol on HUT are marked automatically by Fluclet, were similar to data based on systolic blood pressure obtained in analog processing reported in previous studies.1,2,18,19)

Roles Played by the Autonomic Nervous System on OH
Figure 2 shows changes in blood pressure and heart rate in HUT following administration of hexamethonium. In unstressed rats, decreases in blood pressure after standing were greater than those without hexamethonium, the time-related curve moved downward, and the recovery of blood pressure was poor. Also the tachycardia after standing was observed to be weak. In SART-stressed rats, changes in blood pressure were the same as those in control group without hexamethonium, indicating the rats were not affected by hexamethonium. However, bradycardia after standing became less.

Figure 3 shows the time-related changes in blood pressure and heart rate following administration of isoproterenol. Whereas the blood pressure in unstressed rats was not affected by isoproterenol, the pressure curve in stressed rats moved upward and the recovery curve was similar to that in unstressed rats. As for changes in heart rate, the compensatory tachycardia reflex in unstressed rats became weak and bradycardia in stressed rats became less. These two curves of the changes in heart rate resemble each other.

Effects of hexamethonium and isoproterenol on HUT are summarized in Fig. 4. In unstressed rats, MD and AUC increased following administration of hexamethonium. Hardly any changes occurred by isoproterenol. In stressed rats, on the other hand, hexamethonium hardly affected any indices, and isoproterenol decreased MD, increased %reflex markedly, decreased AUC markedly and improved OH.

Effects of β-Adrenoceptor Antagonists on OH
Effects of β-adrenoceptor antagonists on the blood pressure and heart rate at tilting are shown in Fig. 5 and the indices of OH based on Fig. 5 are summarized in Fig. 6.

As shown in Fig. 5A, following administration of propranolol, both curves of the blood pressure and heart rate moved downward in unstressed rats. The recovery of blood pressure became poor, and the compensatory tachycardia reflex became weak, that is, MD and AUC increased, indicating the tendency of exacerbation. In stressed rats, the blood pressure curve showed no change, and MD and AUC were not affected. However, the %reflex showed the increasing tendency. The changing curve of heart rate moved upward and bradycardia became less.

As for effects of atenolol (Figs. 5B, 6), in either unstressed or stressed rats, both indices of blood pressure and heart rate showed similar changes as those caused by propranolol.

Figure 5C shows effects of a selective β<sub>b</sub>-adrenoceptor antagonist ICI-118,551. Stressed rats showed large drop in blood pressure immediately after standing and weak bradycardia after standing whereas unstressed rats were hardly affected by the drug. The increased %reflex and tendency of decreases in AUC were observed.
Fig. 4. Effects of Hexamethonium and Isoproterenol on Tilting Parameters in Rats

Data show the mean±S.E.M. from 9 hexamethonium- and 10 isoproterenol-treated unstressed rats (U, open and stripe columns) and 10 SART-stressed rats (S, closed and hatched columns). *p<0.05, **p<0.01 vs. respective unstressed group. *p<0.05, **p<0.01 vs. respective control group (Tukey’s test).

Fig. 5. Effects of β-Adrenoceptors on Time-Related Changes in Blood Pressure and Heart Rate Caused by Tilting in Anesthetized Rats

Open symbols: unstressed rats, closed symbols: SART-stressed rats. Triangles: Drug-treated group. Data show the mean±S.E.M. Propranolol, 1 mg/kg (n=7 unstressed and 11 SART-stressed rats), was i.v. administered 45 min before tilting. Atenolol, 1 mg/kg (n=5 unstressed and 7 SART-stressed rats) and ICI-118,551, 0.5 mg/kg (n=4 unstressed and 5 SART-stressed rats) were i.v. administered 15 min before tilting.
DISCUSSION

OH in humans occurs often in hypotensive people, in addition to youth at puberty and elderly people. OH is regarded as one of symptoms of autonomic imbalance and accompanied by circulatory symptoms such as dizziness on standing up, vertigo and cerebral anemia, and autonomic symptoms such as loss of appetite, malaise and headache. In a broad sense, OH belongs to orthostatic dysregulation (OD) along with NMS (neurally mediated syncope) and others. Kaufmann states that severe OH induces NMS. Kenny mentioned OH as the most common cause of NMS.

It has been reported that SART-stressed rats are in the state of continuous hypotension and tachycardia, and have greater drop in blood pressure at standing (MD), lower %reflex and tend to have OH more easily. The compensatory reflex mechanism, which should work against hypotension caused by tilting, is thought to be abnormal in the stressed rats. In the present study, the role played by the autonomic nervous system, particularly β-adrenoceptors, was investigated. Blood pressure and heart rate in this report were measured automatically using an “electrocardiogram and blood pressure waveform recognition frequency change analysis software” (Fluclet) unlike in previous reports which used analog measurement. Basic items had the similar results as in the previous reports.

Effects of drugs are summarized in Fig. 7 for the discussion. Following administration of hexamethonium which has autonomic ganglion blocking action, no apparent changes occurred in SART-stressed rats other than changes induced by stress, whereas the OH symptom exacerbated in unstressed rats. Conversely, following administration of isoproterenol, OH symptoms in stressed rats changed toward improvement, whereas hardly any change occurred in unstressed rats. The patterns of actions of both drugs suggested that sympathetic dysregulation and hypotonicity induced the manifestation of severe OH symptoms in SART-stressed rats, corresponding to the report by Lee C.H. et al. that the most typical or severe OH occurred in the presence of extensive disorders of the sympathetic nervous system.

In summarization of β-adrenoceptor antagonists, in unstressed rats, propranolol and atenolol increased MD and
AUC, turning OH toward exacerbation. A selective β₁-adrenoceptor antagonist ICI-118,551 had little effects. In SART-stressed rats, neither of β₁-adrenoceptor antagonists had hardly any effect. Only %reflex increased following ad- ministration of propranolol and ICI-118,551, indicating the tendency of improvement of OH symptoms.

The above results suggested that β₁-adrenoceptors play a role in %reflex and the sensitivity of β₁-adrenoceptors was elevated in SART-stressed rats. The theory does not contra dict a report [4] that peripheral blood vessels are diluted and the volume of circulatory blood is increased. Changes in blood pressure at tilting resemble those of NMS patients in syncope, corresponding well to a report [28] that the sensitivity of sympathetic activity tachycardia state and it is possible that the myocardial syncope, corresponding well to a report 28) that the sensitivity of sympathetic activity.

Regarding changes in heart rate, administration of hexamethonium and isoproterenol suppressed compensatory tachycardia reflex at tilting in un stressed rats and decreased bradycardia in SART-stressed rats. However, in view of changes in blood pressure, hexamethonium induced OH in un stressed rats, and the changes in blood pressure resembled those in SART-stressed rats at tilting. Isoproterenol improved OH in SART-stressed rats. These facts suggested that SART-stressed rats are in a decreased state in sympathetic tonicity. As mentioned above, continuous injection of isoproterenol at the dose that affected little un stressed rats improved OH in SART-stressed rats. The manifestation is thought to be stimulation to myocardial β₁-adrenoceptors, not stimulation to β₂-adrenoceptors of blood vessels which are already dilated. Also, peripheral blood vessels in SART-stressed rats which are in the dilated state, cancel HUT-induced vasodilatation, causing the difficulty of constriction by reflex activation of sympathetic nerves. In such a state, the pretreatment with β₂adrenoceptor antagonists seems to lead to improvement in reflex function.

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