Effects of Psychological Stress on Autonomic Control of Heart in Rats

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Abstract: The aim of this study was to examine the effects of psychological stress on autonomic control of the heart in rats. For this purpose, we evoked anxiety-like or fear-like states in rats by means of classical conditioning and examined changes in autonomic nervous activity using an implanted telemetry system and power spectral analysis of heart rate variability. Anxiety-like states resulted in a significant increase in heart rate (HR), low frequency (LF) power, and LF/HF ratio, with no change in high frequency (HF) power. Fear-like states resulted in a significant increase in HR and a significant decrease in HF power with no significant change in both LF power and LF/HF ratio, although LF/HF ratio increased slightly. These results suggest that autonomic balance becomes predominant in sympathetic nervous activity in both anxiety-like and fear-like states. These changes in rats correspond to changes which are relevant to cardiovascular diseases in humans under many kinds of psychological stress. Therefore, the experimental design of this study is a useful experimental model for investigating the effects of psychological stress on autonomic control of the heart in humans.

Key words: power spectral analysis of heart rate variability, psychological stress, rat, telemetry system

Introduction

Psychological stress is a risk factor increasing cardiovascular morbidity and mortality in humans [17, 18], and studies have emphasized a relationship between psychologically stressful stimuli and electrical activity of the heart [8, 9, 16, 20]. The effects of psychological stress on such electrical activity of the heart are largely mediated by the autonomic nervous system [20, 21]. Therefore, it seems to be important to investigate the effects of psychological stressors on autonomic control of the heart.

Although rats have been used regularly for studies of the autonomic nervous system, it is difficult to examine responses of autonomic control of the heart to psychological stressors in rats. The main reason is that the conscious and unrestrained state is a necessary condition for studying the effects of psychological stressors on autonomic control, and it is also difficult to record valid cardiovascular parameters in rats because of tech-
nical problems. In recent years, an implanted telemetry system has allowed reliable recordings of electrocardiograms (ECG) from freely behaving rats. Moreover, power spectral analysis of heart rate variability (HRV) has been performed for assessment of autonomic nervous activity in rats. Earlier studies suggested that these methods are powerful tools for obtaining valid cardiovascular parameters of autonomic control of the heart in rats [10, 11].

The aim of this study was to examine the effects of psychological stress on autonomic control of the heart in rats. For this purpose, we evoked a conditioned anxiety-like state in rats using acute physical stress stimuli as unconditioned stimuli, and performed power spectral analysis of HRV from ECG recorded using the telemetry system. In addition, we compared differences in changes of cardiovascular parameters between the anxiety-like state and the fear-like state, evoked just after the acute physical stress stimuli, in the rats.

Materials and Methods

Animals

Seven male Long-Evans rats (Saitama Experimental Animals Supply Co., Ltd., Saitama, Japan), initially weighing 330–390 g, were used in the experiment. All rats were kept individually in wire-topped transparent cages (41 × 25 × 18 cm) with wood shavings for bedding, and the cages were placed in an incubator (MIR-553, Sanyo, Tokyo, Japan) maintained at constant temperature (24°C). Animals were provided with water and food ad libitum and kept on a 12-h light-dark cycle, with lights off at 8:00 p.m.

The experimental procedures followed the guidelines of “Policies Governing The Use of Live Vertebrate Animals” by the University of Tokyo and the 1996 edition of “The National Institutes of Health Guide for The Care and Use of Laboratory Animals”.

Surgery

A telemetry ECG transmitter was implanted in each rat. The body of the transmitter was placed the dorsal subcutanea and paired wire electrodes of the transmitter were fixed under the skin of the dorsal thorax near the right atrium and the apex cordis. Surgery was performed under pentobarbital sodium anesthesia (40 mg/kg, intraperitoneally). Ten days after surgery, rats were used in the experiment.

Experimental apparatus and procedures

Two different stressful states, stress-expecting state and stress-received state, were evoked by means of classical conditioning in the experiment. The experimental testing schedule and protocol are outlined in Fig. 1. The experiment was conducted for 13 consecutive days for each subject. Each session was conducted for 30 min in a day between 10 p.m. and 2 a.m. under dim red illumination (20W).

From Day 1 to Day 10, each subject was moved to the experiment room from its home cage and placed in a transparent test chamber (33 × 24 × 24 cm) for 30 min in order to habituate it to the experiment context; no unconditioned stimulus was delivered. The test chamber was housed in a larger sound-proof box (85 × 60 × 70 cm) with a transparent window. On Day 10, a signal-receiving board (RA1610, Data Science) was set under the same test chamber, and a video camera (DCR-VX1000, SONY, Tokyo, Japan) was placed in front of the window of the box. ECG signals were recorded for the whole session and stored on a computer using an ECG processor (Softron, Tokyo, Japan). Behavior of the rat was simultaneously recorded using the video camera.

From Day 11 to Day 13, each rat was moved to the test chamber as stated above. Such exposure to the context, named “cage-switch stimulus”, was performed as conditioned stimulus. Ten minutes after the cage-switch stimulus, each subject was presented with air jet stress as unconditioned stimulus for one minute, during which the subject was continuously given wind pressure (20 psi, directed at the head from a distance of 3 cm; an intensity was strong enough to part the fur on the rat’s head) using an air pump, and left in the test chamber during the rest of the session. Training sessions were conducted for 2 days and ECG signals and behaviors were recorded on Day 13.

As the conditioning procedures were repeated, we anticipated that a stress-expecting state, like “anxiety”, was evoked immediately before the unconditioned stimulus, and a stress-received state, like “fear”, was evoked immediately after the unconditioned stimulus. Therefore, the data recorded for three minutes before (between 7 and 10 min after the beginning of the session) and three minutes after (between 11 and 14 min after the beginning of the session) unconditioned stimu-
lus were selected for analysis from the whole data recorded on Day 13. In addition, in the same way the data selected from the whole data recorded on Day 10 were analyzed as control data.

**Power spectral analysis of HRV**

An off-line analysis was performed on an ECG processor analyzing system (Softron, Tokyo, Japan) using the recorded ECG data stored on a computer. First, R waves were identified, and both heart rate (HR) and the R-R interval tachogram as the raw HRV were calculated. From the tachogram, data sets of 512 points were resampled at 80 msec. Then, we applied each set of data to the Hamming window and the fast Fourier transform (FFT) to obtain the power spectrum of the fluctuation. For the frequency range of low frequency (LF) and high frequency (HF) in the power spectrum, we set LF at 0.04–1.00 Hz and HF at 1.00–3.00 Hz according to earlier studies [10, 11]. The LF region is considered a measure of both sympathetic and parasympathetic activity. The HF region is associated with respiratory sinus arrhythmia and is almost exclusively associated with parasympathetic activity. The LF/HF ratio was used as a measure of sympathovagal balance.

**Analysis of behaviors**

The videotape recorded for each subject was replayed and analyzed. To examine locomotor activity during each test session, duration of walking and rearing was summed as total locomotor activity: walking was defined as movements involving all four limbs; rearing was defined as raising front paws from the chamber floor and either placing them on the side of the chamber or placing them in front of the body. In addition, duration of freezing responses during each test session was summed as an index of being under stress. Freez-
ing in this study was defined as immobile posture with cessation of skeletal and vibrissae movement except during respiration.

Statistical analysis

All data are displayed as means ± standard error of mean (S.E.M.). Statistical analysis of data was performed using the paired t-test. A value of P<0.05 was considered the criterion for statistical significance.

Results

Power spectral analysis of HRV

Data of power spectral analysis of HRV in stress-expecting states are shown in Fig. 2. In comparison with controls, stress-expecting states showed a significant increase in HR, LF power and LF/HF ratio, with no change in HF power.

Data of power spectral analysis of HRV in stress-received states are shown in Fig. 3. In comparison with controls, stress-received states showed a significant increase in HR and a significant decrease in HF power, with no significant change in both LF power and LF/HF ratio, although LF/HF ratio increased slightly.

Analysis of behaviors

Behavioral data are shown in Table 1. There was no significant difference in locomotor activity between each control session and stress-expecting states or stress-received states. No freezing response was observed in control sessions. On the other hand, considerable duration of freezing responses was observed in both stress-expecting states and stress-received states, with no significant difference between the two.
Discussion

We evoked stress-expecting or stress-received states in rats by means of classical conditioning and examined changes in autonomic nervous activity using an implanted telemetry system and power spectral analysis of HRV. Both states resulted in a significant increase in HR compared with controls, but there was much difference in HRV parameters (LF power, HF power, and LF/HF ratio) between the two states. In stress-expecting states, LF power and LF/HF ratio increased significantly with no change in HF power compared with controls, indicating that the sympathetic activity increased with no change in the parasympathetic activity. In stress-received states, HF power decreased significantly with a slightly increase in LF/HF ratio, indicating that the parasympathetic activity decreased with a little dominant sympathetic regulation.

Although HRV can be influenced by physical activity [2], there was no need to take such an effect into consideration in this study because of little difference in locomotor activity between control and stress-expecting or stress-received states. There were many freezing responses in stress-expecting or stress-received states, although no freezing response was observed in control sessions. Therefore, we concluded that stressful conditions were evoked in the two states because it has been shown that freezing is a typical behavioral response to stressful conditions [3].

In the present study, it was suggested that only psychologically stressful conditions were evoked in rats because they received no physical stressful stimuli during the stress-expecting or stress-received states. The results of power spectral analysis of HRV in this study indicate that dominant sympathetic regulation was induced under both psychologically stressful conditions. Such changes in sympathovagal balance might be attributed mainly to an increase of sympathetic activity in the stress-expecting state, but the same changes of autonomic regulation might be attributed mainly to a decrease of parasympathetic activity in the stress-received state. An explanation of these findings is that the difference in character between the two emotional conditions might cause different autonomic responses. Classical fear conditioning occurs when the animal learns that a certain environmental stimulus, such as cage-switch stimulus, predicts an aversive event [13]. Moreover, air jet stress has been recently utilized for aversive stimulus to investigate the effects of stressful stimuli on the cardiovascular function in rats [14, 15]. Therefore, it is suggested that the stress-expecting state in this study might be equivalent to “anxiety (conditioned fear)” evoked by thinking about aversive situations in the future. In contrast, the stress-received state in this study might be equivalent to “fear (unconditioned fear)” evoked just after stressful stimuli. However, further studies will be needed to clarify the detailed physiological mechanisms behind the different autonomic responses.

Some studies have reported that changes in cardiovascular parameters are very relevant to cardiovascular diseases in humans under psychologically stressful conditions. It was shown that LF power and LF/HF ratio increased in Type A females during a psychomotor task compared with type B females [19]; Type A behavior was defined as a potential risk factor for coronary heart disease in the late 1950s [7]. In addition, HR and LF/HF ratio increased with withdrawal of HF power just after an earthquake [12], considered an acute stress stimuli for humans, and it was suggested that the changes in HRV parameters were important in the induction of myocardial ischemia [12]. Moreover, some mental disorders have been shown to be associated with increasing cardiovascular morbidity [1, 4, 6]. A point in common between such mental diseases is dominant sympathetic regulation in sympathovagal balance, and the main reasons of such dominance in sympathetic regulation have been attributed to an increase of sympathetic activity and/or a decrease in parasympathetic activity [5, 6]. These results correspond with the changes in cardiovascular parameters evoked in rats under the psychologically stressful conditions of this study.

In conclusion, we clearly showed the effects of psychological stress on autonomic nervous activity in rats. The results also suggest the possibility of distinction between two different emotional states in rats: “anxiety” and “fear”. We believe that this experimental model will be useful for investigating the effects of psychological stress on autonomic control of the heart leading to understanding of the details of relevance between cardiovascular disorders and psychologically stressful conditions in humans.
References


