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Acute restraint stress provokes sudden cardiac death in normotensive rats and enhances susceptibility to arrhythmogenic effects of adrenaline in spontaneously hypertensive rats ☆

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ABSTRACT

Background: A high incidence of cardiovascular events and sudden cardiac death (SCD) has been reported following unexpected acute psychosocial stress. The possible pathways by which acute restraint stress (ARS), a kind of acute psychosocial stress, leads to SCD were determined.
Methods: Using 16-week-old male normotensive Wistar Kyoto rats (WKY, n = 24) as controls and spontaneously hypertensive rats (SHR, n = 24) as the hypertensive subjects with left ventricular hypertrophy (LVH), we assessed ARS-related incidence of SCD, cardiac and myocardial autonomic nervous system dysfunction, gap junction connexin-43 (Cx43) channel remodeling, and ventricular repolarization abnormality, based on electrocardiography, an adrenaline test, heart rate variability (HRV), and reverse transcriptase polymerase chain reaction analyses. Rats with ARS were introduced into restrainers that allowed head, limb, and tail movement.
Results: In normotensive hearts without LVH, ARS induced a higher incidence of SCD attributed to lethal bradycardia, increased cardiac and myocardial sympathetic activation, and gap junction Cx43 channel remodeling, as evidenced by the increases in the ratio of low-frequency and high-frequency powers in HRV, the ratio of myocardial neuropeptide Y (NPY) and acetylcholinesterase (AChE) mRNA expressions, and the up-regulation of LV Cx43 mRNA expression; in hypertensive hearts with LVH, ARS enhanced susceptibility to the malignant arrhythmogenic effects of the adrenaline test (a kind of sympathetic stimuli) accompanied by abnormal ventricular repolarization, as evidenced by increased incidence of ventricular tachycardia and/or ventricular fibrillation during the adrenaline test and prolonged QTc immediately after ARS.
Conclusions: ARS may trigger cardiac and myocardial sympathetic predominance, and then induce gap junction Cx43 channel remodeling, finally leading to lethal bradycardia in normotensive WKY. ARS-induced abnormal ventricular repolarization may be responsible for ARS-enhanced susceptibility to sympathetic stimulation in SHR with LVH. Expressions of myocardial NPY, AChE, and Cx43 genes, HRV, QTc and LVH measures showed diagnostic and prognostic potential for predicting ARS-induced SCD.

1. Introduction

High incidences of cardiovascular events and sudden cardiac death (SCD) have been reported following unexpected acute psychosocial stress [1–6]. The acute psychosocial stress caused by immobilization or restraint was associated with a higher incidence of arrhythmia than nonsocial stress [7]. Immobilization or restraint, alone or in combination with other stimuli, induced myocardial necrotic lesions in several animal species, resulting in the release of cytosolic enzyme activities to blood plasma [8]. Restraint stress was shown to induce heart damage related to catecholamine [9] while β-blockers reduced enzyme activities in the restraint stress model [10]. In humans, it was shown that a

Abbreviations: SCD, sudden cardiac death; ARS, acute restraint stress; VT, ventricular tachycardia; VF, ventricular fibrillation; LVH, left ventricular hypertrophy; QTc, corrected QT interval; Cx43, connexin-43; HRV, heart rate variability; CAVB, complete atrioventricular block; WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; PSD, power spectral density; VLF, very low frequency band in HRV; LF, low frequency band in HRV; HF, high frequency band in HRV.

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β-blockade reduced both tissue injury and supraventricular tachycardia induced by acute psychosocial stress [11].

A recent report summarized the various possible pathways by which psychological stress and emotional upset may lead to sudden death [12]. The psychosocial stress-induced neurovegetative changes include the hemodynamic response, autonomic alterations, and neuroendocrine activation as well as inflammatory and prothrombotic responses, all of which are known to have negative influences on the cardiovascular system [1] and may be responsible for the malignant arrhythmia-induced death during psychosocially stressful events [12]. However, the mechanisms underlying psychological stress and emotional upset, such as acute restraint stress (ARS)-related SCD, have not yet been pinpointed.

SCD, a high-priority public health problem necessitating a multi-pronged approach for treatment and prevention, entails a 50% rate of overall cardiovascular mortality [13]. Even with advanced first responder systems, only ~5% of those who suffer out-of-hospital cardiac arrest will ultimately survive [14,15]. Ventricular tachycardia (VT) and ventricular fibrillation (VF), are major causes of SCD [15–17], along with various arrhythmogenic factors such as left ventricular hypertrophy (LVH) [17], abnormalities in autonomic tone [18] and acute psychosocial stress [12]. LVH due to hypertension is characterized by several electro-physiological abnormalities including prolonged duration of the action potential, decreased resting membrane potential, heterogeneous recovery following depolarization and slowed conduction velocity [17–20]. QTc is used as a marker of abnormal ventricular repolarization; it is also one of the potential predictors of SCD in LVH [20] because of its relationship to gap junction connexin-43 (Cx43) channel remodeling [21,22].

Dysfunction of the autonomic nervous system is thought to play an important role in the development of malignant ventricular arrhythmias [23,24], and heart rate variability (HRV) has been recognized as a significant prognostic, noninvasive marker of regional cardiac autonomic nervous system function [25]. Our previous study showed that malignant ventricular arrhythmias were related to a regional cardiac sympathovagal balance shift in an acute ethanol withdrawal rat model using HRV analysis [26]. ARS is an unavoidable stress model, which can elicit several psychosocial and autonomic responses including increases in arterial pressure and heart rate [27,28], the elevation of skeletal muscle vasodilatation and cutaneous vasoconstriction resulting in a drop in skin temperature, and an increase in body temperature [29]. Both circulating and brain catecholamine are involved in the autonomic response elicited by ARS [30].

At present, limited data are available on the ARS-induced malignant arrhythmias such as VF and VF. Moreover, the effects of ARS-induced cardiac autonomic nervous system dysfunction and the resulting ventricular repolarization abnormality on the inducibility of malignant arrhythmias are unclear. Accordingly, in the present study, we used normotensive Wistar Kyoto rats (WKY) as controls and a hypertensive backcross of WKY, the spontaneously hypertensive rats (SHR), as hypertensive subjects with LVH to test hypotheses regarding ARS-induced SCD via cardiac and myocardial autonomic nervous system dysfunction, ventricular repolarization abnormality, and gap junction Cx43 channel remodeling. SHR has been studied extensively as a model of LVH due to hypertension [31].

2. Materials and methods

2.1. Animals

Eight-week-old male WKY (n = 24) and SHR (n = 24) were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). All animals were housed in a climate-controlled room (22 ± 2 °C), with a 12-h:12-h light/dark cycle and fed standard rat pellets and tap water ad libitum. Then, at 16 weeks of age, they were randomly divided into groups with (WKY, n = 18; SHR, n = 18) or without (WKY, n = 6; SHR, n = 6) ARS.

All experiments were approved by the ethics committee on animal experiments of the Yamaguchi University School of Medicine and were controlled by the committee's guidelines for animal experiments. The present research conforms in full to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996).

2.2. Electrocardiographic telemetry and electrocardiography (ECG) recording

We used the same protocol for electrocardiographic telemetry as that of our previous study [26]. Briefly, rats were anesthetized with ~2% isoflurane in oxygen, and an ECG transmitter (Transoma Medic al Data Sciences International, St Paul, MN, USA) was placed into the abdominal cavity with subcutaneous electrodes in a lead II configuration. ECG data was collected and recorded by Dataquest software (Transoma). Malignant ventricular arrhythmia was defined as ventricular tachycardia (VT) or ventricular fibrillation (VF). Ventricular extrasystole was defined as ventricular contraction without atrial depolarization. VT was defined as more than 6 consecutive ventricular extrasystoles; VF was characterized by a loss of synchronicity on ECG plus decreased amplitude; lethal bradycardia was defined as SCD attributed to bradycardia; and CAVB was defined as having P waves that bear no constant relation to the QRS complexes, with the atrial rate being faster than the ventricular rate.

2.3. QTc and HRV analysis

ECG, which was used for the QTc and HRV analysis, was off-line analyzed using Physiostat ECG analysis software, version 7.3.7 (Transoma). All arrhythmic events and compensatory pauses were confirmed and excluded manually before ECG and HRV analysis using the ECG Analysis and HRV Module software (AD Instruments Pty Ltd. Castle Hill, NSW, Australia).

QT intervals were measured from the beginning of the QRS complex to the end of the T wave, and were rate-corrected using Bazett's formula (the QT interval divided by the square root of the heart rate) and expressed as QTc based on the 5-min stable ECG segments of sinus rhythm. QTc was used as the marker of abnormal ventricular repolarization in the present study.

The HRV parameters were assessed on the basis of a short-term ECG recording (5 min). A number of measures derived from the beat-to-beat fluctuations can be obtained from HRV. These measures were simply developed using the time-and/or frequency-domain analyses, such as the calculation of the standard deviation and the power spectral density (PSD) of the HR fluctuations. In the time-domain analysis, it has been widely accepted that the standard deviation of the normal-to-normal interval may serve as diagnostically useful HRV indices. As for the frequency-domain analysis, the conventional fast Fourier transform, in which the individual RR intervals stored in the computer are transformed into bands with different spectral frequencies (very low frequency band (VLF), the low frequency band (LF) and the high frequency band (HF)), is usually applied for PSD calculation [25].

Time- and frequency-domain parameters were measured according to the recommendations of the European Society of Cardiology and North American Society of Pacing and Electrophysiology [32]. However, only frequency-domain parameters, calculated using the fast Fourier transform of the RR interval time series, were used in the present study. Two frequency bands were defined, a