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Electrocardiographic Evaluation of Acute Restraint and Cold Restraint Stress Response in Rats

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ABSTRACT

In this study, we investigated the effects of acute restraint stress and cold restraint stress on electrocardiographic (ECG) parameters in rats. A total of 18 male Wistar albino rats aged 8-10 weeks weighing 180-220 g were divided into three equal groups (n=6/group). No stressors were administered to the rats in the control group. The rats in the restraint group were kept in a restrainer for 2 hours. The rats in the cold restraint group were kept at +4°C for 2 hours in restrainer. ECG was recorded under ketamine and xylazine anesthesia in rats with the Televet II ECG device (Kruuse, Germany), and the Televet 100 program (version® 7.0.0, Kruuse, Heusenstamm, Germany) was used to analyze the leads II. There was no significant difference between the groups in the analysis of PR, QT, QRS intervals, and R-wave amplitude ($p > 0.05$). However, the heart rate was significantly higher in the cold restraint group than in the control group ($p < 0.001$) and restraint group ($p < 0.01$). In addition, the RR interval was significantly lower in the cold restraint group than in the control group ($p < 0.001$) and restraint group ($p < 0.01$). The QTc was increased in rats exposed to cold restraint stress compared to the control group ($p < 0.01$) and restraint group ($p < 0.05$). In conclusion, acute cold restraint stress may cause more pronounced ECG change than restraint stress.

Keywords: Acute Restraint Stress, Acute Cold Restraint Stress, ECG, Rats

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Introduction

Stress is essentially a psychological and physical response to a disturbance in homeostasis. Eustress refers to beneficial stress, whereas distress refers to harmful stress. If the stress is extreme, the organism's homeostatic systems fail, and its survival is jeopardized. Under these conditions, stress generates a wide spectrum of physiological changes known as general adaption syndrome (GAS). Stressors are the stimuli that lead to GAS and might range from physical to psychological factors (Selye, 1973). Acute stress causes a series of biological reactions, mostly by activating two primary pathways, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adreno-medullary system (SAS) (Bali and Jaggi, 2015; Viljoen and Panzer, 2007). SAS can stimulate the central noradrenergic system in response to stress stimuli and has peripheral and central connections with the HPA axis (Viljoen and Panzer, 2007). Responses to stress, on the other hand, can be influenced by brain areas other than the hypothalamus, such as the hippocampus, amygdala, and prefrontal cortex, with which complex connections exist (Herman et al., 2005). The literature on the association between stress and cardiovascular events is considerable. Different responses to various stress protocols were recorded in human and rat studies, such as increased heart rate, decrease or increase in PR and QT interval, prolongation or shortening of corrected QT Interval (QTc) interval, decrease in RR interval, decrease, or increase in QRS amplitude in electrocardiography (ECG) parameters (Bhide et al., 2016; Ghalwash et al., 2021; Park et al., 2017). Variable ECG responses to various stressors, on the other hand, may lead to a misunderstanding of stress-related cardiac mechanisms.

Restraint stress is the most used stressor in rodents (Buynitsky and Mostofsky, 2009). Restraint stress has been reported to cause catecholamine (epinephrine and norepinephrine)-induced cardiac injury (Liu et al., 2016). Experimental animal models of cold stress are being developed to investigate the physiopathological mechanisms and complications of stress (El Marzouki et

al., 2021). Studies show that structural myocardial lesions occur when animals are exposed to extremely low temperatures (Meneghini et al., 2008). Catecholamines are involved in the sympathetic regulation of the heart during cold exposure. It is well known that cold exposure leads to ECG changes. Short-term cold exposure, higher T-wave amplitudes, and shortening of QTc have been reported in humans (Hintsala et al., 2014). Two paradoxical theories have been proposed to explain both the advantage and disadvantages effect of stress on the heart. Interestingly, contrary to the negative effect of acute stress on the heart, cold restraint stress can induce cardiac cell protection and reduce infarct size as the main parameter of cardiac damage (Wu et al., 2004).

Current research reveals that restraint and cold restraint stress have varying impacts on the cardiovascular system. However, no study assessing the impact of different experimental stress procedures on ECG parameters has been reported. The objective of this study is to investigate the effects of experimental acute restraint and cold restraint stress on ECG parameters such as heart rate, PR, RR, QT, QTc intervals, and QRS amplitude.

Material and methods

The study was approved by the Sivas Cumhuriyet University Animal Experiments Ethics Committee (Approval No: 65202830-050.04.04-640). The animals were obtained by Sivas Cumhuriyet University's Experimental Animals Application and Research Centre. The rats were kept in standard conditions (ad-libitum water and pelleted rat food, proper circulation, 12/12 h light/dark cycle, 21-23°C temperature, 35-60% humidity). The experiment was carried out on 18 male Wistar Albino rats, aged 8-10 weeks and weighing 180-220 g. Rats were randomly separated into three groups (n=6/group) after a one-week acclimation period.

Control group: Animals in this group did not be exposed to any stress.

Restraint stress: Restraint group: In this stress protocol, rats were placed in restrictive cylinder-shaped plastics (restrainer, 63.5 x 215.9 mm) designed for the rat as previously described, and their movement was restricted for 2 hours at room temperature (Tu et al., 2019).

Cold Restraint stress: Cold Restraint group: As previously described, rats were kept in the restrainer at +4°C for 2 hours (Zhu et al., 2014).

Rats were transported into the experimental room and allowed 30 minutes to acclimatize to the environment. ECG was recorded with alligator clips in Einthoven mode using Televet II ECG equipment (Kruuse, Germany) under ketamine (60 mg/kg, Keta-Control, Doğa İlaç, İstanbul) and xylazine (10 mg/kg, Ksilazol, Alivira, Ankara) anesthesia. ECG traces were recorded for 5 min. Televet 100 program (Version® 7.0.0, Kruuse, Heusenstamm, Germany) was used to evaluate lead II trace. In addition to the morphological evaluation of the wave, heart rate, RR interval, PR interval, QT interval, corrected QT Interval (QTc, Bazett formula), R-wave amplitude, and QRS interval were analyzed in ECG recordings.

Statistical analysis

Data analysis was performed in GraphPad Prism 8.00 (GraphPad Software, San Diego, CA, United States). ECG parameters were statistically analyzed by a one-way ANOVA test followed by Tukey's multiple comparisons tests. Results are expressed as mean ± SD. Values of $p < 0.05$ were considered significant.

Results

Figure 1 represents ECG recording samples. ECG waves was typical course in the control group rats. On the other hand, in ECG recordings of rats in the restraint group, it was determined that the amplitude of the R wave was generally close to the amplitude of the T wave. In addition, it was observed ST-segment elevation in restraint group rats. In the recordings of rats in the cold restraint group, partial increases in R wave amplitude and a small number of ST segment elevations were observed.

Statistical analysis of ECG parameters is presented in Figure 2. There was no significant difference between the groups in analysis of PR, QT, and QRS intervals ($p > 0.05$). In addition, the amplitude of the R-wave did not differ across groups ($p > 0.05$). However, the heat rate was significantly higher in the cold restraint group than in the

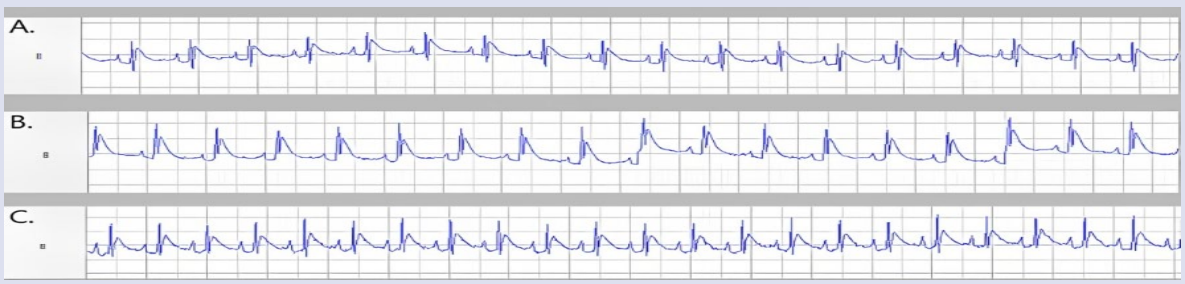

Figure 1. ECG recording samples in study groups. A. Control group, B. Restraint group, C. Cold Restraint group. The ECG trace was recorded in Einthoven mode with a gain of 20 mm/mV and a speed of 50 mm/s using Televet II ECG equipment (Kruuse, Germany) and was showed lead II. The Televet 100 program (Version® 7.0.0, Kruuse, Heusenstamm, Germany)

control group (p <0.001) and restraint group (p <0.01). In addition, the RR interval was significantly lower in the cold restraint group than in the control group (p <0.001) and restraint group (p <0.01). The QTc was increased in rats exposed to cold restraint stress compared to the control group (p <0.01) and restraint group (p <0.05). It was observed that most ECG data from the control group was within the reference range according to previously published literature (Konopelski and Ufnal, 2016).

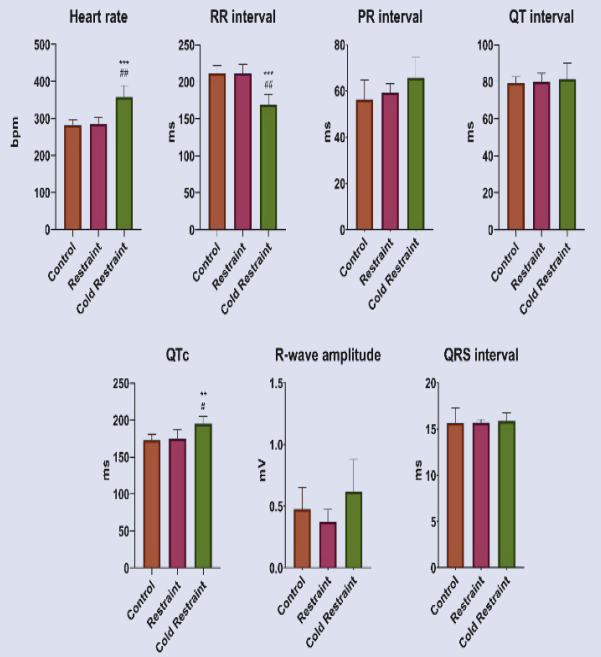

*Figure2. ECG parameters analysis result graphs. **p <0.01 and ***p <0.001 indicate statistical comparison with the control group, #p <0.05 and ##p <0.01 indicate statistical comparison with the restraint group, according to one-way ANOVA with post hoc Tukey analysis.)*

Discussion

Here, we show that acute cold restraint stress affects ECG parameters and that these changes can be seen mainly in heart rate, RR interval, and QTc interval. On the other hand, we showed that acute restraint stress changes the ECG morphology such as ST-segment elevation, R-wave amplitude decreasing.

In a single cardiac cycle, the following intervals and measurements are used to describe different aspects of the electrical activity of the heart:

1. RR interval: The time interval between successive R waves in the ECG signal. It reflects the heart rate and rhythm.
2. PR interval: The time interval between the onset of the P wave and the onset of the QRS complex. It represents the time it takes for the electrical impulse to travel from the atria to the ventricles.
3. QT interval: The time interval between the onset of the Q wave and the end of the T wave. It reflects the duration of ventricular depolarization and repolarization.
4. QTc: QT interval corrected for heart rate since the duration of the QT interval changes with heart rate. It is calculated using different formulas that consider the RR interval and QT interval.
5. R wave amplitude: The height of the R wave in the ECG signal. It reflects the magnitude of ventricular depolarization.
6. QRS interval: The time interval between the onset of the Q wave and the end of the S wave. It represents the time it takes for the electrical impulse to travel from the bundle of His to the Purkinje fibers and ventricular muscle cells, causing ventricular depolarization.

These measurements are useful in diagnosing and monitoring different cardiac conditions, such as arrhythmias, heart block, and prolonged QT syndrome.

Studies have found electrocardiographic alterations during stressful situations. The autonomic nervous system (ANS), which innervates the heart, exhibits heart rate variability through variation in the RR intervals between subsequent heartbeats. This variation reflects the sympathetic and parasympathetic activity of the ANS (Hammoud et al., 2018). Mild cold exposure in rats increased blood pressure, vascular sympathetic activity, and heart rate, whereas overall autonomic activity, parasympathetic activity, and baroreflex sensitivity decreased (Chen et al., 2021). On the other hand, decreased baroreflex sensitivity was reported in an animal model exposed to severe chronic cold weather at ~6 °C (Papanek et al., 1991). Acute restraint stress has been found in a recent study to raise heart rate in mice (Varejkova et al., 2019). It also led to tachycardiac baroreflex response and reduced the bradycardia response in rats (Crestani et al., 2010). Contradictory to expectation, cold restraint stress raised heart rate and shortened RR interval in our study, however acute restraint stress did not affect either. Although in this study the justified restraint stress is not known to have had any effect on heart rate and RR interval, it is possible that the explanation for the increased heart rate and decreased RR interval with cold restraint is that the heart has to work harder to keep the body warm.

ECGs observed in healthy males during acute stress indicate increased heart rate, shortened QT intervals, and longer QTc intervals (Bhide et al., 2016). In contrast, another research found that extended QT intervals were related to the development of ventricular arrhythmias and sudden death under multiple stressors, including mental stress (Andrássy, 2009). In a rat model of depression with myocardial infarction, similarly, extended QT timings were reported, indicating sympathetic hyperactivity (Shi et al., 2014). The QT intervals of rats exposed to both short- and long-term stress were considerably reduced after the recovery time when compared to the QTc intervals assessed following the

stress procedure in rats (Park et al., 2017). In our study, while restraint and cold restraint did not affect the stress QT interval in the groups, interestingly, cold restraint stress caused a prolongation in the QTc interval. Acute cold restraint stress (2 h) suggests that it may cause a more sympathetic tone increase and therefore sympathetic tone imbalance. Moreover, it may increase the risk of cardiac arrest compared to restraint stress (Algra et al., 1991).

Through the stimulation of the sympathetic nervous system, emotional stress causes myocardial ischemia (Rozanski et al., 1988), but it has also been proposed that stress or an excessive release of catecholamines can also cause non-ischaemic heart attacks. Like vasospastic angina, restraint stress in rats generates a fast increase of the ST segment on the ECG, which is recovered following the removal of the stress (Ueyama et al., 2000). In our study, while ST-segment elevation was evident in rats treated with restraint stress, it was partially observed in the cold restraint stress group. It might show that the restraint stress rat model is more capable of simulating a certain sort of stress-related, catecholamine-mediated heart attack in humans.

Without measuring catecholamines, the study may not have been able to fully understand the mechanisms by which stress affects the body, and it may be difficult to generalize the findings to other populations or situations where catecholamines play a significant role. Therefore, the absence of measuring catecholamines is considered a limitation of the study.

In conclusion, compared to acute restraint stress in rats, acute cold restraint stress may have a more noticeable effect on ECG parameters such as heart rate, RR interval, and QTc interval.

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Conflict of Interest

All authors have read and approved the work. There is no conflict of interest between the authors.

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