Original Article

The Validity of Heart Rate Variability Obtained from Electrocardiography and Blood Pressure in Rats Subjected to Isoproterenol-Induced Heart Ischemia

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Abstract

Background: *Heart rate variability (HRV) is calculated by electrocardiography (ECG-HRV) or blood pressure (BP-HRV). The purpose of this study was to determine the validity of the above methods in rats with normal and ischemic hearts during the baroreflex maneuver.*

Methods: *The study was conducted at Shiraz University of Medical Sciences, Shiraz, Iran, in 2021. Sprague-Dawley rats were divided into a sham group and an isoproterenol-mediated cardiac ischemia (ISO) group. Saline and isoproterenol (150 mg/kg) injected subcutaneously for 2 consecutive days in the sham and ISO groups, respectively. Then, the animals were anesthetized with an intraperitoneal injection of sodium thiopental (60 mg/kg), and the femoral artery and vein were cannulated. Baroreflex was activated using an intravenous injection of phenylephrine (10 µg/100 µL saline). ECG, BP, and heart rate (HR) were recorded, and the time domain of HRV and baroreflex gain were calculated.*

Results: *Baroreflex gain in the ISO group (male, weight=275.8±2.8 g, n=8) was lower than that in the sham group (male, weight=258±2.3 g, n=8) (P<0.05). ECG-HRV indicated an increase in the standard deviation of the RR interval (SDRR), the index of overall HRV, and the parasympathetic index of the root mean square of successive differences (RMSSD) in both groups. However, the rise in SDRR and RMSSD in the ISO group was less than that in the sham group (P<0.05). SDRR and RMSSD obtained from BP did not show a difference between the sham and ISO groups, nor did they correspond with the results seen in baroreflex gain.*

Conclusion: *BP-HRV was not as valuable as ECG-HRV in assessing cardiac ischemia.*

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Keywords: *Baroreflex; Blood pressure; Electrocardiography; Heart rate*

Introduction

In ischemic heart disease, the blood flow of the coronary arteries cannot meet the demands of cardiac muscle. Isoproterenol is a β-adrenergic receptor agonist frequently used for cardiac ischemia induction in animal studies.[1] High doses of isoproterenol lead to morphological and functional alterations in the heart, comparable to those occurring in patients with myocardial infarction.[2-5] The overactivity of the α-adrenergic receptors results in an increase in afterload and may worsen

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the conditions of patients with ischemic heart disease.[6-8] The α-adrenergic agonist phenylephrine is constantly used to increase afterload in animal studies. Baroreflex responses to phenylephrine injection include decreases in heart rate (HR), cardiac output, and vascular resistance.[9, 10] The results obtained from phenylephrine injection are comparable with those acquired by many physiological conditions, such as downward tilting in healthy volunteers.[11] Cardiovascular responses to baroreflex strongly support the increase in cardiac vagal nerve activity, even though the neuronal activity has not been directly measured so far.

The variability of consecutive RR intervals of heartbeats is called heart rate variability (HRV).[12] HRV is evaluated using the time domain, frequency domain, and nonlinear measurements.[13-15] The time-domain components of HRV are related to the inter-beat interval variability. These components can be assessed during 1 minute to 24 hours of each event. The standard deviation of the RR interval (SDRR) represents the total HRV, and the root mean square of successive differences (RMSSD) reflects parasympathetic activity.[14, 16] Some studies have reported decreased HRV indices in patients with ischemic heart disease.[17] As a result, HRV can be used as an index for evaluating the effectiveness of therapeutic interventions in cardiovascular disorders. For instance, the standard deviation of normal-to-normal intervals (SDNN) is increased by percutaneous coronary intervention in patients with chronic stable angina.[18] Nonetheless, autonomic dysfunction in cardiac ischemia during increased afterload has not been entirely described by HRV analysis.

HRV can be calculated by electrocardiography (ECG) and pulse pressure intervals. Nevertheless, it is unclear whether data on HRV from blood pressure (BP) is as accurate as HRV from ECG. Studies have revealed that HRV indices obtained from ECG are close to those acquired by finger-tip photoplethysmography in healthy subjects.[19, 20] On the other hand, researchers have reported that HRV data derived from the interval between 2 consecutive pulse pressure waves may not be interchangeable with data derived from ECG, especially during exercise.[21] Some studies have shown reduced HRV in myocardial infarction. A lower HRV is in tandem with a higher risk of severe ventricular arrhythmia and death.[22, 23] Still, there is little evidence to evaluate the aforementioned methods in myocardial ischemia during baroreflex.

Accordingly, in the present study, α-adrenergic agonist phenylephrine was used to induce baroreflex in sham and isoproterenol-mediated cardiac ischemia (ISO) groups. HRV in ECG and BP were analyzed at rest and after increased afterload, and the results of each method considering data on baroreflex gain will be discussed below.

Methods

The current study was approved by the Center of Comparative and Experimental Medicine and the Ethics Committee of Animal Care, according to the Guideline for the Care and Use of Laboratory Animals in Iran (Code No: IR.SUMS.REC.1399.468). Male Sprague-Dawley rats weighing between 250 and 280 g (n=16) were kept in the animal house of the department of physiology with standard cages, controlled temperature (24–26 °C), humidity (40–60%), and 12:12 hours of light/dark cycles. The animals had free access to water and food. The rats were divided into 2 groups: sham and ISO, with 8 animals in each group. The sample size was chosen according to our previous studies.[24]

Isoproterenol (Sigma, Germany) (150 mg/kg dissolved in 0.5 mL of saline) was injected subcutaneously twice at 24-hour intervals for the induction of cardiac ischemia. The same protocol was applied with only saline injection in the sham group. Twenty-four hours after the second injection, the animals were anesthetized with an intraperitoneal injection of 60 mg/kg sodium thiopental (VUAB Pharma Inc, Czech Republic).[25] Repeated doses were used as appropriate. The drug was selected based on previous studies reporting that barbiturates exert negligible effects on the cardiovascular system.[26, 27] After tracheostomy, the right femoral artery and vein were catheterized using a 50-PE tubing cannula. Twenty minutes after the steady state period, phenylephrine (Sigma, Germany) was injected (10 μg/kg dissolved in 100 μL of saline, intravenous). The tubing cannula of the femoral artery was connected to a data-acquisition system (Power Lab, AD instruments, Australia, ML856) through a pressure transducer to record the systemic BP throughout the experiments. At the same time, lead II of ECG was recorded by the Power Lab system. At the end of the experiments, the animals were euthanized with 1 mL of saturated potassium chloride solution intravenously under deep anesthesia.[28] Hemodynamic and ECG parameters were analyzed, and HRV was calculated. Animals with repeated occlusions in the femoral artery catheter or those that died during the experiments (3 of 11 in the ischemic group) were excluded from the study.

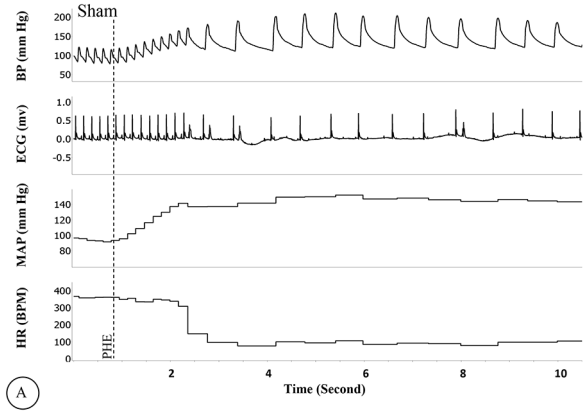
HRV was analyzed by the Fast Fourier Transform method (FFT) using the ECG module of the Power Lab system. Briefly, the recorded ECG was selected. Then, the following HRV setting was applied for the rats: very low frequencies (0–0.2 Hz), low frequencies (0.20–0.75 Hz), high frequencies (0.75–2.5 Hz), and RR intervals ranging between 100 and 200 ms. The accuracy of the calculations was verified in the window of the beat classifier view. The time-domain components of SDRR and RMSSD were also evaluated. SDRR indicates overall HRV, and RMSSD is an index of parasympathetic activity.[14] In the next step, HRV was calculated by the selection of data on pulse pressure from the recorded BP with similar timing as HRV from ECG.

The Shapiro–Wilk test was utilized to determine the normal distribution of the data. The statistical analyses were performed using the SPSS 18 software. All data had normal

distributions and were presented as mean±SE. The noted data were compared using the paired-samples *t* test and the independent samples *t* test. Significance was assumed when the *P* value was below 0.05, and the confidence limits used were 95% intervals.

Results

Representative traces obtained from the Power Lab system recording indicated tachycardia in the ISO group before the administration of phenylephrine (Figure 1). The injection of phenylephrine increased BP and decreased HR in the sham and ISO groups. HR in the sham group remained stable at minimal values, whereas it increased partially in a few seconds in the ISO group (Figure 1).



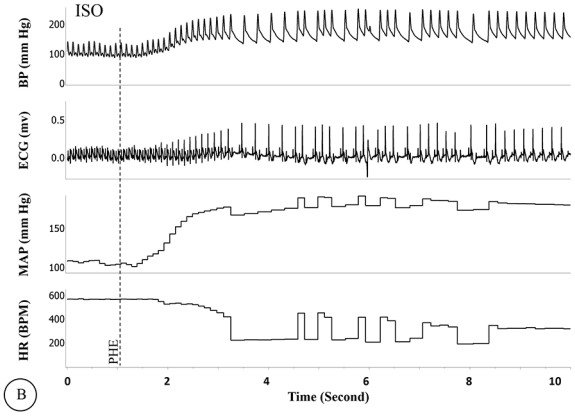


Figure 1. The image depicts a representative graph of blood pressure (BP), electrocardiography (ECG), mean arterial blood pressure (MAP), and heart rate (HR) before and after phenylephrine injection in the sham group (A) and the ISO group (B).
BPM, Beat per minute; ISO, Isoproterenol-mediated heart ischemia; mv, Millivolt; PHE, Phenylephrine

The representative traces of ECG in the sham and ISO groups before and after the injection of phenylephrine are shown in Figure 2. The bradycardia response was much more pronounced in the sham group than in the ISO group. HR in the sham group recovered slowly during 120 seconds. However, HR in the ISO group returned quickly to the baseline values within 30 seconds (data not shown).

The baseline values of HR were 392.64±20.68 and 517.56±19.06 BPM in the sham and ISO groups, respectively. The injection of phenylephrine decreased HR in both

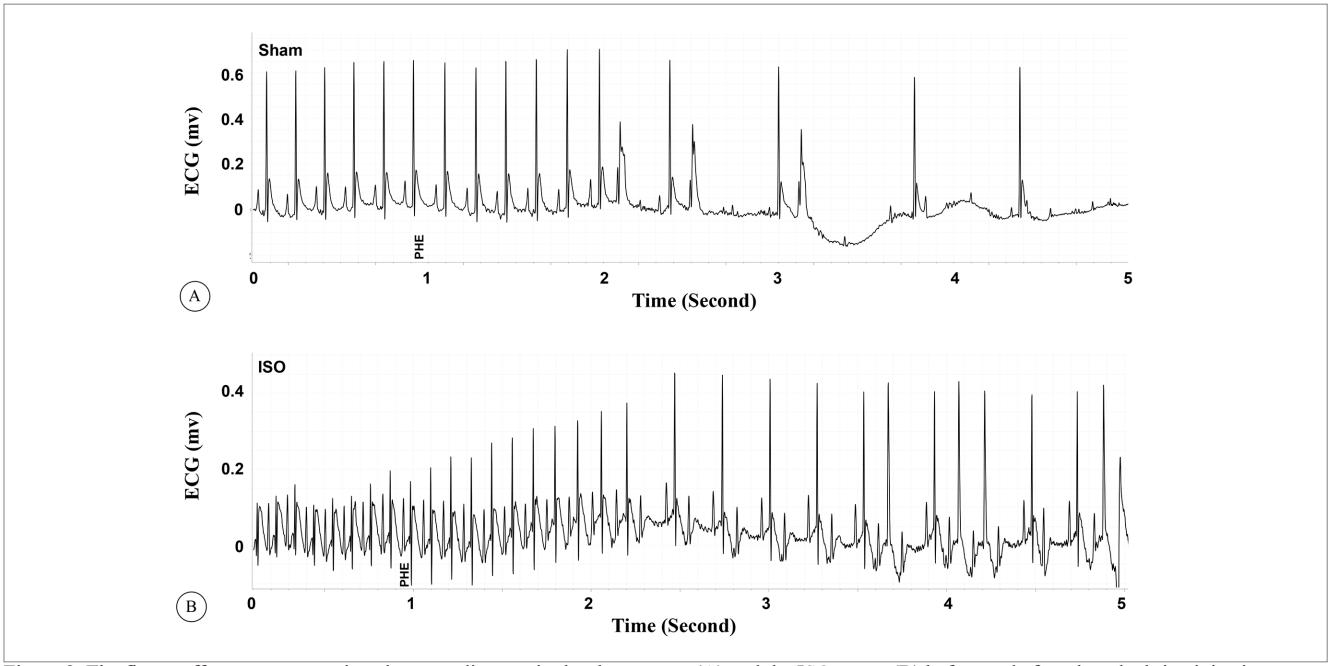


Figure 2. The figure offers a representative electrocardiogram in the sham group (A) and the ISO group (B) before and after phenylephrine injection.
ISO, Isoproterenol-mediated heart ischemia; mv, Millivolt; PHE, Phenylephrine

groups: 320.73±15.12 BPM in the sham group as opposed to 434.21±9.06 BPM in the ISO group. On the other hand, baroreflex gain (ΔHR/ΔMAP) was smaller in the ISO group than in the sham group (*P*=0.009) (Figure 3).

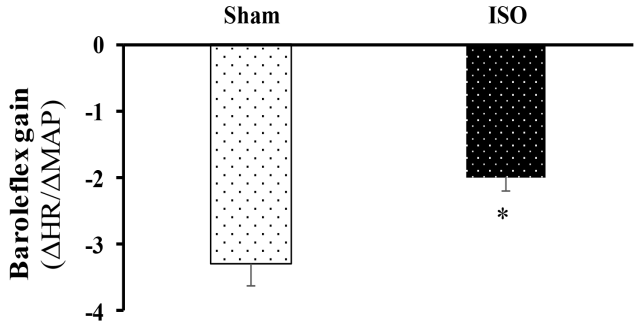


Figure 3. The figure depicts changes in baroreflex gain in the sham and ISO groups. Data are presented as mean±SE (n=8 in each group).
*P<0.05 vs the sham group
ISO, Isoproterenol-mediated heart ischemia; ΔHR, Delta heart rate; ΔMAP, Delta mean arterial blood pressure

ECG-derived HRV was obtained 1 minute before (the baseline) and the first 1 minute after the phenylephrine injection. The mean values of SDRR and RMSSD did not differ between the sham and ISO groups at baseline. The injection of phenylephrine increased SDRR in the sham group (*P*<0.001) and the ISO group (*P*=0.002) compared with their baseline values. RMSSD increased only in the sham group significantly (*P*<0.001). Furthermore, SDRR (*P*=0.018) and RMSSD (*P*=0.001) in the ISO group were significantly lower than those in the sham group (Figures 4A & 5A).

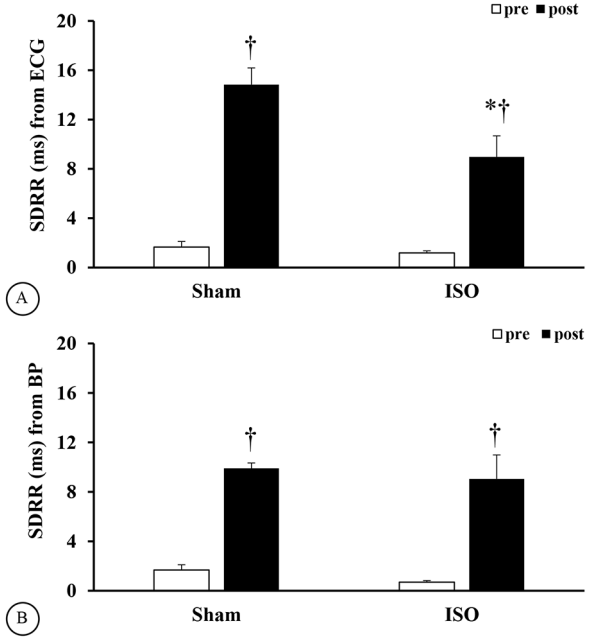


Figure 4. The figure illustrates changes in SDRR. The data were taken from ECG (A) and blood pressure (BP) (B) are presented as mean±SE.
n=8 in each group
*P<0.05 vs the sham group
†P<0.05 vs the pre-injection time in each group
ISO, Isoproterenol-mediated heart ischemia; ms, Millisecond; SDRR, Standard deviation of the RR interval; pre and post, Before and after phenylephrine injection, respectively

BP-derived HRV was calculated at the same time as the HRV-derived ECG 1 minute before (the baseline) and the first 1 minute after phenylephrine injection. The baseline values of SDRR and RMSSD did not differ between the sham and ISO groups. After the injection of phenylephrine, SDRR increased in the sham group (*P*<0.001) and the ISO group (*P*=0.003) compared with the baseline values. Furthermore, RMSSD rose in the sham group (*P*=0.003) and the ISO group (*P*=0.025) compared with their baseline values. However, both variables in the sham group and ISO groups did not statistically differ (Figures 4B & 5B).

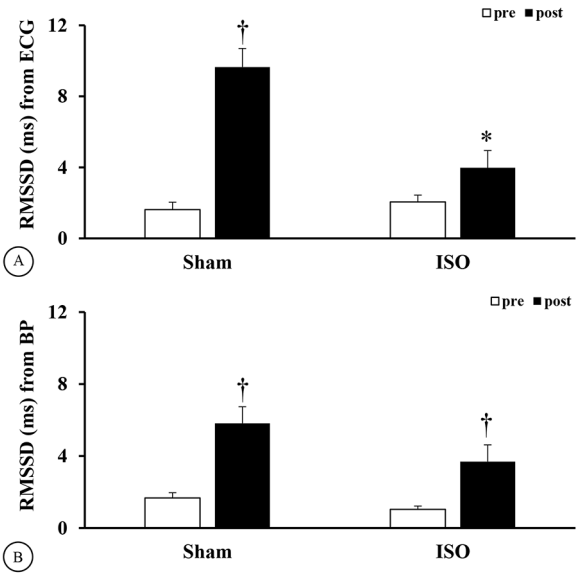


Figure. 5. Changes in RMSSD: data taken from ECG (A) and data taken from blood pressure (BP) (B).
Data are presented as mean±SE (n=8 in each group).
* *P*<0.05 vs the sham group
† *P*<0.05 vs the pre-injection time in each group
ISO, Isoproterenol-mediated heart ischemia; ms, Millisecond; RMSSD, Root mean square of successive differences; pre and post, Before and after injection of phenylephrine, respectively

Discussion

In the present study, we compared HRV parameters obtained from ECG and pulse pressure. Using HRV-derived pulse pressure data, we demonstrated impaired cardiac parasympathetic activity associated with decreased baroreflex gain in isoproterenol-induced cardiac ischemia. Nevertheless, HRV-derived BP did not differ between the sham and cardiac ischemia groups and did not support the

baroreflex gain data.

Isoproterenol-mediated cardiac ischemia is an acceptable animal model of myocardial ischemia.[2-5] Tachycardia in the ISO group was similar to other cardiac ischemia studies.[29] No significant variation was identified in the parasympathetic components of HRV obtained from ECG or BP during the steady-state period. In line with our results, HRV obtained from ECG and pulse pressure indicated identical results in healthy subjects.[19, 20] Nonetheless, it has been shown that HRV-derived ECG is diminished by motion and exercise.[15] As a result, BP derived from HRV could be valuable under such conditions. The increase in cardiac workload during exposure to cardiovascular stresses, along with increased sympathetic activity and decreased parasympathetic activity, exacerbates cardiac ischemia.[15] In our study, phenylephrine increased BP almost equally in the sham and ISO groups. In a normal heart, an increase in BP causes a decrease in HR.[10] Nevertheless, baroreflex gain was lower in the ISO group than in the sham group. Thus, the decrease in baroreflex gain in the ISO group reveals changes in cardiac sensitivity to the autonomic nervous system due to cardiac injury.

Baroreflex increased SDRR in both sham and ISO groups. However, on the HRV-derived ECG, the increase in SDRR in the ISO group was smaller than that in the sham group. SDRR indicates overall HRV.[14] A decrease in HRV may be associated with an increase in sympathetic activity. This compensatory mechanism brings cardiovascular function and homeostasis closer to normal. Still, the condition can adversely affect ischemic hearts during exposure to cardiovascular stresses, such as exercise. A correlation between low HRV levels and mortality rates has been reported in some studies.[23, 30] Low levels of SDRR were also associated with mortality in the ISO group since 3 of 11 rats died after phenylephrine injection and were excluded from the study. Consequently, we did not evaluate HRV for a long period due to limitations associated with increased mortality.

In baroreflex, the increase in RMSSD in the ISO group was smaller than that in the sham group. RMSSD is an index of parasympathetic activity. Our data demonstrated parasympathetic impairment in the ISO group, preventing the normal bradycardia response in baroreflex. RMSSD obtained from BP also increased in the ISO and sham groups after phenylephrine injection, but the data were not statistically different between the groups. Subsequently, the difference in HR and baroreflex gain between the ISO and sham groups could not be explained by data on HRV-derived BP. Our results are consistent with other studies reporting that the use of pulse variability as a surrogate for HRV should be used with caution under certain conditions.[31]

Data on HRV obtained from BP differed from those obtained from ECG. It is hard to differentiate the autonomic activity in the sham and ISO groups through BP-derived HRV. Furthermore, we could not compare our results with others as little attention has been devoted to the comparison of HRV-derived ECG and BP in heart ischemia subjected to baroreflex by other investigators. However, our study, in line with previous investigations, indicates that HRV from BP can be used in stable conditions as a surrogate of HRV obtained from ECG.[19, 20, 24]

Conclusion

In the current study, we found that ECG-derived HRV corresponds to baroreflex gain during cardiovascular stresses induced by phenylephrine. Based on differences in the parasympathetic component of HRV and baroreflex gain, we were able to distinguish the sham group's response to increased afterload from the cardiac ischemia group. In contrast, BP-derived HRV did not differ between the groups and was not consistent with baroreflex gain. Therefore, in emergencies, such as increased afterload, it is still advisable to perform HRV analysis from ECG.

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References

1. Lobo Filho HG, Ferreira NL, Sousa RB, Carvalho ER, Lobo PL, Lobo Filho JG. Experimental model of myocardial infarction induced by isoproterenol in rats. Rev Bras Cir Cardiovasc 2011;26:469-476.
2. Murugesan M, Revathi R, Manju V. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Indian J Pharmacol 2011;43:516-519.
3. Roy SJ, Stanely Mainzen Prince P. Protective effects of sinapic acid on lysosomal dysfunction in isoproterenol induced myocardial infarcted rats. Food Chem Toxicol 2012;50:3984-3989.
4. Soraya H, Khorrami A, Garjani A, Maleki-Dizaji N, Garjani A. Acute treatment with metformin improves cardiac function following isoproterenol induced myocardial infarction in rats. Pharmacol Rep 2012;64:1476-1484.
5. Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G, Muders F, Riegger GA, Kromer EP. Development of heart failure following isoproterenol administration in the rat: role of the renin-angiotensin system. Cardiovasc Res 1998;37:91-100.
6. Chen PS, Chen LS, Cao JM, Sharifi B, Karagueuzian HS, Fishbein MC. Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. Cardiovasc Res 2001;50:409-416.
7. Pilati CF, Bosso FJ, Maron MB. Factors involved in left ventricular dysfunction after massive sympathetic activation. Am J Physiol 1992;263(3 Pt 2):H784-791.
8. Oliveira NL, Ribeiro F, Alves AJ, Teixeira M, Miranda F, Oliveira J. Heart rate variability in myocardial infarction patients: effects of exercise training. Rev Port Cardiol 2013;32:687-700.

9. Estrela HF, Damásio ES, Fonseca EK, Bergamaschi CT, Campos RR. Differential Sympathetic Vasomotor Activation Induced by Liver Cirrhosis in Rats. PLoS One 2016;11:e0152512.
10. Rajanathan R, Pedersen TM, Thomsen MB, Botker HE, Matchkov VV. Phenylephrine-Induced Cardiovascular Changes in the Anesthetized Mouse: An Integrated Assessment of in vivo Hemodynamics Under Conditions of Controlled Heart Rate. Front Physiol 2022;13:831724.
11. Takahashi N, Nakagawa M, Saikawa T, Ooie T, Akimitsu T, Kaneda K, Hara M, Iwao T, Yonemochi H, Ito M, Sakata T. Noninvasive assessment of the cardiac baroreflex: response to downward tilting and comparison with the phenylephrine method. J Am Coll Cardiol 1999;34:211-215.
12. Goldenberg I, Goldkorn R, Shlomo N, Einhorn M, Levitan J, Kuperstein R, Klempfner R, Johnson B. Heart Rate Variability for Risk Assessment of Myocardial Ischemia in Patients Without Known Coronary Artery Disease: The HRV-DETECT (Heart Rate Variability for the Detection of Myocardial Ischemia) Study. J Am Heart Assoc 2019;8:e014540.
13. Wong JS, Lu WA, Wu KT, Liu M, Chen GY, Kuo CD. A comparative study of pulse rate variability and heart rate variability in healthy subjects. J Clin Monit Comput 2012;26:107-114.
14. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health 2017;5:258.
15. Ishaque S, Khan N, Krishnan S. Trends in Heart-Rate Variability Signal Analysis. Front Digit Health 2021;3:639444.
16. McCraty R, Shaffer F. Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health risk. Glob Adv Health Med 2015;4:46-61.
17. Lucini D, Pagani M. Heart rate variability, autonomic regulation and myocardial ischemia. Int J Cardiol 2020;312:22-23.
18. Abrootan S, Yazdankhah S, Payami B, Alasti M. Changes in Heart Rate Variability Parameters after Elective Percutaneous Coronary Intervention. J Tehran Heart Cent 2015;10:80-84.
19. Selvaraj N, Jaryal A, Santhosh J, Deepak KK, Anand S. Assessment of heart rate variability derived from finger-tip photoplethysmography as compared to electrocardiography. J Med Eng Technol 2008;32:479-484.
20. Jeyhani V, Mahdiani S, Peltokangas M, Vehkaoja A. Comparison of HRV parameters derived from photoplethysmography and electrocardiography signals. Annu Int Conf IEEE Eng Med Biol Soc 2015;2015:5952-5955.
21. Carrasco S, González R, Jiménez J, Román R, Medina V, Azpiroz J. Comparison of the heart rate variability parameters obtained from the electrocardiogram and the blood pressure wave. J Med Eng Technol 1998;22:195-205.
22. Buccelletti E, Gilardi E, Scaini E, Galiuto L, Persiani R, Biondi A, Basile F, Silveri NG. Heart rate variability and myocardial infarction: systematic literature review and metanalysis. Eur Rev Med Pharmacol Sci 2009;13:299-307.
23. Fang SC, Wu YL, Tsai PS. Heart Rate Variability and Risk of All-Cause Death and Cardiovascular Events in Patients With Cardiovascular Disease: A Meta-Analysis of Cohort Studies. Biol Res Nurs 2020;22:45-56.
24. Khodadadi F, Bahaoddini A, Tavassoli A, Ketabchi F. Heart rate variability and pulmonary dysfunction in rats subjected to hemorrhagic shock. BMC Cardiovasc Disord 2020;20:331.
25. Khoramzadeh M, Dehghanian A, Ketabchi F. Roles of Endothelin B Receptors and Endothelial Nitric Oxide Synthase in the Regulation of Pulmonary Hemodynamic in Cirrhotic Rats. J Cardiovasc Pharmacol 2019;73:178-185.
26. Eroglu A. The effect of intravenous anesthetics on ischemia-reperfusion injury. Biomed Res Int 2014;2014:821513.
27. Zorniak M, Mitrega K, Bialka S, Porc M, Krzeminski TF. Comparison of thiopental, urethane, and pentobarbital in the study of experimental cardiology in rats in vivo. J Cardiovasc Pharmacol 2010;56:38-44.
28. Cartner SC, Barlow SC, Ness TJ. Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and CO2 inhalation. Comp Med 2007;57:570-573.
29. Khan V, Sharma S, Bhandari U, Sharma N, Rishi V, Haque SE. Suppression of isoproterenol-induced cardiotoxicity in rats by raspberry ketone via activation of peroxisome proliferator activated receptor-α. Eur J Pharmacol 2019;842:157-166.
30. Song T, Qu XF, Zhang YT, Cao W, Han BH, Li Y, Piao JY, Yin LL, Da Cheng H. Usefulness of the heart-rate variability complex for predicting cardiac mortality after acute myocardial infarction. BMC Cardiovasc Disord 2014;14:59.
31. Pernice R, Javorka M, Krohova J, Czippelova B, Turianikova Z, Busacca A, Faes L; Member, IEEE. Comparison of short-term heart rate variability indexes evaluated through electrocardiographic and continuous blood pressure monitoring. Med Biol Eng Comput 2019;57:1247-1263.