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A Comparison of Chronotropic and Dromotropic Properties of Vanillic Acid and Exercise in Aged and Young Rats

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Introduction: Cardiovascular disease represents the proportion of the global burden of disease. Age is by far the most important risk factor in developing cardiovascular or heart diseases, with approximately a tripling of risk with each decade of life. The aim of this study is to compare the effects of exercise and vanillic acid (VA) on heart rate and P-R interval (cardiac health indexes) in aged and young rats.

Materials and Methods: Sixty four male Sprague - Dawley rats were divided into 8 groups. Young and aged: control, VA (10 mg/kg, gavage, 10 days), exercise (1 h, at speed 17 m/min, six times per week) and exercise + VA. Lead II electrocardiogram was recorded by Bio Amp and monitored by a Power Lab system. Heart rate (as chronotropic property) and P-R interval (as dromotropic property) were calculated from ECG recording. ANOVA was used for statistical analysis and $P<0.05$ was considered as significant level.

Results: The results presented in this study demonstrated that heart rate did not change with aging, although there was a significant P-R interval prolongation in aged groups. However, exercise and administration of VA displayed negative chronotropic (with the highest activity in young groups) and dromotropic properties as compared to controls. In the studied groups, physical activity and administration of VA had a heart rate reduction and a P-R interval prolongation effect in aged and young rats.

Conclusions: The results suggest that VA and exercise have a protective role in preventing and treating irregular heartbeats.

Keywords: Vanillic acid, Aging, Exercise, Chronotropic, Dromotropic, Rat.

Introduction

Aging can be defined as a gradual change in an organism that leads to increased risk of weakness, disease, and death. Different system alterations occur during the aging process, as an unavoidable part of life. This process starts with birth and accelerates with advancing age. One of the most widely discussed and investigated processes are change in the heart rate (Ferrari et al., 2003).

Heart rate, or heart pulse, is the speed of the heartbeat measured by the number of heart beats per unit of time - typically beats per minute (bpm) (Diaz et al., 2005). Heart rate is not a stable value and it increases or decreases in response to the body's need in a way to maintain an equilibrium (basal metabolic rate) between requirement and delivery of oxygen and nutrients.

Activities that can provoke change include physical exercise, anxiety, sleep, stress, ingesting, illness, and drugs (Acharya et al., 2006). A number of investigations indicate that faster resting heart rate has emerged as a risk factor for mortality in homoeothermic mammals, particularly cardiovascular mortality in human beings. Faster heart rate may accompany increased production of reactive oxygen species and increased production of inflammation molecules in cardiovascular system, in addition to increased mechanical stress to the heart. There is a direct correlation between increased resting rate and cardiovascular risk. Studies have shown that a resting heart rate (RHR) of over 90 beats per minute doubles heart disease death rates in men and triples that in women (Cheung et al., 2004).

Exercise directly affects the activity, function and health of the heart. Experimental and human studies have shown that exercise training improves survival after myocardial infarction. Regular aerobic exercise makes the heart stronger and more efficient, meaning that the heart pumps more blood each time it contracts, needing fewer beats per minute to do its job. Experimental and human studies have also shown that exercise training improves survival after arrhythmias (Heran et al., 2011).

More rigorous exercise can be made possible with antioxidant supplements. Most recent research indicates the importance of regular, moderate intensity cardiovascular exercise in conjunction with a eating a diet rich in foods high in antioxidants (Kojda and Hambrecht, 2005).

Antioxidants defend against the harmful effect of free radicals, which are associated with heart disease (Dianat et al., 2014a), arthritis, cancer, aging and many other diseases (Valko et al., 2007).

Vanillic acid (VA, 4-hydroxy-3-methoxybenzoic acid) is a phenolic derivative of edible plants and fruits. It is also an intermediate in the production of vanillin from ferulic acid. The largest amount of VA is found in the plant roots of *Angelica sinensis*. It has several medicinal properties including antibacterial and antifilarial antimicrobial activities, free-radical scavenging ability and a chemopreventive effect (Dianat et al., 2014b).

VA reduces lipid peroxidation products (thiobarbituric acid reactive substances, lipid hydroperoxides, conjugated dienes) and significantly restores enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase), non-enzymatic antioxidants (vitamin C, vitamin E, and reduced glutathione) in the plasma (Kumar et al., 2011).

Also, it has been approved that VA decreases ischemic markers and augments endogenous antioxidant and hence, protects myocardium against ischemia–reperfusion induced oxidative stress injuries (Dianat et al., 2014b).

In view of the above facts, we evaluated the chronotropic and dremotropic effect of exercise and vanillic acid in aged and young rats.

Materials and Methods

Animals

In this study Male Sprague-Dawley rats (aged: 18-22 months old / young: 4-6 months old) were purchased from the Animal Lab of Ahvaz Jundishapur University of Medical Sciences. The animals used in this study were treated in accordance with principals and guidelines on animals care of AJUMS and were kept at 20-24ºC under 12 hr light/dark cycle and were allowed free access to tap water and commercial chow. The investigation was approved by the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. ajums. REC.1392.279).

Procedures

The animals were divided into two (young and aged) groups. Each group was randomly divided into four subgroups [control, vanillic acid (VA), exercise (EX), vanillic acid + exercise (VA+EX)].

The 21-day exercise training protocol consisted of running on a motorized treadmill (1 h at speed 17 m/min, six times per week). Very few shocks were administered during a training session within the first week of training.

The sedentary young and aged rats in control groups were handled identically to the treadmill-trained rats. At the same time of day, the sedentary rats were placed on a stationary treadmill, with the shock grid turned off, six days per week for the duration of the treadmill training session (Dianat et al., 2014c) and received normal saline (1 ml), by gavages, once a day for the last 10 consecutive days. In VA groups, the young and aged rats were placed on a stationary treadmill, with the shock grid turned off, six days per week for the duration of the treadmill training session and received vanillic acid (10 mg/kg) (StanelyMainzen et al., 2011), by gavages, once a day for the last 10 consecutive days.

In VA+EX groups, the young and aged rats were trained to run on a motor-driven treadmill (21days) and received vanillic acid 10 mg/kg, by gavages, once a day, during the last 10 days.

Heart rate and P-R interval recording

The animals were operated under anesthesia with combination of xylazine (10 mg/kg) and ketamine (50 mg/kg) via intraperitoneal (ip) route. Lead II electrocardiogram (ECG) was recorded by Bio Amp and monitored by a Power Lab system (AD-Instruments, Australia). Heart rate (as chronotropic property) and P-R interval (as dremotropic property) were calculated from ECG recording in the first day and 21st days after performance (Dianat et al., 2013).

Statistical analysis

Results were analyzed using SPSS and expressed as Mean ± SEM. Comparisons among groups were performed using t-test, one-way and two-way ANOVA followed by Tukey's multiple comparison test. P-values of less than 0.05 were considered significant statistically.

Results

The comparison of chronotropic property in aged and young groups

Statistical comparison of heart rate in young and aged groups showed that there was no significant change in chronotropic property of the rats with aging. On the other hand, the evaluation of the effects of exercise and vanillic acid showed that in both young (Figure 1) and aged (Figure 2) groups, chronotropic effect significantly decreased compared to that in control groups. A comparison of the changes in heart rate in young and aged groups showed that the negative chronotropic property in young group was more than that in aged rats (Figure 3). Furthermore, in this study, exercise + vanillic acid resulted in bradycardia in aged and young groups.

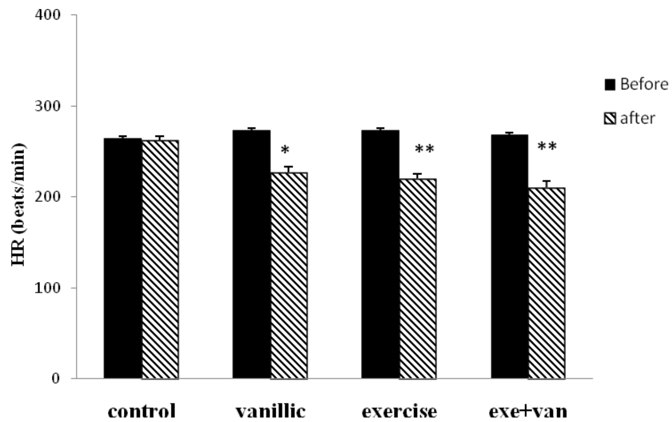

Figure 1. Comparison of Heart rate in young groups.Control, vanillic acid (10 mg/kg), Exercise (21 days), vanillic acid (10 mg/kg) +Exercise (21 days). Results are expressed as Mean ± SEM of 8 rats per group, t-test was used; *P<0.05, **P<0.01 were compared in each group.

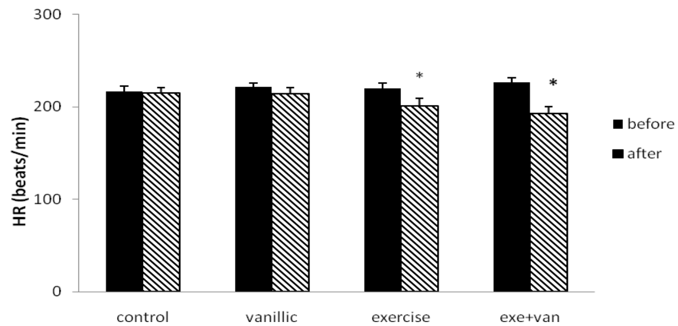

Figure 2. Comparison of Heart rate in aged groups.Control, vanillic acid (10 mg/kg), Exercise (21 days), vanillic acid (10 mg/kg) +Exercise (21 days). Results are expressed as Mean ± SEM of 8 rats per group, t-test was used; *P<0.05, were compared in each group.

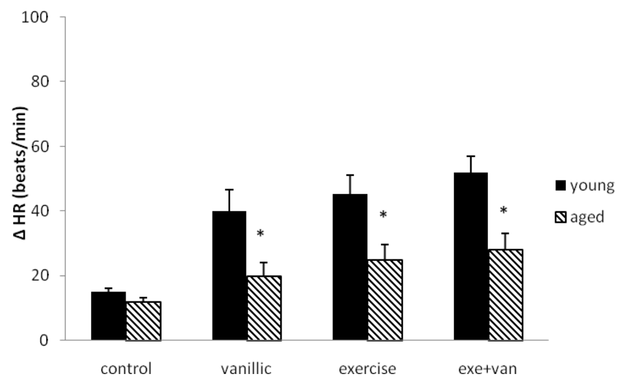

Figure 3. Comparison of the changes in heart rate in young and aged groups. (Control, vanillic acid, exercise and vanillic acid + exercise). Results are expressed as Mean ± SEM of 8 rats per group, ANOVA was used, *P<0.05, were compared between young and aged rats in each group.

Dromotropic property in aged and young groups

The comparison of P-R interval duration in young and aged groups showed that there was significant prolongation in the control group of aged rats (Table 1). Also, the effect of exercise, administration of vanillic acid and exercise + vanillic acid on P-R interval, which represent the conduction time of the impulse through the atrioventicular node, is shown in Table 1. The obtained results in this study showed that in all groups there was a significant increase in duration of P-R interval compared to control groups.

Table 1. Comparison of P-R interval (msec) in different groups

Group	Young		Aged	
	Before	After	Before	After
Control	52±0.4	51±0.8	66±0.5###	65±0.6
Vanillic acid	51±0.5	56±0.5*	63±0.5###	70±0.5*
exercise	50±0.8	55±0.1*	60±0.7###	66±0.2*
Vanillic acid+ exercise	52±0.2	58±0.7*	64±0.1###	68±0.6*

Results are expressed as Mean ± SEM of 8 rats per group. ### P<0.001 vs young rats (Tukey's multiple comparison test) *P<0.05 compared in each group (t-test was used).

Discussion

In our studied groups, heart rate was not affected by aging. In contrast, the results of the present study have shown some alterations of P-R interval with aging. There is evidence that VA has negative chronotropic and dromotropic properties in rats. Also 21-day treadmill exercise acts in the same manner. However, these alterations were reduced in aged groups.

In agreement with previous experiments in the literature (Tanabe and Bunag 1989; Werner et al., 1995; Irigoyen et al., 2000), the obtained results in this study showed that heart rate did not change with aging (Figure 3), Silva et al. also found no heart rate changes in rats aged 219 days or 557 days, whereas they detected bradycardia in older rats aged 851 days and 951 days, which are in agreement with our finding (Silva et al., 2002).

In the present study, exercise and vanillic acid treatment of aged and young rats reduced the basal heart rate and prolonged the P-R interval compared to age and young-matched control rats.

During normoxia, adenosine is released in low amounts at a constant basal rate in the myocardium. In some conditions like exercise, an imbalance between oxygen supply and demand in myocardium corresponds to the breakdown of ATP, and adenosine release can increase up to at least 50-fold (Valko et al., 2007). Adenosine activates acetylcholine-sensitive K^+ current in the atrium, sinus, and AV nodes, resulting in the hyperpolarization, shortening of action potential duration and the slowing of normal automaticity (Mubagwa and Flameng, 2001). Thus, adenosine could be said to have negative chronotropic and dromotropic effects. Also, adenosine antagonizes the response to adrenergic stimulation (Headrick et al., 2000), so it has a profound cardioprotective effect during imbalance of myocardial oxygen supply and demand. Therefore, exercise-induced endogenous releases of adenosine have a negative chronotropic and dremotropic effect (Eltzschig et al., 2013). These results are in agreement with our finding in heart rate reduction and prolongation in P-R interval with 21-day treadmill exercise. However, this hypothesis needs to be further investigated in future studies.

Furthermore, the results presented in this study demonstrate negative chronotropic and dromotropic property of vanillic acid in aged and young rats.

The β-adrenoceptor blocking properties of vanillin and its derivatives were investigated under in vivo and in vitro conditions (Bin-Nan et al., 1994). For example, Bin-Nan showed that vaninolol as well as propranolol inhibited the tachycardia effects induced by isoproterenol and produced a dose-dependent bradycardia response and a sustained pressor action in urethane-anesthetized normotensive rats. This effect is explained by β-adrenergic blocking activity. These findings suggest that vanillic acid as a phenolic compound derived from vanillin probably binds with beta-adrenergic receptors and they prevent increased heart rate which caused by the stimulation of beta-adrenergic receptors. Beta-blockers also bind to beta-adrenoceptors in cardiac conducting system and provide adequate protection from sudden cardiac death resulting from tachyarrhythmias. (Gorré and Vandekerckhove, 2010). However, further studies are necessary to prove this hypothesis.

It is well known that the baroreflex activity decreases with aging. Also, aging is associated with a reduced responsiveness of many hormone receptors. Many studies have been published on age-dependent changes in human alpha- and beta-adrenergic receptors. For example, beta-adrenergic receptor responsiveness declines with age (Kelliher and Conahan, 1980; Poller et al., 1997). The smaller decrease in heart rate observed in aged rats treated with vanillic acid and exercise may be associated with diminution in vagal control of heart rate and reduced responsiveness to adrenergic receptors caused by aging, even though further studies are required to better understand this mechanism.

The results of the present study have shown some differences in P-R interval with aging. The present finding that aging decreases electrical conduction in the atrioventricular node is in accordance with previous findings in experimental animals (Berg, 1955) and humans (Fleg et al., 1990) which is probably related to the same structural and functional changes due to aging. Since the effects of exercise and vanillic acid act in the same way, probably the combined effect of exercise and vanillic acid produces the same effect in heart rate reduction and prolongation in P-R interval.

In previous studies, we documented the protective effects of exercise and vanillic acid on arrhythmias such as ventricular tachycardia (Dianat et al., 2014c). Therefore, we have extended previous findings by documenting that exercise and vanillic acid decrease the heart rate and prolong P-R interval in rats.

Conclusion

Therefore, the obtained results in this study suggest the protective role of VA and exercise in preventing and treating irregular heartbeats. However, further studies are necessary to clarify this issue.

Acknowledgments

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Conflict of Interest

There is no conflict of interest to be declared.

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