Lisinopril indifferently improves heart rate variability during day and night periods in spontaneously hypertensive rats

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Summary

The aim of this work was to investigate the effect of ten-weeks lisinopril treatment to spontaneously hypertensive rats (SHRs) on day/night variations of blood pressure, heart rate and autonomic cardio-regulation parameters. Male SHR with surgically implanted radio-telemetry implant that provided direct measurements of arterial pressure and electrocardiogram wave were used. Animals were allocated to two groups (n=5 each). The first group was treated with lisinopril (20 mg/kg by gavage) daily for 10 weeks (treated group); whereas the second was gavaged daily with tap water (untreated group). Arterial blood pressure, ECG and other telemetry parameters were recorded at the start and at the end of 10-week treatment. Collected data were analyzed using specialized software and were statistically tested. In addition to the expected lowering of blood pressure, spectral analysis of R-R intervals revealed that lisinopril treatment for 10 weeks significantly caused 2-3 fold increase in heart rate variability (HRV) during both active and inactive periods. However, R-R interval durations demonstrated variable distribution patterns during those periods. The cause of observed distribution pattern of R-R intervals during active and inactive periods may be of significance to better understand HRV changes and warrants further investigations.

Key words: heart rate variability, hypertension, circadian rhythm, lisinopril, ACE-inhibitor, telemetry, SHR

Introduction

Cardiovascular system homeostasis is a function of complex and intercalated metabolic, neural, humoral and hemodynamic factors that influence blood vessels as well as cardiac muscle performance and structure (Mancia *et al*. 2007). Disturbance of any of these regulatory factors leads abnormalities in circulatory control, that manifest in various pathological conditions such as essential hypertension (HT) (Singh *et al*. 2010). Pharmacologic treatment of HT targets the same mechanisms that are involved in cardiovascular homeostasis (Mancia *et al*. 2010).

HT is a global epidemic and a major risk factor to cardiovascular morbidity and mortality (Mancia *et al*. 2007). Although no definitive cause of HT has been identified (Singh *et al*. 2010), two systems that are involved in cardiovascular homeostasis have called for more attention than other regulatory systems; *viz.* the autonomic nervous system (ANS) and the rennin-angiotensin system (RAS). The two systems 'crosstalk' in HT (Grassi et al. 2001) and their value is thought to extend beyond reducing blood pressure (Del Colle *et al*. 2007, Grassi *et al*. 2009, Bakris *et al*. 2010).

Autonomic modulation of the cardiovascular system reflects on more than one cardiovascular parameter, one of which is heart rate variability (HRV). HRV is a measure that describes temporal variation between consecutive heart beats and it reflects cardiac autonomic regulation. Measurement of HRV has been shown to be a reliable and reproducible non-invasive technique for assessing autonomic function in health and disease (Khand *et al*. 2006, Rubin *et al*. 2010). Simple analysis of variation in heart rate has been used in clinical practice since the early 1960s and the various methods of analysis used to study the variability of these signals has caught researcher interest in the mid-eighties (Baselliand and Cerutti 1985). At present, HRV has many significant clinical applications (Pumprla *et al*. 2002) and a prognostic significance (Smilde *et al*. 2009, Günther *et al*. 2011).

The autonomic modulation of HRV is associated with day/night cycle variations (Bonnemeier *et al*. 2003). An example of this circadian variation is the demonstration of the sympathetic

dominance on HRV to be in the morning 'active period' compared to the parasympathetic dominance which was shown to be at night 'inactive period' (Ewing *et al*. 1991).

In this study, we investigated the effect of administering lisinopril (for ten weeks) to spontaneously hypertensive rats (SHRs) on day/night variation of blood pressure, heart rate and autonomic cardio-regulation. The rationale was based on the observation that changes in RAS influence HRV (Task Force of the European Society of Cardiology 1996), the observation that angiotensin converting enzyme inhibitors (ACEIs) have been shown to improve HRV in hypertensive patients (Menezes *et al*. 2004), the observation that the autonomic modulation of HRV was associated with day/night cycles and the observation that ANS and RAS 'crosstalk' (Grassi 2001) Hence, we hypothesized that lisinopril as an ACEI may have differential day/night effect on HRV in these animals.

Material and method

Animals

Male SHRs rats weighing 180-220 g (ten weeks old) were obtained from Sultan Qaboos University (SQU) Small Animal House facility. The rats were maintained individually in standard cages with wood shavings. All rats had free access to standard laboratory food pellets and water, and were housed in a temperature-controlled room (21-23° C) with a 12/12-h light/dark cycle with light off at 18:00h.

All procedures were performed in accordance to SQU Animal Ethics Committee regulations and SQU Guidelines for Care and Use of Laboratory Animals.

Surgical Procedure and Telemetry Implants

The implantation of radiotelemetry transmitters (C50- PXT Implants; Data Science Int., St. Paul, MN, USA) which provide direct measurements of arterial pressure (AP), electrocardiogram (ECG) wave, temperature and locomotion activity was preformed as previously described (Gordon et al. 2000, Kramer et al. 2000) with minor modification.

Briefly, rats were anesthetized using intraperitoneal (i.p.) injections of 75 mg/Kg ketamine HCl (Ketasect) and 5 mg/Kg xylazine HCl (Xylajet). A midline incision (5-7 cm) was made along the abdominal muscle. The viscera were pushed aside and the descending aorta was located, exposed, and cleaned from the surrounding connective tissue and fat with a wet cotton swab. The telemetry transmitter was inserted into the lumen of the aorta up to 6 mm above femoral bifurcation and its body was fixed in the abdominal cavity with non-absorbable suture. The abdominal muscle and skin incisions were closed with absorbable suture.

Two ECG leads were routed subcutaneously to the abdominal side of the animal. The negative lead was sutured in the area of the right shoulder and the positive lead in the area of the left groin. The leads were fixed in their position by suturing some muscle around the end of the lead with non absorbable suture.

Prior to the surgery and 5 day thereafter, rats were given enrofloxacin (10mg/kg i.m.). Rats were allowed two weeks to recover from surgery prior to the start of data collection.

Treatment

SHRs were allocated to two groups, the first (n=5) was treated with lisinopril daily (between 16:00 and 17:30 hrs) for 10 weeks with 20mg/Kg/day by gavage, (treated group (T); whereas the second (n=5) was gavaged daily with tap water (untreated group; (UT). The body weight was monitored daily and the dose was adjusted accordingly.

Data Collection

The transmitted signals were received from the implanted radiotelemetry transmitter via data exchange matrix (placed beneath each animal cage) by a radio receiver (model: RPC-1). Data were collected using computerized data acquisition system software (Dataquest A.R.T. 4.0, Data Sciences, Int) at the start and end of experiment i.e. after ten weeks. The telemetry parameters were monitored for 30 min at two different periods (being the most convenient to our setting), day time at 7:00 hrs (inactive period) and night time at 19:00 hrs (active period), with segment duration of 40 sec every minute. The transitional period from ‘active’ to ‘inactive’ period and vice versa was avoided. One day prior the treatment, AP and ECG were recorded from all

groups. Data were collected at the end of weeks one, and ten. The ECG and blood pressure waves were sampled at 1 KHz and 500 Hz respectively.

HRV analysis

Spectral analysis is a quantitative method used to evaluate the interaction of the sympathovagal tone and modulation of cardiovascular functions (Malliani and Pagani 1991, Rubini *et al*. 1993, Montano *et al*. 1994). Power spectral density of each time series was performed on a frequency domain analysis of HRV by using autoregressive approach. Briefly, a first stationary segment, 150-240 beats length, of beat-to-beat series of ECG was selected to calculate power spectrum based on Levinson-Durbin recursion and the model order (range 8-14) was automatically selected using Akaike criterion. The autoregressive spectral method permitted to automatically quantify the center frequency and the power of relevant oscillatory component present in the time serious (Malliani and Pagani 1991, Rubini *et al*. 1993). Threshold-derivative algorithm was used to analyse RRI utilizing HeartScope software (AMPS. llC, USA).

The power spectrum of short term HRV contains three power components: a very low (VLF: below 0.20 Hz), a low (LF: 0.20-0.75 Hz) and a high (HF: 0.75-4 Hz) frequency component (Bertagnolli *et al*. 2008). The power components of LF and HF were expressed in absolute values (ms^2). The ratio of LF/HF was also computed to measure sympathovagal tone. In all analyzed data files there were three exclusion criteria followed. Firstly, the respiratory rate has square coherence value always exceed 0.5 with HF while with LF is always below 0.5. Secondly, all the series had VLF power less than 80%. Thirdly, the locomotion activity of the rats was not more than 2 count/minute.

Statistical Analysis

All data were expressed as mean ±SEM. t-test was used to compare the difference. P value of < 0.05 was considered significant.

Results

Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Heart Rate (HR)

The summary of these data are listed in Table 1. Lisinopril significantly reduced SBP and DBP at 'active' and 'inactive' periods; no changes in blood pressures were observed in untreated SHRs.

The mean values of HR in both groups were not significantly different during active and inactive periods at the start of the experiment. However, after ten weeks, HR was significantly lower compared to their HR values at the start of the experiment only during the inactive period with no changes observed during active period. Treatment did not alter the HR in both periods.

The changes in the RRIs lengths mirrored the changes in HR changes though oppositely being the reciprocal of HR values. We included the RRI (mean values to compare their distribution in accordance to their length in ms (Figure 1). Lisinopril treatment for ten weeks showed a trend to re-distribute the number of RRIs. During the inactive period, lisinopril broadened the intervals distribution i.e. RRIs were less frequent in the center of the histogram indicating a shift from a Gaussian to a binomial distribution (Figure 1B compared to Figure 1A); whereas it caused a shift-to-left during active period i.e. more RRIs were shorter in duration (Figure 1D compared to Figure 1C).

Heart Rate Variability (HRV)

Compared to week zero, both groups showed increase in HRV at week 10. The difference was significant only during active period. The most pronounced effect of lisinopril treatment was observed on HRV. There was about 2-3 fold increase in HRV due to lisinopril treatment in SHRs (Figure 2). The effect was even more obvious during 'inactive' period (6.20±1.03 ms^2 and 24.93±4.2 ms^2) at the start and after ten weeks respectively, while that of the untreated rats showed slight but insignificant increase from 7.85 ±1.4 to 11.36±1.87 ms^2. At the end of 10 weeks of treatment, the variance of treated was significantly higher than that of UT during both periods (24.93±1.77vs 11.36±1.87 ms^2 during inactive and 20.08±2.82 vs 9.8±1.02 ms^2 during active period.

The animals' spontaneous locomotor activity (SLA) was neither significantly different between treated and untreated groups at the start of the experiment nor it was after 10 weeks, yet it

showed different day/night variability. However, it may need to be reiterated that the segment used to analyse HRV was selected to be with less than two counts/min to ensure minimal interference with the ECG signal.

Spectral Analysis

After 10 weeks, the untreated SHRs data showed small increase in HF both in active (2.45±0.31 to 5.74±0.45 ms^2) and inactive (4.94±1.03 to 7.36±1.28 ms^2) periods (values only active periods were significant) (4.94±1.03 and 2.45±0.31ms^2) at the start of the experiment, while these values were slightly higher (7.36±1.28 ms^2 and 5.74±0.45 ms^2) ten weeks after, during inactive and active periods; respectively. The LF component values were 0.39±0.09 ms^2 and 0.34±0.05 ms^2 at the start and 0.82±0.21 and 0.87±0.24 ms^2 ten weeks after; respectively. The VLF component of these rats followed the same pattern with the values 2.53±0.74 ms^2 and 1.3±0.4 ms^2 at the start of the experiment and 3.32±0.74 and 1.30±0.41 ms^2 ten weeks after; respectively (Table 2).

Spectral analysis of data obtained from lisinopril treated SHRs were associated with significant increases (compared to untreated animals) in all components (Table 2). The HF component values were 3.72±0.74 and 3.90±0.87 ms^2 at the start and 15.99±3.24 and 14.48±2.76 ms^2 ten weeks after lisinopril treatment during inactive and active periods; respectively. While the LF components values were 0.31±0.09 and 0.43±0.13 ms^2 at the start, which increased to 1.63±0.44 and 1.59±0.28 ms^2 ten weeks after during inactive and active periods; respectively. The VLF component exhibited the highest increase due to lisinopril treatment. At the start of the experiment it was 2.18±0.78 and 3.19±1.23 ms^2 which increased to 6.95±1.77 ms^2 and 4.01±1.35 ms^2 during inactive and active periods after ten weeks of treatment. The ratio of LF/HF which is indicative of sympatho-vagal tone showed no significant changes between untreated and treated rats.

Discussion

This work compared the effects of lisnopril on blood pressure, HRV and indexes of the ANS involvement in cardiovascular control in SHRs during active (night) and inactive (day) times using telemetric monitoring. The drug significantly reduced blood pressure to normotensive range, which is in line with its antihypertensive properties, had no effect on heart rate which was significantly increased in all rats (treated and untreated) during night, an effect that may be explainable by the increased activity of animals during that period. In addition, lisinopril treatment significantly increased the HRV and its three components namely HF, LF and VLF.

Abnormalities in cardiac autonomic regulation have been shown to contribute to mortality regardless of blood pressure level (Hallstrom *et al*. 2005, Thayer et al. 2010). Therefore, several studies have compared the ability of antihypertensive drugs to better control autonomic cardiovascular parameters independent of hypertension (De Tommasi *et al*. 2003 , Pavithran *et al*. 2010). The most important of these parameters is HRV using its three components *viz.* HF, LF and VLF as indexes of autonomic control of heart rate **(**Stauss and Persson 2006**)**. The HF component is considered to be a product of vagal efferent activity while both vagal and sympathetic modulation are considered to be responsible for LF component. The VLF component is the least defined spectrum of HRV; yet it is speculated to be related to hormonal modulation such as renin-angiotensin system and thermoregulation (Pumrla *et al*. 2002).

In our study, lisinopril caused 2-3 fold increase in HRV (Figure 2). Our results are in agreement with that of Banach *et al*. (2001) and Dias da Silva et al. (2006) who showed that ACE inhibition improves total HRV spectrum and the HF component. Experimental data in humans and animals suggest that the increases in HRV induced by ACE inhibition are mainly due to better modulation of cardiac vagal activity as indicated by increases in HF component (Stauss 2007). However, in our study both HF and LF showed significant increases. Since LF spectral component is indicative of both vagal and sympathetic modulation (Stauss 2007), it is not possible to conclude from our data if sympathetic modulation is also improved. However it may be speculated that the increases in HRV in response to lisinopril treatment are mainly due to increases in vagal modulations since LF/HF ratio showed no significant differences. Yet due to

the lack of BRS sensivity data, it is not possible to speculate if the observed HRV changes were coupled with changes in BRS or with changes of intracardiac origin (Papaioannou *et al* 2012).

Lisinopril significantly increased the VLF component of HRV during active periods only. Circadian rhythm of HR and BP are closely related to circadian fluctuations of ANS function; sympathetic tone being dominant during active periods while vagal tone being dominant during inactive periods (Akita *et al*. 2002). Accordingly, we analysed if the effects of lisinopril on HRV and on its components as markers of ANS were different between inactive and active periods. The drug caused indifferent but significant increases in HF and LF components (and hence HRV) during active and inactive periods. Although our data are in line with the observation that autonomic modulation of HRV is associated with day/night cycle variations (Bonnemeier *et al*. 2003), they are not in complete agreement with what has been previously reported that sympathetic dominance on HRV to be in the morning 'active period' compared to the parasympathetic dominance which was shown to be at night 'inactive period' (Ewing *et al*. 1991).

HRV measures beat-to-beat variance of the lengths of RRIs regardless of its nature i.e. it does not specify "how" was that variance achieved? In this study, to answer this question, at least in part, we plotted the numbers of RRIs versus its length in milliseconds (Figure 2.) As the distribution histogram shows, lisinopril effect on HRV was associated with a tendency to increase the number of "longer" RRIs during inactive period (Figure 1B) but a tendency to increase the number of "shorter" RRIs during active period (Figure D). The shape of the histogram after ten weeks of lisinopril treatment during active period resembles, to a certain degree, the distribution of the untreated rats during the inactive period i.e binormial distribution and *vise versa*. Such may add more insight on how the "variability" in RRIs is induced. Our data, in this respect, were not so decisive having not achieved statistical significance. Probably, administering lisinopril for longer than ten weeks or monitoring HRV for longer periods may make such an effect more evident and may statistically manifest. Nevertheless, no direct explanation or implication of the effect of lisinopril on distribution of RRIs can be provided. However, a number of speculations may be projected. For example, frequent sudden large beat-

to-beat changes in RRIs occur throughout the day and night (Khand *et al*. 2006) and lisinopril may play a role to modulate these changes.

In conclusion, our study was meant to investigate lisinopril effect in the context of day/night cycle since it has been shown that both autonomic modulation of HRV and ANS/RAS 'crosstalk' were associated with day/night cycles (Grassi 2001). However, such makes control of the cardiovascular system more complex (Hoyer 2007, Golombek and Rosenstein 2010). Theoretically, a differential day/night effect on HRV should, probably, have been noticed.

A limitation of our study is the the sampling time of the data which was one hour after changing the light cycle. At that early phase of the light cycle period, the effect of the drug, if any, might not be best demonstrated. An intermediate sampling time during the day/night period, might have shown clearer differential effect of the drug on HRV. On the other hand, the effect of sampling time on the plasma concentration of lisinopril should be negligible since lisinopril concentration, as any other drug, reaches a steady state after 4-5 half-lives, which has been well achieved after the first week of the experiment. Therefore, no influence on HRV should be attributed to lisinopril dosage regimen.

Another limitation of the study is the lack of data from normotensive rats, hence no extrapolation of lisinopril effect could be made to non-hypertensive animals. However, it has been shown that lisinopril antihypertensive properties may be independent of other cardiovascular effects (Porritt *et al* 2010).

Our work showed no differential effect on HRV value but showed a tendency to "differentially" affect its distribution. The complexity of the system warrants further work with longer period of observation and measuring animal's activity to ascertain whether or not the rhythmic variation in behavior had influenced HRV.

Conflict of interest

The authors declared no conflict of interest

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Table 1. Effect of lisinopril treatment on spontaneously hypertensive rats blood pressure and heart rate

	Inactive period				Active period			
	UT		T		UT		T	
	W0	**W10**	**W0**	**W10**	**W0**	**W10**	**W0**	**W10**
SBP (mmHg)	170±7	178±8	163±4	97±5*	166±8	177±6	165±4	84±6*
DBP (mmHg)	110±6	113±7	114±3	59±7*	112±8	118±7	118±4	51±7*
MBP (mmHg)	130±6	135±7	130±3	71±6*	130±8	137±6	134±4	62±7*
HR (bpm)	287±5	260±3*	309±15	262±6*	305±9	307±7	308±11	303±8

Table1. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MBP) and heart rate (HR) values (± SEM) of spontaneously hypertensive rats treated with lisinopril 20/mg/kg/day for ten weeks (T) or untreated controls (UT) during inactive (day) and active (night) periods at the beginning (W0) and the end (W10) of the experiment.* indicates statistically significant difference compared to beginning of experiment (W0) of the same group.

Table 2. Effect of lisinopril treatment on spontaneously hypertensive rats autonomic nervous system indexes affecting heart rate variability

	Inactive period				Active period			
	SHR-UT		SHR-T		SHR-UT		SHR-T	
	W0	W10	W0	W10	W0	W10	W0	W10
HF (ms^2)	4.94±1.03	7.36±1.28	3.72±0.74	15.99±3.24*†	2.45±0.31	5.74±0.45*	3.895±0.87	14.48±2.76*†
LF (ms^2)	0.39±0.09	0.82±0.21	0.31±0.086	1.16±0.44*†	0.335±0.06	0.87±0.24*	0.425±0.13	1.59±0.28*†
VLF (ms^2)	2.53±0.74	3.23±0.95	2.18±0.78	6.95±1.77*†	1.30±0.40	3.19±1.10	3.19±1.23	4.01±1.35
LF/HF	0.079±0.01	0.111±0.02	0.085±0.02	0.134±0.36	0.150±0.03	0.153±0.13	0.105±0.02	0.124±0.03

Table 2. Values(ms^2) ± SEM of heart rate variability components: HF (high frequency), LF (low frequency) VLF; very low frequency and LF/HF ratio recorded from spontaneously hypertensive rats treated with lisinopril 20/mg/kg/day for ten weeks (T) or untreated controls (UT) during inactive (day) and active (night) periods at the beginning (W0) and the end (W10) of the experiment.* indicates statistically significant difference compared to beginning of experiment (W0) of the same group, † indicates statistically significant difference compared to untreated value of the same week.

Figure 1

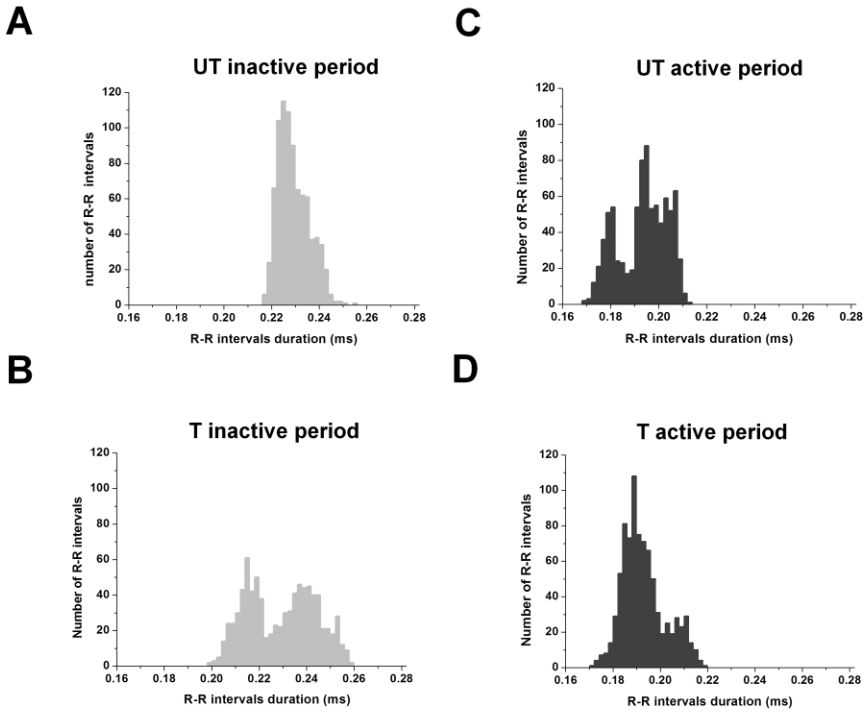

Figure 1. Distribution of R-R intervals (duration *vs.* number) as recorded from spontaneously hypertensive rats ten weeks after treatment with lisinopril 20mg/Kg/day (T) and from untreated controls (UT) during active (night) and inactive (day) periods. Graphs A and C depict data from untreated rats during inactive and active periods respectively, and graphs B and D depict data from treated rats during inactive and active periods respectively

Figure 2

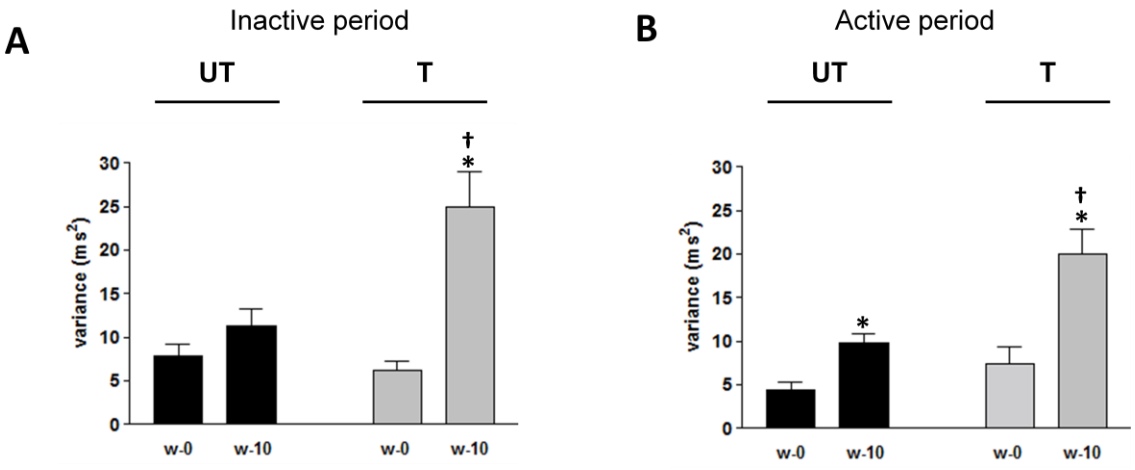

Figure 2. Mean values (±SEM) of heart rate variability (HRV) expressed as variance (ms^2) during inactive (A) and active (B) periods. In untreated rats (UT), variance showed significant increase in week ten (w-10) compared to start of the experiment (w-0) during active periods only. Lisinopril treatment (20mg/kg/day) for ten weeks significantly increased HRV of treated rats (T) compared to untreated rats (UT). *indicates statistically significant difference compared to w-0 of the same group, † indicates statistically significant difference compared to untreated value of the same week.