Heart Rate Variability in Conscious and Anesthetized Rats under the Action of Angiotensin Converting Enzyme Inhibitors

M. M. Fateev, A. V. Sidorov, M. V. Grigor'eva, A. A. Rakov, and K. M. Fateeva

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We studied the effects of three various angiotensin converting enzyme inhibitors (enalapril, lisinopril and quinapril) on heart rhythm variability in anesthetized and immobilized rats. In all cases (except for quinapril in experiments on anesthetized animals), the preparations reduced the total rhythm variability and, according to spectrum analysis, increased activity of the parasympathetic autonomic nervous system to different degrees and decreased sympathetic tone. Quinapril and lisinopril produced the most pronounced influence on heart rhythm in anesthetized rats; enalapril was less potent in this respect. In immobilized animals, quinapril and enalapril showed the greatest activity and lisinopril the lowest. The more pronounced effect of quinapril both under anesthesia and during immobilization appears to be linked to the highest affinity of quinaprilat to circulatory and tissue compartments of the renin-angiotensin-aldosterone system.

Key Words: *heart rate variability; autonomic nervous system; ACE inhibitors; short-term immobilization stress*

Analysis of heart rhythm variability (HRV) is a simple and reliable tool to study humoral and autonomic nervous regulation. The method was derived from the space and sports medicine and acquired special assessment and prognostic significance for patients with cardiovascular pathologies, since impairment of hormone and mediator metabolism underlies pathogenesis of most cardiovascular diseases, in particular chronic heart failure, coronary heart disease, hypertension, *et al.* Modern cardiac drugs comprise a group of so-called neurohumoral modulators (α- and β-adrenergic receptor blockers, inhibitors of angiotensin converting enzyme (ACE), antagonists of angiotensin II receptor, *etc*.) directly affecting the function of the regulatory systems. It is difficult to overestimate the importance of HRV analysis to get more complete idea on the mechanisms of action of these drugs and clarify their central and peripheral effects in various pathological and functional states of the organism.

We previously studied the effect of some β-adrenergic blockers on the heart rhythm of rats in different states such as anesthesia, immobilization stress, as well as in chronic heart failure [5-7].

Here we studied the effects of different ACE inhibitors on HRV in conscious stressed rats and those under anesthesia.

MATERIALS AND METHODS

Two series of experiments were carried out on adult outbred male albino rats weighing 180-210 g. In series I, experiments were performed on three groups of animals (n=15 in each) anesthetized with Nembutal intraperitoneally in a dose of 40 mg/kg and immobi-

Department of Normal Physiology and Biophysics, Department of Pharmacology, Yaroslavl State Medical Academy, Russia. ***Address for correspondence:*** fateev52@mail.ru. M. M. Fateev

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lized in the supine position. Rats of groups 1, 2, and 3 received 1.7 mg/kg enalapril, 3.0 mg/kg quinapril, and 0.8 mg/kg lisinopril in a volume of 1 ml, respectively, through a gastric tube. ECG was recorded immediately before and 1 h after injection of the drugs in rats under previous experimental conditions (anesthesia+immobilization).

In series II, the experiments were performed on conscious rats divided into 4 groups. Group I animals (control, n=40) intragastrically received 1 ml saline; rats of groups 2 (n=15), 3 (n=15), and 4 received enalapril, quinapril, and lisinopril, respectively. The drugs were used in the same doses and volume as in experimental series I. One hour after drug administration, the rats were immobilized in the supine position and ECG was recorded.

The preparations chosen for the study are ACE inhibitors differing by chemical structure and pharmacological properties. In particular, quinapril and enalapril are lipophilic prodrugs, and lisinopril is a hydrophilic drug. The affinity of drugs and their active metabolites to tissue renin-angiotensin-aldosterone system (RAAS) is also different [2]. When calculating the doses of ACE inhibitors, average daily dose for the treatment of chronic heart failure in humans was taken into account as well as the conversion factor for rats equal to 5.9 [3].

ECG was recorded on a 2-channel electrophysiological device (St. Petersburg) connected to a computer via L-CARD E-440 analog-to-digital converter. Sampling frequency was 4.0 kHz. ECG was recorded in lead II for 4 min using L-GRAPH software. The analysis and primary processing of ECG were performed with RRMatch software; final calculation and graphical representation of HRV parameters was performed using CRGraph software [5].

In HRV analysis we used parameters of time domain analysis [1] (HR, standard deviation of normal R-R intervals, coefficient of variation, the square root of the mean of the squared differences between adjacent normal *R-R* intervals), geometric analysis (range of variation, mode, amplitude of the mode, stress index, scattergram area, and scattergram width-to-length ratio) and spectral analysis (power of low-frequency waves [LF], medium [MF] and high frequency waves [HF], total power of spectrum, relative power of LF, relative power of MF, relative power of HF and the index of vagosympathetic interactions). When calculating the geometrical parameters, histogram bin size was equal to 2 msec. In anesthetized rats, LF range corresponded to 0.02-0.15 Hz and HF range to 0.15-2.00 Hz. In conscious stressed animals LF range was 0.02-0.75 Hz and HF range 0.75-3.0 Hz [5].

We calculated the arithmetic mean, standard deviation, error of the mean and used Student's t test. Differences were considered significant at $p<0.05$.

RESULTS

In narcotized rats, some HRV parameters changed after administration of ACE inhibitors (Table 1). Quinapril significantly reduced HR by 40.2% ($p<0.001$) in comparison with initial values, while HR remained virtually unchanged against the background of enalapril and lisinopril treatment. Moreover, quinapril significantly increased the square root of the mean of the squared differences between the adjacent normal *R-R* intervals by 45.6% ($p<0.05$); mode, by 44.8% ($p<0.001$), and scattergram area, 2.3-fold ($p<0.05$). On the contrary, enalapril and lisinopril reduced standard deviation by 1.6 times ($p<0.05$) and 2.3 times ($p<0.01$); coefficient of variation, 1.5-fold ($p<0.05$) and two-fold ($p<0.01$); variation range, by 29.3% ($p<0.05$) and 44.2% ($p<0.01$); scattergram area, by 2.1 times ($p<0.05$) and 3.5 times ($p<0.01$), respectively. In this case, mode amplitude, stress index, and scattergram width-to-length ratio increased significantly only after administration of lisinopril by 1.5 ($p<0.001$), 2.2 ($p<0.001$), and 1.6 times ($p<0.01$), respectively. Other parameters of time domain and geometric analysis did not significantly change under the influence of the test drugs.

Spectral analysis showed that in anesthetized rats, ACE inhibitors virtually did not change the power of LF except of lisinopril, which sharply decreased the power of LF by 9.8 times ($p<0.05$). Only quinapril increased the power of HF by 2.9 times ($p<0.01$). As a result, it was lisinopril, which significantly decreased the total power of spectrum (3.5-fold). Note that all the test ACE inhibitors significantly reduced relative power of LF by an average of 37% ($p<0.05$) in enalapril and quinapril groups and by 57.5% ($p<0.001$), in lisinopril group. On the contrary, enalapril and quinapril increased the relative power of HF by an average of 35% ($p<0.05$), and lisinopril, by 54.2% ($p<0.001$). As a consequence, the index of vagosympathetic interactions decreased in all experimental groups (an average of 3.6 times), but the decrease was significant only under lisinopril (by 6.1 times, $p<0.05$).

Thus, time domain analysis, geometrical analysis and correlation rhythmography indicate that in anesthesized rats, enalapril reduced the activity of parasympathetic system to some extent, and lisinopril also contributed to increased sympathetic tone of the autonomic nervous system. Quinapril, on the contrary, led to activation of parasympathetic regulation without affecting the sympathetic compartment. However, the results of spectral analysis, according to classical interpretation, suggest that all three ACE inhibitors induced qualitatively similar changes in the regulation of HR in anesthetized rats, namely role of the

parasympathetic system increased, and the influence of sympathetic, reduced [1]. Note that enalapril and lisinopril decreased the total HRV in anesthetized rats, while quinapril contributed to its unreliable increase.

General assessment of the intensity of HRV changes allows you to mention that quinapril and lisinopril exerted the greatest influence on HR in anesthetized rats; enalapril, somewhat smaller (Table 1).

The test ACE inhibitors had a greater effect on HRV in conscious rats under short-term immobilization stress (Table 2). Enalapril increased HR by 6.1% ($p<0.01$) and decreased the square root of the mean of the squared differences between adjacent normal *R-R* intervals 1.8-fold ($p<0.001$) compared with controls, whereas quinapril and lisinopril had no effect on this indicator. In addition, enalapril and quinapril significantly reduced standard deviation and coefficient of variation by 2.1 and 2.0 times, respectively, compared with controls ($p<0.01$).

In stressed rats, all ACE inhibitors decreased the variation range, but only enalapril and quinapril significantly changed it by 1.7 and 1.8 times ($p<0.01$), respectively. Only enalapril significantly reduced the mode by 5.8% ($p<0.01$). At the same time, enalapril significantly increased the mode amplitude by 1.6 times; quinapril, by 1.5 times; and lisinopril, by 1.4 times. As a result, the preparations increased stress index by 2.2, 2.3 ($p<0.001$), and 1.5 times ($p<0.05$), respectively.

All the test drugs reduced scattergram area, but only enalapril and quinapril significantly reduced it by 4.1 and 3.3 times ($p<0.01$), respectively. Quinapril significantly increased scattergram width-to-length ratio by 1.8 times ($p<0.001$) compared with controls. Lisinopril had practically no effect on the parameters of correlation rhythmography.

ACE inhibitors also affected spectral parameters in stressed rats. Thus, all the drugs reduced the power of LF. The effect of quinapril was most pronounced: it decreased the power by 15.9 times ($p<0.05$), enalapril by 6.5 times ($p<0.05$), and lisinopril by 2.6 times ($p>0.05$) in comparison with control. In addition, in all experimental groups, the power of HF decreased 3.4-fold ($p<0.01$) against the backdrop of enalapril,

TABLE 1. Effects of ACE Inhibitors on HRV Parameters in Anesthetized Rats (M±*m*)

Parameter	Initial value	One hour after drug administration		
		enalapril	quinapril	lisinopril
HR, bpm	338.0±10.7	350.0±16.1	241.0±19.5^{***xxx}	380.0±16.1^{+++}
Standard deviation of normal *R-R* intervals, msec	2.660±0.225	1.650±0.138*	3.390±0.947	1.180±0.094^{**x+}
Coefficient of variation, %	1.440±0.116	0.940±0.066*	1.110±0.214	0.730±0.048^{**x}
Square root of the mean squared differences between adjacent normal *R-R* intervals, msec	1.60±0.10	1.390±0.108	2.330±0.451^{*x}	1.260±0.101^{+}
Range of variation, msec	14.70±1.02	10.40±1.04*	19.20±4.19^{x}	8.200±0.569^{**+}
Mode, msec	185.40±6.27	175.00±8.02	268.40±25.66^{***xx}	161.00±7.32^{+++}
Amplitude of the mode, %	38.70±2.47	44.50±2.99	36.90±6.13	57.50±4.14^{***x++}
Stress index, arb. units	11,013±1584	16,084±3993	7952±2235	24,711±3950^{***++}
Scattergram area, msec^2	120.80±14.76	58.90±8.49*	278.90±111.8*	34.70±3.87^{**x+}
Scattergram width-to-length ratio, %	41.20±3.59	51.20±5.48	57.80±9.01	67.90±6.57**
LF, msec^2	0.390±0.083	0.110±0.028	0.620±0.353	0.040±0.009^{*x}
HF, msec^2	0.280±0.043	0.220±0.023	0.820±0.365**	0.150±0.023^{x}
Total spectral power, msec^2	0.670±0.099	0.330±0.047	1.440±0.716	0.190±0.029^{*x}
LF, %	48.50±3.61	30.30±4.44*	30.60±4.37*	20.60±2.87***
HF, %	51.50±3.61	69.70±4.44*	69.40±4.37*	79.40±2.87***
LF/HF, arb. units	1.700±0.333	0.500±0.109	0.490±0.089	0.280±0.050^{*+}

Note. Here and in Table. 2: one index, $p<0.05$; two indices, $p<0.01$; three indices, $p<0.001$ in comparison with: *initial values; ^{x}enalapril; $^{+}$quinapril.

3.1-fold under quinapril ($p<0.01$), and 2.1-fold under lisinopril ($p<0.05$). As a result, total spectral power decreased under the effect of enalapril by 5.3 times ($p<0.05$), quinapril by 7.8 times ($p<0.05$), and lisinopril by 2.5 times ($p<0.05$). Only quinapril changed significantly the ratio of relative power of HF to relative power of LF; the LF fraction decreased by 1.6 times and HF fraction increased by 1.7 times. Thus, quinapril sharply reduced the index of vagosympathetic interactions by 4.1 times ($p<0.01$) relative to controls. In turn, lisinopril and enalapril did not significantly alter this indicator.

Thus, based on the time domain analysis, geometric analysis and correlation rhythmography, we can conclude that in stressed animals, enalapril and quinapril administration increased the sympathetic tone to some extent and reduced parasympathetic activity of the autonomic nervous system. However, spectral analysis showed that quinapril increased activity of parasympathetic regulation against the background of decreasing sympathetic activity. Enalapril uniformly reduced the power of LF and HF, as well as the total power of spectrum. Therefore, spectral analysis did not detect significant changes in the prevalence of any division of the autonomic nervous system. In general, HRV in stressed rats was significantly reduced under the influence of enalapril and quinapril. It should be noted that time domain analysis, geometric analysis, and spectral analysis found no significant effect of lisinopril on HR regulation in stressed rats.

Thus, quinapril and lisinopril exerted the greatest influence on heart rate in stressed rats; enalapril, the least influence (Table 2).

Changing the tone of the sympathetic and parasympathetic divisions of the autonomic nervous system affects the sinus rhythm, so that during HR registration, deviations from its mean frequency occurred [4]. These fluctuations measured by HRV indicators are among the most important predictors of life-threatening arrhythmias [4]. Reduced vagal influence providing "antiarrhythmic protection" and increased activity of the sympathetic effects lead to common arrhythmic complications [11]. Spectral analysis of HRV suggests that test ACE inhibitors increased activity of the parasympathetic autonomic nervous system to varying degrees and decreased sympathetic tone both in anesthetized and in stressed rats. In general, this effect can be regarded as favorable. However, the marked changes occurred against the background of pronounced decrease in the overall rate variability (except for the experiment with quinapril in anesthetized animals), that, as is well known [8,12,13], has a negative predictive value. This phenomenon requires further study.

TABLE 2. Effects of ACE Inhibitors on HRV Parameters in Conscious Immobilized Rats ($M\pm m$)

Parameter	Control	Enalapril	Quinapril	Lisinopril
HR, bpm	472.0±4.1	501.0±8.8**	479.0±5.3^{x}	468±6^{xx}
The standard deviation of normal *R-R* intervals, msec	2.290±0.179	1.110±0.047**	1.070±0.088**	1.640±0.192^{x+}
Coefficient of variation, %	1.790±0.133	0.930±0.048**	0.860±0.068**	1.280±0.151^{x+}
Square root of the mean squared differences between adjacent normal *R-R* intervals, msec	1.690±0.087	0.960±0.079***	1.400±0.167^{x}	1.420±0.177^{x}
Range of variation, msec	14.80±1.02	8.60±0.47**	8.40±0.78**	12.30±1.49^{x+}
Mode, msec	127.5±1.2	120.1±2.2**	125.80±1.44^{x}	128.0±1.7^{xx}
Amplitude of the mode, %	38.90±2.07	60.50±2.99***	58.20±3.57***	52.60±3.54**
Stress index, arb. units.	13 829±1533	30 654±2579***	32 092±6342***	20 949±3775*^{x}
Scattergram area, msec^2	110.60±14.12	27.10±2.65**	33.60±5.58**	64.90±16.07^{x}
Scattergram width-to-length ratio, %	45.40±2.92	49.60±4.23	83.20±6.54***^{xxx}	53.10±5.46^{++}
LF, msec^2	1.110±0.206	0.170±0.023*	0.070±0.012*^{xx}	0.420±0.135^{+}
HF, msec^2	0.37±0.04	0.110±0.018**	0.120±0.021**	0.180±0.039*
Total spectral power, msec^2	1.48±0.24	0.280±0.025*	0.190±0.031*	0.600±0.165^{+}
LF, %	63.80±3.08	62.80±5.12	38.80±2.98***^{xxx}	58.80±5.78^{++}
HF, %	36.20±3.08	37.20±5.12	61.20±2.98***^{xxx}	41.20±5.78^{++}
LF/HF, arb. units	2.73±0.34	2.61±0.80	0.670±0.089**^{x}	2.100±0.528^{+}

We revealed unequal activity of ACE inhibitors in various states of experimental animals. Thus, quinapril exerted a pronounced effect on both anesthetized and stressed rats. In anesthetized animals, enalapril showed the lowest activity and lisinopril virtually did not change HRV parameters during stress.

Prodrugs quinapril and enalapril are metabolized into active quinaprilat and enalaprilat, which differ in lipophilicity [2]. It is believed that high lipophilicity determines greater affinity to tissue RAAS (also in the heart and vessels), which accounts for 90% of its volume [9]. ACE radioligand binding method shows [10] that among the test drugs quinaprilat had the highest affinity to both the plasma and tissue RAAS. This fact probably explains high activity of quinapril not only under conditions of stress-induced hyperactivation of the circulatory RAAS when its "emergency" compensatory effects on the myocardium and blood vessels dominate over the action of tissue RAAS, but under anesthesia at rest, when local RAAS have the leading role. Lisinopril has the same affinity to plasma RAAS as enalaprilat, but its affinity to tissue RAAS is more than 2 times higher [10]. This apparently explains why the more pronounced effect of lisinopril compared with enalapril was found by us in anesthetized animals. The fact that under stress conditions enalapril is more active than lisinopril can be explained by its higher bioavailability as a lipophilic substance during intragastric administration.

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