Heart Rate Variability in Rats with Experimental Chronic Heart Failure and Long-Term Exposure to β-Adrenoblockers

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 2, pp. 139-143, February, 2009
Original article submitted December 25, 2007

Simulation of chronic heart failure in rats led to an increase in heart rate variability and decrease in heart rate. All β-blockers also promoted the heart rate variability augmentation in ill animals. The most potent effects on heart rate variability were produced by pindolol, atenolol, and propranolol. Bisoprolol and metoprolol affected heart rate and heart rate variability in less extent, and nebivolol normalized a part of heart rate variability parameters.

Key Words: *experimental chronic heart failure, heart rate variability, autonomic nervous system, β-adrenoblockers, short-term restrain stress*

Application of the heart rate variability (HRV) method for cardiovascular system state estimation in various pathological conditions including chronic heart failure (CHF) is more and more common in clinics [3,12]. β-Adrenoblockers, various in their pharmacokinetic and pharmacodynamic properties, hold one of central positions in contemporary CHF therapy. There are only few studies carried out to evaluate effects of β-adrenoblockers on HRV in humans with various cardiovascular pathologies [4,6,13] and on HRV in healthy animals after single dose [1,10,11,14,15]. Previously we showed that β-adrenoblockers significantly increase 6 month survival in rats with experimental CHF [9] and assessed its effects on HRV of healthy anesthetized animals and awake animals under conditions of short-term restrain stress [7].

The subject of this study was a comparative analysis of HRV characteristics in rats with experimental CHF, treated with β-adrenoblockers of different types.

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MATERIALS AND METHODS

All experiments were carried out on adult nonpedigree male rats weighted 180-210 g. CHF was simulated by functional heart overload [8]. In this purpose rats under hexenalum anesthesia (100 mg/kg intraperitoneally) using method of N. N. Pyatnitskiy and Yu. A. Blinkov in our modification [8] were twice injected with silicon oil into the pleural cavity: firstly 1.5 ml of oil per 100 g of rat body weight and 30 days later 1.0 ml per 100 g of body weight in each pleural cavity. 20-40 days after rats had a dyspnoe, face and paw cyanosis. With CHF aggravation the intensity of dyspnoe and cyanosis increased, animals became hypodynamic, untended, frequently took orthostatic position (with front paws on the feeder edge), the sings of fluid retention (face puffiness, especially of upper lip, and paw puffiness) were noted. Under morphological investigation the cardiomyocyte hypertrophy in both ventricles, an increase in nucleus size, and the hypertrophy and hyperplasia of stroma fiber structures were observed. Signs of lesser circuit hypertension on the background of venostasis were identified in lungs. In liver venostasis and hepatocyte

0007-4888/09/1472-0181

adipose degeneration on the peripherical lobe part were observed, what reflects right ventricle failure [8].

Rats with CHF were divided into 7 groups: in 1st group (n=40) rats received daily from Day 31 to Day 100 saline solution intragastrically; animals in other groups in the same manner received medical products: propranolol (anaprilin, ZAO Medisorb) 10 mg/kg (n=10), pindolol (visken, Novartis Pharma) 2.5 mg/kg (n=14), atenolol (Pliva) 5 mg/kg (n=11), metoprolol (metokard, Polpharma) 10 mg/kg (n=16), bisoprolol (konkor, Nicomed) 5 mg/kg (n=10), nebivolol (nebilet, Berlin Chemie) 0.5 mg/kg (n=14). The conversion coefficient of 5.9 for rats have been taken into account for dose determination [5]. Control group (healthy animals; n=54) received 1 ml of saline intragastrically during the whole experiment.

Chosen medical products represent the main types of β-adrenoblockers (different in selectivity to cardiac β_1-adrenoreceptors and in intrinsic adrenomimetic activity (IAA), physicochemical properties and additional vasodilatational effect) [2].

Upon completion of mentioned period the ECG was recorded in awake immobilized animals. At the moment of ECG recording the age of all animals was the same. ECG registration was performed using dual-channel electrophysiological facility, connected through ADC (L-CARD E-440) with IBM PC Pentium II. Sampling frequency was 4.0 kHz. ECG was recorded in second standard lead for 4 minutes using program L-GRAPH provided with ADC. Viewing and primary processing of ECG data were carried out using software RRMatch, and final calculation of HRV parameters and their graphic rendition were performed in program CRGraph [7].

For HRV analysis were used characteristics of temporal analysis [7] (heart rate, standard deviation of normal *R-R* intervals, coefficient of variation, square root from sum of squared differences of successive *R-R* intervals values), geometry analysis (range, mode, mode amplitude, stress-index, skatterogram area, ratio of skatterogram width to it's length) and spectral analysis: power of low frequency waves (LF), power of high frequency waves (HF), total spectrum power, relative value of LF wave power, relative value of HF wave power, and vagosympathetic interaction index. During calculation of geometric characteristics the histogram step was 2 msec. LF range was 0.02-0.75 Hz, HF range was 0.75-3.0 Hz [7].

Data processing was carried out in Statistica 6.0. Arithmetical mean, mean square deviation, error of mean and Student's t test were calculated. Differences considered to be statistically significant at $p<0.05$.

RESULTS

Animals with CHF were different from the healthy ones (controls) in majority of investigated markers (Tables 1, 2). Heart rate at an average was significantly 1.1 fold lower in that animals compared to control. Values of mean square deviation, coefficient of variation, square root from sum of squared differences of successive *R-R* intervals, range, mode, skatterogram area significantly increased in 1.3, 1.2, 1.2, 1.4, 1.1, and 1.7 fold consequently, and the stress-index decreased in 1.5 folds. All this changes indicated the increase in tonus of parasympathetic department of autonomous nervous system during this pathology, what coincides with results of our previous studies with CHF simulation in rats [8] and conforms to possibility of CHF development in humans by this type [12]. Spectral analysis revealed significant 2.1 fold increase in LF power in rats with CHF compared to healthy animals and consequent 1.7 fold increase in total spectrum power and in vagosympathetic balance, and 1.3 fold increase in relative value of LF power.

All investigated b-adrenoblockers had almost similar beneficial effects on clinical course of experimental CHF: decreased severity of dyspnoe, cyanosis and puffiness disappeared, but changes in HRV were not so well-defined. Following changes in HRV characteristics were noted in rats treated with β-adrenoblockers. Heart rate was greater reduced by atenolol and pindolol (at an average in 1.3 fold in comparison with control group and in 1.2 fold ($p<0.001$) in comparison with 1st one) and weaker reduced by bisoprolol and metoprolol (at an average of 1.1 fold ($p<0.01$) only in comparison with control group). Other parameters of temporal and geometry analyses had similar changes (Tables 1, 2). Values of mean square deviation, coefficient of variation, square root from sum of squared differences of successive *R-R* intervals, range, mode, skatterogram area had a greater increase during treatment with atenolol and pindolol, and stress-index values (in 5.7 fold in comparison with control group and in 3.8 fold in comparison with 1st one, $p<0.001$) and ratio of skatterogram width to it's length (in 1.6 and 1.5 fold consequently, $p<0.01$) were greater reduced under the influence of atenolol. Mentioned HRV parameters were weaker affected by bisoprolol, metoprolol and nebivolol. All this data gives an evidence of parasympathetic autonomous system tonus predominance and coincide with our previous data [7] and with results of another authors concerning effects of β-adrenoblockers to HRV in healthy animals [1,10,11,14, 15]. Moreover, such parameters like coefficient of

TABLE 1. Effects of Long-Term Treatment with β-Adrenoblockers on HRV Parameters in Rats with Experimental CHF (*M±m*)

Parameter	Control	HRF	Propranolol	Atenolol
HR, min^{-1}	493.0±4.3	456.0±4.4***	425.0±6.6***^^	376.0±9.6***^^^°°°
SDNN, msec	1.550±0.067	2.020±0.147**	3.130±0.262***^^	3.860±0.439***^^^
CV, %	1.270±0.055	1.530±0.108*	2.200±0.173***^^	2.370±0.233***^^^
RMSSD, msec	1.400±0.059	1.670±0.103*	1.890±0.161**	2.290±0.245***^^
MxDMn, msec	9.20±0.39	13.10±0.96***	18.40±1.57***^	21.10±1.95***^^^
Mo, msec	122.40±1.04	132.40±1.33***	142.40±2.44***^^	161.00±4.21***^^^°°
AMo, %	49.10±1.49	45.70±1.93	29.10±3.49***^^^	24.30±2.42***^^^
SI, arb. units	25360±1819	16958±1649**	6473±1329***^^	4429±909***^^^
EllSq, $msec^2$	56.00±3.99	97.50±13.18**	166.30±24.18***^	259.30±49.98***^^^
EllAs, %	55.00±2.65	49.70±2.61	32.90±2.76**^^	33.50±3.49**^^
LF, $msec^2$	0.270±0.036	0.580±0.110**	1.550±0.306***^^^	2.330±0.689***^^^
HF, $msec^2$	0.230±0.021	0.270±0.032	0.410±0.089	0.510±0.102***^^
TP, $msec^2$	0.500±0.050	0.850±0.135**	1.960±0.367***^^	2.840±0.743***^^^
LF%	48.60±2.51	61.30±2.56***	78.50±2.34***^^	77.00±3.88***^^
HF%	51.40±2.51	38.70±2.56***	21.50±2.34***^^	22.00±3.88***^^
LF/HF	1.250±0.128	2.060±0.203***	4.090±0.464***^^^	5.290±0.931***^^^

Note. Here and in table 2: SDNN — standard deviation of normal *R-R* intervals, CV — coefficient of variation, RMSSD — square root from sum of squared differences of successive *R-R* intervals, MxDMn — range of variation, Mo — mode, AMo — mode amplitude, SI — sress-index, EllSq — skatterogram area, EllAs — ratio of skatterogram width to it's length, TP — total spectrum power. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the control; $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}p<0.001$ compared to the CHF group; $^{\circ}p<0.05$, $^{\circ\circ}p<0.01$, $^{\circ\circ\circ}p<0.001$ — propranolol in comparison with other β-adrenoblockers; $^{x}p<0.05$, $^{xx}p<0.01$, $^{xxx}p<0.001$ — atenolol in comparison with other β-adrenoblockers; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ — pindolol in comparison with bisoprolol, metoprolol and nebivolol; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ — nebivolol in comparison with bisoprolol and metoprolol.

variation (nebivolol) and square root from sum of squared differences of successive *R-R* intervals (bisoprolol and nebivolol) corresponded to values of healthy animals.

Spectral analysis demonstrated (Tables 1, 2) that LF power significantly greater increased during treatment with propranolol, atenolol and pindolol (in 5.7, 7.9, 8.6 fold, respectively, in comparison with control group and in 2.7, 3.7, 4.0 fold in comparison with 1st one). LF power under influence of bisoprolol and metoprolol was in average 3.2 fold higher ($p<0.001$) only in comparison with control group, and virtually was the same in control and 1st groups during nebivolol treatment (Tables 1, 2). HF power significantly increased in comparison with control group (averagely in 2.3 fold) and with CHF group (averagely in 1.9 fold) only during treatment with pindolol and atenolol. Another β-adrenoblockers had virtually no effect on this parameter in comparison with control animals and with rats from 1st group. Total spectrum power significantly increased in comparison with control under influence of all β-adrenoblockers except nebivolol, but in comparison with 1st group — only during treatment with propranolol, atenolol and pindolol. Similar changes were noted in percent of LF and HF, and vagosympathetic balance was significantly higher under influence of all β-adrenoblockers in comparison with control, and in comparison with CHF group — in all treated animals except nebivolol group. Increase in LF power and, subsequently, in vagosympathetic balance value during treatment of immobilized animals with CHF with β-adrenoblockers completely coincide with our previous data concerning effects of single doses of propranolol and atenolol on HRV characteristics of healthy immobilized animals [7]. It one more time indicates that LF power value reflects not onlysympathetic department of autonomous nervous system, but also another (probably histamine and serotonin systems) neurohumoral mechanisms, which are activated during short-term restrain stress under influence of β-adrenoblockers.

During comparison of β-adrenoblocker effects on HRV in rats with CHF was noted that the most potent effects on HRV characteristics had atenolol and pindolol (their effects did not differ between each other), propranolol in comparison with them had significant changes in heart rate (1.1 fold higher in average) and in mode (1.1 fold less in average; Table 1). Bisoprolol, metoprolol and nebivolol affected HRV parameters in less extent in com-

TABLE 2. Effects of Long-Term Treatment with β-Adrenoblockers on HRV Parameters in Rats with Experimental CHF (*M±m*)

Parameter	Control	Bisoprolol	Pindolol	Metoprolol	Nebivolol
HR, min^{-1}	493.0±4.3	388.00±7.46***^^^^oo	455.0±8.2***oxxx###+	466.0±7.5**ooooxxx###++	435.0±5.7***^xxx###
SDNN, msec	1.550±0.067	3.560±0.587******^^	2.150±0.202**ooxx	2.430±0.243***xx	2.140±0.235**oxx#
CV, %	1.270±0.055	2.250±0.351***^	1.620±0.131*ox	1.870±0.179***	1.550±0.169ox
RMSSD, msec	1.400±0.059	2.340±0.278******^^	1.630±0.114x	1.920±0.225**	1.640±0.098x#
MxDMn, msec	9.20±0.39	22.30±3.59******^^	14.00±1.63***x	15.0±1.1***xx#	12.90±1.04***ooxxx#
Mo, msec	122.40± 1.04	155.70± 2.91***^^^^oo	132.60± 2.36***xxx###	129.90± 2.12**ooooxxx###++	138.60± 1.88***^xxx###
AMo, %	49.10±1.49	33.40±4.32******^^	38.80±3.05**ooxx	36.8±3.7***^x	39.10±2.39**oxxx
SI, arb. units	25360±1819	7967±1867******^^	12348±2274**oxx	11539±2111***x	12311±1443***ooxxx
EllSq, $msec^2$	56.00±3.99	263.70±66.88*******^^	94.10±15.12**oxx#	120.20±18.33***xx#	94.20±15.72**oxx#
EllAs, %	55.00±2.65	41.90±4.14*	44.10±4.55o	50.60±6.54o	47.20±4.68ox
LF, $msec^2$	0.270±0.036	2.120±0.975***^	0.830±0.291***	0.900±0.239***x	0.430±0.094ooxxx
HF, $msec^2$	0.230±0.021	0.530±0.140***^	0.220±0.040x	0.310±0.053	0.220±0.027oxx#
TP, $msec^2$	0.500±0.050	2.650±1.071******^^	1.050±0.322**x	1.210±0.259***x	0.650±0.109ooxxx
LF%	48.60±2.51	64.60±5.27**o	71.90±4.46***	65.80±5.07**	60.20±4.69*oox
HF%	51.40±2.51	35.40±5.27**o	28.10±4.46***	34.20±5.07**	39.80±4.69*oox
LF/HF	1.250±0.128	3.390±0.935***^	3.51±0.75***^	3.450±0.842***^	2.080±0.394*oox

parison with above mentioned β-adrenoblockers (Table 2). Bisoprolol and metoprolol effects on heart rate were practically the same, and nebivolol differed from them only in effects on heart rate (1.1 fold low in average) and mode (1.1 fold higher in average). Moreover, such HRV characteristics like coefficient of variation, square root from sum of squared differences of successive *R-R* intervals, LF power and total spectrum power during nebivolol treatment matched values of control animals.

Analysis of data obtained allows to make a conclusion that high-selective lipophilic compounds of 2nd and 3rd generation, like bisoprolol, metoprolol, and vasodilating nebivolol have a mild effect on heart rate in comparison with nonselective lipophilic atenolol and nonselective pindolol. Our previous studies showed that in particular high-selective lipophilic β-adrenoblockers more significantly increased survival of rats with CHF. The most potent clinical activity of bisoprolol, metoprolol and nebivolol in CHF treatment seems to be associated with their normalizing effect on catecholamine metabolism in myocardium [8,9].

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