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Influence of Central Neurotransmitters on Heart Rate Variability in Outbred Rats at Rest and during Acute Stress: Nature of Very-Low-Wave Spectrum Component Revisited

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We studied the effect of blockade of neurotransmitter monoamine reuptake and inhibition of catecholamine synthesis on heart rate variability in outbred albino rats at rest and during acute stress. Amitriptyline increased, while α-methyltyrosine reduced the power of VLF waves in heart rate variability spectrum. The effect of amitriptyline manifested at rest and was most striking under stressful conditions, while the effect of α-methyltyrosine was observed only under conditions of acute stress. This suggests that VLF-band waves in the heart rate variability spectrum have catecholaminergic nature and are determined by activity of the central monoaminergic systems; the role of peripheral adrenergic influences mediated by humoral and nervous regulation in the formation of these waves cannot also be excluded.

Key Words: *heart rate variability; VLF-band of spectrum; amitriptyline; α-methyltyrosine; stress*

Modern physiological interpretation of heart rhythm wave structure is based on the concept of multilevel heart regulation system [2]. Respiratory arrhythmia, also known as high-frequency (HF) component of parasympathetic nature, is considered to be the basic component of heart rate variability spectrum (HRV) [2,3,5,11]. It is assumed that slow LF-waves are associated with vascular tone regulation, and both sympathetic and parasympathetic neural inputs associated with baroreflex control of heart rate participate in their formation [4,5,12]. The nature of VLF-component of the heart rate wave structure is still least studied [2,11]. A theory of humoral-metabolic origin of VLF-waves was proposed [2]. At the same time, functional probes prove the sympathetic nature of VLF in humans and the rise of VLF at the peak of the sympathomimetic phase of epinephrine action was demonstrated [5]. Some authors believe that VLF waves have neurogenic nature and reflect the degree of activation of cerebral subcortical structures [3] or the influence of higher autonomic centers on the cardiovascular center in the brainstem and the segmental level of blood circulation regulation [2]. Here we studied heart rate regulation and changes in the power of very slow waves of HRV spectrum in outbred albino rats at rest and under conditions of acute stress during simulation of high and low activity of the brain monoamine systems.

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MATERIALS AND METHODS

Awake mature outbred male rats were used in the study. The animals were kept under standard vivarium

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conditions with free access to food and water. Rats with high activity of the autonomic regulation circuit and dominance of high-frequency waves (HF) in HRV spectrum were selected for the experiment on the basis of preliminary HRV analysis in quiet awake state [5]. For activation of the central monoaminergic system, group 1 rats were intraperitoneally injected with amitriptyline, a non-selective transmitter reuptake blocker, in a dose of 2.5 mg/kg body weight for 7 days. This dose of amitriptyline constitute $^1/_4$ of its maximum effective dose [9], the possibility of intraperitoneal and intravenous administration of amitriptyline to rats in comparable doses was shown previously [10,13]. For reducing the concentration of adrenergic transmitters in the brain, catecholamine synthesis was blocked by intraperitoneal administration of D,L-α-methyl-P-tyrosine methyl ester hydrochloride (α-MT; Sigma; group 2) in a dose of 180 mg/kg body weight for 3 days. The dose was selected on the basis of previous data [14]. Control group consisted of intact rats receiving 0.9% NaCl intraperitoneally (0.5 ml/100 g of body weight) for 3 days before the main experiment. The main experiment included ECG recording in awake rats after completion of drug administration (3 h after the last injection). For evaluation of specific features of stress-induced HRV changes under normal conditions and against the background of stimulation and blockade of the monoaminergic system, each rat was subjected to acute emotional painful stress (EPS) for 1 h including immobilization of the animal and electric painful stimulation according to a stochastic scheme [8].

ECG in quiet wake state [6] and on minutes 15, 30, 45 и 60 of EPS was recorded via miniature electrode clamps using Varicard hardware-software complex (Ramena). Measurement of *R-R* intervals and primary data processing were performed using ISKIM6 software (Ramena). Continuous 1-min series of *R-R* intervals were used for the analysis. Parameters of variation pulsometry were calculated: heart rate (HR), *R-R* interval mode (Mo), stress index according to R. M. Bayevsky [2]. Spectral analysis of HRV was performed in HF (0.9-3.5 Hz), LF (0.32-0.90 Hz), VLF (0.18-0.32 Hz) ranges. Total spectral power was determined (TP, msec^2) and index of centralization was calculated (IC=(LF+VLF)/HF). The data were processed statistically by Student *t* test and analysis of variation (ANOVA) using Statistica 6.0 software.

RESULTS

Administration of physiological saline had no effect on parameters of HRV (control; Table 1). Amitriptyline increased heart rate ($p<0.05$) and stress index ($p<0.01$), which attests to strengthening of simpathoadrenal influences on the heart [2]. Similar effect of amitriptyline on heart rate was reported previously [1]. Administration of amitriptyline also increased slow wave spectral power, especially in VLF range ($p<0.05$): these rats had higher VLF ($p_F=0.003$) and centralization index ($p_F=0.003$) at rest compared to controls. Administration of α-MT reduced heart rate ($p<0.05$) and Mo ($p<0.05$), increased TP ($p<0.01$) at the expense of increased HF range power ($p<0.01$) and trends to an increase in other components; this determined higher HRV in these rats compared to control animals (Table 1). Analysis of variances showed that the increase in VLF wave power and their domination in the HRV spectrum at rest were not fortuitous, but most likely were a result of action of an organized factor, aminotriptyline administration (Wilks $\lambda=0.45$, $p=0.008$), while the effect of α-MT on spectral properties of heart rhythm was less pronounced (Wilks $\lambda=0.81$, $p=0.253$).

In control rats, the major changes in HRV, increase in heart rate ($p<0.001$) and stress index ($p<0.05$) at the early EPS stage (15 min), were driven by reduction of cardiointerval Mo ($p<0.001$, $r=-0.99$). Stress index remained high during the entire EPS procedure, predominantly due to low Mo value ($p<0.001$). By minute 30 of EPS, the power of LF-waves reflecting baroreflex activity [5,6,13] increased almost twice and VLF-waves tended to increase; these shifts determined the increase in centralization index on minutes 30 ($p<0.001$) and 60 ($p<0.05$) of the stress procedure.

In group 1 rats, the stress-induced tachycardia peaked on minute 30 of EPS ($p<0.001$). In contrast to the control, stress against the background of amitriptyline administration induced an increase in HRV. Sharp growth of TP during the first minutes of EPS was determined by enhancement of oscillations in all frequency ranges: 3.3-fold in HF range ($p<0.05$), 6.2-fold in LF range ($p<0.01$), 8.2-fold in VLF range ($p<0.01$); the latter which became predominant in the HRV spectrum. Centralization index during this period maximally increased ($p<0.05$) and stress index decreased ($p<0.01$). On minutes 45-60 of stress, VLF wave power returned to the level observed at rest, but HF and LF wave powers remained elevated ($p<0.05$), which determined higher TP values ($p<0.01$) and low stress index values ($p<0.01$) compared to the control. Analysis of variances showed that enhancement of VLF waves on minutes 15-30 of EPS is largely associated with preliminary administration of amitriptyline to animals (Table 1).

In group 2, stress-induced tachycardia was weaker than in group 1 and control group, it peaked on minute 15 of EPS ($p<0.001$). Stress index increased by minute 15 of stress procedure ($p<0.05$) and remained elevated during the entire EPS with a peak

TABLE 1. Parameters of HRV in Male Outbred Albino Rats at Rest and during Acute Stress (*M*±*m*)

Parameter	Rest		EPS			
	before drug administration	after drug administration	15 min	30 min	45 min	60 min
Control group (*n*=10)						
HR, bpm	318.8±4.7	315.2±7.4	436.2 ± 9.4^{xxx}	446.6 ± 8.4^{xxx}	422.1 ± 5.6^{xxx}	411.4 ± 5.8^{xxx}
Mo, msec	189.5±4.5	192.7±4.5	139.4 ± 3.6^{xxx}	135.3 ± 2.7^{xxx}	140.8 ± 3.8^{xxx}	146.4 ± 3.1^{xxx}
SI, rel. units	18.46±3.65	24.66±2.25	44.19 ± 3.01^{xxx}	35.34 ± 1.50^{xxx}	39.33 ± 3.25^{xxx}	37.43 ± 3.49^{xx}
TP, msec^2	14.13±1.99	15.10±2.09	18.01±3.64	18.21±3.33	21.85±3.36	16.08±2.83
HF, msec^2	6.76±1.79	8.75±1.49	11.08±3.15	5.37±0.81	9.80±1.81	7.79±1.52
LF, msec^2	4.02±0.79	3.57±0.58	3.74±0.51	7.96 ± 1.15^{x}	7.96 ± 1.07^{x}	5.98 ± 0.55^{x}
VLF, msec^2	3.34±0.48	2.76±0.40	2.14±0.39	4.88±1.00	4.09±0.65	4.08±0.61
IC, rel. units	1.18±0.43	0.75±0.10	0.63±0.08	2.97 ± 0.55^{xxx}	1.37 ± 0.18^{x}	1.39 ± 0.15^{x}
Spectrum ratio	HF>LF>VLF	HF>>LF>VLF	HF>>LF>VLF	LF>HF>VLF	HF>LF>VLF	HF>LF>VLF
Rats with transmitter reuptake blockade (*n*=7)						
HR, bpm	315.0±5.2	$334.7\pm5.4^{+}$	414.5 ± 5.7^{xxx}	420.2 ± 5.3^{xxx}	407.2 ± 4.9^{xxx}	413.2 ± 6.4^{xxx}
Mo, msec	190.6±4.1	180.8±3.4	145.3 ± 4.6^{xxx}	137.2 ± 3.6^{xxx}	146.3 ± 2.9^{xxx}	141.8 ± 4.2^{xxx}
SI, rel. units	18.07±3.63	$30.28\pm4.12^{+**}$	$14.21\pm2.85^{xx**}$	$11.38\pm2.25^{xx***}$	$16.50\pm2.93^{x**}$	$14.78\pm3.51^{x***}$
TP, msec^2	19.05±2.56	18.93±2.48	$54.11\pm4.72^{xxx***}$	$113.9\pm18.8^{xxx**}$	28.95 ± 3.88^{x}	$35.21\pm4.65^{xx**}$
HF, msec^2	7.28±0.75	5.87±1.75	12.00 ± 2.04^{x}	$19.65\pm4.98^{x**}$	11.39±2.14	$15.48\pm2.93^{x*}$
LF, msec^2	4.21±0.56	5.98±1.14	$20.06\pm3.0^{xxx***}$	$38.42\pm7.98^{xx***}$	11.23 ± 1.80^{x}	$12.54\pm1.98^{x**}$
VLF, msec^2	3.91±0.78	$6.79\pm0.97^{+***}$	$22.04\pm3.18^{xxx***}$	$55.89\pm14.68^{xx***}$	6.21±1.30	7.18±1.14*
IC, rel. units	1.11±0.27	2.34±0.51**	$3.92\pm0.43^{x***}$	$7.37\pm1.87^{xxx*}$	2.04±0.28*	2.46±0.59
Spectrum ratio	HF>LF>VLF	VLF>LF=HF	VLF≥LF>HF	VLF>LF>>HF	HF=LF>VLF	HF>LF>VLF
ANOVA results		Wilks λ=0.45, *p*=0.008	Wilks λ=0.48, *p*=0.016	Wilks λ=0.29, *p*=0.009	Wilks λ=0.69, *p*=0.112	Wilks λ=0.66, *p*=0.089
Rats with catecholamine synthesis blockade (*n*=7)						
HR, bpm	325.0±6.6	$304.0\pm4.8^{+}$	$381.0\pm7.8^{xxx***}$	$367.7\pm2.5^{xxx***}$	$355.0\pm6.9^{xxx***}$	$353.4\pm5.9^{xxx***}$
Mo, msec	182.6±2.1	$197.1\pm4.3^{++}$	160.3 ± 7.3^{xxx}	167.2 ± 5.5^{xxx}	173.0 ± 4.4^{xxx}	173.0 ± 4.7^{xx}
SI, rel. units	21.25±2.61	14.79±2.91	29.83 ± 2.91^{x}	43.33 ± 5.59^{xxx}	25.07 ± 3.56^{x}	29.86 ± 4.76^{x}
TP, msec^2	14.59±1.58	$29.19\pm3.62^{++***}$	18.10 ± 2.88^{x}	35.82±6.26*	33.87±5.44	26.11±3.78*
HF, msec^2	6.63±0.33	$11.57\pm1.23^{++}$	10.61±1.72	$21.17\pm2.16^{x***}$	$25.49\pm3.95^{x***}$	15.95±2.49**
LF, msec^2	4.71±0.56	5.91±0.42	5.21±1.17	10.96±2.58	6.72±1.31	4.78±1.13
VLF, msec^2	3.20±0.87	4.27±0.96	2.27±0.50	3.68±0.75	$2.03\pm0.27^{x*}$	$1.20\pm0.23^{xx*}$
IC, rel. units	1.34±0.17	1.11±0.22	0.76±0.13*	0.76±0.16***	$0.59\pm0.11^{x**}$	$0.56\pm0.09^{x**}$
Spectrum ratio	HF>LF>VLF	HF>>LF>VLF	HF>>LF>VLF	HF>>LF>>VLF	HF>>LF>>VLF	HF>>LF>>VLF
ANOVA results		Wilks λ=0.81, *p*=0.253	Wilks λ=0.76, *p*=0.227	Wilks λ=0.63, *p*=0.126	Wilks λ=0.52, *p*=0.027	Wilks λ=0.35, *p*=0.005

Note. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared to rats at rest before drug administration; $^{x}p<0.05$, $^{xx}p<0.01$, $^{xxx}p<0.001$ compared to rats at rest after drug administration; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the corresponding control (Student *t* test). ANOVA results: results of analysis of variances of the effects of amitriptyline and α-MT on VLFabs and VLF% in the course of the experiment. SI: stress index, IC: index of centralization.

on minute 30 ($p<0.001$). Reduction of TP occurred in the beginning of EPS ($p<0.05$) and was associated with the formation of a trend to power attenuation in VLF range. At later stages, wave structure of the heart rhythm markedly changed: domination of EPS HF wave increased starting from minute 30 and the tendency towards attenuation of VLF oscillations gave way to a decrease in the contribution of these waves into HRV spectrum from 12.5%, observed on minute 15, to 4.5% on minute 60 of EPS ($p<0.05$). Therefore, heart rate regulation during stress was characterized by minimum centralization of control ($p<0.05$). Analysis of variance showed that attenuation of VLF wave power and decrease of their input into total HRV on minutes 45 and 60 of EPS are largely determined by preliminary administration of α-MT (Table 1).

These data and results of analysis of variance showed that amitriptyline, activator of neurotransmitter monoamines in the brain, potentiates heart rhythm sympathization and VLF wave power growth in rats in quite awake state. Under these conditions, acute stress could induce burst activity of supersegmentary ergotropic structures, which are a component of the central element of the stress-realizing system of the body [7]; this manifested in increased contribution of VLF oscillations into wave structure of the heart rhythm during the initial 30 min of stress. Subsequent attenuation of VLF waves against the background of high power of FF and HF oscillations most likely reflects reciprocal type of interaction between the central and autonomic contours of heart rhythm regulation; this possibility was previously discussed by other authors [2,3]. α-MT decreased heart rate and increases HF wave power at rest. Under physiological conditions, catecholaminergic mechanisms probably participate in the maintenance of high heart rate and restrict cardiointerval variability by reducing the amplitude of high-frequency spectrum component (HF). The decrease in catecholamine concentration in CNS and blood induced by α-MT [14] can explain weakening of stress-induced changes of HRV (tachycardia and increase in LF wave power). Sharp decrease in VLF wave power during stress in animals treated with α-MT attests to an important role of the central catecholaminergic (monoamine) system in the formation of oscillations in this range of HRV spectrum, while increased contribution of HF waves into HRV spectrum confirms reciprocal relationships between the levels of regulation of the chronotropic function of the heart.

In general, basing on known data on the effects of amitriptyline and α-MT on brain monoamine systems, we believe that the opposite shifts of VLF wave power under conditions of acute stress against the background of administration of these substances confirm the concept about the role of central ergotropic structures in the formation of VLF oscillations in HRV wave structure [3].

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