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Features of the Effects of Serotonin and Dopamine on Changes in Heart Rate Variability in Non-Linear Rats under Conditions of Acute Stress

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Changes in indicators of heart rate variability after a single injection of serotonin (200 μg/kg) or dopamine (60 μg/kg) were analyzed in male non-linear rats at rest and during acute stress. At rest, administration of serotonin is accompanied by an increase in the role of the vasomotor center in the formation of heart rate variability; administration of dopamine causes a moderate weakening of HF waves. In both cases, no sharp shifts in the sympathetic–parasympathetic relations are seen, but a more variable rhythm is formed under the effect of serotonin. Under conditions of acute stress, the increase in HR after injection of serotonin is half as much as in the control series, a trend towards weakening of the power of VLF- and even LF-waves is observed. After dopamine injection, a sharp increase in HR and strain index and a decrease in the power of waves of all spectral ranges and the centralization index are observed at the beginning of stress, but by the 30th min, tachycardia becomes moderate, the powers of all waves are restored; HF waves dominate in the spectrum, but potentiation of VLF- and LF-waves and growth of the centralization index are not observed in contrast to the control series. In general, serotonin and dopamine exhibit stress-limiting properties and moderate activation of sympathoadrenal influences and the suprasegmental level of regulation under stress conditions, but after dopamine injection we observed increased reactivity at the beginning of stress and less variable HR. Therefore, serotonin has more pronounced stress limiting effects, which can be revealed by heart rate variability analysis.

Key Words: *heart rate variability; serotonin; dopamine; acute stress*

Serotonin and dopamine are very important and actively studied regulatory amines in the CNS and at the periphery. Many authors find some similarities in their effects. Serotonin and dopamine through specific receptors on cardiomyocytes trigger signal cascades leading to an increase in the frequency and strength of heart contractions [1-5], through receptors in the vascular walls affect vascular tone and BP [2,3,5]. Both monoamines affect the release of norepinephrine from sympathetic terminals [5-7] and acetylcholine from cholinergic terminals [2,8]. These and other effects of serotonin and dopamine in the CNS and at the periphery determine their ability to limit manifestations of the stress response [9-11]. However, the possibility and specificity of changes in the systemic response to stress under the action of exogenous monoamines is not fully understood. There is evidence that changes in the body after the administration of serotonin can be systemic [12].

Here we studied the effect of serotonin and dopamine on changes in the HR regulation in male non-linear rats under conditions of acute stress.

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MATERIALS AND METHODS

The studies were carried out on 36 mature male non-linear rats weighing 270-300 g. The experiments were carried out in compliance with Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes). The animals were kept under standard vivarium conditions with free access to water and food. The experiments were carried out in summer.

A single injection of serotonin was used to stimulate peripheral serotonin receptors (200 μg/kg, Sigma) [12] (serotonin group; n=12). Peripheral dopamine receptors were stimulated by a single injection of dopamine (60 μg/kg, Sigma) [1] (dopamine group; n=12). Animals of the control group (n=12) received a single injection of saline in a volume of 1 ml/kg. In all animals, ECG was recorded during the first 10-15 min after drug administration, and parameters of heart rate variability (HRV) calculated on their basis were considered as reflecting the state of regulatory systems during calm wakefulness against the background of the test influences.

Immediately after ECG recording, the animals were subjected to acute stress as described elsewhere [13]. The rats were fixed for 1 h in individual plexiglass boxes. Immobilization was combined with five 5-sec electrocutaneous stimulation of the tail with alternating current (4-6 V, 50 Hz) applied at stochastic time intervals.

ECG was recorded on the Varicard hardware-software complex (Ramena) using miniature clamp electrodes under local anesthesia with lidocaine [13] in non-fixed rats in a state of calm wakefulness against the background of stimulation of serotonin and dopamine receptors or after saline injection (control), as well as at 15, 30, and 60 min of stress. HRV analysis was performed on segments consisting of 350 *R-R* intervals using the ISKIM6 software (Ramena) in the specified states. HR (bpm), strain index (SI, rel. units) were calculated taking into account the width of the histogram class (7.8 msec): SI=(AMo/2×ΔX×Mo)×(50/7.8)×1000 [13,14]. Spectral analysis of continuous series of *R-R* intervals was performed in the following wave bands: high-frequency (HF; 0.9-3.5 Hz), low-frequency (LF; 0.32-0.9 Hz), very low-frequency (VLF; 0.17-0.32 Hz) [13]. The absolute (in msec^2) and relative (in %) wave power were determined. The centralization index (IC, rel. units) was calculated using the formula: IC=(LF+VLF)/HF [14].

Statistical processing of the results was performed using Microsoft Excel 2003 and Statistica 10.0 (StatSoft, Inc.). The statistical significance of changes in HRV parameters in the dynamics of acute stress in each series was assessed using the nonparametric Wilcoxon's test, differences between the control and experimental groups at each stage of the study – using the non-parametric Mann–Whitney U test. The data are presented as $M\pm m$ calculated using descriptive statistics. The differences between the means were considered significant at $p<0.05$.

RESULTS

In male rats of the control group, stress modeling caused an increase in HR (by 39-30% $p<0.001$), which remained within 400-450 bpm during the entire stress (Table 1). SI increased moderately (by 43-45%, $p<0.05$) and was maintained at the level of 45-50 rel. units. The HRV spectrum showed a trend towards a decrease in the power of HF waves by 35-15%, but the values remained in the range of average values. A trend towards an increase in the power of LF waves by 50% ($p<0.1$) was revealed only by the 60th minute. The increase in VLF waves turned out to be more significant (by 78-118%, $p<0.05$) and manifested itself from the 30th minute of stress (Fig. 1). Accordingly, the proportion of HF waves in the spectrum decreased from 55-60 to 35-38% ($p<0.1$) and the proportion of VLF-waves increased from 16-20 to 33% ($p<0.05$), a trend appeared to an almost 2-fold increase in IC ($p<0.1$) (Table 1). It was previously reported that stress leads to a sharp increase in adrenergic influences on the background HR, a moderate increase in sympathetic influences on HRV, and an increase in the centralization of control due to the activation of the suprasegmental level of regulation [14].

In serotonin-treated male rats in the state of calm wakefulness, the absolute and relative power of LF waves and IC exceeded the control values, but the differences were insignificant; nevertheless, this indicated an increase in the role of the vasomotor center in the formation of HRV. In these animals, stress caused an increase in HR by only 14% or less ($p<0.05$) (Table 1). At all stages of observation, HR did not exceed 400 bpm and was lower than in control males ($p<0.01$ and $p<0.05$). SI increased insignificantly. Under stress conditions, in male rats treated with serotonin, there was a tendency to an increase in HF waves, and at the 15th minute this increase was significant ($p<0.05$), as well as a tendency to a decrease in LF and VLF waves (Fig. 1). At the same time, the absolute powers of HF and LF waves corresponded to average values (3.5-10 msec^2), the power of VLF waves corresponded to low values (below 3.5 msec^2). The greatest changes in the structure of the HRV spectrum occurred on the 15th minute of stress, when HF% increased from 42 to 63% ($p<0.05$), LF% decreased from 34 to 25%, VLF% decreased from 23 to 11% ($p<0.05$). IC also

TABLE 1. Changes in HRV Parameters in Rats Treated with Serotonin and Dopamine under Conditions of Acute Stress (*M*±*m*)

Parameter	Group	Calm wakefulness	Stress		
			15 min	30 min	60 min
HR, bpm	Control	318.7±7.4	443.8±10.2^{++}	426.1±15.0^{++}	416.2±10.4^{++}
	Serotonin	319.3±17.6	364.5±15.4^{**+}	354.1±14.4**	370.0±15.2^{*+}
	Dopamine	274.8±15.7**	410.5±12.0^{*++}	361.3±18.5^{**++}	345.5±12.4^{***++}
SI, rel. units	Control	32.4±5.1	46.8±3.8^{+}	46.1±4.9^{+}	46.1±7.3
	Serotonin	44.3±6.5	54,0±8,8	68.7±20.5	45.7±7.2
	Dopamine	46.9±13.2	100.8±15.4^{*++}	56.8±21.0	42.8±6.6
HF%	Control	54.7±5.1	57.7±6.0	38.4±6.2	38.3±4.4
	Serotonin	42.2±5.7	62.9±5.4^{+}	55.6±4.4^{+}	50.7±4.8
	Dopamine	56.8±6.8	66.5±6.6	61.1±9.2**	50.9±5.3
LF%	Control	23.4±5.7	25.5±3.5	28.7±4.0	28.2±3.4
	Serotonin	34.1±5.0	25.3±2.7	27.0±3.0	29.7±2.6
	Dopamine	23.1±5.6	20.5±2.5	25.9±6.2	25.1±3.5
VLF%	Control	21.9±4,4	16.8±3.7	32.9±3.4^{+}	33.5±4.2^{+}
	Serotonin	23.3±3.0	11.7±3.2^{+}	17.4±3.3	19.6±4.3
	Dopamine	20.0±3.4	13.0±5.3	12.9±5.3**	23.9±4.4
IC, rel. units	Control	0.9±0.1	2.0±1.0	1.5±0.5	1.8±0.6
	Serotonin	2.0±0.4	0.8±0.3^{+}	0.9±0.2^{*+}	1.3±0.3^{+}
	Dopamine	0.9±0.2	0.6±0.2	0.8±0.3**	1.1±0.2

Note. *p<0.05, **p<0.01, ***p<0.001 in comparison with the corresponding control (Mann—Whitney U test); ^{+}p<0.05, ^{++}p<0.01 in comparison with calm wakefulness in each group (Wilcoxon's test).

decreased by 2.5 times in the beginning of stress exposure (p<0.05) and remained below the level of calm wakefulness (Table 1). Thus, stress in serotonin-treated animals was associated with increased variability of the heart rhythm due to an increase in conjugation with the respiratory rhythm. At the same time, there was some weakening of conjugation with BP fluctuations and rhythms of suprasegmental influences. The heart rhythm was predominantly controlled by the structures of the autonomous regulation circuit, the influence of the structures of the central circuit weakened. Consequently, serotonin administered to animals before acute stress contributed to less activation of sympathoadrenal influences and the central circuit of regulation, which made it possible to maintain the autonomic balance and the leading role of the autonomic circuit in regulation. Hence, the reaction to stress proceeded more economically against the background of serotonin treatment.

Dopamine-treated male rats in a state of calm wakefulness were characterized by a lower HR (p<0.01) and a somewhat lower power of HF waves in the HRV spectrum. These animals responded to stress by a strong increase in HR (by 49%; p<0.001), which was even more pronounce than in the control series. In absolute terms, at the 15th minute of stress, HR increased by 135 bpm (Table 1). At later stages, tachycardia decreased by almost 2 times, HR remained at the level of 360-340 bpm, *i.e.* was lower than the control. SI at the 15th minute of stress exceeded 100 rel. units (p<0.05) and was higher than in the control (p<0.01). However, by the 30th minute, SI decreased by almost 50%, and by the 60th minute it returned to the values at rest. The sharp rise in SI was due to a decrease in the power of all waves of the spectrum: HF by 43% (p<0.1), LF by 78%, and VLF by 83% (p<0.05) (Fig. 1). This indicated that the influences of all levels of regulation were realized at this stage against the background of increased activity of the sympathetic channel. Nevertheless, by the 30th minute, and even more so by the 60th minute of stress, the variability of cardio intervals in the LF and VLF ranges increased to the initial values, in the HF range it became even surpassed the initial one by 61-14%. The proportion of HF waves exceeded 65% at 15-30 min, but returned to the 50% level by 60 min. The proportion of LF waves most stable during stress (20-25%), the proportion of VLF decreased at the beginning of stress, but by the 60th minute it again reached 20-23%, *i.e.*, there was no significant increase in the power of LF waves. IC reached the lowest values at the 15th minute of stress (0.6 rel. units), which corresponded to a drop

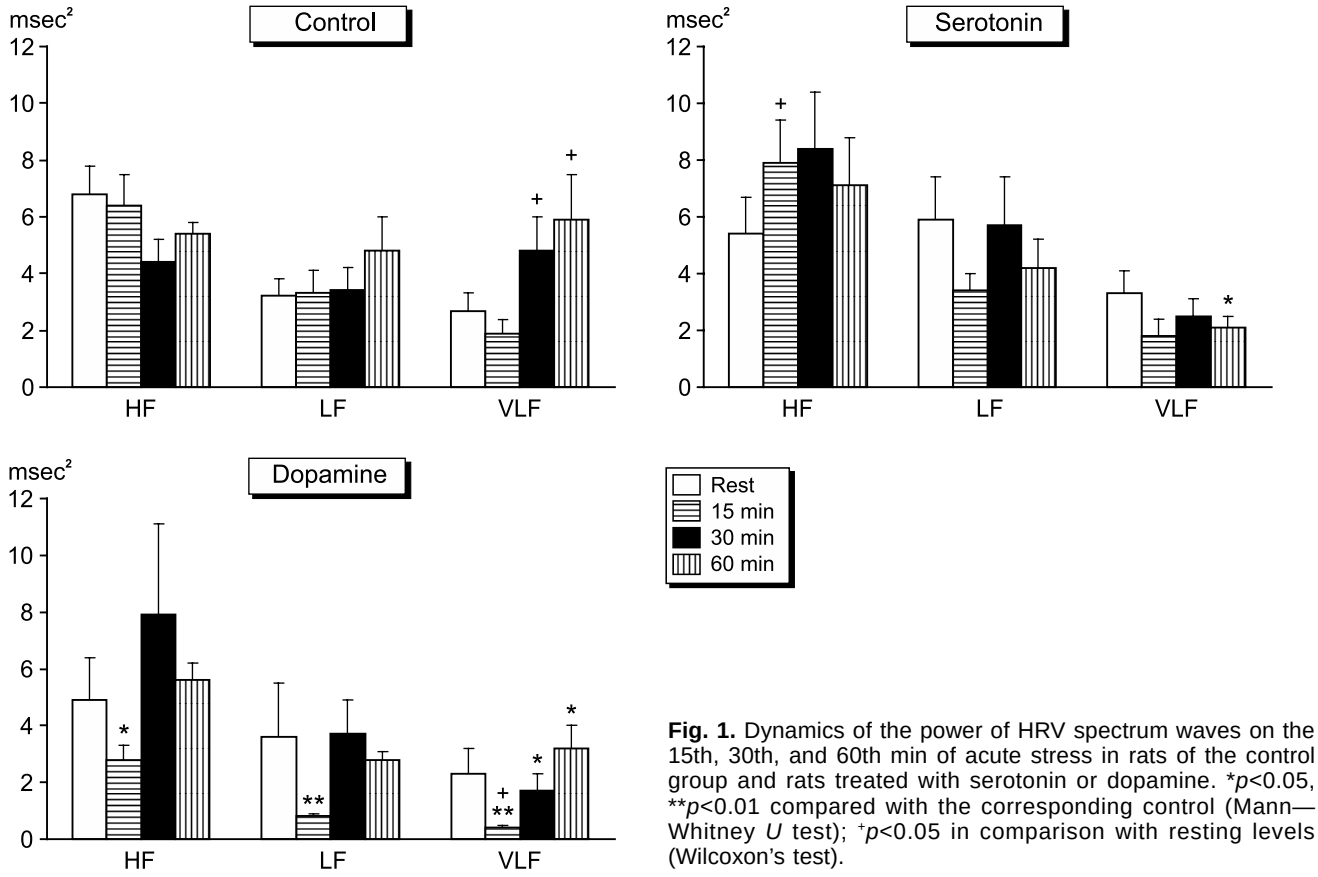

Fig. 1. Dynamics of the power of HRV spectrum waves on the 15th, 30th, and 60th min of acute stress in rats of the control group and rats treated with serotonin or dopamine. **p*<0.05, ***p*<0.01 compared with the corresponding control (Mann—Whitney *U* test); ^{+}p<0.05 in comparison with resting levels (Wilcoxon's test).

in the power of all waves, especially LF waves, but as the variability recovered, IC increased to 0.8-1 rel. units. Thus, in male rats treated with dopamine, high reactivity to stress was observed at the initial stage; it manifested itself in a sharp increase in HR, SI, and a drop in the power of all frequency ranges of the HRV spectrum. Consequently, dopamine potentiates the increase of sympathoadrenal influences at the onset of stress. However, the severity of the reaction quickly weakened, by the 30th minute the variability of cardiointervals recovered in all spectral ranges, and tachycardia became moderate. HF waves dominated in the spectrum that is the heart rhythm was primarily influenced by the respiratory function, no significant activation of the suprasegmental influences was noted. In general, under stress conditions against the background of dopamine administration, the autonomic balance was quickly restored after a sharp increase in sympathetic influences, the leading role in the regulation of heart rhythm was still played by structures of the autonomic circuit.

It should be noted that the characteristic changes in the heart rhythm under stress are sharp tachycardia and a trend towards a decrease in HF waves with an increase in the power of LF and especially VLF waves due to increased baroreflex modulation and the influence of the suprasegmental level of regulation [14,15]. The injection of serotonin and dopamine to some extent weakens stress-induced tachycardia and prevents strengthening of centralization of heart rhythm control. These effects of both monoamines can be interpreted as stress-limiting. In this case, the action of serotonin may be determined by serotonergic modulation of the acetylcholine release from cholinergic terminals. It can be hypothesized that at rest serotonin through 5-HT_4, 5-HT_7 receptors predominantly inhibits the release of acetylcholine thereby limiting excessive rhythm variability, while during stress in a combination to action of epinephrine, serotonin through 5-HT_{2C}, 5-HT_3 receptors stimulates the release of acetylcholine [2]. There is also evidence that serotonin regulates the release of epinephrine from the adrenal glands during stress [11].

Unlike serotonin, dopamine had a stress-potentiating effect at the initial stage of stress, because stress caused a very sharp increase in HR and a decrease in the power of the spectrum waves in dopamine-treated animals. Increased stress reactivity against the background of dopamine can be explained by the fact that this monoamine has effects by binding not only to dopamine, but also to α_1- and β_1-adrenergic receptors of cardiomyocytes [4]. However, rapid recovery of

variability in the HF and LF ranges and weakening of tachycardia can be determined by inhibition of norepinephrine release from sympathetic terminals by dopamine and a decrease in the sympathetic tone [6,7].

Serotonin and dopamine receptors are present in the vessels, participate in the modulation of not only efferent, but also afferent signaling, in particular, from the chemo- and baroreceptors of blood vessels [2,5]. We believe that potentiation of autonomic reflexes by monoamines with participation of the parasympathetic channel of regulation provides an increase in the conjugation of the heart rhythm with respiration and fluctuations in vascular tone, which limits stress tachycardia and the dominant role of sympathoadrenal influences during stress.

In general, both serotonin and dopamine contribute to the weakening of stress-induced tachycardia, heart rhythm tension, and participation of the suprasegmental structures in the control of the chronotropic function of the heart, that is corrects the reaction of regulatory systems to stress. At the same time, dopamine provokes an increase in the reaction at the initial stage of stress, which increases the risk of arrhythmias and heart function in general. Serotonin has a stress-limiting effect at all stages of stress; more variable heart rhythm is formed against the background of serotonin treatment.

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