Peculiarities of Autonomic Regulation Assessed by Variability of Hemodynamic Parameters in Rats with Different Stress Resistance

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Analysis of contribution of sympathetic and parasympathetic systems into heart rate variability carried out using atenolol and atropine showed that August rats are characterized by enhanced tone of the sympathetic system and reduced tone of the parasympathetic system compared to Wistar rats. Reduced tone of the parasympathetic system is also confirmed by lower sensitivity of the baroreflex. Blockade of NO synthesis with N^{ω}-nitro-L-arginine more markedly increased blood pressure variability in August rats compared to Wistar rats. The data attest to a certain rigidity of the autonomic cardiovascular regulation in August rats.

Key Words: *heart rhythm; spectrum analysis; N^{ω}-nitro-L-arginine; baroreflex; Wistar rats; August rast*

Variability of the parameters of systemic hemodynamics reflects activity of many regulatory systems [1]. Clinical studies showed that different pathological processes are accompanied by a decrease in heart rate (HR) variability. That is why this parameter is an important diagnostic and prognostic sign in many pathologies [1,7]. Close interrelations between HR variability and pathological states are also established in animals. In rats, HR variability markedly decreases during myocardial infarction [14]. In addition, a close negative correlation exists between HR variability and vulnerability to arrhythmias [14]. At the same time, genetic peculiarities of the interrelations between HR variability and resistance to damaging factors are poorly studied. For example, in August rats the spectrum power density of HR variations at rest is far lower, while that of blood pressure (BP) higher than in Wistar rats [3], which probably indicates their low resistance to damaging factors [1]. However, the data obtained in comparative studies of the resistance of both rat strains to stress-induced and ischemic damages are ambiguous. August rats are less resistant to long-term immobilization stress due to dramatic drop in BP [8], but more resistant to stress-induced ulceration of the gastric mucosa [6] compared to Wistar rats. Moreover, August rats demonstrate lower mortality during acute myocardial infarction and lower incidence of severe arrhythmias than Wistar rats [2]. An important role in the pathogenesis of these diseases is played by activation of sympathetic nervous system (SNS). The resistance to pathological damages is greatly determined by the balance between SNS and parasympathetic nervous system (PNS). The study of sympathovagal balance by assessment of HR variability showed that in Wistar rats, HR at rest is predominantly regulated by PNS. β-Adrenoblockers (atenolol and propranolol) produce no significant effect on the spectrum power of HR oscillations, while atropine significantly depresses the low- and middle-frequency oscillations and completely eliminates high-fre-

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quency ones, which mainly reflects the effect of PNS [12]. BP variability is determined by a more complicated ensemble of neural and humoral factors, NO being one of them. In this respect, it should be taken into account that August rats differ from Wistar rats by increased level of NO, which not only provides the most efficient regulation of BP, but can also limit activity of SNS [15]. In Wistar rats, blockade of NO synthesis increases BP variations, which attests to a buffer function of this transmitter in respect to BP variations [9]. However, similar studies were not carried out on rats of different genetic strains. To analyze the differences between variability of hemodynamic parameters and resistance to stress-induced damage in Wistar and August rats, we examined the peculiarities of autonomic regulation of the cardiovascular system using pharmacological "probing" of HR and BP variability. In addition, the sensitivity of cardiotropic branch of the baroreflex to BP was compared in both rat strains.

MATERIALS AND METHODS

The experiments were carries out on male August and Wistar rats weighing 238-250 g and 372-420 g, respectively. BP was recorded in awake unrestricted rats via a catheter in the femoral artery, which was inserted under intraperitoneal sodium thiopental narcosis (40 mg/kg) 2 days before the experiment. BP was measured with a Statham 8200 P23AA tensometric transducer and converted by a precise 16-bit digitizer (L-Card) at a sampling rate of 500 Hz. Original software (developed by A. S. Borovik) was used to calculate the mean beat-to-beat BP and beat-to-beat cardiac interval. The spectrum analysis was used to examine BP and HR variations. Linear interpolation was used to calculate the above parameters with 0.1-sec intervals. The examined interval (1 h) was divided into non-overlapping 51.2-sec segments (512 points). In each segment, the mean value and linear trend were subtracted. The power spectrum was calculated using fast Fourier transform. The resulting spectra were averaged. The power spectra of BP and cardiac intervals were assessed in three frequency domains: low-frequency (0.0195-0.2500), middle-frequency (0.25-0.75), and high-frequency (0.75-2.00). Variability of BP was also assessed by the value of standard deviation calculated in 30-60 min segments.

To examine the sympathovagal balance, the cardiac interval power spectrum was analyzed pharmacologically by selective blockade of sympathetic or vagal influences on the heart with β-adrenoblocker atenolol (2 mg/kg) and atropine (1 mg/kg), respectively. These agents produced no effect on BP. In the next experimental series, we examined the effect of NO-synthase blocker N^{ω}-nitro-L-arginine (L-NNA, IGN, 10 mg/kg) on BP variations. All blockers were injected into the jugular vein via a preliminary implanted catheter. The measurements were performed in a noiseless black-out room for 1 h before and 1 h after injection of the preparations. The sensitivity of the chronotropic component of the baroreflex was assessed by changes in beat-to-beat interval in response to BP increment caused by intravenous phenylephrine (5 μg/kg). In this series, BP was recorded in the femoral artery with a Mingograph-34 (Siemens) under urethane anesthesia (1.5 g/kg). Baroreflex sensitivity was calculated as Δ (beat-to-beat interval)/Δ(BP), where Δ are the relative changes of the respective parameters (in brackets) measured at the peak of the L-NNA effect and during 10 sec preinjection (the baseline value).

The data were processed statistically using Student's t test and Mann—Whitney U test.

RESULTS

In all experiments, HR and mean BP in August rats were higher than in Wistar rats (422-464 and 402-439 min^{-1}, respectively, $p<0.001$; 125-127 and 114-118 mm Hg, respectively, $p<0.001$). Spectrum power of oscillations of beat-to-beat interval in August rats was significantly lower, while mean BP was higher than the corresponding values in Wistar rats, which agrees with previous observations [3]. In the studied rat strains, atenolol equally reduced HR (from 464 to 384 min^{-1} in August rats and from 439 to 351 min^{-1} in Wistar rats), but produced different effects on the power spectra of beat-to-beat interval (Fig. 1). In Wistar rats (n=10), the low-frequency power density decreased by 50%, while in August rats (n=10) it remained unchanged. At the same time, the middle- and high-frequency power density in Wistar rats did not change, which agrees with previous data [12], while in August rats the corresponding values increased by 72 and 49%. The observed elevation of the high-frequency spectrum power in August rats was similar to that observed in Wistar—Kyoto rats for the same frequency range (about 1.5 Hz) [13]. This phenomenon probably results from activation of PNS due to inhibition of cardiotropic sympathetic influences and indicates greater contribution of SNS to HR variability in August rats in comparison with Wistar rats. This fact attesting to increased sympathetic tone in August rats agree with the data increased catecholamine content in the adrenals and blood in these rats [5].

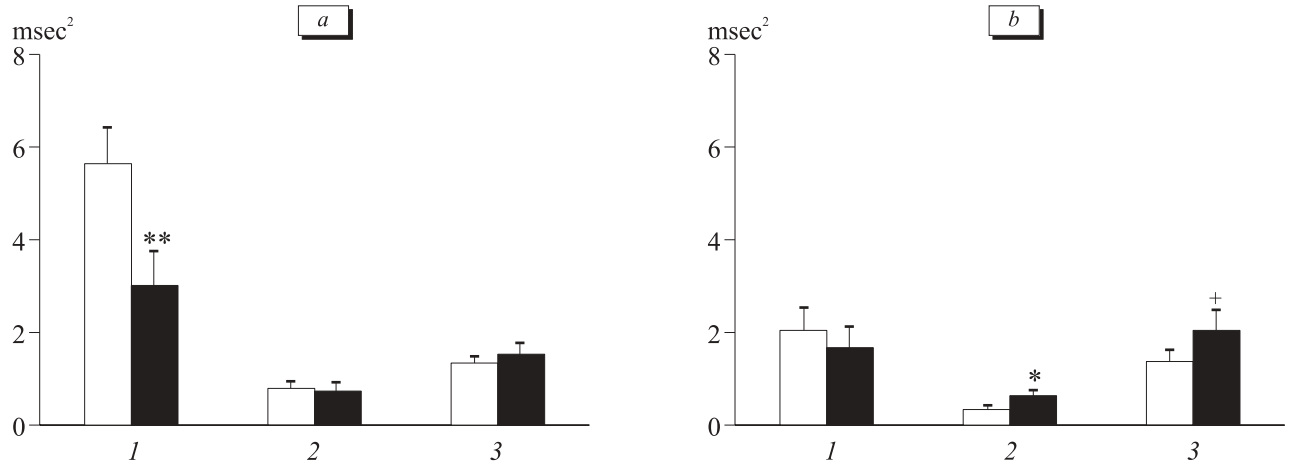

Fig. 1. Effect of atenolol on spectrum power of beat-to-beat interval oscillations in Wistar (*a*) and August (*b*) rats during the first 30 min postinjection. **p*<0.05 and +*p*<0.05 compared to initial level by *t* and *U* tests. Here and in Figs 2 and 3: low-frequency (*1*), middle-frequency (*2*) and high-frequency (*3*) spectrum power. Open and filled bars correspond to pre- and postinjection data.

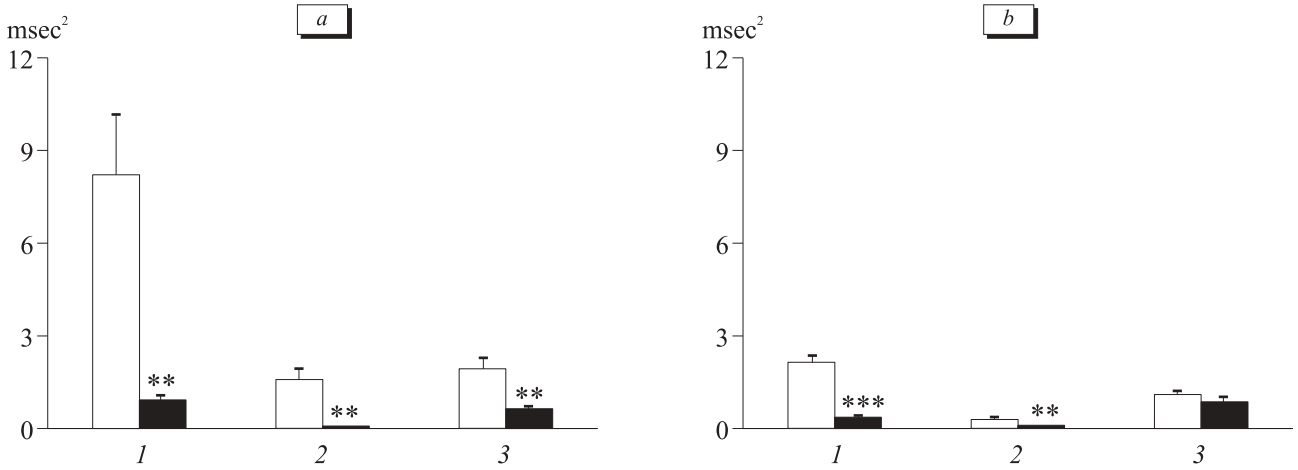

Fig. 2. Effect of atropine on spectrum power of beat-to-beat interval oscillations in Wistar (*a*) and August (*b*) rats during the first 30 min postinjection. **p*<0.05 and ***p*<0.01 compared to initial level by *t* test.

Atropine increased HR in August and Wistar rats from 448±6 to 492±9 min^{-1} and from 423±9 to 465±12 min^{-1}, respectively. Low-frequency spectrum power density in both strains decreased by 80% (Fig. 2). At the same time, middle-frequency spectrum power in August rats (n=9) decreased to a lesser extent (by 58% *vs.* 94%, p<0.02, Student t test) than in Wistar rats (n=9). High-frequency power in Wistar rats decreased by 50% (p<0.01), but remained unchanged in August rats. Thus, the effect of atropine on middle- and high-frequency oscillations of beat-to-beat interval (reflecting predominantly activity of PNS) was more pronounced in Wistar rats compared to August rats, which attests to decreased tone of PNS in the latter case. When analyzing the effects of atenolol and atropine on low-frequency beat-to-beat interval oscillations, it should be taken into consideration that in addition to SNS, this frequency domain is also affected by CNS and the humoral factors. Moreover, atropine modulates activity of CNS. These features impede the analysis of the effects of the test blockers on beat-to-beat interval spectrum.

When analyzing the sensitivity of the chronotropic component of the baroreflex depending mostly on reflex potentiation of the vagal activity, we found that in August rats not only the tone, but also activation capacities of PNS are decreased. In this series, body weights of August (n=9) and Wistar (n=10) rats were 210±11 and 315±16 g, respectively. Under narcosis, the initial systolic BP an August and Wistar rats was 92±7.0 and 111±4.6 mm Hg, respectively, and the initial HR was 325±9 and 370±23 min^{-1}. Phenylephrine increased BP in August and Wistar rats by 49 and 38%, respectively (p<0.001). Bradycardia in August rats was less pronounced than in Wistar rats: beat-to-beat interval increased by 13.7±3.7 and 39.2±4.9 msec,

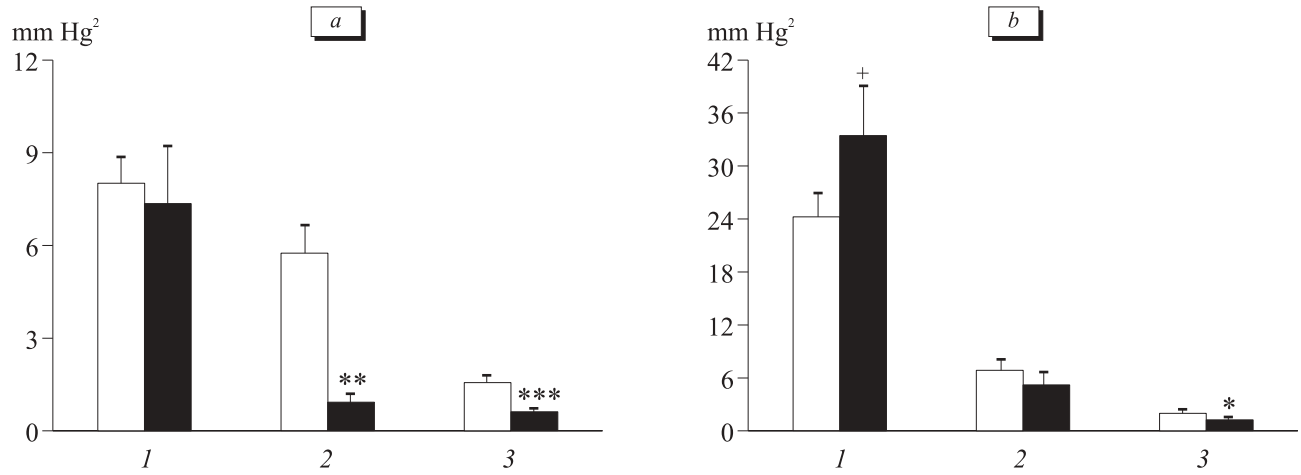

Fig. 3. Effect of L-NNA on spectrum power of mean beat-to-beat blood pressure oscillations in Wistar (*a*) and August (*b*) rats during the first 30 min postinjection. **p*<0.05 and ***p*<0.01 compared to initial level by *t* test. ^{+}p<0.05 compared to initial level by *U* test.

respectively (p<0.001). As a result, the baroreflex sensitivity in August rats was almost 4 times lower than in Wistar rats (0.27±0.05 and 1.06±0.16, *p*<0.001). Therefore, the shift of sympathovagal balance in August rats towards predominance of SNS is related to decreased sensitivity of the baroreceptors resulting in inhibition of PNS, which attests to certain rigidity of cardiovascular regulation in these rats.

The decreased sensitivity of baroreflex in August rats can be related to enhanced variability of BP in these animals. This hypothesis is corroborated by the fact that decreased baroreflex sensitivity (for example, during arterial hypertension) is associated with increased BP variability [11].

In the series with NO synthesis blockade with L-NNA, the power of low-frequency spectrum of mean BP in August rats (*n*=6) surpassed that in Wistar rats (*n*=7) by 3 times (*p*<0.001), while their middle- and the high-frequency spectrum powers were similar (Fig. 3). At rest, BP variability in August rats was slightly higher than in Wistar rats (Table 1). L-NNA increased the mean BP (over 30 min) in August and Wistar rats by 18 and 33%, respectively, but the absolute BP increased to the same values (143±3.4 and 150.0±4.2 mm Hg, respectively). Despite less pronounced pressor reaction, BP variability in August rats increased to a greater extent than in Wistar rats (83 and 47%, *p*<0.05 by *U* test). In Wistar rats, this parameter completely recovered 60 min after injection of the blocker, while in August rats BP variability remained significantly elevated by 32%. In August rats, L-NNA increased low-frequency BP oscillations by 49%, but in Wistar rats it did not change the power spectrum in this frequency domain, which disagree with the data reporting elevation of low-frequency spectrum power in Wistar—Kyoto rats at similar frequencies of 0.02-0.20 Hz [9]. This disagreement is probably related to genetic differences between these subpopulations of Wistar rats. Many factors affect the low-frequency spectrum power of BP oscillations: blood level of angiotensin II and kinins, rhythmic activity of blood vessels [10], and locomotor activity of the animal. The effects of L-NNA on these factors need further investigation. The middle-frequency power spectrum of BP oscillations predominantly reflects the effects of SNS. In August rats, blockade of NO synthesis did not change this spectrum band, although in Wistar rats this blockade dramatically decreased it (by 90%). We assume that the applied dose of L-NNA was insufficient for complete inhibition of NO synthesis in August rats, but was enough to block it in Wistar rats. The decrease in middle-frequency BP oscillations in Wistar rats is probably related to activation of SNS known to damp variations of the blood pressure. In both groups, L-NNA significantly (by 48-60%) decreased high-frequency variations of BP reflecting respiratory waves and oscillations of the intrathoracic pressure, which agrees with other data. In August rats, the pressor reactions induced by phenylephrine or L-NNA were accompanied by less pronounced bradycardia in comparison with Wistar rats. Thus,

TABLE 1. Effect of L-NNA on BP Variability in Wistar and August Rats (mm Hg, *M*±*m*)

Index		Wistar (*n*=5)	August (*n*=6)
Prior to injection		6.36±0.28	7.1±0.27*
Postinjection	30 min	9.36±0.91^{+}	13.0±1.43**$^{++}$
	60 min	6.22±0.74	9.38±0.36**$^{+}$

Note. **p*<0.05, ***p*<0.01 compared to Wistar rats. ^{+}p<0.05, ^{++}p<0.01 compared to initial level (before injection).

NO produces different modulating action on blood pressure variations in Wistar and August rats. It seems that in August rats the NO system damps oscillations of BP, while in Wistar rats it rather potentiates them.

Our experiments revealed less labile regulation of the cardiovascular system in August rats compared to Wistar rats. However, the spectrum analysis of NO contribution into BP oscillations and higher blood NO level in August rats suggest that insufficient activity of PNS in these animals can be compensated by enhanced activity of their NO system. These genetic determinants of the cardiovascular regulation in August rats explain their selective resistance to some damaging factors. Probably, enhanced resistance of August rats to stress-induced ulceration is primarily related to moderated activity of PNS, which plays an important role in this pathology. In addition, August rats demonstrate enhanced resistance to ischemia, which can be related to increased activity of the NO system known to protect the heart against ischemic damages [2]. Thus, the pathogenetic role of HR and BP oscillations is different in various genetic strains of the same animals. While the peculiarities of cardiac intervals and BP variations can be prognostically unfavorable, some animals like August rats can possess enhanced resistance to stressor and ischemic damages. The phenomenon can be explained by rigidity of some regulatory systems manifested in moderated variations of heart rate and enhanced oscillations of blood pressure, which can be compensated by enhanced activity of other regulatory systems.

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