Development of Regulation of the Cardiac Chronotropic Function in Albino Rats during the Early Postnatal Ontogeny according to the Results of Spectral Analysis of Heart Rhythm Variability

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Regulation of the cardiac chronotropic function was studied by spectral analysis of cardiac rhythm variability in HF, LF, and VLF bands in rats at various stages of the early postnatal ontogeny. The inadequacy of the regulatory mechanisms during the first days of life manifested by low power of all waves (particularly HF) in the cardiac rhythm variability spectrum. On day 14 of life, the cardiointerval variability was formed by HF waves, their low power together with increasing heart rate indicating more intense sympathetic effects. On day 21 of life, a potent elevation of the VLF power reflected a stronger centralization of regulation from higher autonomic centers. The age of 28 days was characterized by a sharp increase of HF activity and could be regarded as the turning point in the development of parasympathetic effects and activity of the autonomic regulation contour. From the age of 35 days, the wave power and the proportion of the spectral components of cardiac rhythm variability in albino rats corresponded to the adult pattern; a trend to the central regulation predominance and to greater rigidity of cardiac rhythm formed only with the onset of sexual maturation.

Key Words: *cardiac rhythm variability; spectral analysis; ontogenesis; rats*

The statistical method for analysis of cardiac rhythm variability (CRV) of rats has made an important contribution to understanding of the age-specific development of autonomic regulation of the cardiac chronotropic function [3,10]. Intensification or reduction of the sympathetic and parasympathetic nervous effects on the heart at various stages of the ontogeny are evaluated by changes in heart rate (HR) and Baevsky's indexes. However, the statistical parameters are based on just general CRV, while its intensification or attenuation are determined by changes in the fluctuations of cardiointerval lengths for various frequency bands (HF, LF, VLF). It is assumed that the CRV wave power at a certain frequency reflects the cardiac chronotropic effects of not only certain channels, but of regulation levels as well. Studies of the spectral analysis of cardiac rhythm at various stages of the ontogeny are scanty [5,11]. We studied the regularities of cardiac rhythm regulation development in albino rats during the early postnatal ontogeny by spectral analysis of CRV.

MATERIALS AND METHODS

The study was carried out on conscious animals aged 3, 7, 14, 21, 28, 35, 42 days and 3.5 months, kept under standard vivarium conditions. ECG was recorded on a Varicard device (Ramena) via miniature clamp electrodes in conscious rats at rest. In young nude (before hair growth) rats, ECG was recorded using

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wick electrodes wetted in warm saline and contact gel. Young rats were taken for the experiment only after the mother was removed from the cage. Rats aged 3-14 days were put onto cotton wool into a deep ceramic box placed into warm water bath in order to maintain the temperature of a nest. Measurements of *R-R* intervals and primary data processing were carried out using ISKIM6 software (Ramena).

Continuous series of 400 *R-R* intervals from each record in little rats and 300 *R-R* intervals in adult rats were processed. The HR, *R-R* interval mode (Mo), standard deviation (SD), and strain index (SI) after R. M. Baevsky [1] were calculated with consideration for the histogram class width (7.8 msec): $SI=(AMo/2\times\Delta X\times Mo)\times(50/7.8)\times1000$. Spectral analysis of CRV was carried out for HF, LF, and VLF bands, their borderline determined with consideration for respiration rate (for HF band), threshold migration of the wave peaks in respective bands for each age group, and published data [4,12,13] (Table 1). The total power of the spectrum (TP, $msec^2$) and wave power for each band were evaluated and the centralization index was calculated: IC=(LF+VLF)/HF [1]. The results were statistically processed using Student's *t* test and analysis of correlations using Statistica 6.0 software.

RESULTS

The lowest HR was recorded in 3-day-old rats (Table 2). The CRV spectral power during the first days of life was 8-fold lower than in adult rats ($p<0.001$) and HF waves were weakest (15-fold lower than in adult rats; $p<0.001$); this indicated low activity of parasympathetic effects and autonomic regulation contour [1,2]. In 3-day old rats, the CRV was formed by mainly VLF waves reflecting activity of suprasegmentary structures [1,2]. On day 7 of life, HR increased by 9.4% ($p<0.05$) due to reduction of cardiointerval Mo ($p<0.05$), this presumably indicating more intense adrenergic effects through the humoral regulation channel [1]. The summary CRV power was also low ($p<0.001$), while the contribution of respiratory arrhythmia to the formation of CRV somewhat increased ($p<0.05$). The percentage of rats with predominating HF reached 74 *vs.* 40% during the previous age period. Heart rate increased significantly (by 36.6%; $p<0.001$) at the age of 14 days, as did SI (4-fold; $p<0.001$). The cardiac rhythm rigidity was caused by low spectral power ($p<0.01$) and its concentration mainly in the HF band. The HF waves became predominating in 90% rats. The power of VLF fluctuations and centralization index decreased ($p<0.01$ and $p<0.1$, respectively). By the age of 21 days, the HR reached the maximum values, which was in line with a previous report [9]. This was paralleled by the first increase of spectral power (2.6 times; $p<0.001$) due to more intense slow waves: 5.8 times for LF ($p<0.001$) and 19.8 times for VLF ($p<0.001$), which could indicate stimulation of the cerebral CAergic systems [6] and of suprasegmentary regulation level [1]. Due to more complex wave structure, the cardiac rhythm became less strained (in comparison with that in 14-day-old rats), SI decreased by 1.8 times ($p<0.01$), but remained significantly higher than 100 arb. units. Heart rate decreased at the age of 28 days ($p<0.001$). In parallel with this, SI dropped by 4.7 times ($p<0.001$). These changes in heart rhythm correlated strongly ($r=-0.74$, $p<0.01$) with increase of CRV spectral power ($p<0.001$). The HF waves increased most of all (13.6 times; $p<0.001$), while LF wave power increased only 2-fold ($p<0.01$), and hence, the regulation centralization decreased significantly ($p<0.001$). The percentage of rats with predominating HF at the age of 28 days was 79% (*vs.* just 30% during the previous period). By the age of 35 days, the CRV and spectral power decreased ($p<0.001$) to the level characteristic of adult animals. The proportion of components in the CRV spectrum also reached the level characteristic of adult age. By day 42 of life, HR further decreased (by 9% *vs.* that at 35 days; $p<0.05$), but still remained higher than in adult rats ($p<0.001$). The SD decreased by almost one-third ($p<0.001$), while SI increased ($p<0.01$), that is, cardiac rhythm remained more strained because of HF waves low power in comparison with that in adult rats ($p<0.05$). Despite this, the proportion of CRV spectral components corresponded to the adult type (Table 2), the centralization index indicating the predominance of the central regulation contour over autonomic contour. HR in adult rats decreased to 310-350 bpm ($p<0.001$). The cardiointerval variability parameters increased significantly in comparison with the age of 42 days: SD by 44% ($p<0.05$), spectral

TABLE 1. Frequency Bands for Which Spectral Analysis of CRV Was Carried out in Rats of Different Age

Age	HF, Hz	LF, Hz	VLF, Hz
3 days	3.6-1.0	1.00-0.32	0.32-0.13
7 days	3.6-0.7	0.7-0.3	0.30-0.18
14 days	4.0-1.3	1.3-0.3	0.30-0.22
21 days	4.0-1.0	1.00-0.32	0.32-0.17
28 days	4.0-1.3	1.3-0.3	0.3-0.2
35 days	3.5-0.9	0.90-0.32	0.32-0.20
42 days	3.5-0.9	0.90-0.32	0.32-0.20
3.5 months (adult)	3.5-0.9	0.90-0.32	0.32-0.18

power by 68.4% ($p<0.05$); SI decreased ($p<0.001$), this, together with more rare HR and increase of spectrum wave power indicating weakening of sympathetic effects. The level of parasympathetic effects increased in adult rats, judging from the power of HF waves ($p<0.05$) steadily predominating in the spectrum (~50% spectral power) in conscious rats at rest.

Hence, low HR and CRV spectral power of 3-day-old rats seemed to indicate general weakness of regulatory mechanisms, and high SI at this age could not be regarded as an indicator of high sympathetic effects on the heart, an had been assumed previously [10], but was a result of low variability of cardiointervals. High centralization of heart rhythm regulation during the first days of life was presumably caused by activity of the thermoregulation center and by spontaneous motor activity (startling) periodically emerging in rats with a rhythm of about 1 min according to our observations and other data [11]. The spectral power remained very low until the end of the first week of life, which reflects weakness of regulatory mechanisms. However, a trend to 1) more intense adrenal effects on the heart through the humoral regulation channel, 2) an increase of the respiratory wave contribution to CRV formation, 3) weaker centralization of cardiac activity regulation could be traced by day 7 of life. At the age of 14 days, all these trends grew stronger, the low power of CRV spectrum waves together with intense tachycardia and high SI presumably indicating more intense sympathetic effects on the heart. It is known that by the end

TABLE 2. Parameters of CRV in Outbred Rats at Different Stages of Ontogeny (*M*±*m*)

Parameter	Age of rats							
	3 days (*n*=15)	7 days (*n*=17)	14 days (*n*=18)	21 days (*n*=21)	28 days (*n*=19)	35 days (*n*=22)	42 days (*n*=27)	3.5 months (*n*=26)
HR, bpm	289.0±8.7**	316.4±5.4^{+}	431.2±17.2^{+++***}	481.8±9.2^{++***}	392.7±5.5^{+++***}	418.6±11.4***	383.9±8.9^{+***}	325.6±7.2^{+++}
Mo, msec	216.6±7.9**	190.8±3.2^{+}	145.1±6.6^{+++***}	125.6±2.5^{++***}	156.1±2.0^{+++***}	148.5±4.7***	159±3.7^{+***}	187.6±4.5^{+++}
SD, msec	4.66±0.62*	3.41±0.26***	1.78±0.34^{++***}	4.14±0.56^{+***}	9.00±0.39^{+++***}	6.09±0.38^{+++}	4.44±0.31^{+++***}	6.42±0.48^{+++}
SI, arb. units	68.8±27.3	75.3±7.02***	311.5±51.71^{+++***}	156.2±26.63^{+***}	33.32±2.29^{+++}	41.25±4.67	64.02±7.05^{++***}	30.91±3.98^{+++}
TP, msec^2	2.05±0.56***	1.83±0.34***	1.88±0.58***	7.78±1.70^{+***}	41.58±2.73^{+++***}	12.18±1.57^{+++}	9.82±1.51*	16.54±2.98^{+}
HFabs, msec^2	0.52±0.14**	0.89±0.22***	1.23±0.41**	1.69±0.31**	23.01±2.24^{+++***}	5.27±0.85^{+++}	3.89±0.68*	8.28±2.00^{+}
LFabs, msec^2	0.51±0.14***	0.29±0.07***	0.48±0.21***	2.91±0.69^{+++}	6.34±1.07^{++}	3.33±0.85^{+}	2.62±0.48	3.96±0.82
VLFabs, msec^2	1.02±0.35**	0.64±0.16***	0.18±0.08^{++***}	3.18±0.82^{+++}	2.74±0.39	3.57±0.63	3.31±0.72	4.29±0.79
IC	2.33±0.39**	1.33±0.23^{+}	0.76±0.28*	2.76±0.33^{+++***}	0.87±0.06^{+***}	1.74±0.21^{++}	1.94±0.34	1.31±0.16
Proportion of spectral components	VLF>HF=LF	HF>VLF>LF	HF>>LF>>VLF	VLF>LF>HF	HF>>>LF>>VLF	HF>VLF=LF	HF>VLF>LF	HF>VLF>LF

Note. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ in comparison with the previous period; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with rats aged 3.5 months.

of week 2 of life the rats open their eyes and the light is a potent stimulus for the sympathetic nervous system. According to morphological findings [7], active functioning of the sympathetic neurocytes starts from the end of week 2 — beginning of week 3 of life in rats. At the age of 21 days, when the rats start leaving the nest, use common fodder, and actively explore the environment, activity of the suprasegmentary regulation level has to increase. The slow wave power really increased significantly in the CRV spectrum, the activity of the sympathoadrenal system increasing as well. The spectral analysis of CRV showed that the age of 28 days was a critical point in the development of regulatory effects on the heart. A drastic increase of power in the HF band indicated an increase of the autonomic regulation contour contribution to CRV formation. This was in line with the data on changed activities of the cholinergic mechanisms in the rat heart tissue [9]. However, HR still remained high ($p<0.001$). Rats aged 35 days could be considered independent; during this period the nervous regulation system started functioning in a mode typical of an adult organism. However, the regulation centralization increased again during the prepubertal period, the sympathoadrenal effects predominating over parasympathetic, though not so much as in younger age. A sort of balance between the regulation contours with predominating autonomic one was maintained in adult rats; the HF wave power increased. The most significant shift of that period was a lower HR, indicating attenuation of the adrenergic effects through the humoral regulation channel.

Hence, spectral analysis of CRV in young rats demonstrated: 1) very low power of CRV spectrum during the very first days of life of albino rats, more likely indicating inadequacy of all regulatory mechanisms but not a high level of sympathetic effects; 2) a jump-wise increase of cardiac rhythm wave power: all waves were weak during the first 2 weeks of life, while by day 21 the first elevation of spectral power was recorded due to VLF and LF waves intensification and by 28 days a second elevation was recorded at the expense of HF waves amplification; by day 35 the power of all waves and the CRV spectrum structure reached the level of adult animals; 3) heterochronous increase of cardiac rhythm waves power: VLF wave power was the first to reach the level of adult age (by 21 days), while the HF and LF waves power reached the adult level later (by 35 days); the most significant increment of fluctuation power was recorded for HF band; 4) alternation of periods of predominating activities of regulation contours: periods of high centralization of regulation with slow waves predominating (at 3, 21, 42 days) are followed by periods of higher autonomic contour activity, when HF waves predominated in the spectrum (at 14, 28 days and 3.5 months). These regularities are in good agreement with stages in the morphofunctional maturing of rats [8].

REFERENCES

1. R. M. Baevsky, G. G. Ivanov, L. V. Chireikin, *et al.*, *Vestn. Aritmol.*, No. 24, 1-23 (2001).
2. *Autonomic Disorders: Clinical Picture, Diagnosis, Therapy*, Ed. A. M. Vein [in Russian], Moscow (2003).
3. T. L. Zefirov, N. V. Svyatova, and N. I. Ziyatdinova, *Byull. Eksp. Biol. Med.*, **131**, No. 6, 612-616 (2001).
4. T. N. Kirillina, M. A. Usachyova, and L. M. Belkina, *Ibid.*, **142**, No. 10, 376-381 (2006).
5. O. V. Korkushko, V. B. Shatilo, T. V. Shatilo, and E. V. Korotkaya, *Fiziol. Chel.*, **17**, No. 2, 31-39 (1991).
6. E. V. Kurjanova and D. L. Teplyj, *Byull. Eksp. Biol. Med.*, **149**, No. 1, 14-17 (2010).
7. P. M. Maslyukov, V. V. Shilkin, and Zh. P. Timmermans, *Morfologiya*, **128**, No. 5, 41-44 (2005).
8. V. I. Makhin'ko and V. N. Nikitin, *Molecular Physiological Mechanisms of Age-Associated Development* [in Russian], Kiev (1975), pp. 308-326.
9. F. G. Sitdikov, T. A. Anikina, and R. I. Gil'mutdinova, *Byull. Eksp. Biol. Med.*, **126**, No. 9, 318-320 (1998).
10. F. G. Sitdikov and V. F. Savin, *Autonomic Parameters of Adaptation to Exercise* [in Russian], Kazan (1984), pp. 100-109.
11. O. P. Timofeeva, A. V. Bursian, V. A. Sizonov, and N. D. Vdovichenko, *Zh. Evol. Biokhim. Fiziol.*, **44**, No. 2, 187-193 (2008).
12. A. E. Aubert, D. Ramaekers, F. Beckers, *et al.*, *Comput. Methods Programs Biomed.*, **60**, No. 3, 197-213 (1999).
13. Y. Z. Yoon, J. Kim, B. C. Lee, *et al.*, *Gen. Physiol. Biophys.*, **24**, No. 2, 147-159 (2005).