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Correlation and Spectral Analysis of Vascular Tone Regulator Mechanisms in Paired Formations during Postnatal Ontogenesis in Rats

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The study focused on the changes of microcirculation indices and components of the vascular tone of endothelial, neural, and myogenic nature in symmetrical skin sites of temples, forehead, auricles, scapulae, and groin during postnatal ontogeny. Initially, microcirculation indices in the symmetrical regions decreased to postnatal day 10, thereafter they surpassed the initial level at the age of 30 days. The endothelial, neural, and myogenic indices of vascular tone changed differently in the symmetrical left and right sites. On the right side, all indices increased by the postnatal day 21 ("redundancy" phase), and by postnatal day 30 surpassed the initial level by two times. On the left side, the redundancy phase manifested in the endothelial component on postnatal day 10, but to postnatal day 30 all three indices dropped below the initial level.

Key Words: *microcirculation; asymmetry; postnatal ontogenesis*

In the microcirculatory bed, the blood flow is characterized by spontaneous fluctuations. A number of correlation and spectral analysis methods are used to analyze the processes involved in microcirculation, which can differentiate the contributions of endothelial, neural, and myogenic mechanisms of vascular tone control into the total spectral power of these "spontaneous" fluctuations [3-5,10,11]. The studies of statistical and spectral microcirculatory parameters of paired organs revealed functional differences between symmetrical sites [5-7]. The problem of functional asymmetry of various organs belongs to fundamental physiological enigmas [2]. However, the researchers focus mostly on asymmetry of the autoregulatory mechanisms, which control the tone of blood vessels in the brain where the functional differences (including the ontogenetic ones) are well studied [1,9,12]. Thus, the problems of asymmetry and age-related changes of microcirculatory parameters in the peripheral organs as well as description of the sequence of nature-determined structural and functional alterations need the detailed study.

This work focuses on the changes of microcirculatory parameters in the symmetrical vessels of the paired structures during postnatal ontogenesis in rats.

MATERIALS AND METHODS

Experiments were carried out on rat pups (n=25) aging 1-30 days. The pups were narcotized with nembutal (5 mg/100 g). The age-related periods were chosen according to the time of manifestation of the antigravity muscles' reactions during the early postnatal ontogenesis, *i.e.*, the postnatal days 1-3, 9-10, 21, and 30. The capillary blood flow was measured with laser Doppler flowmetry employing a LAKK-01 (Lazma) flowmeter. The microcirculatory parameters in symmetrical skin sites of temples, forehead, auricles, shoulder blades, and groin were recorded with the sampling rate of 10 Hz, the epoch length being about 2 min comprising more than 1000 sampled points. The data were fed into

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PC for off-line statistical processing as well as for correlation and spectral analysis. The maximum sampling rate in the periodograms was 5 Hz. To assess the contribution of various mechanisms into the integral vascular tone, we calculated the maximal amplitudes of spectral harmonics of periodograms in the following frequency bands: 0.009-0.02 Hz (NO-related endothelial mechanisms) and 0.02-0.2 Hz (low frequency [LF] band, where 0.02-0.06 Hz and 0.06-0.2 Hz harmonics are considered to be of neural [LFn] and myogenic [LFm] origin, respectively). The high-frequency, respiratory, and cardiac oscillations were not analyzed since they are indicative of the passive mechanisms of blood flow modulation [3,4,10,11]. Vascular tone was assessed by σ/ALF ratio, where σ and ALF are mean square deviation and maximum amplitude of LF harmonics. Interrelations between various microcirculatory parameters were assessed with Spearman's rang correlation coefficient.

The results were analyzed statistically using Statistica 6.0 software. Significance was assessed by Student's *t* test and *F*-test for a difference in dispersion.

RESULTS

During ontogenesis, the microcirculatory parameters of the symmetrical sides of the body demonstrated the reduction phase (postnatal day 10), but on day 30 they attained the greater values in comparison with initial level, which were more pronounced at the left side. Figure 1 shows the course of various microcirculatory parameters in the symmetrical sides during postnatal ontogenesis normalized to the postnatal day 1, including the microcirculatory index (MCI, Fig. 1, *a*) as well as the endothelial, neurogenic, and myogenic components of vascular tone (Fig. 1, *b*, *c*). At the left side, the endothelial tone increased to postnatal day 10 (the redundancy phase) but dropped below initial level to day 30. At the same side, the neurogenic and myogenic tone components passed through redundancy phase to postnatal day 21. To postnatal day 30, the neurogenic tone decreased below initial level, while the myogenic tone remained elevated. On the right side, all tone components increased to postnatal day 21 and remained elevated thereupon to postnatal day 30.

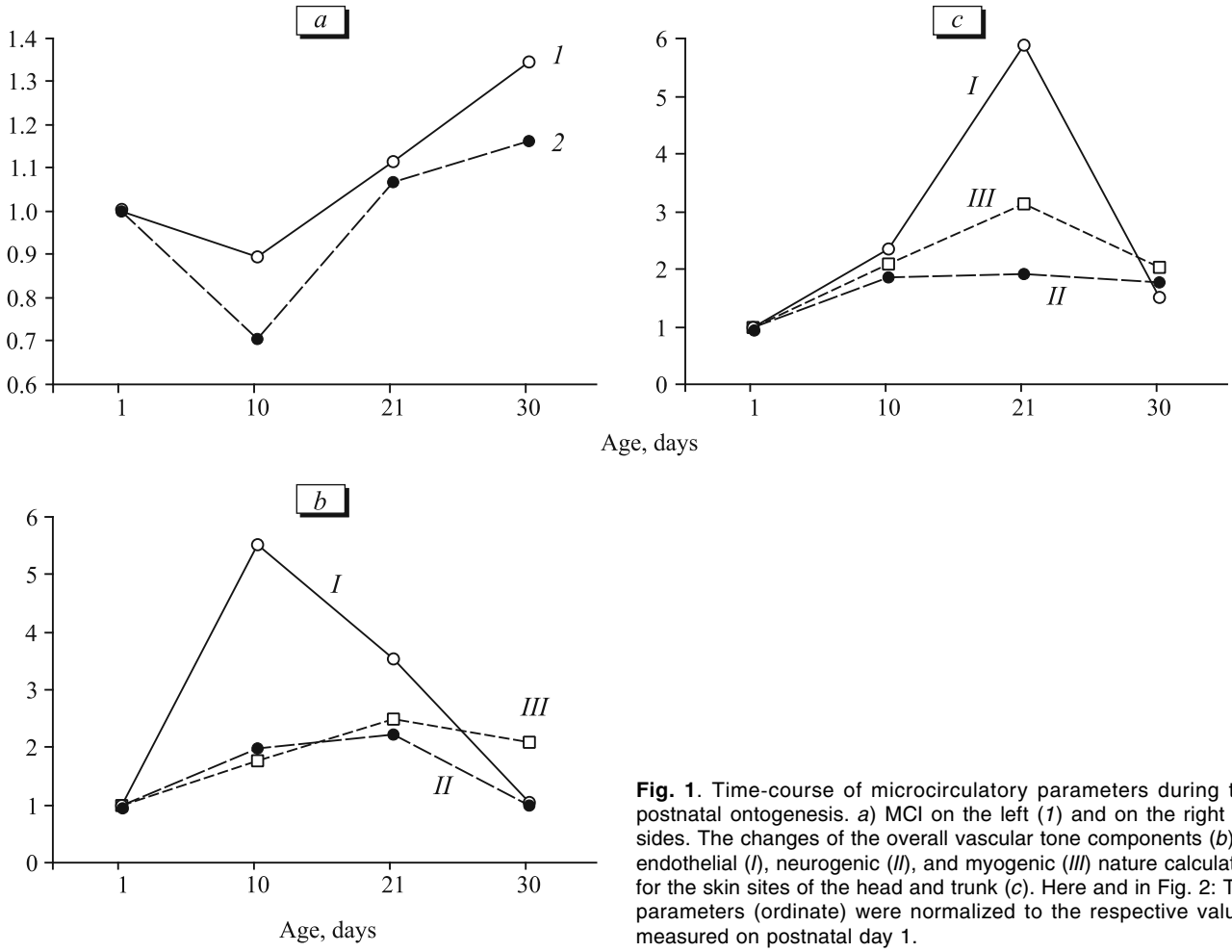

Fig. 1. Time-course of microcirculatory parameters during the postnatal ontogenesis. *a*) MCI on the left (*1*) and on the right (*2*) sides. The changes of the overall vascular tone components (*b*) of endothelial (*I*), neurogenic (*II*), and myogenic (*III*) nature calculated for the skin sites of the head and trunk (*c*). Here and in Fig. 2: The parameters (ordinate) were normalized to the respective values measured on postnatal day 1.

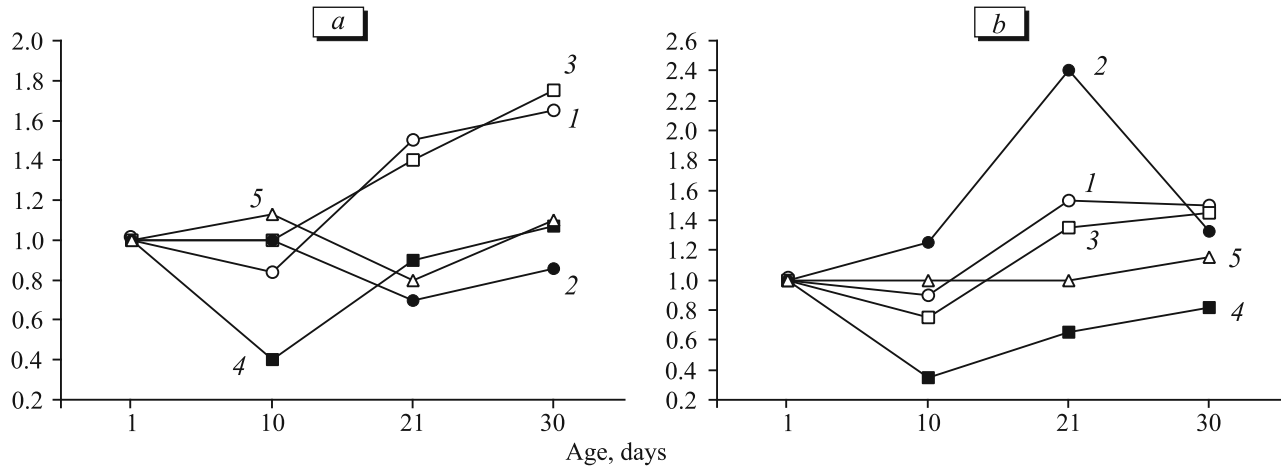

Fig. 2. Time-course of MCI on the left (*a*) and right (*b*) skin sites of rat pups measured for temples (*1*), auricles (*2*), forehead (*3*), shoulder blades (*4*), and groin (*5*) during postnatal ontogenesis.

At both sides, the most pronounced changes observed during postnatal ontogenesis were characteristic of endothelial component of vascular tone.

Table 1 shows the data of correlation analysis reflecting the interrelations between the microcirculatory parameters during postnatal ontogeny. Persistent negative correlation was observed between MCI and endothelial tone at the left side, while similar negative correlation between MCI and myogenic tone was characteristic of the right side. At the right side, there was persistent positive correlation between endothelial and neurogenic vascular tone components. Interrelation between neurogenic and myogenic tone components was observed only in newborn rat pups.

Correlation analysis performed for the first five days of postnatal ontogenesis at the left side concluded that during the early postnatal period, σ can represent the blood flow ($R>0$). In addition, the endothelial tone component and MCI were inversely proportional ($R<0$): smaller values of endothelial tone corresponded to greater MCI values (in other words, endothelial-related dilation 'maintained' the blood flow at the left side). It is important that positive correlation between endothelial and neurogenic tone components can be indicative of dramatic changes of vascular tone (in both directions). The value of σ is inversely proportional to neurogenic and myogenic tone components ($R<0$): smaller values of these tone components corresponded to greater MCI variability. Correlation analysis carried out for the first five days in the right side concluded that endothelial tone component can be indicative of the blood flow: the greater its value, the larger MCI ($R>0$). Moreover, there were the positive correlations: between MCI in symmetrical sides during the first postnatal days and between the endothelial and neural tone components.

Correlation analysis performed on postnatal days 8-10 shown that the correlations of MCI with tone components were of the same sign at both sides except that the left side demonstrated the negative correlation between dispersion and the neural tone component, while the right side showed negative correlation between dispersion and the myogenic tone component. In addition, there was correlation between the vascular parameters measured at the symmetrical sides. On postnatal day 21, the correlations between MCI with endothelial or neural tone components were negative irrespective of the body's side. At the right side, the negative correlation was also observed for the myogenic tone component. The positive correlation between the endothelial and neural tone components was observed only for the microcirculation parameters at the right side. On postnatal day 30, the negative correlation between MCI and the right myogenic tone component myogenic was characteristic of the both sides.

The examined parameters revealed common peculiarities of microcirculation in the symmetrical sides of rat's body. The study demonstrated the typological features of microcirculation in the symmetrical organs. Figure 2 shows the data collected for the skin sites of temples, forehead, auricles, scapulae, and groin. At the left side, the most augmented microcirculation parameters were those of temple and forehead, while at the right side, the inflated valued were those of temple and auricle. The scapular microcirculation parameters decreased on the postnatal day 10; thereupon, they set above and below the initial level at the left and right sides, respectively. On postnatal day 21, the auricular parameters changes in the opposite directions at both sides: they decreased at the left side and increased at the right side, while in other organs (except for the groin) the changes were unidirectional. On this day, the inguinal microcirculation parameters decreased at the left side, although they remained unchanged at the right side.

TABLE 1. Spearman's Correlation Coefficients (*R*) for Microcirculatory Parameters in Symmetrical Sides of Rat Pups during Postnatal Ontogenesis

Parameter		Correlation coefficients on the left side					Correlation coefficients on the right side				
		MCI	δ	E	N	M	MCI	δ	E	N	M
Pups aging <5 days	left MCI	1									
	δ	0.412*	1								
	E	-0.388*		1							
	N	-0.477*	-0.337*	0.464*	1						
	M		-0.408*		0.652*	1					
	right MCI	0.676*	0.367*		-0.465*	-0.414*	1				
	δ		0.664*					1			
	E			0.333*			440*		1		
	N		-0.317*		0.409*	0.415*	-0.405*	-0.356*	0.326*	1	
	M				0.363*	0.598*	-0.358*	-0.371*		0.717*	1
Pups aging 8-10 days	left MCI	1									
	δ	0.395*	1								
	E	-0.381*		1							
	N	-0.658*	-0.356*	0.578*	1						
	M	-0.579*				1					
	right MCI	0.732*	0.377*	-0.371*	-0.624*	-0.435*	1				
	δ	0.384*					0.479*	1			
	E	-0.468*					-0.533*		1		
	N	-0.504*					-0.630*		0.602*	1	
	M	-0.398*	-0.425*				-0.564*	-0.591*			1
Pups aging 21 days	left MCI	1									
	δ	0.403*	1								
	E	-0.361*		1							
	N	-0.396*			1						
	M					1					
	right MCI	0.559*		-0.30*	-0.311*		1				
	δ	0.468*					1				
	E	-0.289*			0.292*		-0.422*		1		
	N				0.496*		-0.401*		0.453*	1	
	M					0.496*	-0.486*				1
Pups aging 30 days	left MCI	1									
	δ	0.299*	1								
	E	-0.389*		1							
	N				1						
	M		0.281*			1					
	right MCI	0.583*			-0.290*	-0.279*	1				
	δ		0.384*		0.265*		0.281*	1			
	E				0.265*				1		
	N				0.393*				0.329*	1	
	M	-0.348*		-0.284*		0.544*	-0.314*				1

Note. E, N, and M denote the endothelial, neurogenic, and myogenic vascular tone components, respectively. *$p<0.05$.

This study attests to asymmetry in the development of regulatory mechanisms of vascular tone in various paired structures during postnatal ontogenesis. In these paired structures, MCI decreased to postnatal day 10, and they stabilized to day 30 above initial levels, the overshoot being more pronounced for the left organs. During postnatal ontogenesis, the greatest changes concerned the endothelial component of vascular tone. At the left side, it pronouncedly increased to postnatal day 10, although it decreased below initial level to postnatal day 30. At the right side, the endothelial tone component also markedly increased to postnatal day 21, thereupon it dropped below initial level to postnatal day 30. Thus, the endothelial component of vascular tone demonstrated a pronounced redundancy phase, which developed in different periods at both symmetrical sides. Our data agree with common views on redundancy of the biological systems [8]. They also attest to the fact that during postnatal ontogenesis, the endothelial component of vascular tone plays an important if not the dominant role in the control of vascular tone in microvessels in full agreement with our previous report [5]. One can reasonably conclude that namely the endothelial vascular tone mechanisms in microvessels are responsible for manifestation of the fundamental property of biological redundancy during the postnatal development of structural and functional organization in biological systems.

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