Spectral Analysis of Blood Velocity Fluctuations and Characterization of the Mechanisms Controlling the Tone of Individual Microvessels in Frog Tongue

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 152, No. 9, pp. 355-360, September 2011
Original article submitted April 27, 2011

Intravital microscopy and high-frequency ultrasonic system equipped with a microtransducer working at 38.5 MHz were employed to record blood velocity in arterioles and venules in the symmetrical fragments of the microvascular bed in frog tongue. The diameters of the vasculature ranged 75-200 μ. The record length was 2-5 min. The range of blood velocities was wider in the left arterioles than in the right ones, and inversely, it was greater in the right venules than in the left ones. The high-frequency and respiratory harmonics (0.2-1.5 Hz) dominated in the velocity spectrum of microvessels at each side, the greatest values being 64.6 and 72.3% for the left venules and the right arterioles, respectivelyly. The low-frequency harmonics (0.009-0.02 Hz) were rare observed (7.6-15.4% examined vessels). They appeared in vessels with pronounced difference between the maximum and minimum velocities, in the cases with velocity variations looking like batches (bursts), *etc*. Correlation of velocities in arterioles and venules was positive at the left side (0.451) and negative at the right side (-0.574) of the tongue.

Key Words: *blood velocity; spectral analysis; venules; arterioles; vascular tone*

Fluctuations of the parameters describing the physiological systems accompany their normal work, and analysis of these fluctuations opens the way to reveal the mechanisms, regulatory principles, and stability of such systems [1]. Information on the fluctuations makes it possible to apply various mathematical methods to study the physiological systems: spectral analysis, analysis of the non-linear dynamic systems, chaos theory, *etc*. [2,3,9-11]. Fluctuation analysis and assessment of the mechanisms, which modulate the tone in microcirculation system underlies laser Doppler flowmetry (LDF) now widely used in clinics [4]. Moreover, LDF measures the average parameters of the pulsatile blood flow and assesses the regulatory mechanisms of vascular tone in the examined region of microvascular bed (MVB) or tissue. The development of ultrasonic Doppler methods opened novel vista to assess the blood velocity in individual microvessels, to determine the range of blood velocities, and to examine dynamic and transient processes during various interventions [8].

This study was designed to measure the blood velocities in frog lingual arterioles and venules and to characterize the nature of regulatory mechanisms controlling vascular tone with the help of high-frequency ultrasonic technique [5,6].

MATERIALS AND METHODS

The experiments were carried out on spinal frogs. The lingual microvessels were observed in transmitted light with an MBI-15 microscope equipped with objective lenses of ×3.5 and ×9. The study employed an ultrasonic system working at 38.5 MHz and a sin-

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0007-4888/12/1523-0382

gle space-saving transducer (0.7 mm height, 200 mg weight). The transducer housing incorporated a piezoelectric cell with a diameter of 0.5 mm directed at 50° to the vasculature plane. The ultrasound was focused with an acoustic lens. The ultrasonic system had an acoustic output used to adjust the transducer position relatively to direction of blood flow in the examined vessel. Under the microscope, the transducer was positioned above the vessel with a micromanipulator to assure the maximum of the recorded ultrasonic signal (Fig. 1). The visual field with the transducer and microvessel was photographed; thereafter the size of

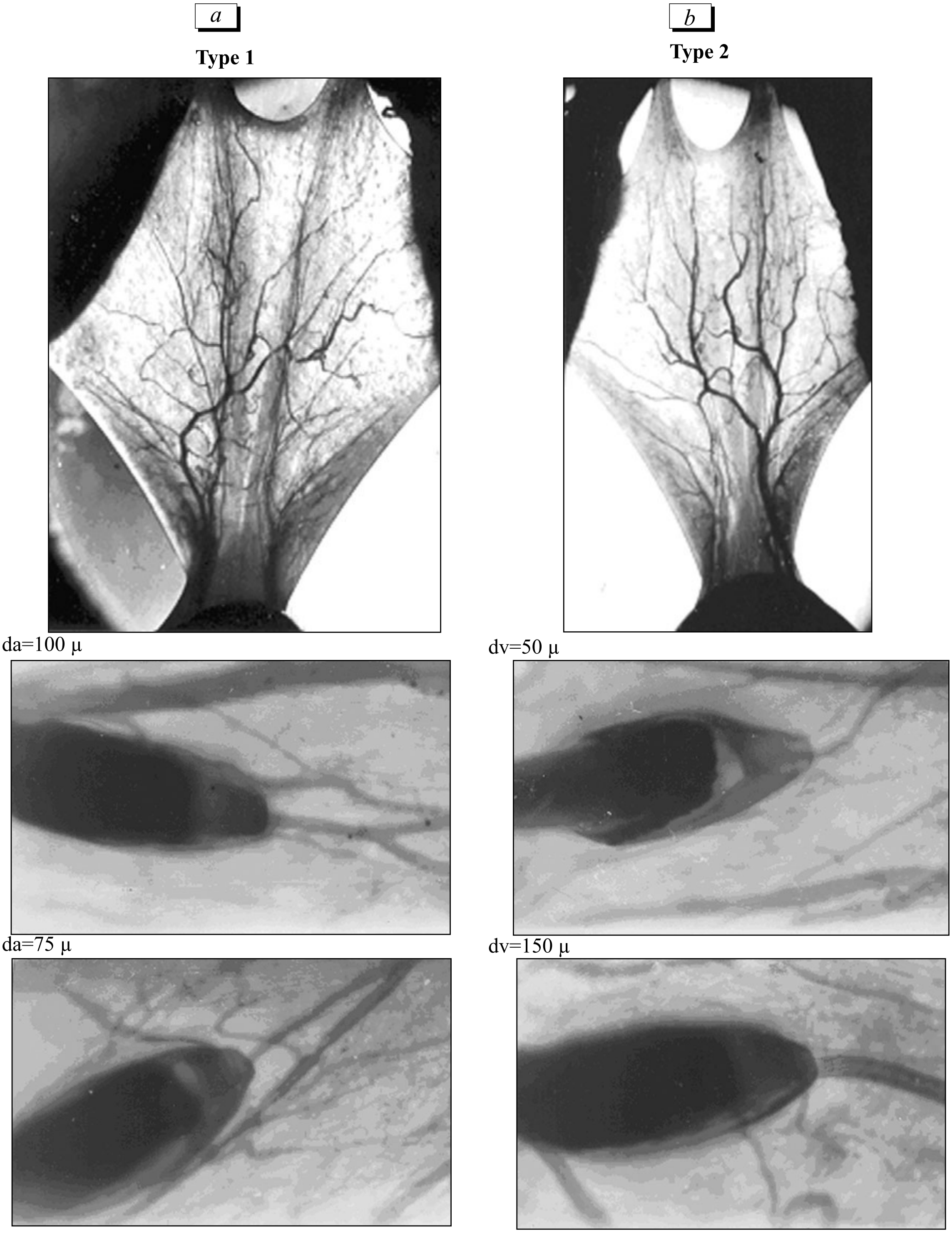


Fig. 1. The basic types of MVB in frog tongue and the transducer mounted on microvessels. d_a, diameter of arteriole; d_v, diameter of venule.

the vessel was measured. The blood velocity was recorded for 2-5 min. The data were digitized with an LA20 USB converter at a sampling rate of 10 Hz. The spectrum ranges for the harmonics were specified according to the accepted routine of LDF [4]. Dopplerographic data were analyzed in the frequency domain with the standard spectral technique. The frequency range of the analyzed data was 0.003-5 Hz. Vascular tone of MVB was assessed by spectral harmonics in the following frequency ranges: *1*) low-frequency (LF) range of 0.009-0.02 Hz corresponding to contribution of endothelial factor to the regulation of the muscular tone caused by NO activity; *2*) LF range of 0.02-0.06 Hz corresponding to neurogenic influences (LFn); *3*) LF range of 0.06-0.2 Hz corresponding to myogenic influences (LFm); 4) high-frequency (HF) range corresponding to HF oscillations (0.2-0.6 Hz), respiratory rate (RF, 0.6-1.5 Hz), and the cardiac rhythm (CF, 1.5-5.0 Hz). According to LDF routine [4], the contribution of the endothelial (V_{tonE}), neurogenic (V_{tonN}), and myogenic (${}_{VtonM}$) mechanisms into resulting vascular tone was assessed according to σ/ALF ratio, where σ is root-mean-square deviation and ALF maximum amplitude of the low-frequency harmonics in the corresponding frequency band.

RESULTS

In the study of MVB in frog tongue, we most frequently observed two types of asymmetric arrangement of the vessels (Fig. 1) and rarely met the tongues with symmetrical MVB [7]. In this paper, the first type of MVB dominated (Fig. 1, *a*). The diameter of the examined vessels ranged 75-200 μ. Blood velocities in the arterioles and venules located in the symmetrical regions of lingual MVB (Fig. 1) were recorded for 2-5 min. According to modern views, spectral analysis of fluctuations of the blood velocity reveals peculiarities of the mechanisms regulating the tone of microvessels. Especially interesting are the data on major harmonics in such spectrum during physiological control of vascular tone (Table 1). Initially, the high-frequency and respiratory-rate harmonics dominated in the blood velocity spectrum independent on the side of the tongue. Tables 2 and 3 show the mean parameters of arterioles and venules in lingual MVB obtained by analysis of blood velocity in time- and frequency-domains.

Calculation of the correlation between blood velocity values in arterioles and venules performed for the symmetrical sides of lingual MVB showed that the left and right correlations coefficients were $R(V_a,V_v)_{left}=0.451$ ($p<0.05$) and $R(V_a,V_v)_{right}=-0.574$ ($p<0.05$), respectively. The positive correlation of blood flow in arterioles and venules could result from unidirectional changes in the blood flow in MVB vessels at the left side of the tongue; in case of drastic changes in the input blood flow (increase or decrease), no less pronounced changes could occur in the output flow.

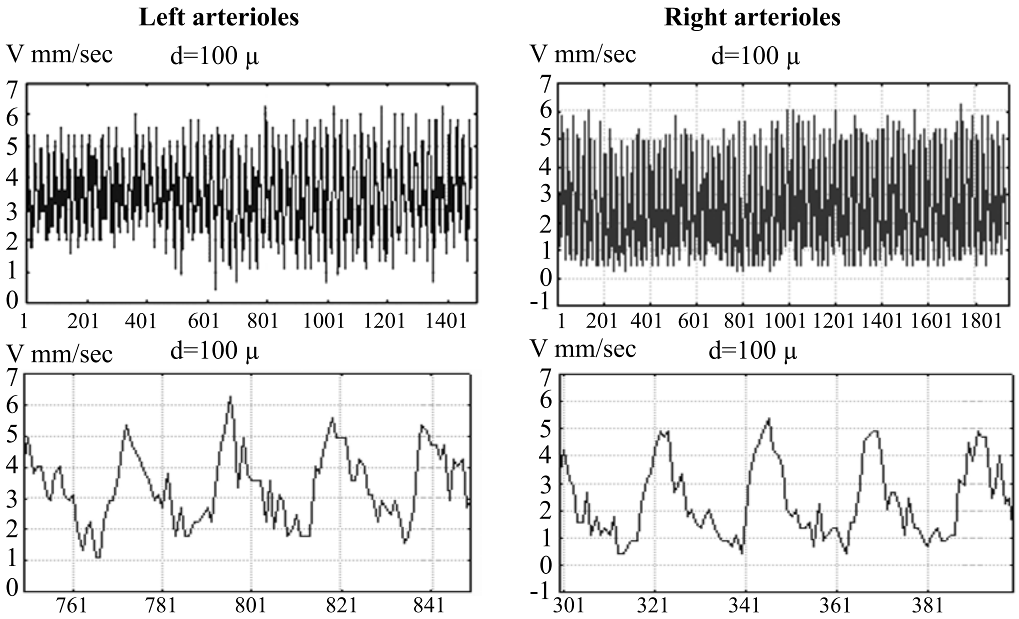
The applied method revealed various types of the velocity distribution in blood microvessels (Fig. 2, 3). It is noteworthy, that when velocity fluctuations looked like ‘batches’ (bursts) in the time-domain, the low-frequency harmonics dominated in the corresponding spectrum. The study showed that the velocities of blood flow in arterioles and venules in the examined type of MVB were greater in the left side of the tongue than in its right side (Figs. 2, 3). The high-frequency harmonics of blood velocity variations were dominant in both sides with possible difference in their power. The assessments of the vascular tone components for the vessels of approximately the same diameter could differ by several times (Tables 2 and 3).

Application of high-frequency ultrasonic Doppler system equipped with a microtransducer makes it pos-

TABLE 1. Incidence (in %) of Various Type Harmonics in Blood Velocity Spectrum of Individual Microvessels

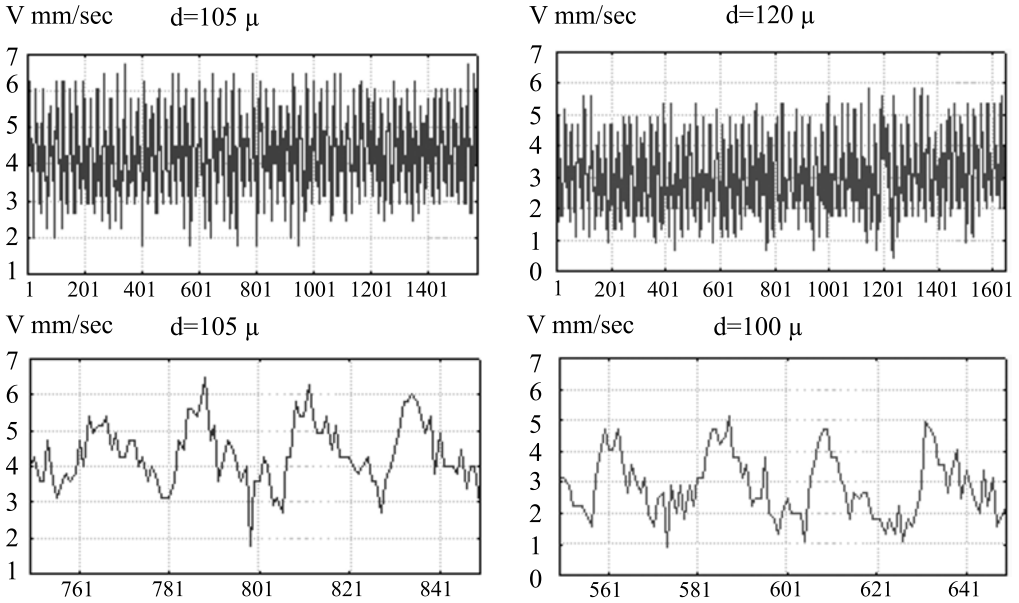
Range	Arterioles		Venules	
	left	right	left	right
LF_e	13.9	7.6	15.4	13.5
LF_n	1.7	5.7	13.8	14.6
LF_m	12.2	13.3	5.4	5.6
HF	45.2	39.0	24.6	44.9
RF	25.2	33.3	40.0	21.3
CF	1.7	1.0	0.8	-

Note. Calculation was performed on 5 harmonics with the largest amplitudes. The frequency ranges of the specified harmonics are given in “Materials and Methods”.



V=3.51±1.12 mm/sec; d=100 μ;
5 harmonics:
937.50:0.440; 121.2:0.447; 94.14:0.886;
60.23:0.880; 54.77:0.453
Vtone: 0.069E; 0.218N; 0.396M

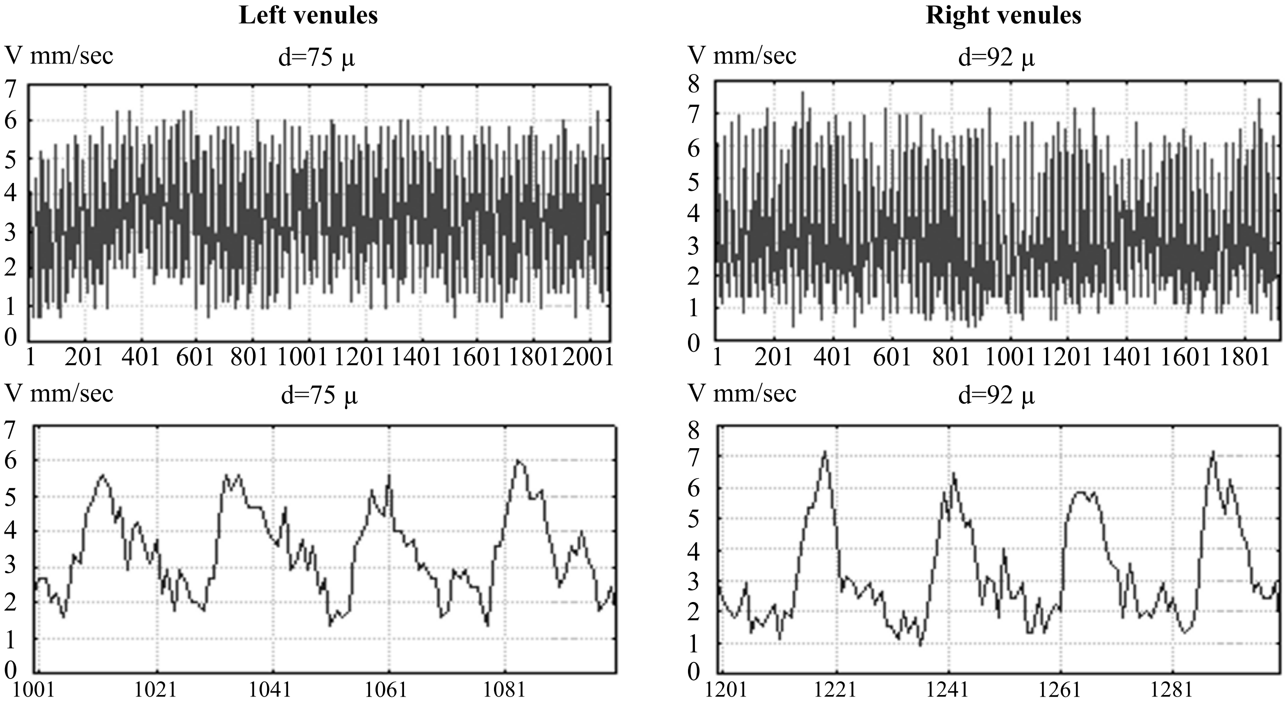
V=2.65±1.53 mm/sec; d=100 μ;
5 harmonics:
1437.0:0.435; 702.1:0.440; 502.3:0.430;
123.2:0.876; 114.0:0.446
Vtone: 0.658E; 0.340N; 0.336M



V=4.36±0.94 mm/sec; d=108 μ;
5 harmonics:
336.0:0.427; 242.4:0.420; 82.62:0.847;
56.61:0.433; 53.47:0.414
Vtone: 0.159E; 0.041N; 0.433M

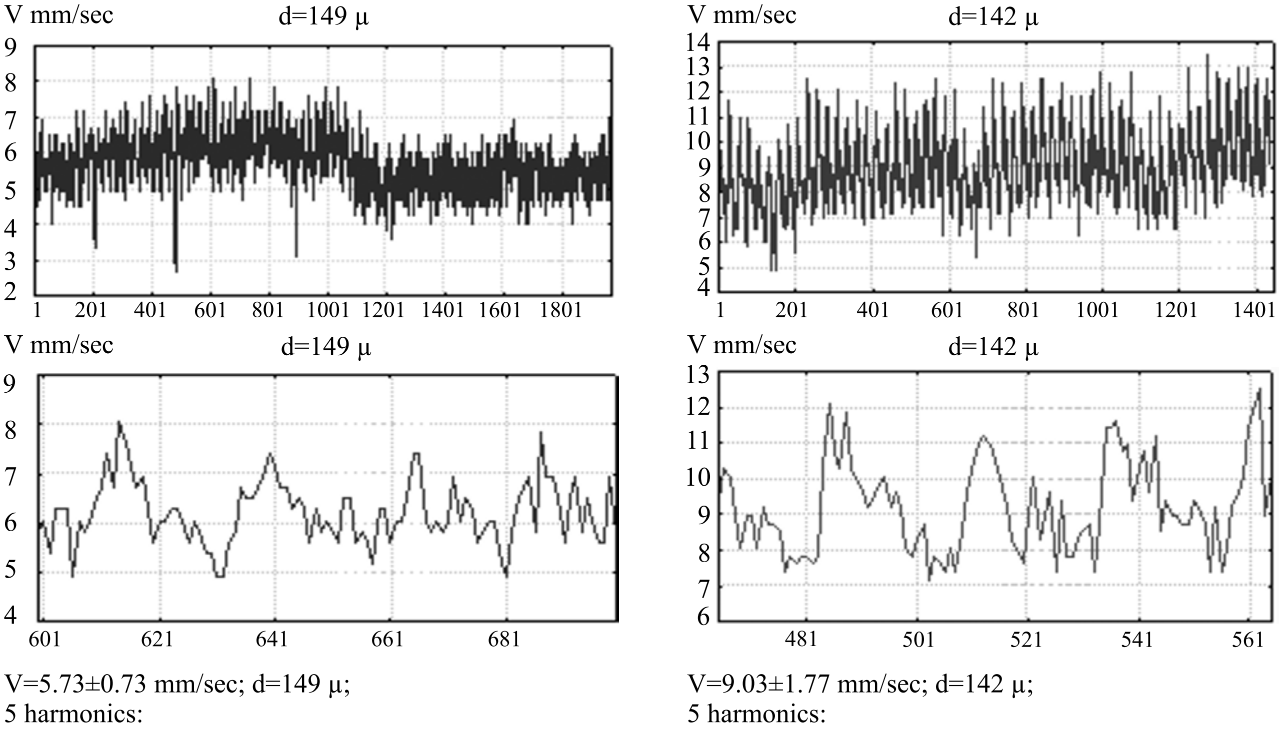
V=3.05±1.03 mm/sec; d=122 μ;
5 harmonics:
784.0:0.413; 165.7:0.826; 25.25:1.239;
20.55:0.419; 12.28:0.820
Vtone: 0.091E; 0.599N; 0.140M

Fig. 2. Fluctuations of blood velocity (full-length records and typical fragments) in arterioles and the corresponding spectrum parameters. Here and in Fig. 3: abscissa, the number of data points harvested at the sampling rate of 10 Hz; ordinate, blood velocity in mm/sec. V, mean blood velocity; d, vascular diameter in μ; amplitude:frequency numbers refer to the most large spectral harmonics (in rel. units and in Hz, respectively). The bottom string gives the vascular tone parameters of various nature.



V=3.40±1.23 mm/sec; d=75 μ;
5 harmonics:
947.5:0.412; 515.8:0.417;
229.4:0.829; 155.7:0.422; 95.4:0.407
Vtone: 0.029E; 0.048N; 0.209M

V=3.19±1.53 mm/sec; d=92.5 μ;
5 harmonics:
2329.0:0.421; 454.1:0.843;
145.8:0.427; 128.9:0.848; 105.8:0.416
Vtone: 0.136E; 0.186N; 0.326M



V=5.73±0.73 mm/sec; d=149 μ;
5 harmonics:
84.92:0.401; 84.21:0.005;
78.61:0.010; 62.62:0.406; 12.08:0.035
Vtone: 0.009E; 0.060N; 0.156M

V=9.03±1.77 mm/sec; d=142 μ;
5 harmonics:
519.0:0.389; 407.5:0.396;
258.9:0.786; 147.2:0.021; 55.83:0.382
Vtone: 0.076E; 0.0096 N; 0.066 M

Fig. 3. Fluctuations of blood velocity (full-length records and typical fragments) in venules and the corresponding spectral parameters.

TABLE 2. Mean Blood Velocity and Velocity Spectrum Parameters of Frog Lingual Arterioles

Parameter	Left arterioles (*n*=22)	Right arterioles (*n*=22)
V, mm/sec	4.600±1.849 (0.927-8.900)	3.790±1.123 (1.75-5.50)$^+$
σ	1.423±0.596 (0.756-3.454)	1.237±0.310 (0.658-1.699)$^+$
ALF$_e$	121.30±164.95 (1.42-608.30)	76.26±161.30 (3.240-731)
ALF$_n$	61.22±79.69 (1.99-287.90)	33.44±44.22 (1.71-181.80)$^+$
ALF$_m$	122.97±313.04 (1.71-1348)	96.82±257.10 (2.29-1185)
σ/ALF$_e$	0.099±0.158 (0.002-0.658)	0.121±0.120 (0.002-0.395)
σ/ALF$_n$	0.158±0.186 (0.008-0.697)	0.149±0.179 (0.009-0.679)
σ/ALF$_m$	0.171±0.188 (0.002-0.787)	0.155±0.130 (0.001-0.433)$^+$

Note. The left side demonstrated greater heterogeneity of myogenic arterial tone than the right side. Here and in Tables 3: V, the mean velocity; ALF$_e$, maximal amplitude of endothelial low-frequency harmonic; ALF$_n$, maximal amplitude of neurogenic low-frequency harmonic; ALF$_m$, maximal amplitude of myogenic low-frequency harmonic. σ/ALF$_e$, σ/ALF$_n$, σ/ALF$_m$ – the spectrum parameters evaluating the control of vascular tone by the corresponding mechanisms.

TABLE 3. Mean Blood Velocity and Velocity Spectrum Parameters of Frog Lingual Venules

Parameter	Left venules (*n*=28)	Right venules (*n*=16)
V, mm/sec	5.610±2.217 (2.27-11.00)	3.99±1.84 (1.928-9.030)*
σ	1.391±0.550 (0.542-2.720)	1.156±0.301 (0.679-1.572)$^+$
ALF$_e$	157.3±274.7 (0.77-1368)	84.8±150.5 (0.980-609)$^+$
ALF$_n$	140.7±276.1 (2.34-1439)	56.5±65.6 (3.22-186.30)$^+$
ALF$_m$	62.8±104 (2.63-477.50)	30.8±44.8 (2.48-153.70)$^+$
σ/ALF$_e$	0.150±0.396 (0.002-1.951)	0.120±0.198 (0.003-0.785)$^+$
σ/ALF$_n$	0.057±0.074 (0.002-0.296)	0.078±0.076 (0.007-0.234)
σ/ALF$_m$	0.123±0.121 (0.006-0.428)	0.182±0.169 (0.010-0.458)

Note. Blood velocity was larger in the left side of the tongue than in its right side. The left side demonstrated greater heterogeneity of endothelial venular tone than the right side.

sible to examine the character of blood flow in various segments of MVB, which is important for preventive and therapeutic purposes.

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