DOI 10.1007/s10517-022-05539-7

Chronic Effects of Modeled Myocardial Infarction, Thoracic Trauma, and Denervation of Hind Limb on Rheographic Parameters of Rat Femoral Arteries *In Situ*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 173, No. 3, pp. 294-300, March, 2022
Original article submitted January 13, 2022

In experiments on narcotized male rats (n=85), the mean electroimpedance Z and peak-to-peak magnitudes (the swing ranges) of passive (ΔZ_p) and active (ΔZ_a) pulsatile electroimpedance oscillations of isolated segment of femoral arteries were determined *in situ*. These rheographic parameters (RP) were measured in intact animals and in those with modeled chronic myocardial infarction, chronic denervation of the right hind leg, as well as in rats subjected to sham operations to mimic denervation or infarction (with thoracic trauma). The rats with modeled myocardial infarction demonstrated decreasing trends of all RP. In sham-operated rats with thoracic trauma, ΔZ_p increased significantly on postsurgery months 2-4 by 4.3 times in comparison with the control. No essential correlation was found in denervated rats between RP of any femoral artery and severity of neuropathic pain syndrome assessed by autotomy of the operated leg. In these rats, the mean electroimpedance Z of any femoral artery was significantly greater than the control level. They demonstrated especially high values of ΔZ_p with significant difference between ΔZ_p of innervated and denervated hind leg. In denervated rats, ΔZ_a was significantly greater than the control value without significant difference between ΔZ_a of both femoral arteries. The paradoxically great increase of ΔZ_p (100- and 50-fold for innervated and denervated legs, respectively) and a significant 3-fold increment of ΔZ_a in both hind legs provoked by denervation of one of them are discussed in relation to searching for the ways of systemic influences on vascular network in clinics and experiments.

Key Words: *electroangiology; rat femoral arteries; active and passive pulsing; denervation; myocardial infarction*

Previous studies revealed an unknown property of major arteries to pulse in active mode, which differs from the passive one by synphase changes in BP and the current value of arterial electroimpedance reflecting parallel elevation/decrease of BP and corresponding constriction/dilation of the artery during a cardiocycle, generation of arterial action potentials, and augmented peak-to-peak magnitude (the swing range) of electroimpedance oscillations by an order of magnitude [8-10]. A strong positive correlation between the swing ranges of passive (ΔZ_p) and active (ΔZ_a) pulsatile oscillations of arterial electroimpedance made it possible to extend the Frank–Starling law of the heart onto the arteries and to consider this extension as reflection of evolution-consolidated feature in the work of the cardiovascular system [10]. Since active pulsing of arteries is a diagnostic sign of healthy

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vessels [6,7], while pronounced changes in the swing range of pulsatile oscillations were observed in some pathologies [2,13], it seems important to assess the effects of diverse factors on rheographic parameters (RP) of the arteries such as mean electroimpedance Z as well as ΔZ_p and ΔZ_a.

Here we employed the electrophysiological methods to reveal the chronic influences of various factors (denervation of a hind leg, experimental myocardial infarction, and thoracic trauma) on the state of femoral arteries in rats. The study is aimed to assess the chronic functional alterations of the arteries as well as to broaden the views on peculiarities of vascular diseases and the ways to affect the vascular network with therapeutic vista.

MATERIALS AND METHODS

Experiments were carried out *in situ* on femoral arteries of outbred male albino rats (n=85). The animals were maintained under vivarium conditions at room temperature with *ad libitum* water and food supply. The study was conducted under the guidance of Ethical Committee of the Research Institute of General Pathology and Pathophysiology in compliance to Order No. 199n of Ministry of Health of the Russian Federation (On Approval of the Rules of Good Laboratory Practice; April 1, 2016), Requirements of International Association of Study of Pain (IASP), and Directive 2010/63/EU of the European Parliament and of the Council (On the Protection of Animals Used for Scientific Purposes; September 22, 2010).

The rats were anesthetized with intraperitoneal Nembutal (50 mg/kg). The body temperature was maintained at 38.0±0.5°C using an infrared lamp and heated surgery operating platform. Euthanasia was performed with overdose of Nembutal. The animal state was assessed by monitoring BP in the left femoral or left carotid artery, ECG in the second standard lead, and the depth and rate of respiration derived from the pneumotachogram recorded with differential manometer connected to intratracheal tube. When necessary, anesthesia was maintained with supplementary intravenous Nembutal (10% initial dose).

In situ recording of RP of a segment of femoral artery with the length of about 4 mm was described elsewhere [8-10]. The data were analyzed in the segments of electroimpedance records corresponding to the stable fragments of BP and pneumotachogram traces. In these segments, the mean value of electroimpedance Z was calculated over 10 cardiac cycles. The passive or active pulsatile modes of artery was established by applying alternating (100 kHz) probe current with the amplitude of 0.2 or 2 mA, respectively [8]. In each of these modes, the maximal and minimal electroimpedance values were measured in a cardiac cycle during the expiration phase, thereupon the swing ranges of pulsatile oscillations ΔZ_p and ΔZ_a were calculated as the difference between the corresponding maximal and minimal values [10].

The experiments were carried out on 5 groups of rats. The intact control group (n=30) consisted of healthy rats weighing 250-550 g and aging 3-15 months. Myocardial infarction (n=8) was modeled in the group of rats weighing 150-180 g and aging about 2 months. To this end, the anesthetized animals were intubated and artificially ventilated with a small animal ventilator. After thoraco- and pericardiotomy, a lavsan ligature (3-0) was passed below the anterior branch of the left coronary artery 1 mm downstream its emergence from under the atrial auricle, thereupon this artery was single-stage ligated. The wound was closed in layers. The experiments were conducted on postsurgery days 80-90 taking into consideration that major alterations in the cardiovascular system characteristic of chronic heart failure should be consolidated to this time [4]. The group of sham-operated (SO) rats with surgical thoracic trauma (n=13) consisted of the rats weighing 350-400 g and aging 4-6 months. When these rats were aging about 2 months and weighing 150-180 g, they were subjected to SO for myocardial pseudo-infarction. In these rats, the surgery was the same as in the myocardial infarction group except for ligation of the left coronary artery.

The group of rats with denervated right hind leg (n=25) comprised the mature animals weighing 190-240 g prior to the surgery. Denervation was performed by transection of the right sciatic nerve in *popliteal fossa* with routine method [1,3]. The experiments with denervated rats were carried out in 4-6 weeks after surgery. The severity of neuropathic pain syndrome (NPS) in these rats was determined according to specialized scale of autotomy in the affected leg [5]. In rats without NPS (*i.e.*, without the autotomy signs) the severity of pain syndrome was assumed to be zero. RP of the right and left femoral arteries were determined in denervated rats with NPS of 1-5 points (n=17) and in such rats without NPS (n=8). SO rats were subjected to the same surgical manipulations except for neurotomy of the sciatic nerve (n=9).

The data were statistically processed using Statistica 6.0 (StatSoft, Inc.) and SigmaPlot 11.0 (Systat Software, Inc.) software. The groups were compared with Mann–Whitney U test. If the analyzed parameters of both femoral arteries of denervated rats did not significantly differ from each other, they were combined into a common group. Association of two parameters was determined with Spearman's rank correlation test. The data are summarized as Me (Q1-Q3).

RESULTS

In intact rats (n=30), BP was 126 (115-138) mm Hg. In other groups of rats, BP did not significantly differ from this control level, which made it possible to determine this parameter for all examined rats (n=85) as 126 (114-138) mm Hg. Probably, the virtually identical BP values in all groups resulted from regulation of narcosis depth in order to maintain anesthesia. This peculiarity made it possible to characterize the state of femoral arteries without correction for BP.

There were no significant intergroup differences between all RP of intact and SO rats, so these rats were combined into a common control group (n=39). The denervated rats demonstrated no essential correlation between RP of femoral artery in each leg, on the one hand, and NPS severity, on the other hand (the corresponding correlation coefficients were <0.3), therefore the data on each leg were combined into the common groups irrespective of NPS severity.

Table 1 shows the mean electroimpedance Z of the segments of femoral arteries in all groups of rats. Only Z values of arteries in both hind legs of denervated rats significantly differed from the control level, although there were no significant differences between Z values in denervated leg and innervated one (Fig. 1, *a*). Thus, denervation of one hind leg induced a chronic elevation of Z value of femoral arteries in both hind legs attesting to their constriction. Interestingly, this chronic effect of denervation was opposite to that observed in classical acute experiments of Claude Bernard with transection of cervical sympathetic nerve. It should be remembered that the present data relate to major arteries instead of peripheral circulation as in the above classical experiments.

Another interesting finding was a significant difference (p<0.05) in Z values between the groups of rats with infarction and SO rats with thoracic trauma (Fig. 1, *a*; Table 1). These groups demonstrated the opposite trends in the changes of Z values against the control level. Thoracic trauma insignificantly increased Z in SO rats, but insignificantly decreased this parameter in rats with modeled infarction. Since the modeled myocardial infarction combined thoracic trauma and the surgery on the heart, it is reasonable to expect that the real infarction not accompanied with thoracic trauma would lead to a more pronounced drop in Z value reflecting arterial dilation, which would decrease the mechanical load to the heart.

TABLE 1. Mean Electroimpedance Z of Rat Femoral Arteries (Me (Q1-Q3))

Experimental conditions	Mean electroimpedance Z, Ω
Control (n=39)	1580 (1420-1910)
Thoracic trauma (SO group) (n=13)	1890 (1500-1910)
Modeled myocardial infarction (n=8)	1380 (1340-1470)$^{+}$
Artery of innervated leg in denervated rats (n=16)	2430 (2100-2480)$^{***++}$
Artery of innervated leg (n=25)	2140 (1900-2500)$^{***++}$
Artery of both hind legs in denervated rats (n=41)	2370 (1900-2480)$^{***+++}$

Note. Here and in Tables 2 and 3: $^{*}p$<0.05, $^{***}p$<0.001 in comparison with control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 in comparison with RP of the arteries in SO rats with thoracic trauma; ^{o}p<0.05 in comparison with RP of the artery in innervated hind leg of denervated rats.

The swing range of passive pulsatile oscillations ΔZ_p is an important hemodynamic parameter capable to essentially affect the arterial hydroimpedance, which grows with ΔZ_p [12]. The rats with modeled myocardial infarction demonstrated a decreasing trend in ΔZ_p, whereas ΔZ_p significantly increased in SO rats with thoracic trauma in comparison with the control level (Fig. 1, *b*; Table 2). As in the above, it can be hypothesized that in contrast to modeled infarction, which combined thoracic trauma and myocardial ischemia, the real infarction (without the thoracic trauma) would differ from the control not by a trend, but by significant drop in ΔZ_p. In myocardial infarction, simultaneous decrease of Z (corresponding to arterial dilation) and ΔZ_p (contributing to a drop of arterial hydroimpedance) should diminish the load to the heart [12].

Especially interesting is a paradoxically high increase of the swing range of passive pulsatile oscillations of electroimpedance ΔZ_p of femoral arteries in both hind legs after denervation of one of them (Fig. 1, *b*; Table 2). Approximately, in the innervated leg ΔZ_p increased 100-fold, while in the denervated one the elevation of ΔZ_p was 50-fold. It is worthy to stress that despite such spectacular rise of the swing range of passive pulsatile oscillations, the changes in total (mean) electroimpedance Z during such pulsations did not surpass 1%, which corresponded to variations of arterial diameter of merely 0.5%. Virtually, such small oscillations cannot affect the hydroimpedance of a major artery and overall blood flow in it, but it should be taken into consideration, that they can possibly affect the near-wall flow of the blood and arterial glycocalyx [11]. The significant difference in ΔZ_p between both femoral arteries in unilaterally denervated rats attests to the fact that denervation of a rather large region in the organism provoked both local and systemic vascular reactions. Importantly, the increment of the swing range of passive pulsatile oscillations was greater in the leg with intact innervation than in the denervated one. This fact favors the view that the revealed systemic angiotropic reaction could include a local neurogenic component. Exclusively high swing

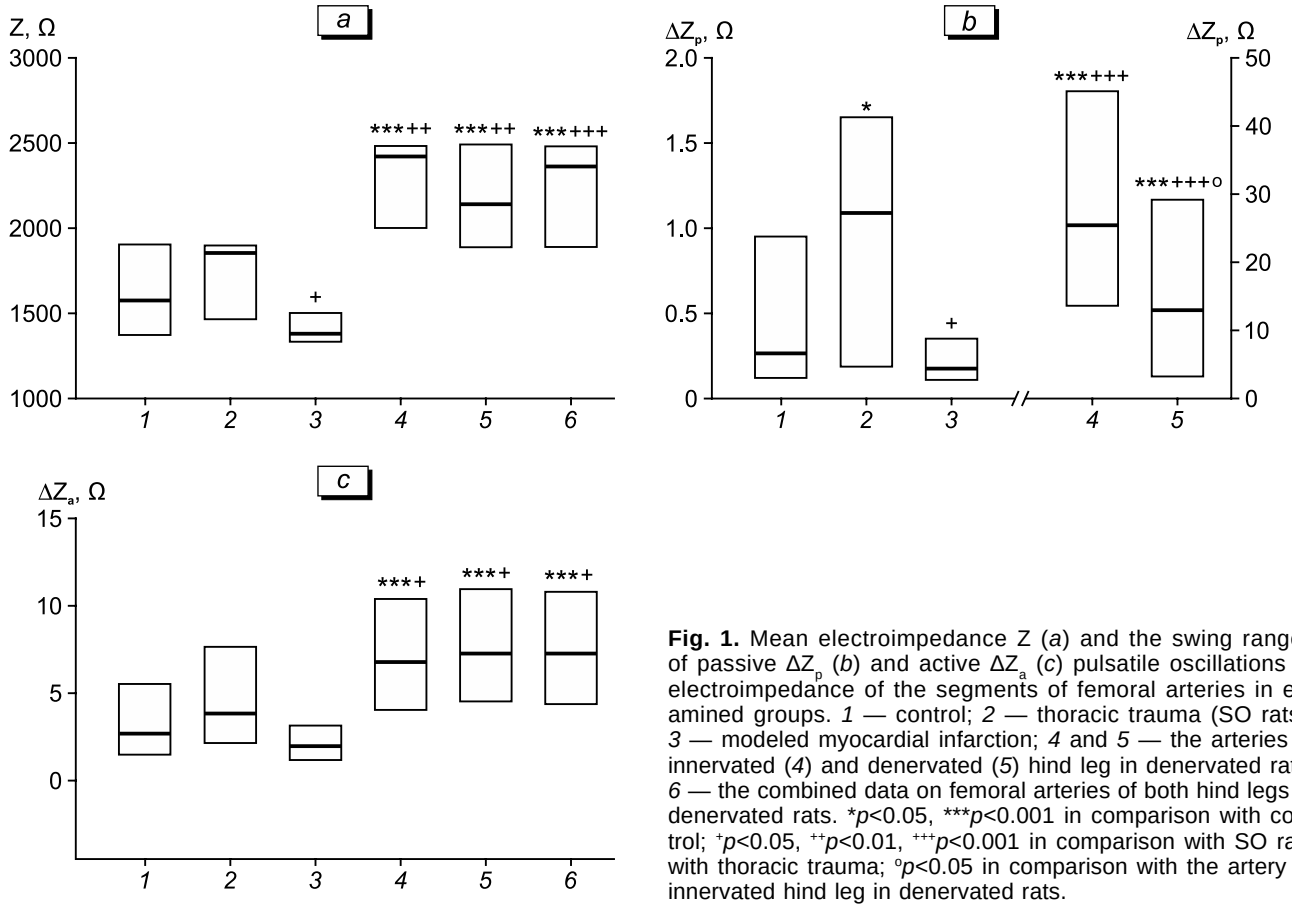

Fig. 1. Mean electroimpedance *Z* (*a*) and the swing ranges of passive ΔZ_p (*b*) and active ΔZ_a (*c*) pulsatile oscillations of electroimpedance of the segments of femoral arteries in examined groups. *1* — control; *2* — thoracic trauma (SO rats); *3* — modeled myocardial infarction; *4* and *5* — the arteries in innervated (*4*) and denervated (*5*) hind leg in denervated rats; *6* — the combined data on femoral arteries of both hind legs in denervated rats. **p*<0.05, ****p*<0.001 in comparison with control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 in comparison with SO rats with thoracic trauma; ^{o}p<0.05 in comparison with the artery of innervated hind leg in denervated rats.

range of passive pulsatile oscillations in the arteries of denervated animals can be an important cue in understanding the peculiar features in the state of blood vessels during neurogenic pathologies and the organismal response to neurotropic influences.

Previously, a positive correlation between ΔZ_p and ΔZ_a in femoral arteries of healthy rats was established [10]. Here, it was expected that the changes in ΔZ_p would be accompanied with the parallel ones of ΔZ_a. Overall, the experiments corroborated this assumption (Fig. 1, *b*, *c*; Tables 2, 3). However, elevation of ΔZ_a in denervated rats was far less pronounced (although significant) than that of ΔZ_p. Probably, this fact attests to certain restrictions in the work of smooth muscles of arterial wall due to energy limitations. It should be noted that neither pseudo-infarction with thoracic trauma, nor chronic modeled myocardial infarction induced significant changes in the swing range of active pulsatile oscillations of arterial electroimpedance, although these changes demonstrated the same trends as observed in the swing range of passive oscillations (Fig. 1, *b*, *c*). It is noteworthy that a weak influence of modeled infarction on RP of rat arteries could be related to the fact that these parameters were determined postsurgery after a rather long time (2-4 months), which needed to match the age and the size of arteries of rat pups to those of control animals. It cannot be excluded that during the acute period after myocardial infarction, the changes of arterial RP were more pronounced.

It is important that ΔZ_a values of both hind legs of unilaterally denervated rats were virtually identical (Fig. 1, *c*). This observation shows that the segmental neural influences play a minor role in determining the swing range of active pulsatile oscillation, which seems to be mostly controlled by the central mechanisms common for the whole organism.

TABLE 2. The Swing Ranges of Passive Pulsatile Oscillations of Electroimpedance ΔZ_p of Rat Femoral Arteries (Me (Q1-Q3))

Experimental conditions	ΔZ_p, mΩ
Control (*n*=39)	250 (110-900)
Thoracic trauma (SO group) (*n*=13)	1080 (180-1540)*
Modeled myocardial infarction (*n*=8)	160 (100-290)+
Artery of innervated leg in denervated rats (*n*=16)	25,280 (14,660-44,800)$^{***+++}$
Artery of innervated leg (*n*=25)	12,800 (3480-27,780)$^{***+++o}$

TABLE 3. The Swing Ranges of Active Pulsatile Oscillations of Electroimpedance ΔZ_a of Rat Femoral Arteries (Me (Q1-Q3))

Experimental conditions	ΔZ_a, mΩ
Control (*n*=39)	2580 (1490-5390)
Thoracic trauma (SO group) (*n*=13)	3900 (2190-7360)
Modeled myocardial infarction (*n*=8)	1900 (1120-3020)
Artery of innervated leg in denervated rats (*n*=15)	6680 (4040-10,310)***+
Artery of innervated leg (*n*=24)	7190 (4500-10,880)***+
Artery of both hind legs in denervated rats (*n*=39)	7180 (4360-10,660)***+

The present data can be useful in understanding the processes in various fields of physiology and medicine related to neurotropic influences. For instance, it cannot be excluded that the therapeutic effectiveness of Vishnevsky procaine blockade of peripheral nerves includes a component associated with systemic vascular reaction. Similar reaction can be expected in response to various types of neurotomy or nerve ablation. Remembering that the first researcher observing in 1941 a pronounced rise of the swing range of pulsatile electroimpedance oscillations of brachial artery in patients with Basedow's disease was Russian physiologist Alexei Kedrov [2], it would be reasonable to name the discussed systemic vascular reaction after him.

One of the challenges to modern medicine is the pandemia provoked by coronavirus SARS-CoV-2. An important achievement related to the present study was a recently established fact of enhanced resistance to this virus in humans with low velocity of the pulse wave in major arteries [13]. It is known that this velocity depends on arterial elasticity, which correlates with the swing range of passive pulsatile oscillations: elevation in ΔZ_p corresponds to a drop in velocity of pulse wave. With due account for the above facts, elevation of the swing range of pulsatile oscillations can strengthen the antiviral defense in arteries. This possibility indicates necessity to study the effects of the pulsatile oscillations on the structure of arterial glycocalyx, which is viewed as a protective barrier against the detrimental influences onto the vascular wall [11]. In addition, a possible effect of the swing range of vascular wall pulsing on arterial thrombosis should be taken into consideration.

Thus, the revealed systemic vascular reaction induced by denervation of an extensive region in the organism puts a number of questions in physiology and medicine. There is no doubt on necessity of further detailed study of this reaction.

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