Effect of Aerobic Training on Innervation Density and Neurogenic Responses of Skin Afferent Blood Vessels

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We studied the effect of 8-week aerobic training (treadmill running) on neurogenic responses and density of sympathetic innervation of subcutaneous artery in rats. In trained rats, the artery response to stimulation of intramural sympathetic nerve decreased, but the sensitivity of vascular smooth muscles to norepinephrine was not changed. The density of adrenergic nerve fibers in the arterial wall after training was also lower than in the control group. This effect of training can be explained by the need in increased heat emission during physical activity.

Key Words: *aerobic training; subcutaneous artery; sympathetic innervations; rats*

Regulation of blood flow in the skin is closely related to body temperature control. Body temperature elevation is followed by an increase in cutaneous blood flow primarily due to reduction of vasoconstrictor influences of sympathetic nerves. Aerobic training leads to activation of oxidative phosphorylation in muscular fibers (MF) along with elevation of heat production in the body. Thus, activity of heat emission mechanisms should also increase. It was demonstrated that blood flow in the skin of trained individuals at the same core temperature is higher than in untrained subjects [4]. This phenomenon can be explained by reduction of basal efferent sympathetic activity [6,8]. Moreover, attenuation of nervous influence on blood vessels could be associated with changes in peripheral mechanisms of sympathetic control, but this hypothesis was not experimentally proved.

In contrast to humans, whose heat exchange with the environment is mediated by whole body skin, in rats only skin regions not covered with fur serve as heat exchange organs. They include the tail, ears, and distal parts of the limbs. In rats, subcutaneous artery (*a. saphena*) is located on the shank and brings blood to the foot. This artery is an adequate model for investigation of nervous regulation of skin blood supply, because cutaneous blood flow is regulated by changes in the vascular tone of relatively large vessels [9].

Here we studied the effect of aerobic training on neurogenic responses and density of sympathetic innervations of subcutaneous artery in rats.

MATERIALS AND METHODS

Experiments were performed on 1.5-2 month-old male Wistar rats (age at the beginning of the experiment).

Training was conducted on Exer-4 treadmill (Columbus instruments) 6 days per week for 8 weeks. The load was gradually increased and by the end of the 4th week trained rats (n=9) ran 60 min per day at 25 m/min tape speed and 10° slope. In order to maintain running experience, the animals from the control group (n=7) ran along a horizontal path 2 times per week for 10 min at 10 m/min tape speed.

Level of training was estimated by the index of maximum oxygen consumption (MOC) and activity of SDH, oxidative pathway marker in energy metabolism, in MF from the central part of gastrocnemius muscle. MOC was measured using an Oxymax colorimeter

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(Columbus instruments) at the start and end of the training cycle during running in a closed treadmill.

For evaluation of plastic changes in muscular tissue, 12-14-μ serial cross-sections of muscular fibers were prepared from frozen (-25°C) specimens on a microtome (Leica). Slow and fast MF were identified using NCL-MHCf (a+b) and NCL-MHCs antibodies (Novocastra Laboratories) and FITC-labeled secondary antibodies (Jackson Immunochemicals). SDH activity was detected by the tetrazolium method.

Section visualization was done using an Axiovert-200 microscope with ×20 objective and AxioCam HiRes digital camera (resolution 1300×1030 pxls, 8 bit/pxl). Photographs of the same fragments on sections were taken first in UV light using an N10 filter (450-490 nm; for identification of MF types) and then in the light of halogen lamp (to estimate SDH activity). Image processing was performed using WCIF ImageJ software. No less than 100 MF of each type were analyzed for each specimen.

After completion of the training cycle, the rats were intraperitoneally anesthetized with nembutal (40 mg/kg) and then decapitated. Segments of the subcutaneous artery (10-13-mm long, near the site of separation of the subcutaneous artery from the femoral artery) were isolated on both hindlimbs. One segment was used for investigation of vasomotor responses under conditions of constant flow rate perfusion. To this end, the segment was cannulated at both ends and placed into a thermostatically controlled camera (37°C) with Krebs—Henseleit solution containing in (mM): 119 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO4, 1.17 $MgSO_4 \times 7H_2O$, 0.026 EDTA, 5.5 D-glucose, and 2.5 $CaCl_2 \times 2H_2O$. The solution was aerated with a mixture of 95% O_2+5% CO_2 in order to maintain pH 7.3-7.4. Constant flow through the vessel (2 ml/min) was provided by a peristaltic pump (LKB), pressure before the cannula was constantly measured with DDA-2 detector. Flow stability was controlled using an ultrasonic flow meter (Transonic System Inc., T106, detector 1N).

For stimulation of intramural nervous fibers with electrostatic field, electric impulses with alternating polarity (200 mA, 0.2 mses, 5-20 Hz impulse frequency) were delivered to platinum electrodes placed at both sides of the vessel segment. Vessel response to stimulation was fully blocked with tetrodotoxin (3×10^{-6} M), *i.e*. the constriction was neurogenic. Nervous fibers were stimulated for 60 sec with 4-min intervals. Sensitivity of smooth muscle of the vessel to sympathetic mediator norepinephrine was also investigated. To this end, norepinephrine in various concentrations was added to the perfusion solution. In all experimental series, the effect of norepinephrine on β-adrenoreceptors was blocked with propranolol (10^{-6} M).

The data were recorded and processed using original software on an IBM PC computer using 16-bit analog-to-digital converter (L-Card, digitalization frequency 10 Hz). The vasoconstriction response was estimated by the increase in perfusion pressure, which is proportional to the increase in flow resistance in the blood vessel under condition of constant flow.

Another fragment of the subcutaneous artery was used for investigation of adrenergic nervous fibers. The vascular segment was placed into 0.1 M PBS (pH 7.2) containing glyoxalic acid (2%), sucrose (10%) and pontamine sky-blue (0.03%). After 30-min incubation, the segment was spread on a slide, dried for 30 min with hot air (5 min at 100°C), embedded into mineral oil, and covered with a coverslip. Investigation was performed using the same microscope, but at higher magnification (×40) and using S25 filter (excitation wavelength 380-440 nm, the studied luminescence wavelength 440-480 nm). The density of nervous fibers was evaluated by counting their intersections with a grid laid over the specimen microphotographs. The grid consisted of 40 markers uniformly distributed in the field of view (326×258 μ). For evaluation of the intensity of fluorescence (IF), the images were processed using WCIF ImageJ software. Total IF was measured in each field of view and normalized to the background IF level (in areas between MF). Calculation was performed in 10 areas for each specimen.

All pharmacological agents were purchased from Sigma.

Statistical analysis was performed using Mann—Whitney test.

RESULTS

Initially body weight in the two experimental groups was similar, but by the end of the experiment it was lower in trained animals than in untrained rats (Table 1). Initial MOC level did not differ either. During the experiment, MOC index increased in both groups, which reflects age-related changes. In trained animals MOC value was by 15% higher than in control group by the end of the experiment (Table 1). Moreover, training significantly (16%) increased SDH activity in fast and slow MF. These changes confirm the efficiency of the chosen aerobic training regimen.

In the absence of constrictor influences, perfusion pressure was 20-30 mm Hg in both groups, *i.e*. the anatomical lumen of the subcutaneous artery was not altered by training. Stimulation of sympathetic nerves led to rapid increase in perfusion pressure (Fig. 1, *a*). In trained animals neurogenic responses were by 43% lower than in control group at stimulation frequency of 20 Hz (Fig. 1, *b*). Since the response to exogenous norepinephrine did not differ in the control and ex-

TABLE 1. Effect of Aerobic Training on Body Weight, SDH Activity in Slow and Fast MF, and on MOC in Rats (*M*±*m*)

Parameter	Control (*n*=7)	Training (*n*=9)
Body weight, g		
start of the experiment	215.3±18.8	226.4±20.7
end of the experiment	440.8±11.3^{+}	395.2±8.1^{*+}
MOC, ml/kg×min		
start of the experiment	60.2±0.6	61.1±0.3
end of the experiment	75.9±0.4^{+}	87.2±0.5^{*+}
Content of slow MF in gastrocnemius muscle, %	17.3±4.1	21.9±3.8
SDH activity, arb. units		
in slow MF	0.51±0.03	0.59±0.02*
in fast MF	0.46±0.02	0.53±0.02*

Note. $p<0.05$ compared to: *control, $^{+}$start of the experiment.

perimental groups (Fig. 1, *c*), it can be expected that the revealed reduction of neurogenic responses was caused by changes in presynaptic mechanisms of sympathetic neurotransmission.

The results of morphological study indicate that decreased artery response to nerve stimulation results from reduced density of periarterial sympathetic nerve fiber plexus. The total IF of nervous plexus (Fig. 2,

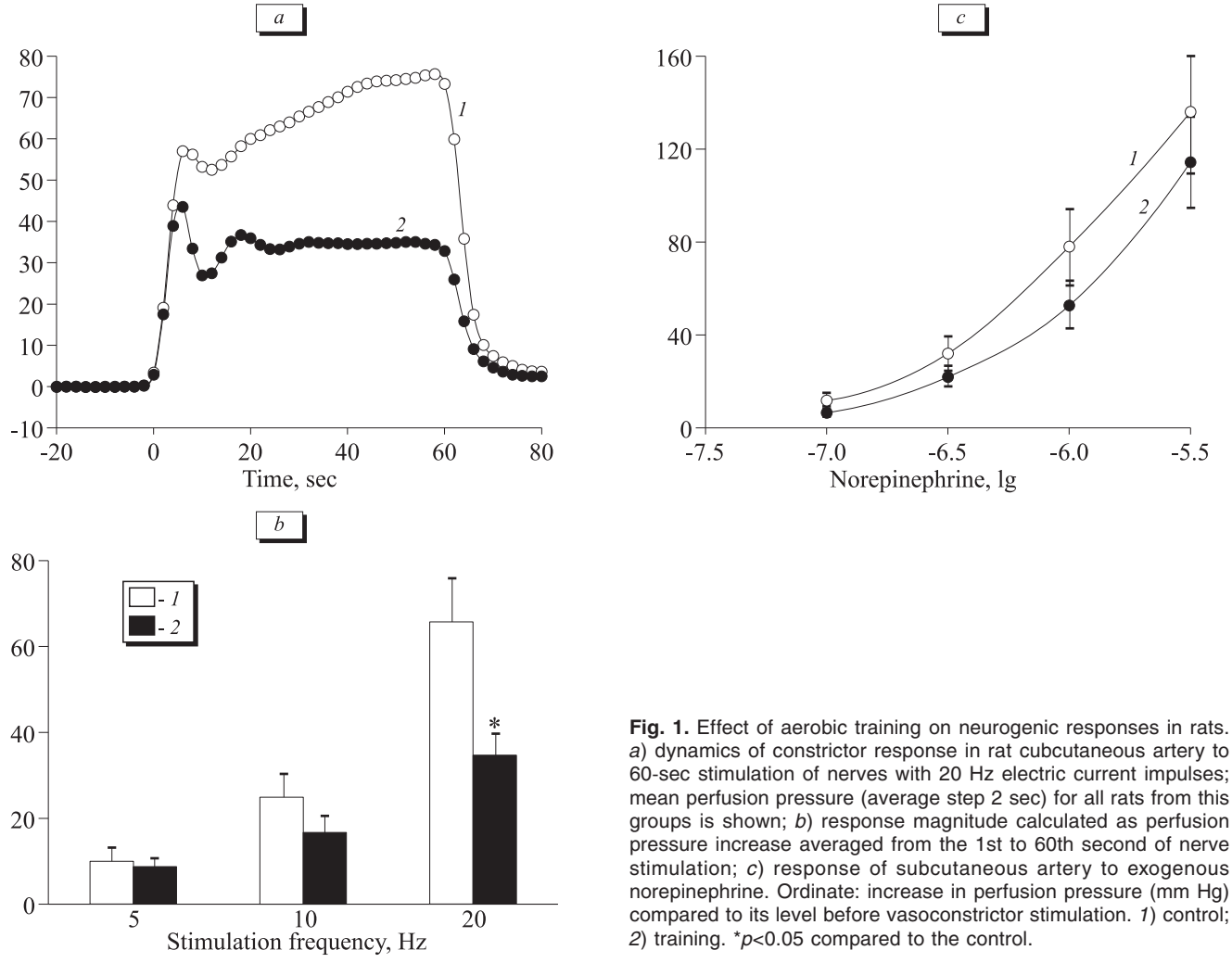

Fig. 1. Effect of aerobic training on neurogenic responses in rats. *a*) dynamics of constrictor response in rat cubcutaneous artery to 60-sec stimulation of nerves with 20 Hz electric current impulses; mean perfusion pressure (average step 2 sec) for all rats from this groups is shown; *b*) response magnitude calculated as perfusion pressure increase averaged from the 1st to 60th second of nerve stimulation; *c*) response of subcutaneous artery to exogenous norepinephrine. Ordinate: increase in perfusion pressure (mm Hg) compared to its level before vasoconstrictor stimulation. *1*) control; *2*) training. *$p<0.05$ compared to the control.

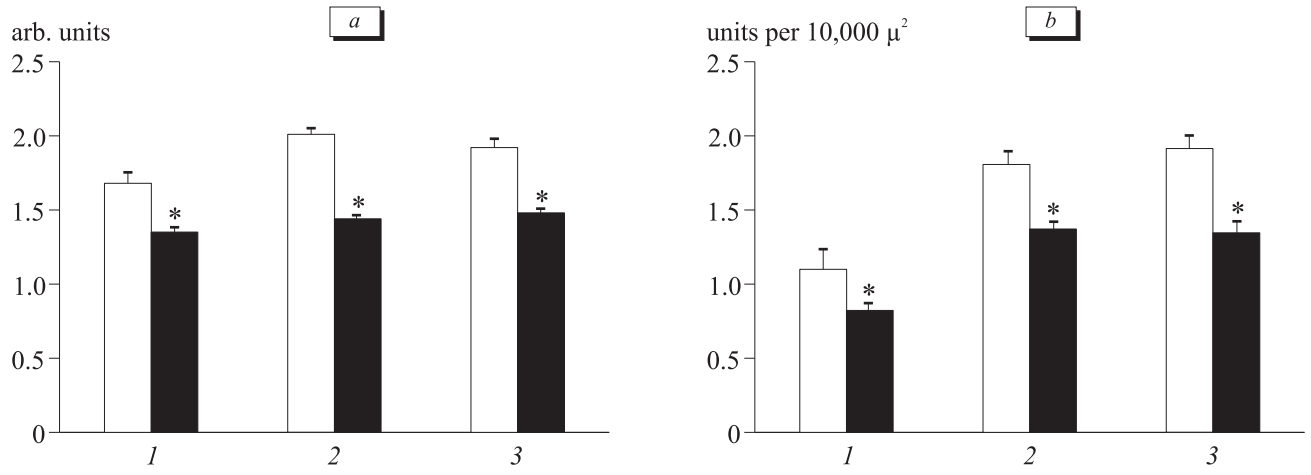

Fig. 2. Properties of adrenergic nerve fiber plexus in the wall of rat subcutaneous artery: total IF (*a*) and plexus density (*b*). *1*) proximal part; *2*) medial part; *3*) distal part. Light bars: control; dark bars: training. **p*<0.05 compared to the control.

a) and innervations density (Fig. 2, *b*) in trained rats were reduced to the same extent as compared to the control. Due to such coherence of changes, specific IF (ratio of total IF to nervous plexus density) is similar in control and trained animals, *i.e*. the content of the transmitter (norepinephrine) in nerve fibers was not altered by training.

These data suggest that aerobic training reduces innervations density in rat subcutaneous artery and decreases nervous responses of this artery. This together with reduced efferent sympathetic activity should lead to reduction of the neurogenic tone of skin blood vessels, increase in blood flow in the skin, and consequently, to an increase in heat emission.

Despite the functional role of the observed phenomenon is obvious, the mechanisms of its development remain unclear. It is known that nerve plexus in vascular wall can undergo reorganization not only in case of pathology, but also in different physiological processes: pregnancy [7], chronic decrease of blood pressure [1], and ageing [10]. These processes are controlled by growth factors secreted by the target organ; nerve growth factor is one of the most important among them. Rearrangement of vascular innervation usually correlates with changes in the expression of this neurotrophin or its receptors [7].

We found no reports about the effects of training on the production of nerve growth factor. However, it is known that training affects production of various humoral factors which can directly or indirectly modulate the effects of neurotrophin. For instance, endothelin directly promotes the growth of postganglionar sympathetic fibers and potentiates the effects of nerve growth factor [2,3]. It is known that aerobic training markedly reduced endothelin secretion [5]; hence, restriction of its neurotropic effect can lead to reduction of nervous plexus density in blood vessels of the skin.

Results of this study suggest that changes in the peripheral mechanisms of sympathetic control could be a mechanism responsible for maintenance of constant body temperature in people adapted to long-term work with aerobic metabolic supply.

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