RELATIVE VOLUME OF OPEN CAPILLARIES IN RAT SKELETAL MUSCLES IN RATS WITH CHRONIC LOCAL ARTERIAL HYPOTENSION

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KEY WORDS: skeletal muscles; chronic arterial hypotension; capillaries.

It has been shown that a fall of arterial pressure (BP) in the vessels of the hind part of the body in rats for 14-90 days causes a marked decrease in the hydraulic resistance of the resistive vessels of that region [7, 8]. One cause of the decrease in hydraulic resistance in the region of chronic arterial hypotension is an increase in the lumen of the vessels due to a substantial decrease in thickness of the wall of vessels of all calibers [4, 5]. However, the possibility likewise cannot be ruled out that the decrease in hydraulic resistance in the region of hypotension is due not only to an increase in the lumen of the resistive vessels, but also to an increase in the number of vessels with parallel function. The basis for this hypothesis appeared all the firmer because, as several workers have shown [3, 10, 11], only a fraction of precapillary vessels is open simultaneously in a resting muscle. Nevertheless, a special investigation did not yield data which could indicate that the decrease in hydraulic resistance of the vessels developing during prolonged lowering of BP was connected with any increase in the number of vessels with parallel function [1]. However, the possibility had to be considered that when fresh muscle tissue is removed and fixed with formalin, changes may take place in the state of its microcirculation. Accordingly, a method of determining the relative volume of erythrocytes in skeletal muscle capillaries was developed [2]. This parameter was taken as the measure of the number of functioning capillaries.

In the investigation described below the improved method of determining the relative volume of erythrocytes was used to study the effect of a prolonged fall of BP on the number of functioning vessels in red and white skeletal muscles of the rat limb.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing initially 180-200 g. BP in the vessels of the hind part of the body of the rats was lowered by applying a nichrome coil to the abdominal aorta, distally to the origins of the renal arteries, which constricted the vessel to such a degree that the pressure in the femoral artery was 50-70% of the pressure in the carotid artery. The operation was performed under pentobarbital anesthesia (30 mg/kg).

Animals of three groups were used in the experiments: 5 control rats, 7 rats with arterial hypotension lasting 14 days, and 7 rats with arterial hypotension lasting 3-4 months. The technique of freezing the muscles, preparing the specimens, and staining them was described in detail previously [2]. A special feature of the method was intravital freezing of the organ in situ with liquid propane, cooled with liquid nitrogen, followed by lyophilization of the specimens, their fixation with gaseous formaldehyde, and staining by Pickworth's method [6]. By this method it is possible to detect open (functioning) capillaries because of the dense black staining of the erythrocytes contained in them. The soleus (red) and gastrocnemius (white) muscles of control and experimental rats were studied.

The relative volume of the erythrocytes was determined stereologically by Hauge's method [12]. A 16× objective, 12.5× ocular, and a grid with 440 intersections were used for counting. Only capillaries falling on intersections of the grid were counted. Counting was carried

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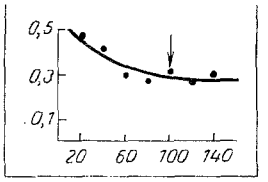

Fig. 1. Dependence of standard error of means (ordinate) on number of fields of vision counted (abscissa).

TABLE 1. Relative Volume of Erythrocytes in Rat Skeletal Muscles

Experimental conditions	Soleus muscle (SM)	Gastrocnemius muscle (GM)	SM/GM
Control	1,15±0,08	0,52±0,04*	2,2
Hypotension for 14 days	1,16±0,08	0,56±0,1*	2,1
Hypotension for 3-4 months	1,13±0,08	0,59±0,12	1,9

Legend. $P < 0.05$.

out in 100 fields of vision, for preliminary experiments showed that the standard error of the mean stabilizes only if 100 fields of vision or more are counted (Fig. 1).

The number of capillaries filled with erythrocytes falling in each field of vision within intersections of the grid was counted, and the values obtained were then added together. Next, having distinguished 9 points in the grid, the number of points falling on muscle fibers was measured. The number of points on which there was no muscle fibers was calculated (in per cent), after which the total number of capillaries was reeuced by an amount corresponding to the volume of nonmuscle tissue.

EXPERIMENTAL RESULTS

The relative volume of erythrocytes in the skeletal muscles obtained by intravital freezing was found to be much lower than when ordinary fixation of the skeletal muscles in formalin was used [1].

Nevertheless, this investigation showed that the relative volume of erythrocytes in the soleus (red) muscle was significantly greater than in the gastrocnemius (white) muscle (Table 1). These data are in agreement both with the results of a previous investigation [1] and with the results of other investigations [9, 13, 14] which showed that the blood flow in the soleus muscle is much greater than in the gastrocnemius muscle.

It will be clear from Table 1 that the relative volume of erythrocytes, reflecting the number of open capillaries, in the soleus and gastrocnemius muscles of rats with local arterial hypotension lasting 14 days and 3-4 months was virtually indistinguishable from the corresponding values in control animals.

The results of this investigation thus confirm the correctness of the previous conclusion that there are no grounds for connecting the decrease in hydraulic resistance developing during prolonged arterial hypotension with an increase in the number of vessels with parallel function.

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DISSECTION OF PERIPHERAL NERVE SHEATHS WITH AN ULTRASONIC MICROSCALPEL WITHOUT IMPAIRING NERVE CONDUCTION

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In neurophysiological research it is often necessary to dissect nerves without disturbing conduction of nervous impulses. An important step in the operation is incision of the nerve sheaths — the outer (epineural) sheath to isolate a separate nerve branch from the main trunk, and the inner (perineural) sheath to isolate microbundles or single nerve fibers [3]. Usually the sheath is incised in the longitudinal direction under the microscope by means of microscissors or, in the case of a thin branch, with a piece of razor blade [4]. Both methods require many micromovements with the cutting edges and they are accompanied by traction on the nerve along the path of the instrument. During operations on nerves *in vivo* a further difficulty is bleeding from the divided epi- and perineural vessels. These complications may lead to injury to nerve fibers. The writers have developed a method of dissecting the epineurium and perineurium by means of an ultrasonic microscalpel (USM) which is free from the defects described above and have determined the level of impairment of conduction of excitation along the nerve caused by the use of this method.

EXPERIMENTAL METHOD

The USM was made on the basis of the UZKh-201 apparatus for ultrasonic surgery, manufactured commercially, operating on a frequency of 44 kHz [1]. For this purpose, a pointed fragment of safety razor blade 8-10 mm long was soldered to the tip of the vibrator. The amplitude of the longitudinal oscillations of the razor blade was selected to be 2-5 μ.

In five cats anesthetized with chloralose and urethane the saphenous nerve in the hind limb was freed from connective tissue for a length of 5-7 cm from the knee to the hip joint. Folds of skin were formed into a well along the nerve which was filled with mineral oil. In the distal part the nerve was placed on stimulating electrodes, and in the proximal part on recording electrodes. Bipolar (differential) derivation was used to record the composite action potential. The signal was amplified by 10^4 times with a PARC (model 113) amplifier and filtered in the 20 Hz—1 kHz band. A segment of nerve 10 mm long was chosen between the recording and stimulating electrodes, where the epi- and perineurium were incised by means of the USM (Fig. 1A).

EXPERIMENTAL RESULTS

The control composite action potential of the nerve is illustrated in Fig. 1B. Waves of the composite action potential, formed by impulses in Aβ and Aδ fibers, were recorded during stimulation of the nerve with square pulses (amplitude 1.0-1.2 V, duration 0.1 msec). Thin unmyelinated C fibers were stimulated by square pulses (amplitude 10-12 V, duration 1 msec). The amplitudes of the impulses were three times the threshold values for excitation of Aδ

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