CHANGES IN VASCULAR PERMEABILITY IN THE MICROCIRCULATORY SYSTEM OF SKELETAL MUSCLES DURING WORKING HYPEREMIA

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UDC 612.135:612.74

Vascular permeability of the microcirculation in the quadriceps femoris and gastrocnemius muscles of albino rats at rest and during working hyperemia was studied by intravital luminescence microscopy. Bovine globulin labeled with fluorescein (FITC) was used as the indicator of permeability. Quantitative luminescence analysis was carried out with a microspectrofluorometer. During working hyperemia the FITC concentration in the muscle was 40-45% higher than at rest, evidence of an increase in transendothelial transport of materials. Experiments to measure the FITC concentration around a single capillary gave results suggesting that under the conditions of working hyperemia the permeability of the vessels of the microcirculation is increased.

KEY WORDS: permeability; microcirculation; working hyperemia; luminescence biomicroscopy.

Among the factors controlling transendothelial transport of materials, one of the most important is the permeability of the vascular wall [7, 13]. Meanwhile the state of vascular permeability of the microcirculatory system during functional activity of an organ, accompanied by intensification of the blood flow through the organ, remains virtually unstudied.

Experiments on rats and frogs have shown [2] that during fatigue caused by prolonged swimming, vascular permeability in the muscles of T-1824 is increased. It has also been shown [3] that vasomotor and rheological responses are not always accompanied by changes in permeability. During electron-microscopic investigations [1] direct stimulation of the diaphragm was found to be accompanied by activation of pinocytosis which, in the modern view

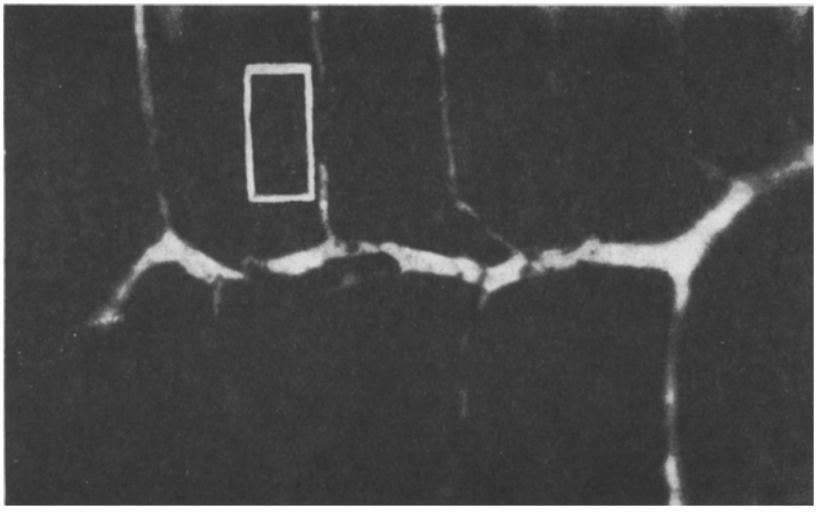

Fig. 1. Fragment of microcirculatory system of quadriceps femoris muscle of albino rat after intravenous injection of FITC (intravital photomicrograph, objective 20, ocular 10). Rectangle indicates location of spectrofluorometer probe.

Research Institute of Child and Adolescent Physiology, Academy of Pedagogic Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 369-371, April, 1979. Original article submitted April 11, 1978.

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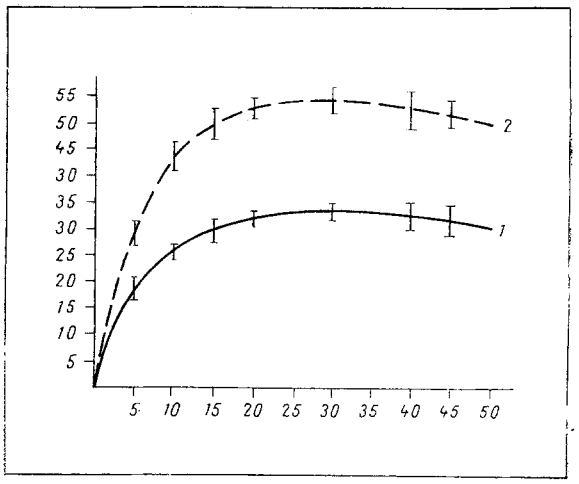

Fig. 2

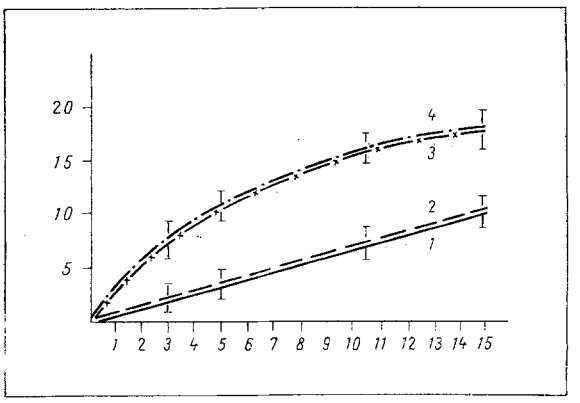

Fig. 3

Fig. 2. Dynamics of changes in FITC concentration in quadriceps muscle at rest (curve 1) and during working hyperemia (curve 2). Here and in Fig. 3: abscissa, time (in min); ordinate, FITC concentration (in luminescence units).

Fig. 3. Dynamics of changes in FITC concentration near a single capillary in gastrocnemius and quadriceps femoris muscles of albino rat at rest and during working hyperemia. 1) Quadriceps femoris muscle at rest; 2) gastrocnemius muscle at rest; 3) quadriceps femoris muscle during working hyperemia; 4) gastrocnemius muscle during working hyperemia.

[10-12, 14, 15], is one mechanism of transport of materials. The results of these investigations suggest that a change in vascular permeability is one of the mechanisms responsible for the higher level of metabolism in skeletal muscles during their contraction.

The object of the present investigation was to assess by means of intravital luminescence microscopy the state of vascular permeability in the microcirculatory system in skeletal muscles at rest and during working hyperemia.

EXPERIMENTAL METHOD

Experiments were carried out on 50 albino rats weighing 150-180 g. A model of working hyperemia was created by stimulation of the divided femoral nerve (for investigation of the quadriceps femoris muscle) or sciatic nerve (for investigation of the gastrocnemius muscle) with square pulses of a frequency of 5 Hz and a duration of 0.2 msec for 5 min. According to some observations [6, 8], the strength of stimulation with pulses of the chosen duration was sufficient to excite motor fibers but did not exceed the threshold of excitation of vasomotor nerves.

Intravital microscopy of the quadriceps and gastrocnemius muscles was carried out in incident light by means of the ML-4 or LUMAM-P3 luminescence microscope. Under pentobarbital anesthesia the above-mentioned muscles were exposed surgically and the surface of the muscles irrigated with warm Ringer's solution. To abolish the direct effect of operative trauma on the microcirculation, the rats were given a preliminary injection of diphenhydramine hydrochloride in a dose of 5 mg/100 g body weight. The study of vascular permeability began not less than 20-25 min after preparation of the animals.

As an indicator of vascular permeability, bovine globulin labeled with fluorescein isothiocyanate (FITC) was injected intravenously in a dose of 0.5 ml/100 g body weight.

Quantitative luminescence analysis was carried out on a microspectrofluorometer [4], the design of which was based on the ML-4 microscope and included a type UM-2 monochromator with automatic drive, giving linear scanning of the spectrum on a PDS-021M automatic x-y writer; the outlet slit of the monochromator was focussed on the input of an FÉU-68 photoelectric multiplier. The dimensions of the probe varied from 20×40 to 60×80 μ, so that both the total concentration of FITC in the muscle and its concentration around individual arterioles, capillaries, and postcapillaries could be estimated (Fig. 1).

EXPERIMENTAL RESULTS

Under resting conditions after intravenous injection of FITC its concentration in the muscle began to rise gradually and reached a maximum after 25-30 min (Fig. 2). The FITC concentration remained at the same level for 10-12 min and then began to fall slowly.

During postcontraction hyperemia the FITC concentrations in the muscle tissue rose more rapidly. After only 5 min the FITC concentration in the muscle during working hyperemia was significantly higher than at rest, and after 25 min the difference amounted to 40-45% (Fig. 2). These results indicate that under conditions of working hyperemia the transendothelial transport of materials was increased. The increase in the FITC concentration of the muscle during working hyperemia could be explained, on the one hand, by an increase in the number of functioning capillaries observed during hyperemia, and on the other hand by changes in vascular permeability itself. To differentiate between these two possible mechanisms of the increase in transendothelial transport of materials, a special series of experiments was carried out in which the FITC concentration was measured near one capillary by the use of a spectrofluorometric probe of small area. Since the measurements were made in the surface layer of the quadriceps muscle, which consists mainly of white muscle fibers, and in the gastrocnemius muscle, which is a mixed muscle in a composition of its fibers, the permeability of capillaries in different types of muscles could be compared.

Analysis of the FITC concentration in the quadriceps and gastrocnemius muscles at rest showed that the concentration of labeled protein near a single capillary was on average the same in the two muscles. During working hyperemia the FITC concentration near a single capillary increased more rapidly than in the resting state. According to visual observations the velocity of the blood flow in the capillaries, which was increased during working hyperemia, returned to its original level about 3 min after the end of stimulation. Since an increase in the FITC concentration took place even after the velocity of the blood flow had returned to its original level (Fig. 3), this suggests that intensification of the transendothelial transport of materials during working hyperemia is associated with changes in vascular permeability proper.

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