STRUCTURE AND SOME FUNCTIONAL PROPERTIES OF THE ARTERIAL SYSTEM OF THE FROG SUBMAXILLARY MUSCLE AS AN OBJECT FOR THE STUDY OF WORKING HYPEREMIA

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KEY WORDS: architectonics of intramuscular arteries; smooth-muscle cells; vasomotor activity; working hyperemia.

Changes in the blood supply of a skeletal muscle (including working hyperemia) are usually judged by changes in its volume blood flow. However, the total blood flow of an organ reflects only the integral changes in the lumen of all blood vessels within the organ. Meanwhile arteries of skeletal muscles constitute a complex system, the separate parts of which may react differently. The necessity for knowledge of the particular features of the system as a whole and of each of its separate parts is evident.

The object of this investigation was to study the architectonics of the arterial system and and the structure of the walls of arteries of different caliber in the frog submaxillary muscle, which is a convenient object with which to study working hyperemia of skeletal muscles. Besides the morphological investigation, intravital observations also were made on the arterial vessels of this muscle at rest and during contraction.

EXPERIMENTAL METHOD

The submaxillary muscles were exposed in frogs *(R. temporaria)*, anesthetized with viadril (3.7 mg, intravenously). The pressure in the dorsal aorta was measured with an electromanometer and recorded on the KSP-4 potentiometer. Branches of both trigeminal and facial nerves innervating this muscle were divided and the peripheral ends of branches of the trigeminal nerves were placed on unipolar silver electrodes for stimulation (stimulus duration 10 μsec, frequency 4 Hz, amplitude 3 times the threshold level for muscular contraction). Observation on the state of the intramuscular arteries and photographic recording were carried out under the MBI-6 microscope in transmitted light from an ISSh-100 flash lamp. During contraction of the muscle, the method of stroboscopic illumination was used [3]. To study the architectonics of the vascular system a suspension of lead carbonate in 5% gelatin solution was injected by Arutyunov's method [1] through the great cutaneous artery [1]. The required artery or vein was found for light and electron microscopy intravitally under the MBS-2 microscope, the order of branching to which it belonged was determined, and its diameter was measured. Next, fixative (2% paraformaldehyde solution in phosphate buffer, pH 7.6) for light microscopy or Karnovsky's fixative for electron microscopy was applied to the region chosen for testing. Pieces of tissue were excised, kept for 2 h in the corresponding fixative, then washed and postfixed for electron microscopy in 1% OsO_4. Material for light microscopy was glued with chick albumin with the wide part of the muscle to a piece of Whatman's filter paper, so that after embedding in paraffin wax, a series of sections parallel to the flat part of the muscle could be cut. These sections were stained with hematoxylin and eosin, by Van Gieson's method, and for elastic fibers with counterstaining by Van Gieson's method. Material for electron microscopy was embedded in Araldite. Ultrathin sections were studied in the JEM-7A microscope.

EXPERIMENTAL RESULTS

Each of the two submaxillary muscles is supplied with arterial blood by the external mandibular artery and the internal mandibular artery which anastomoses with it. An intramuscular

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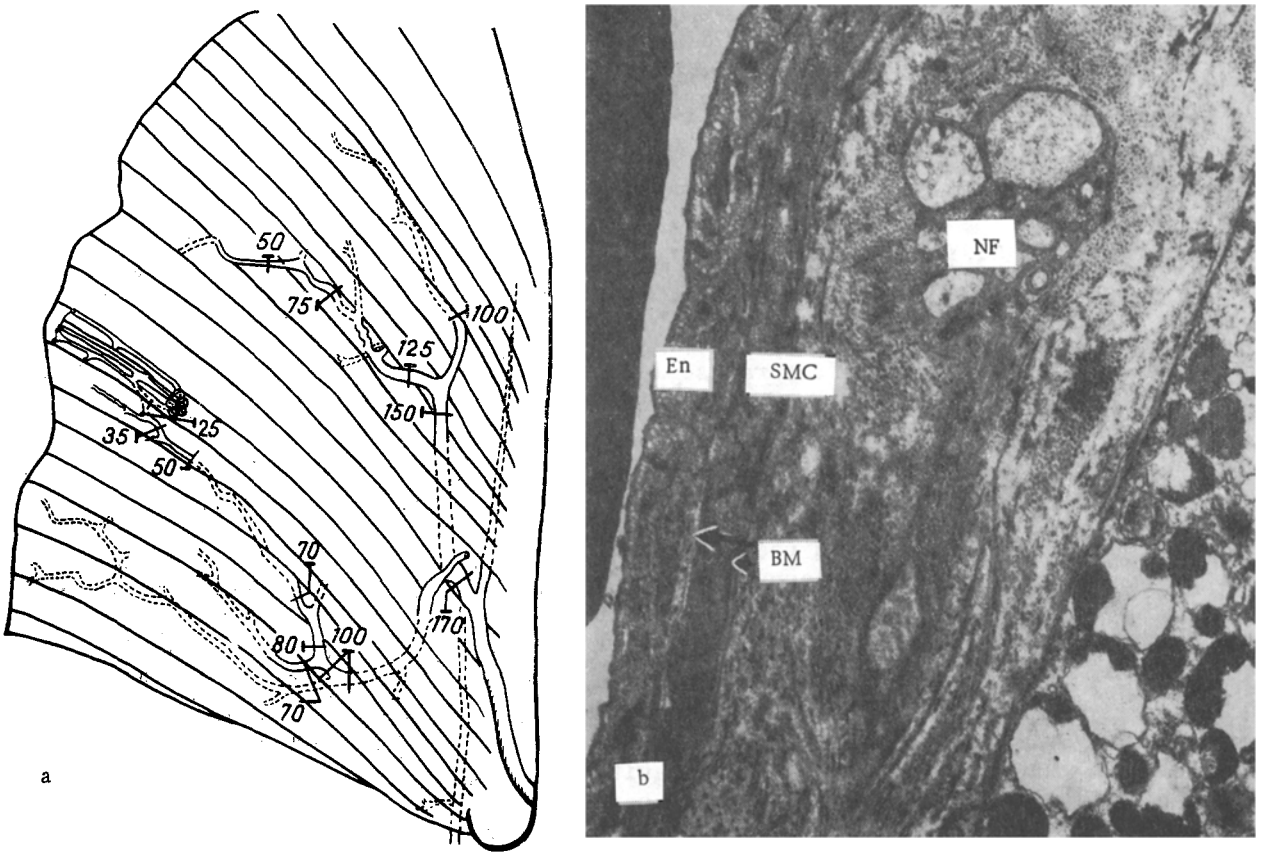

Fig. 1. Scheme of branching of arteries of frog submaxillary muscle (a) and ultrastructure of wall of an artery 100 μ in diameter (b). a) Scheme based on drawing of submaxillary muscle preparation in which arteries were filled with injection material. Continuous lines — direction of bundles of cross-striated fibers. Vessels indicated by broken lines located in thickness of muscle tissue, those indicated by continuous lines are superficial. Numbers show approximate diameter of vessels: vessels of the I order 170 μ, II order 150-100 μ, III order 125-100 μ, IV order 80-50 μ, and V order 35-25 μ; b) endotheliocytes (En) with numerous vesicles have large dense granules of chromaffin type. Smooth-muscle cells (SMC) form one layer, and nerve fibers (NF) are clearly visible in the adventitia. BM) Basement membrane.

artery of the largest caliber (about 170 μ) was counted as an artery of the I order. Daughter branches of this artery, of about equal diameter, arising by its dichotomous branching, were regarded as vessels of the II order, and so on. Five orders of arteries ranging from 170 to 24 μ in diameter could be distinguished in a preparation of the submaxillary muscle in which the arteries were filled with injection material (Fig. 1a). Arteries from 8 to 12 μ in diameter could be identified only in muscle sections under the microscope. Depending on the microstructure of their wall they were classed as vessels of the VI or VII order of branching.

The wall of the intramuscular arteries consisted of three membranes. The inner membrane of arteries of the I-III order (170-100 μ) was formed by endothelium and a subendothelial layer, consisting of ground substance and elastic and collagen fibers. Endothelial cells could form valve-shaped projections into the lumen of the vessel. Junctions between endothelial cells as a rule were of the tight or gap type, with single zones of obliteration (zonula occludens). The endotheliocytes contained many pinocytotic vesicles, single dense bodies of chromaffin type, and groups of filaments of intermediate type, most of which were oriented along the artery although individual bundles of filaments could also be circular in arrangement. These skeletal formations also were found in endotheliocytes of arteries of smaller caliber. A thin inner elastic membrane was clearly visible in arteries of the I-III order. The muscular membrane of the initial part of an intramuscular artery of the I order consisted of smooth-muscle cells (SMC), arranged in two layers. Further along the course of the same artery SMC began to be arranged in two rows. In this case a cross section of the vessel wall

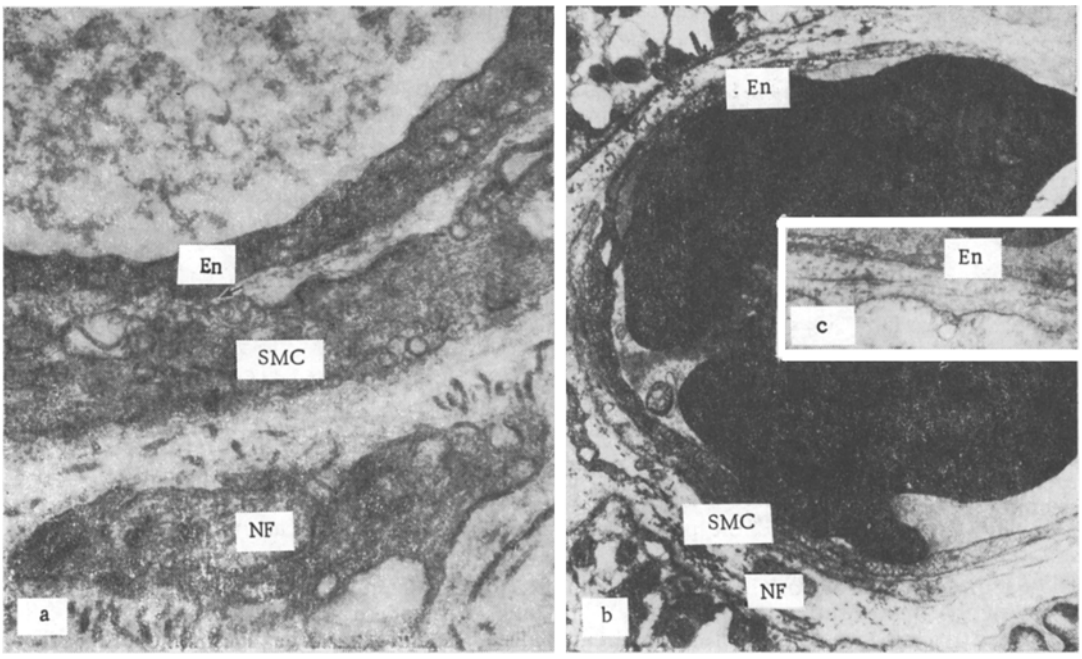

Fig. 2. Ultrastructure of wall of arteries of different caliber. a) Artery 24 μ in diameter. Arrow shows tight junction between endotheliocyte and SMC. 20,000 ×; b) artery 12 μ in diameter. Artery shows distance between endotheliocyte and striated muscle fiber amounting to 1 μ. 5000 ×; c) capillary wall. 7000 ×. Remainder of legend as to Fig. 1.

revealed the main part of one SMC, covered by the terminal (tapering) part of another SMC. The other intramuscular arteries were characterized by one layer of myocytes, circular in their arrangement (Fig. 1b; Fig 2a). The exceptions were bifurcations of arteries of the I-V order (170-30 μ), where the SMC also lay in several layers, evidently forming something like a crest. It must be pointed out that these areas had a particularly well developed innervation. The SMC were in contact with each other both with their ends and with their sides; in the zone of a tight gap junction there were many vesicles. Special note must be made of the presence of a common basement membrane between contacting SMC in arteries of the II-III order (Fig. 1b).

Pigmented cells were clearly visible in the outer membrane of an artery of the I order. Sometimes they were arranged along the course of arteries penetrating into the depth of the muscle. Besides ground substance and obliquely oriented collagen fibers, unmyelinated nerve fibrils sometimes with vesiculated axons, large vesicles (10 nm) with a dense core, and fusiform connective-tissue cells also were found in the outer membrane of arteries of the I-III order.

The inner membrane of vessels of the IV-VII order (80-8 μ) was characterized by a thin subendothelial layer, consisting of ground substance and single collagen fibers. In places where this layer was much thinner, myoendothelial junctions were formed (Fig. 2a).

The SMC of these vessels appeared to be less well differentiated. In particular, they had no well defined basement membrane, nor were thick filaments and dense bodies developed in them. Meanwhile bundles of thin filaments and intracellular vesicles were well represented. The fact must be emphasized that in nearly every muscle cell a single nerve fiber could be found, often with vesicular expansion of the axon, and large vesicles with a dense core (Fig. 2a, b).

SMC in vessels of the VI-VII order were arranged at a small angle to the axis of the vessel; their nuclei appeared to vary: Denser and narrower nuclei alternated with paler and larger. In vessels 12-8 μ in diameter SMC consisted of single cells arranged in a spiral form. The length of the SMC, measured on cells cut through longitudinally in the section, was 60 μ or more. It can accordingly be concluded that the SMC must make 1.5-2 turns around a vessel 12 μ in diameter. In vessels of this caliber the distance between an endotheliocyte and the plasmalemma of a striated fiber was short (about 1 μ), whereas in the largest arteries it was 5-8 μ.

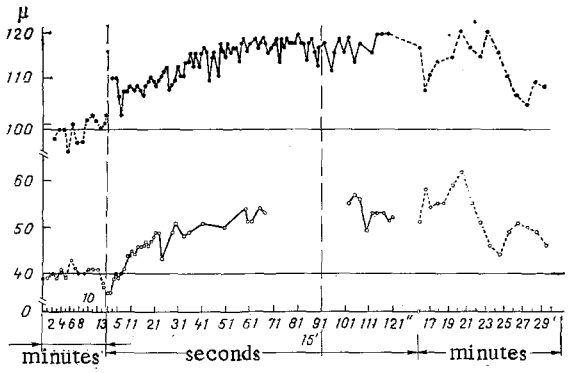

Fig. 3. Changes in diameter of arteries of different caliber before, during, and after muscle contraction. Horizontal lines indicate mean value of background diameter of vessels. Vertical broken lines indicate boundaries of period of muscle contraction. Each point on graph represents diameter of corresponding vessel measured on photomicrographs obtained with single flashes.

Separate subendothelial cells with fusiform (but not branched, as in the case of pericytes) shape and with filaments in the cytoplasm, were seen in the arteriolar part of the capillaries.

The wall of the venular part of the capillaries contained endothelium and a subendothelial layer (Fig. 2c). The distance between the plasmalemma of the striated-muscle cell and endotheliocyte was 1-0.8 μ.

Venules and small veins had a wide lumen (40-50 μ) and, in the structure of their wall, they were similar to arteries of the VII order. In particular, SMC of these vessels do not form a continuous layer.

In the resting decentralized submaxillary muscle arteries of all levels spontaneously change their lumen [2]. Vasomotion was irregular. The diameter of these vessels in the dilator phase could increase by 90% of its value in the maximally constricted state. The duration of the latter exceeded the duration of the dilator phase. During constrictor phases the blood flow in some vessels could stop.

Contraction of the submaxillary muscles evoked by simultaneous stimulation of both branches of the trigeminal nerve led to appreciable dilatation of arteries of the III-VI order, and the blood flow in them increased. The degree of increase in diameter was 12-118% of the mean diameter of a given vessel measured 12-30 min before the beginning of contraction. The increase in diameter during contraction was invariably greater than the magnitude of spontaneous dilatations at rest. The magnitudes of the dilator responses were inversely proportional to the average background diameter of the vessels.

The latent period of responses of arteries of the III-VI order was short, about 5 sec. Meanwhile the time course of dilatation of different vessels, and even of different but nearby regions of the same vessels differed (Fig. 3). At the end of muscle contraction the lumen of the arteries was reduced for 3-12 min, after which a second period of vasodilatation could take place.

A characteristic feature of virtually all arterial vessels of the frog submaxillary muscle is thus the presence of only one layer of SMC, and the SMC in most vessels (80-8 μ) appear to be flattened. We found no structures similar to those found in the retrolingual membrane of the frog [4], to which the function of regulation of the blood flow through single capillaries could be ascribed, namely precapillary sphincters. Their function in the submaxillary muscle may perhaps be performed by arterioles, as has been postulated also for *m. tenuissimus* in the cat [5]. Meanwhile the anatomical structure of the points of division of the large and medium-sized arteries indicates that activation of these regions, which have a double layer of SMC, can sharply restrict or completely interrupt the blood flow through considerable areas of muscle without contraction of distal arterioles. Arterial vessels of all calibers in the rest-

ing acutely decentralized muscle are in a state of visible contraction, evidence of their considerable myogenic tone. In fact, during contraction of the muscle with a frequency of 4 Hz the diameter of its arteries may be doubled. We found no differences in the latent period of responses of arteries of different caliber during muscle contraction, as has been observed for vessels of *m. cremaster* of the hamster [6]. Differences in the time course of responses of different vessels, or even different parts of the same vessel, may depend on their state immediately before contraction, which varies because of the presence of vasomotion.

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EFFECT OF CYSTEINE HYDROCHLORIDE AND SULFATE IONS ON MORPHOLOGICAL CHANGES IN THE LIVER IN CHRONIC YELLOW PHOSPHORUS POISONING

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Yellow phosphorus, which is widely used in the national economy, is a highly toxic substance which may cause poisoning. In phosphorus poisoning the liver is particularly severely damaged. Hence the need for effective measures of treatment of phosphorus poisoning, but despite much research, no pathogenic treatment for this serious disease has yet been devised. The search for effective methods of treatment and prevention of phosphorus poisoning that are safe for long-term use is thus a very important task. Cysteine hydrochloride and sodium sulfate have proved promising in this direction [6, 10].

This paper describes a study of the effect of cysteine hydrochloride and sodium sulfate on morphological changes arising in the liver in experimental chronic yellow phosphorus poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on 163 albino rats of both sexes weighing 150-180 g, of which 30 rats served as the control. Animals of group 1 received yellow phosphorus daily by the intragastric route in the form of a solution in sunflower oil in a dose of 1 mg/kg body weight (1/3 LD_{50}), animals of group 2 received phosphorus in the same dose and cysteine hydrochloride in the form of an aqueous solution in a dose of 50 mg/kg body weight, and animals of group 3 received phosphorus in the same dose and an aqueous solution of sodium sulfate in a dose of 25 mg/kg body weight, calculated as sulfate ion. This quantity of sulfate was taken with the drinking water; the concentration of sulfate ions did not exceed the MAC level (500 mg/liter, State Standard 2874-73 for drinking water), and the quantity of cysteine and of sulfate ions was equivalent in sulfur content. For each group there was a corresponding control,

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