ROLE OF THE ENDOTHELIUM IN DEVELOPMENT OF FUNCTIONAL HYPEREMIA OF SKELETAL MUSCLES

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Functional hyperemia of skeletal muscles is one of the main vascular reactions whose mechanism, despite much research, has not been finally explained [4, 5]. The substances which, in accordance with the metabolic hypothesis, participate in the development of this reaction, have not been finally established. As a rule, the discovery of new endogenous substances with vasodilator action is accompanied by attempts to explain their involvement in the development of working hyperemia. In the last decade the role of the endothelium in vasodilatation has been established [9, 10], for it has been shown that endothelial cells synthesize a smooth muscle relaxation factor, identified as nitric oxide [12]. Endothelial relaxation factor (ERF) can be secreted by endothelial cells when acted upon by mechanical factors [1, 6, 7] and by various metabolic agents [10, 16], which are regarded as possible participants in this reaction. It must be pointed out that vasodilator compounds secreted by endothelium satisfy the demands presented to substances of this type [5] or are closely similar to them in their properties, and as a rule, they can be involved in the development of reactive hyperemia [2, 3].

In accordance with the considerations expressed above, the aim of the present investigation was to study the role of endothelium and of vasodilator substances secreted by its cells in the development of functional hyperemia of the skeletal muscles of the dog limb.

EXPERIMENTAL METHOD

Experiments were carried out on 25 mongrel dogs (14-25 kg) anesthetized intravenously with chloralose and urethane (0.05 and 0.5 g/kg respectively). Working hyperemia was produced in the gastrocnemius muscle. The hind limb was rigidly fixed in the horizontal position. To prevent a collateral blood flow the branches of the femoral artery and vein were ligated throughout the length of the thigh as far as the popliteal fossa, and additionally, wire tourniquets were applied to the thigh muscles. The blood flow in the femoral artery was determined by means of an RKÉ-2-BI electromagnetic blood flowmeter. The pressure in the femoral artery and vein was monitored by means of strain-gauge transducers (746, from "Elema," Sweden). The force of isometric contractions of the gastrocnemius muscle was recorded by connecting the peripheral end of the muscle to a strain-gauge transducer. Contraction of the gastrocnemius muscle was evoked by direct electrical stimulation of the muscle with a frequency of 8 and 40 Hz, a voltage of 20 V, and pulse duration 5 msec; contractions lasting 30 sec were produced by an ÉSL-2 electrostimulator. All the data were recorded on a multichannel Mingograf-82 automatic writer (Siemens-Elema, West Germany and Sweden).

To assess the degree of hyperemia, which depended on the amount of work done, the maximal increase in the blood flow was divided by the force of muscular contractions.

To determine the role of the endothelium in the development of working hyperemia, the effect of de-endothelization, of blockade and stimulation of biosynthesis, and also inhibition of the action of vasodilator biologically active substances secreted by the endothelium, was studied.

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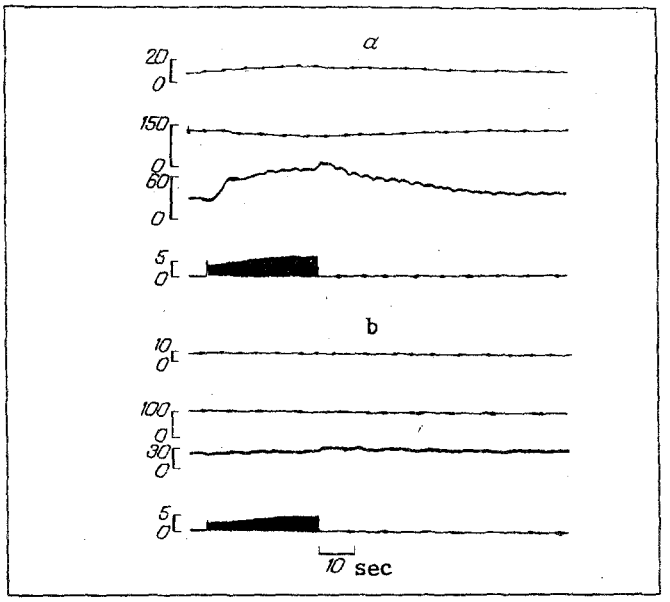

Fig. 1. Effect of chemical de-endothelization of vessels of the femoral arterial system on development of working hyperemia of gastrocnemius muscle during stimulation with a frequency of 8 Hz. Here and in Fig. 2: a) control reaction; b) reaction after de-endothelization. From top to bottom: mean pressure in femoral vein (in mm Hg); mean pressure in femoral artery (in mm Hg); blood flow in femoral artery (in ml/min), force of muscular contractions (in N).

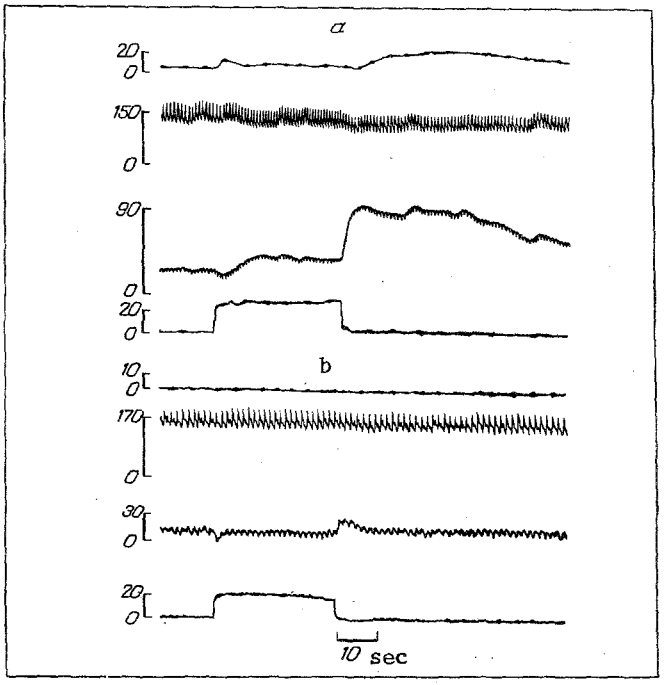

Fig. 2. Effect of chemical de-endothelization of vessels of femoral arterial system on development of working hyperemia of gastrocnemius muscle stimulated at a frequency of 40 Hz.

When the response had been recorded in the initial state, and in two work schedules (with a frequency of muscle stimulation of 8 and 40 Hz) [5], de-endothelization, blockade, or stimulation of BRF formation was carried out and working hyperemia was again produced. De-endothelization was performed by injecting a solution of saponin (1 mg/ml) into the system of the femoral artery, with the blood flow arrested for 5 min [3, 14]. Prostacycline biosynthesis was blocked by intravenous injection of a solution of indomethacine (3 mg/kg), and ERF biosynthesis was blocked by injection of gossypol solution (10 mg/kg) [8]; ERF synthesis was stimulated by a solution of L-arginine (50 mg/kg) [15]. The action of ERF on vascular smooth muscles was inhibited by intravenous injection of a solution of methylene blue (10 mg/kg) [11, 13]. The completeness of de-endothelization, the degree of blockade, stimulation, or inhibition of the action of vasodilator biologically active substances of endothelial origin, and the functional state of the vascular smooth muscles were monitored by recording changes in the vascular reaction of the region studied to intra-arterial injection of the endothelium-dependent vasodilator acetylcholine (10 μg) and the endothelium-independent dilator papaverine (2 mg). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

At a frequency of stimulation of the gastrocnemius muscle of 8 Hz the blood flow in the femoral artery increased rapidly in the first 6-10 sec after the beginning of working of the muscle, and thereafter for the next 20 sec of stimulation it continued to increase very slightly, to reach the peak value at the end of working of the muscle (Fig. 1a). The increase in blood flow at the peak of the response exceeded the initial level by 173 ± 10.2%, and when expressed per unit of developed force of the contracting muscle, the value was 6.8 ± 0.7. Immediately after the end of stimulation, smooth recovery of the blood flow rate to the background level took place in the course of 2-3 min.

At a frequency of stimulation of 40 Hz, during the first few seconds from the beginning of muscular contraction the blood flow decreased a little, after which it rose very slightly, and remained at that level until the end of stimulation of the muscle (Fig. 2a). Immediately after the end of stimulation the blood flow increased sharply to reach its peak values, the increase amounting to 325 ± 32% of the initial level. Recovery of the original blood flow took place after 3-6 min. Although the reaction of working hyperemia under these conditions was stronger than with stimulation at a frequency of 8 Hz, the ratio of the peak blood flow to the force of muscular contractions was smaller, namely 2.5 ± 0.2. Reduction of this value during stimulation of the muscle with a frequency of 40 Hz led to a more rapid (compared with stimulation at 8 Hz) increase in the force of muscular contractions than in the peak blood flow.

After de-endothelization of the vascular bed of the femoral artery, the response of increased blood flow to injection of acetylcholine virtually disappeared. Meanwhile the vasodilator response to papaverine remained unchanged. The resistance in the femoral arterial system increased but the blood flow was reduced by 2-2.5 times. The reaction of working hyperemia was significantly reduced after de-endothelization. An increase in the blood flow rate in response to stimulation at 8 Hz compared with the control reaction began later (at the 15th-20th second) and developed very slowly (Fig. 1b). The peak blood flow averaged 36.2 ± 14.8% of the initial values of the blood flow, and its ratio to the force of muscular contractions was reduced compared with the control reaction by 5.5 times ($p < 0.001$), to 1.2 ± 0.3 (Fig. 3). During stimulation at 40 Hz, the blood flow after de-endothelization did not increase before the end of stimulation, by contrast with the control reaction (Fig. 2b). Immediately after the end of muscular contraction the blood flow increased, by an amount equal to 84.5 ± 22% of the initial level. In this case the ratio of the peak blood flow to the force of muscular contractions averaged 0.46, which was 5.5 times less than in the control ($p < 0.001$) (Fig. 3). The time taken for the initial level of the blood flow to be restored was shortened to 10-40 sec (3-6 min in the control). The results of this series of experiments indicate that the endothelium can take part in the development of working hyperemia.

The endothelium is known to be involved in vasomotor reactions through the secretion of biologically active substances by its cells. Among the vasoactive substances produced by endothelial cells, prostacycline and ERF possess a vasodilator action. To study the role of prostacycline in the development of working hyperemia a series of experiments was carried out in which prostacycline biosynthesis was blocked by inhibiting cyclooxygenase activity by indomethacin. After preliminary injection of indomethacin the reaction of working hyperemia was not reduced during muscular work on either mode (Fig. 3) and the character of the reaction did not differ from the control. This indicates that prostacycline evidently has no essential role in the development of working hyperemia, and it suggests that ERF is involved in this reaction.

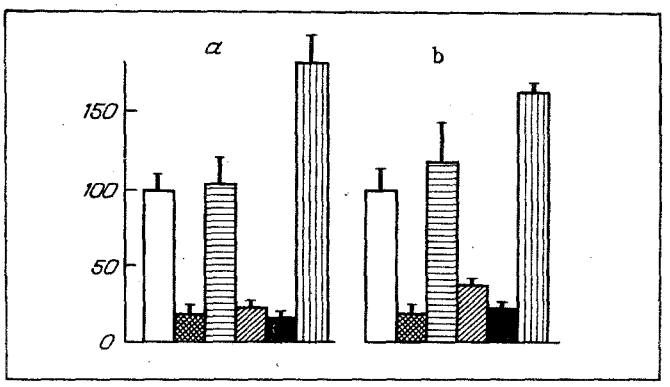

Fig. 3. Effect of saponin (cross-hatched columns), indomethacin (horizontally shaded columns), methylene blue (obliquely shaded columns), gossypol (black columns), and L-arginine (vertically shaded columns) on reaction of working hyperemia of gastrocnemius muscle. Unshaded columns – control; a) 8 Hz, b) 40 Hz. Ordinate, ratio of peak blood flow to force of muscular contractions (in % of control).

Endothelium-dependent vascular reactions mediated by ERF are inhibited by gossypol, which blocks its biosynthesis [8]. After blockade in this way the reaction of working hyperemia was significantly reduced for both modes of muscle stimulation (Fig. 3). Meanwhile the response of vasodilatation to acetylcholine injection was virtually absent, and the response to papaverine was unchanged. In response to stimulation at 8 Hz, the increase in the blood flow rate was delayed, it rose slowly, and the ratio of the peak blood flow to the force of muscular contractions was reduced by 6.6 times ($p < 0.001$). During stimulation of the muscle at 40 Hz, the blood flow at the beginning of muscular contraction was reduced a little, after which it returned to its initial level, and remained unchanged thereafter until the end of muscular work. After the end of stimulation the blood flow increased somewhat, but the ratio of its peak value to the force of muscular contractions was 4.5 times less than in the control ($p < 0.001$). The recovery time of the blood flow was 10-35 sec.

Secretion of ERF by the endothelial cells and its action on vascular smooth muscles are realized through activation of the soluble fraction of guanylate cyclase, and through an increase in the intracellular cGMP concentration in the muscles. Guanylate cyclase blockade by the specific inhibitor methylene blue inhibits endothelium-dependent vascular reactions [13]. In our experiments, blockade of this kind with a frequency of muscle stimulation of 8 Hz reduced the peak blood flow, as a ratio of the force of muscular contractions, by 4.2 times ($p < 0.001$), whereas during muscle stimulation at 40 Hz the decrease was 2.6 times ($p < 0.01$; Fig. 3). The character of the change in the reaction was the same as after blockade of ERF biosynthesis or de-endothelization of the vascular bed in the test region. Injection of acetylcholine in these animals induced hardly any vasodilatation.

Preliminary injection of L-arginine, which promotes ERF formation by the endothelium [15], led in our experiments to an increase in the response to injection of acetylcholine by $30 \pm 6.5\%$. The resistance in the femoral arterial system was reduced but the blood flow was increased by 1.5-2 times. The reaction of working hyperemia was increased after injection of L-arginine when the muscle was stimulated at either 8 Hz or 40 Hz. The ratio of peak blood flow to force of muscular contractions (frequency of stimulation 8 Hz) was increased by 1.8 times ($p < 0.01$). Postcontraction hyperemia (frequency of stimulation 40 Hz) also exceeded the control by 1.6 times ($p < 0.001$).

The considerable inhibition of working hyperemia after de-endothelization of the vascular bed in the test region indicates that the endothelium is involved in the development of this reaction. Experiments with cyclooxygenase blockade indicate that involvement of prostacycline, which is synthesized by the endothelium, in the development of this reaction is unlikely, despite evidence that during prolonged muscular work (5 min or more) it may participate in the development of functional hyperemia [5]. Inhibition of working hyperemia by blockers of ERF biosynthesis, and by blockers of its secretion and action on vascular smooth muscles, leads to the conclusion that the endothelium participates in the development of working hyperemia through involvement of ERF. The increase in working hyperemia after preliminary injection of the ERF precursor confirms this conclusion.

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