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Responses of the Arterial and Venous Vessels of the Skeletal Muscle to Norepinephrine Following Damage to Their Endothelium

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The functional activity of vascular endothelium in a muscle preparation from the feline gastrocnemius is impaired with ethanol, which results in an increase of an adrenergic responsiveness of the arterial compartment of the vascular bed. The exchange function of the microvessels changes little. Veins exhibit nonuniform changes in their responsiveness after exposure of their endotheliocytes to ethanol.

Key Words: *norepinephrine; blood vessels; skeletal muscle; endothelium; ethanol*

In studies carried out on strips and segments of major blood vessels, mainly arteries, endothelium has been shown to contribute to the pattern of their responses to humoral factors acting on vascular myocytes [7,10,11,14]. The role of endothelium in the vasomotor reactions of an organ as a whole has to be studied by a dosed, selective damage to the endothelium in consecutive portions of the vascular bed, while fully preserving the exchange function of the microvessels [1]. A dosed impairment of the function of endothelium without damage to its structural integrity in microvessels of the organ may be achieved using chemical agents [7].

The objective of this study was to examine the norepinephrine (NE)-induced responses of the arterial and venous vessels of the gastrocnemius muscle for different degrees of endotheliocyte dysfunction caused by ethanol.

MATERIALS AND METHODS

The experiments were carried out on 23 urethane-anesthetized (1 g/kg) cats of both sexes. The vas-

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cular bed of the hemodynamically and neurally isolated gastrocnemius muscle (shin preparation [4]) was perfused with heparinized (2000 IU) autologous blood of the animals [1,9] with a constant flow pump. NE (Spofa, Czechoslovakia) in a dose of 10 μg was instantaneously injected in *a. poplitea* in 0.1 ml of dextran solution (polyglucin). Changes in the arterial resistance to a constant blood flow in the muscle preparation were determined by resistography [9], and shifts in the intramuscular venous resistance by the earlier described method of venous resistography [5,13]. The mean hydrostatic capillary pressure (P_c) and the capillary filtration coefficient (CFC) were determined as described elsewhere [1]. The hydrostatic pressure of the venous efflux was maintained at a level of 10 mm Hg, which was determined in previous studies to be the optimal pressure for the venous vessels to exhibit their contractile responses [4,8,9]. The temperature of the blood entering the preparation was maintained at 37±0.5°C using an ultrathermostat and recorded with the transducer of a TPEM-I electrothermometer. The parameters were recorded on an H-327-8 ink recorder. The results were statistically processed using Student's test.

The parameters of vascular functions were measured before and after a short-term (15-20 sec) infusion of 3 ml of 6, 12, 24, and 48% ethanol into the vascular bed of the muscle preparation. For this purpose, the blood flow was discontinued, and blood was removed from the gastrocnemius preparation by a 15-20 sec infusion of 10 ml physiological saline; ethanol was removed in a similar manner.

The morphological control of the state of the structural elements in the walls of the arteries, veins, and capillaries was performed after the end of the experiments by fixing the muscle preparation in 2.5% glutaraldehyde in a 0.135 M sucrose-containing phosphate buffer (pH 7.4) and isolating segments of the arterial and venous vessels, as well as sections of the muscle tissue with microvessels. Tissue fragments were prepared routinely for electron-microscopic examination and embedded in Epon. Ultrathin sections were examined in a JEM-100B electron microscope at an accelerating voltage of 80 kV.

RESULTS

Our studies showed that after a short-term (15-20 sec) infusion of 6, 12, 24, and 48% ethanol into the vascular bed of the muscle, the arterial and venous resistance to the blood flow remained virtually unchanged. For instance, even after the infusion of 24 or 48% ethanol changes of the resistance to the blood flow constituted, respectively, 4.5±5.6 or 3.1±3.8% of the baseline values in the arterial and 3.0±11.6 or 2.7±4.1% in the venous compartments of the vascular bed.

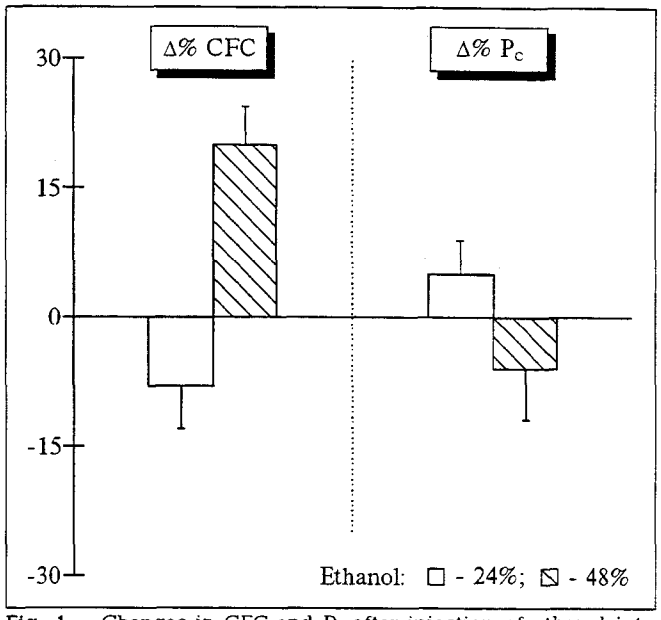

Fig. 1. Changes in CFC and P_c after injection of ethanol into vascular bed of gastrocnemius muscle, % of initial values.

The parameters of microhemodynamics and of the transcapillary fluid exchange - P_c and CFC -

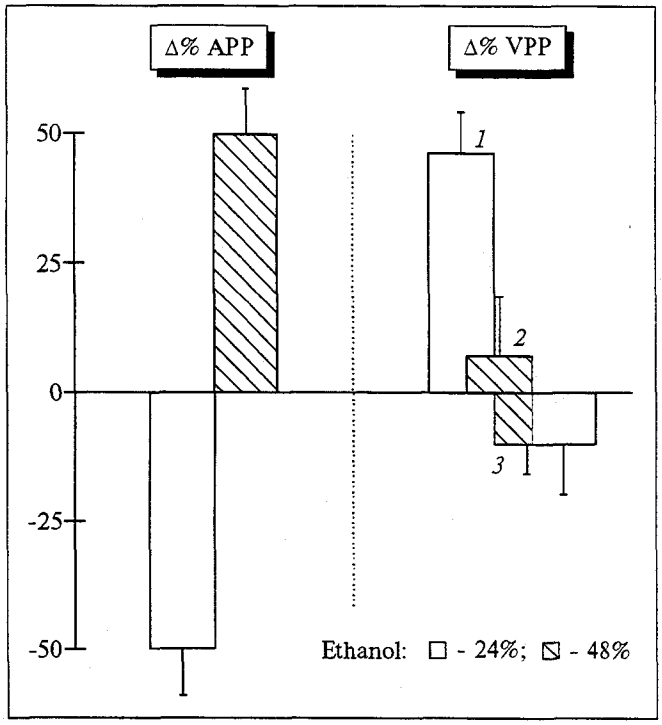

Fig. 2. Changes in arterial (*APP*) and venous (*VPP*) perfusion pressure in response to NE (10 μg) after ethanol impairment of vascular endothelium in the gastrocnemius muscle, % of initial values. *1*, *2*, and *3*) groups of animals with similar adrenergic responses.

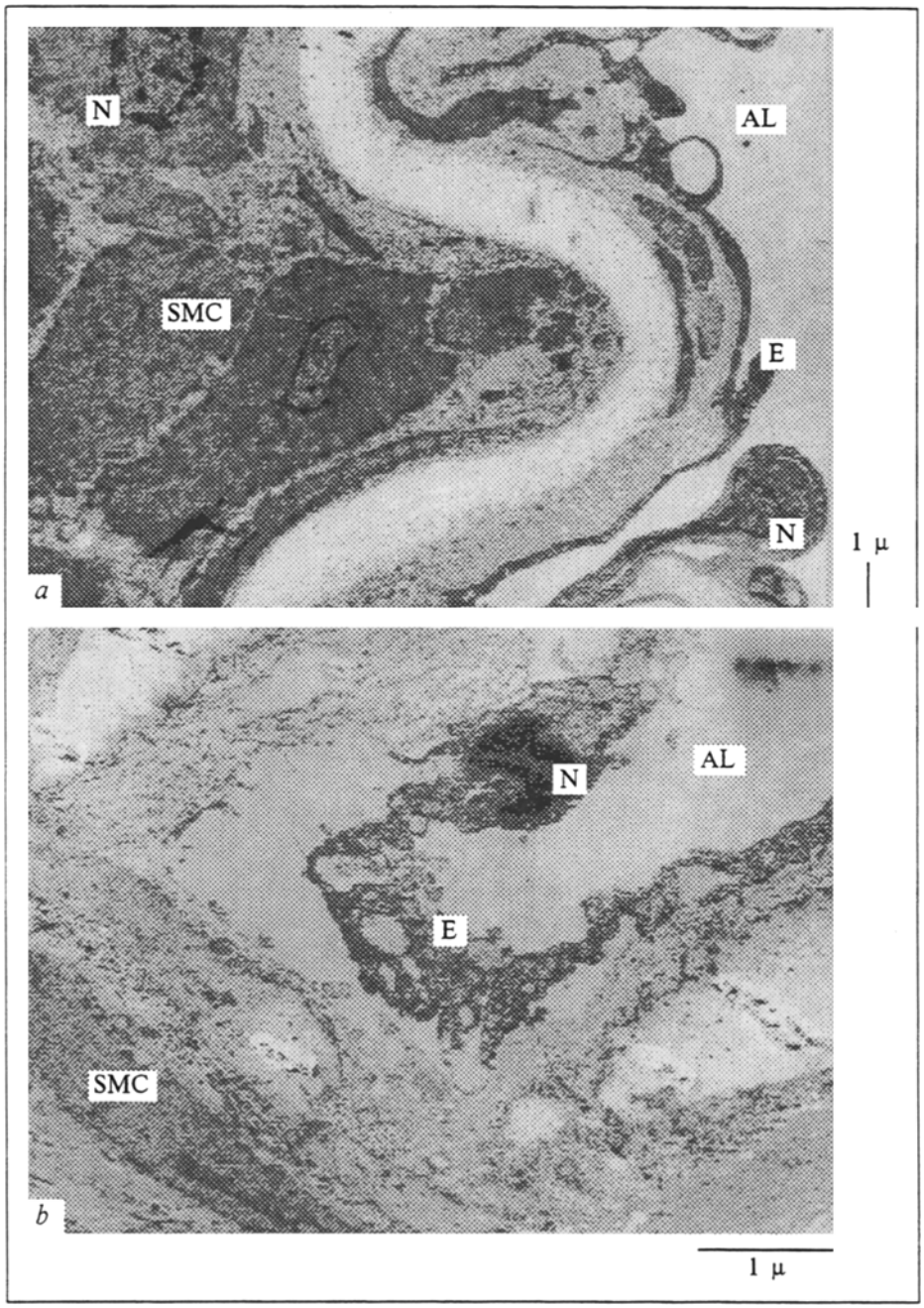

Fig. 3. Ultrastructural changes in arterial wall after exposure to ethanol. Injection of 24% (*a*) and 48% (*b*) ethanol. *SMC*: smooth muscle cell; *AL*: arterial lumen; *E*: endotheliocyte; *N*: cell nucleus.

were also virtually unchanged after the infusion of 6, 12, and 24% ethanol (Fig. 1). For example, following infusion of 24% ethanol into the preparation, P_c rose just 3.0±3.4% above the baseline value (19.7±0.4 mm Hg), and CFC dropped 5.0±6% below the baseline (0.036±0.0045 ml× ×min^{-1}×mm Hg^{-1} per 100 g); only after injection of 48% ethanol into the circulation did CFC markedly rise (18.0±1.36%, $p<0.05$), the changes of P_c still being insignificant (3.0±6.5%) (Fig. 1).

Our findings led to the conclusion that the resistive function of the arterial and venous compartments of the vascular bed of the gastrocnemius muscle, as well as the exchange function of its microvessels, were virtually unchanged after the infusion of ethanol solutions (up to 24%) into the preparation, since the above-mentioned shifts remained within the limits of baseline variation [1].

In studies of the arterial and venous responsiveness of the muscle preparation, after a short-term (15-20 sec) exposure of the vascular bed to 24% ethanol, we noted a change in this parameter. The infusion of 6 and 12% ethanol elicited no changes in the responsiveness of these vascular compartments to NE. The use of 24% ethanol markedly enhanced the constrictive responses of the arterial compart-

ment of the gastrocnemius to 10 μg NE, which manifested itself in increased shifts of the integral (arterial) perfusion pressure (by 60.0±17.1% of the baseline values). The use of 48% ethanol markedly reduced (by 52.0±10.8%) the constrictive responses of arteries to NE (Fig. 2).

In our experiments changes in the constrictive responses of the veins to NE after exposure to 24% ethanol, which were judged from shifts of the venous perfusion pressure, were qualitatively nonuniform. It was found that overall (a total of 18 animals were used) three equal-sized groups could be distinguished: group I, in which the adrenergic constrictive responses of the venous vessels increased (by 69.0±35.1%); group II, in which the venous resistance changed slightly (6.0±26.0%); and group III, in which the venous responses to NE diminished (by 65.0±4.2%) (Fig. 2). The constrictive responses of the intramuscular venous bed to NE after exposure to 48% ethanol declined by 71.2±10.6% as compared to the initial values. Thus, our findings lead to the conclusion that the use of 24% ethanol enhances the constrictive responses of the arterial vessels of the gastrocnemius muscle to NE and produces nonuniform changes in the adrenergic responses of the intramuscular venous vessels, while the use of 48% ethanol markedly reduces the NE-induced constriction of the arterial and venous compartments of the muscle's vascular bed.

Published data [10,12] obtained *in vitro* suggest that NE, acting via the β-adrenoceptors of endothelial cells, may cause the relaxation of vascular myocytes, which, on the whole, mitigates the contractile adrenergic effect. Hence, impairment of the functional activity of endothelium must result in an enhancement of the vascular contractile response to NE. Our findings attesting to enhanced NE-induced constrictive responses of the arteries of the feline gastrocnemius following injection of 24% ethanol into the vascular bed may be attributed to impairment of the functional activity of the endothelial layer in the arterial vessels by ethanol. The reduced constrictive responses of the arteries after exposure to 48% ethanol were due to damage to the smooth muscle elements of the vascular wall.

The results of morphological investigations corroborated this assumption. For instance, after the vessels were exposed to 24% ethanol, the shape of endotheliocytes changed: the bulk of the cytoplasm with the nucleus evaginated into the vascular lumen, and the marginal portions of cells became much thinner. Deep invaginations of the karyolemma and an increase in the electron density of the perimembrane chromatin were observed. The number of microvilli and outgrowths on the surface of the endothelial cells increased. Endothelial contacts were broken and the gaps between cells widened (Fig. 3, *a*). These ultrastructural changes were in accord with published data on the stress response of vascular endothelium to damaging factors [2,3,6]. Ethanol in a higher concentration (48%) caused destructive changes not only in the endothelial layer (a marked increase in the electron density of the nucleus and cytoplasm of endotheliocytes and wrinkling and detachment of the latter) (Fig. 3, *b*), but also in the smooth muscle elements.

Our findings show that after exposure to 24% ethanol shifts in the adrenergic responsiveness of the intramuscular venous vessels were nonuniform. Only 48% ethanol markedly reduced the NE-induced constrictive responses of the venous vessels in the muscle preparation. In this case electronmicroscopic investigations demonstrated reactive reorganizations of venous endotheliocytes, this being attended by enhanced pinocytosis, the emergence of a few foci of destruction, and, more rarely, by the widening of cell-cell gaps. The nonuniformity of the results with respect to the shifts in the responsiveness of the venous vessels following exposure to 24% ethanol may be attributed either to the fact that their sensitivity to ethanol differed from that of the arteries, or to the fact that the dose of ethanol needed to exert an effect upon the arterial bed was not delivered to this compartment, due to ethanol binding by proteins of the arterial endothelium.

Thus, ethanol provided for a dosed impairment of the functional activity of the vascular endothelium in the gastrocnemius muscle, which enhanced the adrenergic responsiveness of the arterial bed of the muscle preparation. Meanwhile, the exchange function of microvessels changed little. The venous vessels exhibited nonuniform changes in responsiveness after exposure of their endotheliocytes to ethanol, a feature which calls for further study, including the development of new experimental approaches.

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The Asymmetry Phenomenon in Responses of Frog Lingual Microvessels

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Qualitative evaluations and measurements of morphometric parameters (area, length, and diameter) performed in symmetrical fragments of the vascular bed of 30 frog tongues before, during, and after the production of local ischemia on the ipsi- or contralateral side reveal morphological and functional asymmetries in microvessels on the two sides of the tongue. Two groups of individuals, tentatively designated as "right-dominant" and "left-dominant," are identified.

Key Words: *microcirculation; morphometry; asymmetry; ischemia*

In studying postischemic disturbances of the microcirculation in paired organs [6], our attention was drawn to one object in which the right and left microcirculatory beds could be observed in a single experiment, namely the microcirculatory bed (MCB) of the tongue. It goes without saying that the tongue has an axis of symmetry - this is evidenced by anatomical data. The blood supply to the left half of the tongue and that to its right half are largely independent of one other, as was demonstrated when lingual vessels were stained *intra vitam* [7]. The property of autonomy shown by the symmetrical halves of the tongue has been utilized in chronic experiments with cleft tongues of frogs to study how the activity of the taste receptors is regulated [4,5], and also in experiments with tongues of rats to gain information on the relationship between the gustatory response and blood flow [7, 8]. In these studies no attempt was made to identify the symmetrical sides of the tongue. However, when the right and left halves of the human tongue were compared, a considerable asymmetry in the distribution density of gustatory papillae was found [3]. That the human system of blood vessels is functionally asymmetrical was known even to the school of Salerno in medieval times [2]. The present study was undertaken to identify the lingual MCB on the right and on the left.

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MATERIALS AND METHODS

The study was conducted on 30 male frogs with destroyed spinal cord. All measurements were made

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