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Nonactomyosin Component of Thermo-Induced Contractions of the Vascular Wall in Hypertension

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It is determined that arterial hypertension induced by deoxycroticosterone results in the almost total disappearance of the nonactomyosin components of the thermomechanical responses of the thoracic section of the rat aorta wall. This may explain the known effect of the decreased mechanical stress generated by the vascular wall during hypertension. It is assumed that this decrease in the mechanical stress generated by nonactomyosin mechanisms is due to an increase in the conformational stability of collagen of the connective-tissue matrix.

Key Words: *vascular wall; collagen; nonactomyosin contractile mechanisms; hypertension*

Previously we found that thermo-induced contractions of the walls of a number of vessels are provided by thermomechanical reactions of the connective-tissue matrix [1]. Further investigations allowed us to identify two types of nonactomyosin mechanisms of vascular smooth-muscle tissue: "connective tissue independent" and "connective tissue dependent" [2]. On the other hand, it is known that cardiovascular diseases are, as a rule, accompanied by pronounced changes in the structure and features of connective tissue of the vascular wall. This is clearly manifested, for example, in arterial hypertension [9,12]. In view of this, it was of interest to study the hypertension-induced changes of the nonactomyosin, "connective tissue" contractile mechanisms of the vascular wall.

MATERIALS AND METHODS

Experiments were carried out with male Wistar rats aged 7 months. Hypertension was induced by daily intramuscular injections of deoxycorticosterone (DC) during 16 days in a dose of 2 ml 0.5% solution per kg [7,8]. The mean arterial pressure in hypertensive rats was 165±5 mm Hg and in control normotensive rats 115±5 mm Hg. Arterial pressure was measured with an EMT-31 electromanometer (Elema-Shonander, Sweden).

Strips of the wall of the thoracic aorta measuring 7×1 mm were cut out parallel to the large axis of the vessel, and also in the shape of rings, and were fixed to the experimental chamber, one end to a Mioton TsA-012-MT dynamometer (Akademicheskii Center, Ekaterinburg), and the other end to a Mioton TsA-012-ZD generator of mechanical deformations (the same manufacturer). Initial length (L_i) was determined after [11]. The preparations were then stretched to length equal to 1.4 L_i and washed in Krebs solution for 60 min before the beginning of the experiments [1]. The experimental chamber was kept at temperatures from 20 to 40°C by means of an automatic thermostat, which provided accuracy of control of the solution temperature ±0.2°C.

The rigidity of preparations was studied by exposing them to the action of sinusoidal oscilla-

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tions with 30 Hz frequency and an amplitude equal to 0.5% of L_i [4].

Total and irreversible suppression of the contractile activity - denaturation - of the preparations of smooth muscles was carried out using a previously described method [2].

All reagents used were by Sigma. Evaluation of the mechanical stress generated by the test strips was performed after [6].

In each study, the changes in rigidity of the strips and the stress produced by this rigidity under the action of temperature were investigated. The cells of smooth muscles were then subjected to total suppression and parameters of the thermomechanical reactions of denaturated vessel strips were evaluated.

RESULTS

The data are presented in Fig. 1. In accordance with our concept of nonactomyosin mechanisms of contraction of smooth muscles, themomechanical reaction *1* (Fig. 1, *a*) is provided by a "connective tissue independent" contractile mechanism [1,2]; thermo-induced response *2* (Fig. 1, *a*) by "connective tissue independent" and "connective tissue dependent" mechanisms; contractile reaction *3* (Fig. 1, *a*) by the two above-mentioned nonactomyosin contractile mechanisms and by the actomyosin complex of the smooth-muscle cells [2].

The nature of the thermo-induced responses of the aorta wall changes markedly during hypertension. Thermomechanical reaction *1* (Fig. 1, *c*) diminishes almost 80% in amplitude, and thermo-induced response *2* (Fig. 1, *c*) markedly decreases as well. The amplitude of contractile reaction *3* (Fig. 1, *c*) also markedly decreases. However, the component of the latter which belongs to the actomyosin complex and serves as the difference between the total amplitude of contractions and the sum of the contributions of the two nonactomyosin components does not change so markedly. The alterations in rigidity of aorta preparations of hypertensive animals caused by the functioning of the actomyosin complex (Fig. 1, *d*), as well as the corresponding alterations in mechanical stress (Fig. 1, *c*), do not differ so much from those for aorta strips of normotensive animals (Fig. 1, *a, b*).

Qualitatively analogous data were obtained for preparations cut out in the shape of rings.

The values of the Young modulus of preparations of normotensive and hypertensive animals were similar to each other and were about 2.5×10^{-5} N/m^2 for native strips and 2.0×10^{-5} N/m^2 for denatured strips.

The results obtained indicate the almost total disappearance of both nonactomyosin components of the thermomechanical reactions of the aorta wall under the influence of hypertension and the slight decrease of the actomyosin component. We consider that these data can elucidate the causes of the decrease of amplitude of the changes in mechanical stress in the contractile reactions of the vascular wall in hypertension [3,6,10,12]. Despite the rise of sensitivity of the contractile apparatus of the vascular tissue to Ca^{2+} and the increase in the force of the agonist-induced contractions during hypertension [8], the mechanical stress generated by the vascular walls of hypertensive animals increases in comparison with that of normotensive animals [3,12]. It is obvious that the exclusion of the two nonactomyosin mechanisms from the process of generation of mechanical stress by the contractile system of the smooth muscles will result in a decrease of the amplitude of changes in mechanical stress of smooth muscle tissue during contraction. The two nonactomyosin components of contraction of the smooth muscle tissue disappear almost simultaneously, a fact we consider to be of considerable importance, as it can serve as indirect evidence of the presence of a common executive apparatus of the two nonactomyosin contractile mechanisms - "connective tissue independent" and "connective tissue dependent" - and as further indirect evidence of the presence of a "connective tissue dependent" mechanism, based on chemomechanical reactions of connective-tissue structures (the tree-dimensional network of collagen fibril I and III), which are provoked by the action of transmitter factors produced by smooth muscle cells.

Apparently, the disappearance of predenaturation changes in the conformation of collagen in the tested range of temperatures is due to an increase in its conformation stability. What could be the reason for such a pronounced rise in collagen stability of the vascular wall under the action of hypertension? Probably, this phenomenon could be the result of at least two processes: an increase (caused by the rise of arterial pressure) of the mechanic stretching force applied to the wall of the vessel and the influence of DC, which stimulates the maturation of collagen.

As is known, both these factors activate the processes which enhance the structural stability of the connective tissue: an increase in the total mass of collagen, an increase in the quantity of transverse covalent sutures in collagen, and the alteration of its isomolecular composition [12], i.e., the biological "age" of collagen increases, since analogous alteration in structures of connective tissue

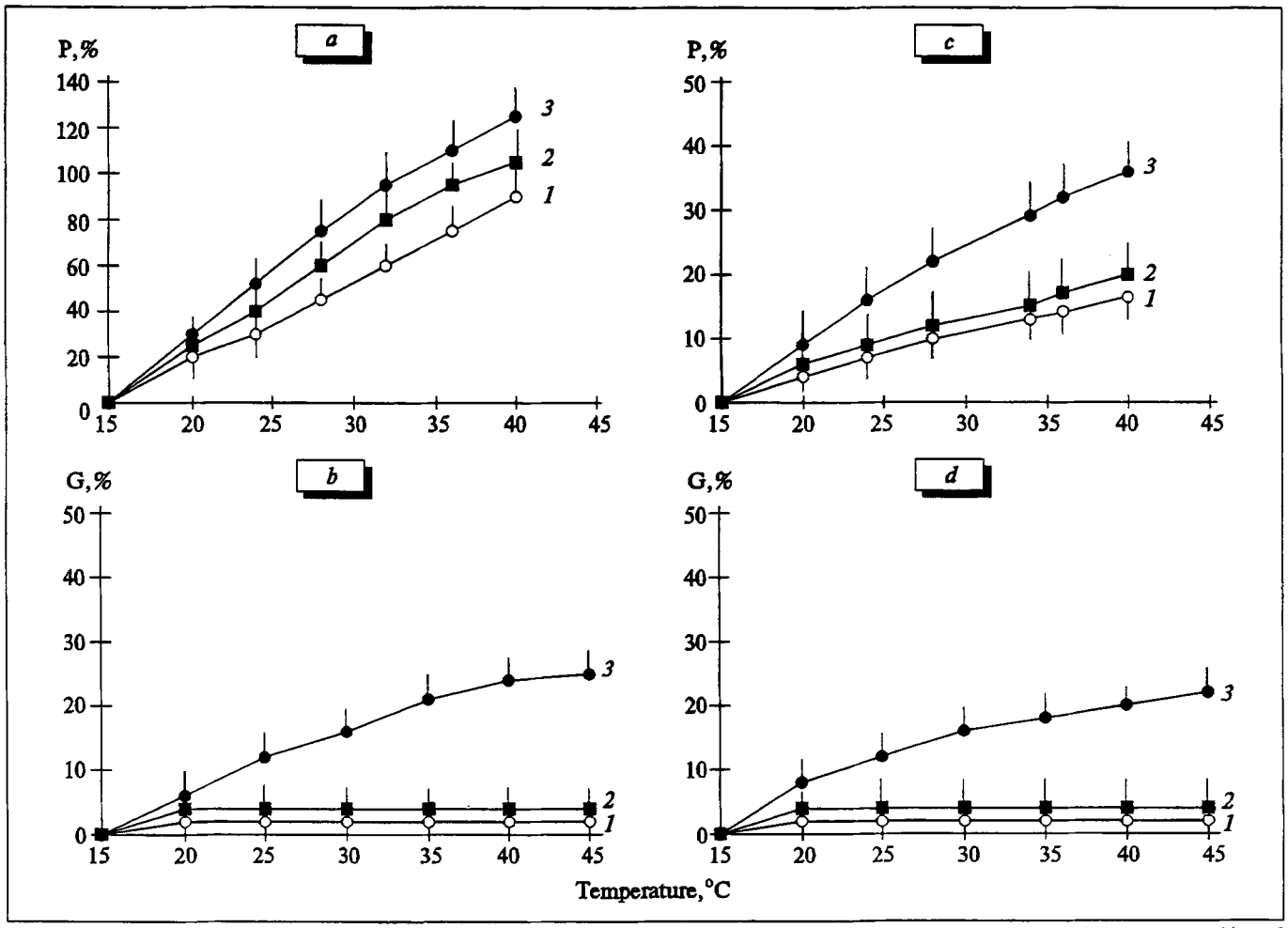

Fig. 1. Changes in mechanical stress (*P*) and the Young modulus (*G*) of isolated longitudinal strips of aorta: control (*a*, *b*) and hypertensive animals (*c*, *d*); *1*) denaturated; *2*) native (before noradrenalin treatment); *3*) native, treated with noradrenalin. Changes in *P* and *G* of preparations of normotensive and hypertensive animals, respectively, during contractions induced by noradrenalin treatment (10^{-5} M) taken as 100%. The data are presented as the arithmetic mean values with an indication of the confidence interval ($p=0.05$, $n=7$).

occur during the aging of the organism. It is thought that the complex of changes in mechanical and chemomechanical properties of the connective tissue caused by aging of the organism is identical to the complex of corresponding changes induced by DC and by the increased mechanical stress. At least two marked differences concerning the alteration of properties of the connective tissue matrix during hypertension and aging can be noted: 1. The rigidity of the tissue of the vascular wall does not change much in hypertension (while during aging, it increases markedly). 2. The ability of structures of the connective tissue matrix of the native smooth muscles to generate mechanical stress almost vanishes in hypertension but hardly changes during aging.

Since the total rigidity of the collagen-glycosaminoglycan-elastin complex is determined by the consecutive elastic component with respect to the collagen elements, it can be assumed that the first difference is due to the fact that this elastic component does not change so much during hypertension, whereas during aging it changes markedly. The existence of the second difference testifies that there is an important factor in the complex of the influence of DC-induced hypertension on smooth muscle tissue which changes the chemomechanical properties of collagen and is absent in the complex of the influence of aging. These speculations lead us to the important conclusion that the degree of transverse covalent bonding of collagen (markedly increased during aging) does not greatly influence its capacity for predenaturation changes in conformation.

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Lipophilic Antioxidant U-18 and Superoxide Dismutase Prevent Cultured Hippocampal Neurons from Destruction during Hypoxia and in the Posthypoxic Period

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The lipophilic antioxidant U-18 from the class of hindered phenols prevents the destruction of cultured hippocampal neurons during hypoxia and also in the posthypoxic reoxygenation period, apparently by being stably incorporated into their phospholipid membranes and by safeguarding these from free-radical damage in the course of reoxygenation. On the other hand, the protection afforded to the cultured hippocampal neurons by superoxide dismutase is probably due to its ability to interfere with the posthypoxic neuron-degrading processes mediated through hyperproduction of superoxide radicals in the neuronal cytoplasm.

Key Words: *nerve cell culture; hippocampus; hypoxia; antioxidants*

Results of numerous *in vivo* and *in vitro* experimental studies attest to important roles of free radicals and lipid peroxidation (LPO) in causing damage to brain neurons during hypoxia/ischemia [5,6,8,15]. One of the factors initiating these conditions is activation of several intracellular enzymes (phospholipase A_2, xanthine oxidase, NO synthase) by calcium ions. The hyperstimulation of glutamate receptors resulting from increased presynaptic release of glutamate and impairment of the mechanisms of its reuptake under conditions of energy deficiency causes calcium to accumulate in the cytosol. As a consequence, the level of low-molecular substrates and the activity of enzymes involved in the generation of reactive oxygen species become greatly elevated during hypoxia.

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