METHODS

Role of the Connective Tissue Matrix in Thermomechanical Reactions of the Aortic Wall

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Mechanical thermosensitivity of the aortic wall is studied using transverse and longitudinal preparations. The major contribution of the connective tissue matrix to the heat-induced contraction is demonstrated. Transverse preparations develop greater tension than longitudinal preparations. A model with two active elements is proposed.

Key Words: *aortic wall; thermosensitivity; mechanical tension; modulus of elasticity; connective tissue matrix*

Mechanical behavior of smooth and cross-striated muscles is described by Hill's model [6]. According to this model, the muscle consists of the active force-generating element (actomyosin complex) and the passive elastic element (intra- and extracellular elastic structures [3,4,10]). However, it was shown that in heat-induced responses of the aortic wall generation of mechanical tension is provided predominantly by "passive" extracellular matrix. Therefore, it is interesting to study in detail mechanical thermosensitivity of the aortic wall and develop a rheological model with two active elements that reflect the participation of both actomyosin complex and connective tissue in force generation in response to high temperature.

MATERIALS AND METHODS

Circular and longitudinal [3] preparations of isolated adult rat thoracic aorta were used. One end of aortic strip was attached to a sensor and the other to a generator of mechanical deformations. The initial value of the strip (L_{ini}) was measured as described [9]. Mechanical tension (σ) and rigidity (S) of the strip were measured in isometric regime at 1.2 L_{ini} [2,7].

In each experiment, σ and S of native strip were measured in the temperature range 20-40°C. Then the preparations were treated with smooth muscle cell metabolism inhibitors [8], and mechanical properties of the connective tissue matrix were studied in the same temperature range. Krebs' solution contained (mM): NaCl 120.4, KCl 5.9, $NaHCO_3$ 15.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11.5, and $CaCl_2$ 2.5; pH of the solution was maintained at 7.4 throughout the temperature range. Before measurements, each preparation was left in the working chamber filled with Krebs' solution for 30 min at 1.2 L_{ini}.

RESULTS

Figure 1, *I*, *a* and *II*, *a* shows that the shapes of the curves of heat-induced responses of both preparations do not differ considerably. Both transverse and longitudinal preparations have high heat sensitivity ($\delta\sigma/\delta\tau$). Statistically significant changes in this parameter were observed only between native and inhibitor-treated transverse preparations (Fig. 1, *I*, *a*). It is reasonable to regard the difference between the tensions developed by native and inhibitor-treated preparations as a contribution of smooth muscle cells

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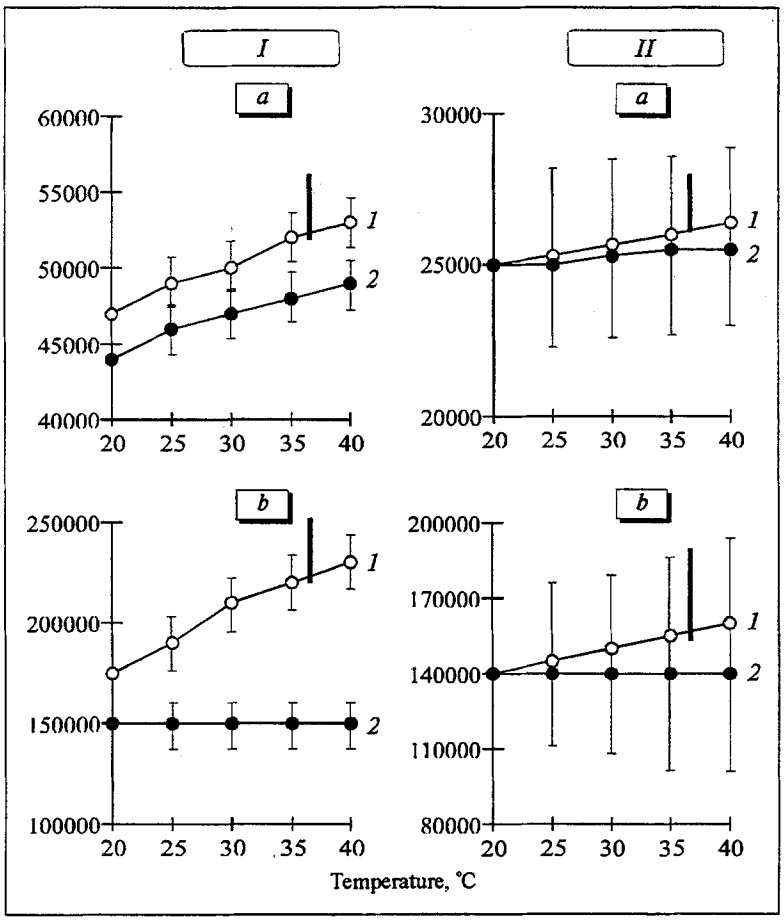

Fig. 1. Effect of temperature and norepinephrine on mechanical tension (σ, *a*) and modulus of elasticity (*E*, *b*) of transverse (*I*) and longitudinal (*II*) isolated preparations of rat aorta. Ordinate: tension or modulus of elasticity ($N \times m^{-2}$). *1*) native preparations; 2) inhibitor-treated preparations. Vertical line — amplitude of changes in tension and modulus of elasticity in response to 10^{-5} M norepinephrine at 37°C.

to the heat-induced reaction of the aortic wall. Consequently, it can be concluded that in the studied temperature range the contribution of the actomyosin complex to mechanical thermosensitivity is small in transverse preparations and practically absent in longitudinal preparations.

The involvement of smooth muscle cells in heat-induced contraction of aortic wall is confirmed by changes in the rigidity of native longitudinal and transverse preparations, which were more pronounced in transverse preparations (Fig. 1, *I*, *b*). Treatment with the inhibitor led to statistically insignificant decrease in the rigidity of longitudinal preparations at 20°C (Fig. 1, *II*, *b*) and a substantial decrease in the rigidity of transverse preparations (Fig. 1, *I*, *b*).

These results indicate that the connective tissue matrix is the major contributor to heat-induced contractions of the aortic wall, while the contribution of the smooth muscle cells is much lower, being greater in transverse preparations.

It should be noted that there were considerable differences in mechanical characteristics of transverse and longitudinal preparations. The initial resting tension at 20°C and rigidify at equal stretch force were greater in transverse preparations (both native and inhibitor-treated). Mechanical thermosensitivity (Fig. 1, *I*, *a*, *II*, *a*) and the corresponding changes in rigidity (Fig. 1, *I*, *b*, *II*, *b*) of native and inhibitor-treated transverse preparations were also higher than those of longitudinal preparations. From this finding it can be concluded that the differences in mechanochemical properties of the aortic connective tissue matrix in transverse and longitudinal directions correspond to the differences in thermomechanical responses of aortic wall. We think that this regularity is a component of the mechanism responsible for the vascular tone formation. The physiological significance of this mechanism is confirmed by the fact that the amplitude of heat-induced mechanical responses of the aortic wall in the studied temperature range is comparable to that of its response to the vasoconstrictor agonist norepinephrine.

On the basis of experimental data we propose the following mathematical model of heat-induced mechanical response of the aorta. This model is based on Hill's three-component model which assumes that both extracellular matrix and the contractile apparatus—consecutive elastic element complex are heat sensitive. In Hill's model, the extracellular matrix plays the role of a parallel component. Since inac-

tivated smooth muscle cells lose their elasticity [5,11], inhibitor-treated strip was regarded as a parallel elastic component. Since in Hill's model the parallel elastic element is connected in parallel to contractile and consecutive elastic element, which are connected in series, it can be assumed that the forces generated by these components in isometric regime are additive. This allows one to interpret the difference between the forces (and rigidities) of native and inhibitor-treated muscles as a contribution of the contractile apparatus to the consecutive passive elastic component.

According to Hooke's law, the following equation can be written:

$$\delta\sigma=\sigma(t_2)-\sigma(t_2)=E\delta\varepsilon+\varepsilon\delta E, \quad (1)$$

where: t is the temperature ($t_2>t_1$), σ is the force per transverse area, E is the modulus of elasticity, ε the lengthening determined as [$\varepsilon=(L-L_o)/L_o$, where L_o length of muscle in a resting state ($\sigma=0$)].

As applied to all the components, equation (1) can be written as:

$$\delta\sigma=E_c\delta\varepsilon_c+\varepsilon_c\delta E_c+E_{ca}\delta\varepsilon_{ca}+\varepsilon_{ca}\delta E_{ca}, \quad (2)$$

where E_{ca} and ε_{ca} are the parameters of the contractile apparatus—consecutive elastic element system and E_c and ε_c are the parameters of the parallel elastic component. Experimental data show that $\delta E_c=0$ ($\varepsilon=0.2$), therefore equation (2) can be written as follows:

$$\delta\sigma=E_c\varepsilon_c+E_{ca}\delta\varepsilon_{ca}+\varepsilon_{ca}\delta E_{ca}. \quad (3)$$

Then we have assumed that for isometric regime $\delta\varepsilon_{ca}=0$. In this case:

$$\delta\sigma=E_c\delta\varepsilon_c+\varepsilon_{ca}\delta E_{ca}. \quad (4)$$

In order to check up this assumption, equation (4) can be written as $Y=Ax+B$, (5)
where: $Y=\delta\sigma$, $A=\delta E_{ca}$, $x=\varepsilon_{ca}$, and $B=\delta\varepsilon_c E_c$.

The coefficients A and B obtained by the linear regression were analyzed using the χ^2 test. Statistical significance of these coefficients indicates that equation (4) is correct, consequently, the assumption that $\delta\varepsilon_{ca}=0$ is also correct.

The following conclusions were made from the results obtained in this study:

- The amplitude of heat-induced mechanical tensions developed by the aortic wall is comparable to an almost maximum amplitude of agonist-induced contractions.
- Thermomechanical reactions of the aortic wall are determined predominantly by the connective tissue matrix.
- The contribution of smooth muscle cells to heat-induced generation of mechanical tension is small in transverse preparations and negligible in longitudinal reparations.
- Thermomechanical responses of transverse and longitudinal preparations of the aortic wall differ in the amplitude: tension generated by transverse preparations is greater than that generated in longitudinal preparations. This holds true for both native and inhibitor-treated preparations.
- Heat-induced mechanical responses of the aortic wall can be described with the use of a mathematical model with two active elements that reflect the participation of the actomyosin complex and connective tissue in force generation. The relationship between the main mechanical parameters of the model is described with the following equation:

$$\delta\sigma=E_c\delta\varepsilon_c+\varepsilon_{ca}\delta E_{ca}.$$

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