Mechanical Properties of Lymph Node Capsule

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We studied the relationship between the length and tension in isolated strips of the capsules of bovine mesenteric lymph nodes. The deformation–strain and radius–pressure relationships were established in the lymph node. The capsule possesses high distensibility, modulus of elasticity at optimal tension was 0.09×10^5 N/m^2. Smooth muscle activation produces a 6-fold increase of modulus of elasticity. Maximum active stress in the capsule was recorded at a length of 1.1 L_0 and maximal active pressure (4.5 cm H_2O) at 0.9 L_0.

Key Words: *lymph node capsule; mechanical properties; length-tension relationship; strain; pressure*

The lymph flowing in lymphatic vessels from tissues where it is generated to the place where the main lymphatic collector flows into large cervical veins usually passes through several lymph nodes (LN) [3,11]. While lymph vessels are chains of single-compartment pumps with multiple-circuit regulation [1,2,13-15], LN is rather a serious barrier for the lymph flow, because its inner space is filled with lymphoid tissue. At the same time, lymphoid tissue possesses sinuses presented by slit-like spaces; lymph slowly passes through them from the place, where afferent lymphatic vessels flow into it, to the LN hilus.

The capsule of LN contains a considerable number of smooth muscle cells [6]. Spontaneous contraction of the entire LN and capsule strips (CS) was demonstrated in previous studies [7,12]. During synchronous contractions of LN capsule smooth muscles, intranodal pressure can increase, thus pumping the lymph from the LN into afferent lymph vessels. The values of intranodal, or transmural pressure at rest (or at diastole in the language accepted for lymph vessels [9,14]) and during maximal contraction (at systole) are so far unknown.

Here we studied passive and active mechanical properties of LN CS and capsule strain and calculated transmural pressure during contraction and relaxation of smooth muscles in LN capsule.

MATERIALS AND METHODS

Experiments were carried out CS from bovine mesenteric LN. Round LN were dissected (n=17) from the mesentery of animals (body weight 320-400 kg) 15 min after bleeding. Round LN (equal in three dimensions) were selected for the experiments (diameter ~22.1±2.57 mm) and CS (15×2 mm) were prepared. The first group (n=23) consisted of CS oriented from the node hilus to its convex side (the entrance of afferent lymphatic vessels). CS of the same size (n=16) were cut from opposite LN side in the direction perpendicular to that of the first group of strips. CS were placed in physiological saline containing albumin at 2-4°C. CS thickness was measured using MC-2 ZOOM microscope with micrometer-type ocular lens. LN CS thickness in the absence of tension was 0.290±0.032 mm.

One end of the strip was rigidly fixed in a thermocontrolled chamber (37±0.1°C, BT5-1 thermostat, Termex); the other end was attached to the rod of a FORT-10 force transducer (WPI) with resolution power <1 mg. The force transducer was rigidly fixed to a micromanipulator connected with a micrometer; length measurement error was 0.1% of the strip length. The signal from the force transducer entered a Labmas-

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ter block (an amplifier+an ADC, Pavlov Institute of Physiology, Russian Academy of Sciences) and then a computer where it was processed using the Labmaster software. CS with minimum tension (200 μN) were incubated for 45 min in the chamber perfused with physiological saline containing (in mM): 120.4 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 15.5 $NaHCO_3$, and 11.5 glucose supplemented with 10 g/liter purified bovine serum albumin at 37±0.1°C. Physiological saline was saturated with 95% O_2 and 5% CO_2) for oxygenation and pH maintenance (7.36-7.40).

In the beginning of the experiment, CS length was increased using micromanipulator until 5 mN tension was reached and 5 min later it was reduced to zero tension was recorded [4]. Then, the length of the strip was increased step by step (with 0.5 mm intervals) and the tension was recorded. When stable tension was reached, physiological saline in the chamber was replaced with a solution where Na^+ was replaced with K^++10^{-5} M of norepinephrine hydrochloride (Sigma) [7,12]. When a new stable CS tension level was attained, physiological saline was added to the chamber. CS tension was returned to 0. The strip was stretched again 10 min later, now by 1 mm above the tension taken as 0, and tension was recorded. Stepwise increase in strip length with consequent high-potassium solution was performed until passive tension of the strip reached 100 mN.

Statistical processing of the data was performed using ANOVA, the significance of differences between the capsule strip tensions was estimated using Student's *t* test. Differences were considered significant at $p \le 0.05$.

RESULTS

The increase of CS length led to rapid tension elevation with subsequent relaxation. A new stable tension level was attained within 60-90 sec. In high-potassium solution with norepinephrine, LN CS tension rapidly increased at a rate typical of spontaneous phase contractions [12] and reached a new steady level over 45-60 sec.

Length–passive tension, length–total tension, and length–active tension diagrams for differently oriented LN CS are shown (Fig. 1). The length–tension diagrams were similar to the curves describing this dependence in veins [4,8].

Then, we calculated CS strain at various lengths basing on the data on LN (initial diameter) and CS dimensions (length, width and thickness) and recorded values of passive and total tension. Strain was calculated according to the formula:

$$S=\frac{T}{A},$$

where T is CS tension at the specified length and A is CS cross-section area at the given tension. Diameter of LN at various strip length was calculated according to the formula

$$D=Di\times L/Li,$$

where Di is LN diameter before preparation, L is CS length at the given tension, Li is initial CS length before it was cut from the LN. The data obtained on perpendicularly oriented strips made it possible to

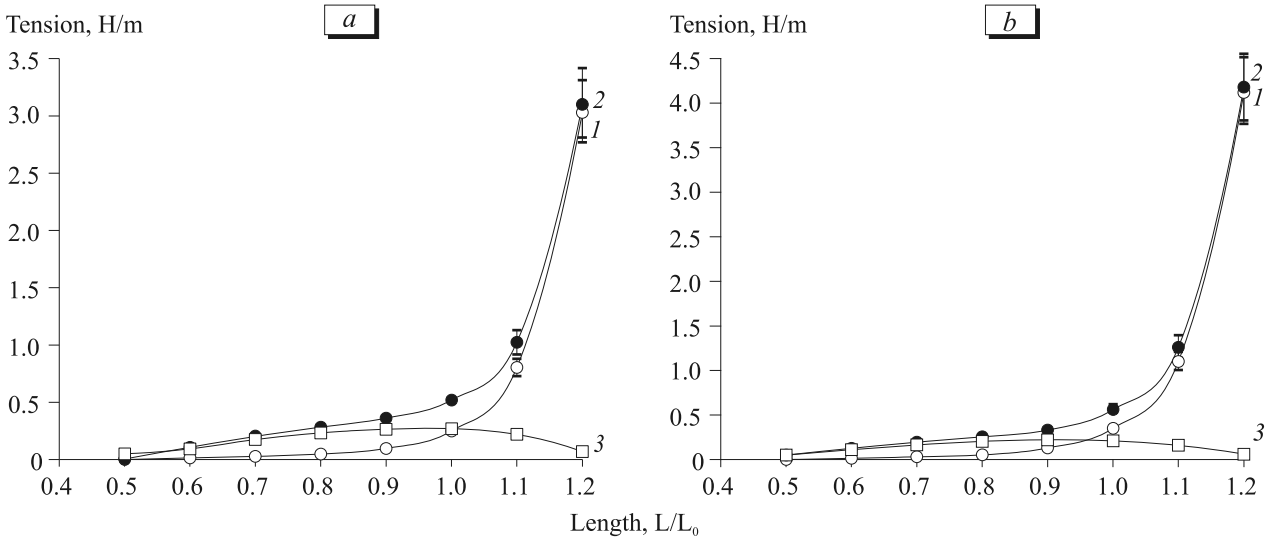

Fig. 1. Length–tension relationship in the LN CS oriented in the direction from the node hilus to the entrance of afferent lymphatic vessels (*a*) and in perpendicularly oriented strips (*b*). *1*) length–passive tension; *2*) length–total tension; *3*) length–active tension. The first two plots were plotted on the basis of direct measurements of the node capsule tension. The length–active tension relationship was plotted on the basis of active tension values calculated by subtraction of passive tension from the total tension. The length of LN CS is shown as L/L_0 for data averaging. Strip length at which maximal contraction was developed was taken as L_0.

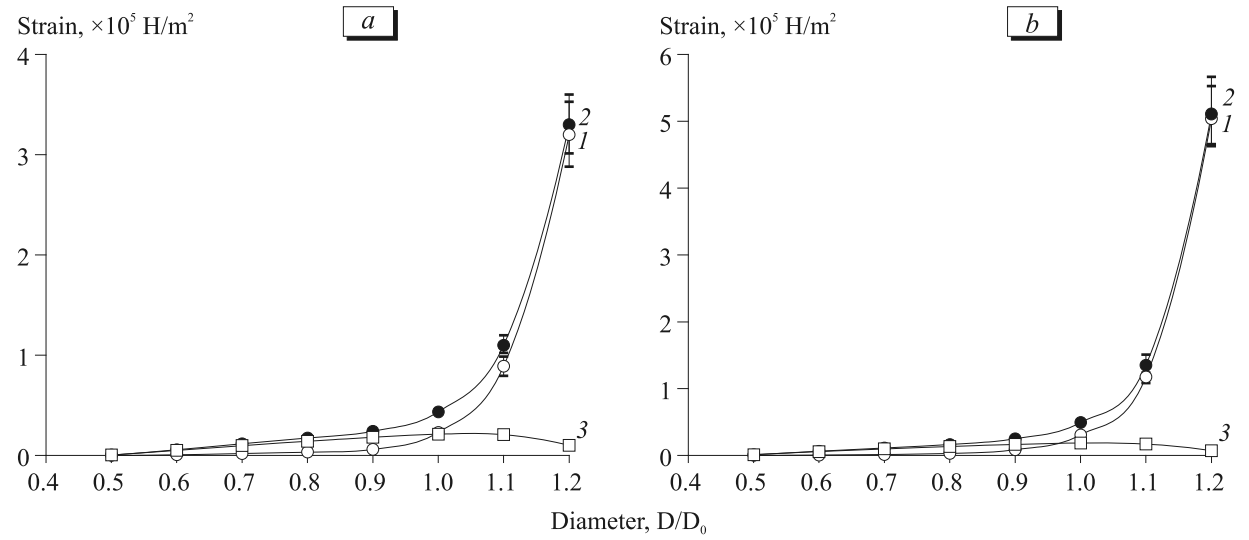

Fig. 2. Diameter–strain relationship in the LN CS oriented in the direction from the node hilus to the entrance of afferent lymphatic vessels (*a*) and in perpendicularly oriented strips (*b*). *1*) diameter–passive strain; *2*) diameter–total strain; *3*) diameter–active strain. For data averaging, the diameter of LN CS is presented as D/D_0, where D_0 is node diameter at maximum active strain of the capsule fragment (strip). Active tension value was calculated by subtraction of passive strain from the total one.

calculate circumferential stress in LN capsule in two perpendicular directions. Plots describing the dependence of passive, total, and active LN CS tension on LN diameter are shown (Fig. 2). Presented tension values were averaged over the wall thickness, however, considering that initially the capsule thickness was less than 1.5% of the node diameter, the stress in the inner layer of the capsule should slightly differ from the stress in the outer layer.

We calculated effective Young elasticity modulus in the node capsule during passive tension and application of high-potassium solution (Fig. 3, *a*). CS stretching from 0.6 to 0.8 L_0 almost did not alter Young modulus value both in physiological saline (about 0.05×10^5 N/m^2) and high-potassium solution (about 0.3×10^5 N/m^2). After subsequent stretching, elasticity modulus of the node capsule increased rapidly and at a length of 1.0–1.1 L_0 reached the value typical of veins [4,5].

Since there are no published data on mechanical properties of LN capsule and intranodal pressure has never been measured, we determined transmural pressure in the node at the state of capsule muscles relaxation and activation on the basis of obtained data. To determine the transmural pressure in the node we used Laplace law for a sphere taking into consideration spherical LN organization and heterogeneity and anisotropy of the capsule material (from morphological data [3] and our data on mechanical properties of

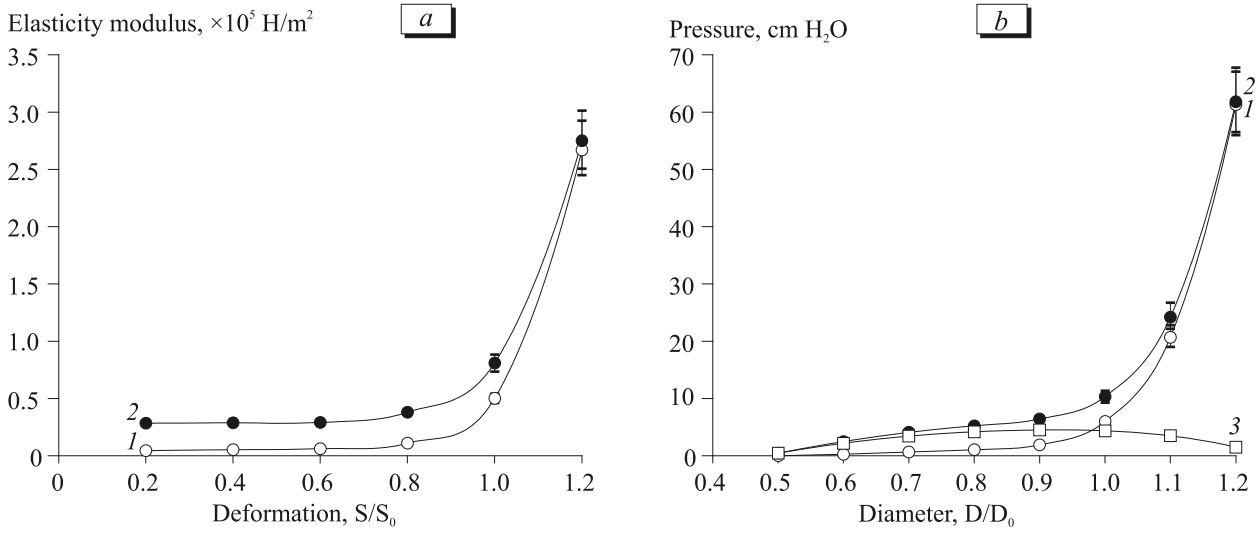

Fig. 3. Relationship between the modulus of elasticity of the LN CS oriented in the direction from the node hilus to the entrance of lymphatic afferent vessels and the strain (*a*) and relationship between the diameter and pressure in the lymph node (*b*). *a*: *1*) in physiological saline; *2*) in high-potassium solution. *b*: *1*) diameter–passive pressure; *2*) diameter–total pressure; *3*) diameter–actively generated pressure.

differently oriented LN CS). Transmural pressure was calculated by the formula:

$$P=(S1+S2)\times h/r,$$

where S1 is circumferential stress in the capsule in the direction from the hilus to the convex side (entrance of afferent lymphatic vessels), S2 is circumferential stress in the capsule in the perpendicular direction, h is capsule strip thickness, and r is node radius at a given capsule tension. Results are presented as a function of normalized radius (R/R_0), which was calculated on the basis of L and L_0 values similar to the D and D_0 calculation (Fig, 3, *b*). We found that even after a nonsufficient increase in the diameter and passive pressure, LN smooth muscles develop a strain which produces a high pressure. Maximum actively generated pressure (4.5 cm H_2O at a diameter 0.9 R_0) was obtained at capsule strip tension corresponding to diastolic pressure of 1.9 cm H_2O. These values of active pressure in LN considerably differ from the parameters recorded previously on a single lymphangion (actively generated pressure 22 cm H_2O at passive pressure 5 cm H_2O [10]). This could come from both lower smooth muscle density in the capsule and from lower ratio between the capsule thickness and lymph node diameter compared to that in the lymphatic vessel.

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