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LENGTH-TENSION CHARACTERISTICS OF BOVINE TRACHEOBRONCHIAL LYMPHATIC SMOOTH MUSCLE

M.K. Ferguson, U. Williams, A.R. Leff, R.W. Mitchell

Departments of Surgery and Medicine, The University of Chicago, Chicago, Illinois, USA

ABSTRACT

Current information regarding the physiology of lymphatic smooth muscle is derived from experiments on mesenteric and thoracic duct lymph trunks. We hypothesized that tracheobronchial lymphatics share many of the same properties possessed by the mesenteric lymphatics, and examined the passive and active length-tension characteristics of the two. Fresh isolated lymph vessel rings were prepared from bovine mesenteric and tracheobronchial lymphatic collectors, mounted in organ baths, and connected to force-displacement transducers. Isometric contractions were induced by exposure to 65mM KCl-substituted perfusate after intermittent ring length changes. Active tension was calculated. Optimal vessel length was greater in tracheobronchial vessel rings, averaging 4.9±0.4mm vs 2.8±0.3mm in mesenteric rings ($p<0.001$). Optimal resting tension and AT_{max} were similar for both truncal types, measuring 738±95mg and 2379±289mg in tracheobronchial vessel rings, and 625±108mg and 2501±320mg in mesenteric vessel rings, respectively. Stress developed at L_0 (optimal length) was similar for tracheobronchial (35.4±4.3mN mm^{-2}) and mesenteric (26±4.3mN mm^{-2}) lymphatics (P=N.S.). The data demonstrate that tracheobronchial lymph vessels are similar to mesenteric lymph vessels in their ability to generate significant stress, and suggest that these lymphatics participate in the regulation of lymph flow.

Mesenteric lymphatics exhibit contractile activity that promotes lymph flow (1). They are primarily under myogenic control, but also respond to neural and pharmacologic stimulation (2-5). In contrast, the thoracic duct in some species displays only moderate smooth muscle activity (6,7). Whether this topographical heterogeneity in the activity of lymphatics is caused solely by differences in gross structure, by variations in their innervation, or by non-uniformity in the character or concentration of pharmacologic receptors on smooth muscle myocytes is not known. It has been hypothesized that the lymphatics are active in regulating lymph flow from the lung and other mediastinal structures (8-10). We postulated that tracheobronchial lymph vessels share contractile properties found in mesenteric lymph vessels.

We compared passive and active length-tension characteristics of bovine tracheobronchial and mesenteric lymphatic vessel rings *in vitro*. We found that tracheobronchial lymphatics are similar to mesenteric lymphatics in that both possess contractile activity with length-tension characteristics similar to those found in other vascular smooth muscle. We demonstrated that normalized force production in tracheobronchial lymphatic smooth muscle is similar to that in mesenteric lymphatics. These findings have important implications regarding the function of tracheobronchial lymphatics in the regulation of lymph flow.

MATERIALS AND METHODS

Tissue Preparation

Blocks of mediastinal and mesenteric tissue from freshly slaughtered cattle (200 to 300kg; male or female) were immersed in saline at 37°C. A 1% solution of Evans blue in saline was injected into lymph nodes in the mesentery and at the tracheobronchial junction, and the tissue blocks were allowed to incubate for 30 min to permit staining of efferent lymphatics. Lymph vessels measuring 2-5mm diameter were ligated downstream and dissected sharply from surrounding tissue using microscissors under a binocular dissecting microscope. The lymph vessels were cut into 5mm width rings (n=14 mediastinal rings from 4 cattle; n=11 mesenteric rings from 3 cattle), each of which was suspended in a water-jacketed bath (10ml) containing buffered Krebs solution (NaCl, 118mM; $NaHCO)_3$, 24mM; KCl, 4.7mM; KH_2PO_4, 1.2mM; $CaCl_2$, 1.6mM; $MgCl_2$, 0.4mM; dextrose, 5.5mM; pH 7.4) at 37°C continuously aerated with 95% O_2 and 5% CO_2. Each vessel ring was mounted onto two rigid stainless steel wires. One wire was attached to a rigidly held glass rod within the bath. The other wire was fixed with a loop of 0000 braided silk ligature to a Grass FT.03 force-displacement transducer that was mounted on a rack and pinion to stretch the tissue preparation to desired lengths. At the conclusion of each experiment, the length of the stretched vessel ring was measured with a micrometer. The rings were gently blotted and weighed (wet weight).

Determination of Length-Tension Relationships

Following a 1 hr equilibration period, resting tension was set at 200mg. Isometric contractions were produced by bathing the lymph vessel rings in 65mM KCl-substituted Krebs perfusate following intermittent tissue ring length increases of 100μ. Resting tension and total tension were measured, and active tension was calculated as the difference between the two. Vessel rings were discarded if spontaneous contractions were present, as these prevented accurate measurement of tensions. Optimal vessel ring length (L_o; one-half the vessel ring circumference) was determined using a micrometer at the point where maximum active tension (AT_{max}) was generated. Vessel ring length was expressed as a percent of L_O, and active tension was expressed as a percent of AT_{max}.

Calculation of Stress Generation

Vessel ring cross-sectional area was calculated as $A=W/L_o\rho$ where A=area in mm^2, W=vessel ring wet weight in mg, L_o=optimal vessel ring length in mm, and ρ was assumed to be 1.06mg mm^{-2} (11). Stress was calculated by dividing AT_{max} by the calculated vessel ring cross-sectional area.

Analysis of Data

Data are expressed as mean ± S.E.M. Differences between means were examined using unpaired t-tests. Statistical significance was claimed when $p<0.05$. Curves for resting tension and total tension were fit to data using an exponential function, while curves for active tension were fit using a third order polynomial function.

RESULTS

Length-Tension Relationships

Changes in resting tension and total tension in response to KCl following successive increases in vessel ring length produced similar non-linear curves in each lymphatic examined *(Fig. 1)*. All lymph vessel rings demonstrated stress-relaxation in response to stretch. Mean resting tension at L_o was 738±95mg for tracheobronchial vessels and 625±108mg for mesenteric vessels (*Table 1*; P=N.S.).

TABLE 1
Comparison of Physical Properties and Stress Generation in Tracheobronchial and Mesenteric Lymphatic Vessel Rings

	Lymphatic Trunks	
	Tracheobronchial	Mesenteric
Optimal length (mm)	4.9 ± 0.4	2.8 ± 0.3*
Weight (mg)	3.88 ± 0.50	2.98 ± 0.40
Resting tension (mg)	738 ± 95	625 ± 108
AT_{max} (mg)†	2379 ± 289	2501 ± 320
Generated stress (mN mm^{-2})	35.4 ± 4.3	26.0 ± 4.3

*$P<0.001$
†AT_{max}=maximum active tension generated in response to 65mM KCl-substituted perfusate

Tracheobronchial vessel circumference was significantly greater than that in lymphatic vessels from the mesentery. Optimal vessel length averaged 4.9±0.4mm for tracheobronchial vessel rings and 2.8±0.3mm for mesenteric vessel rings (*Table 1*; $p<0.001$).

The relationships between active tension and vessel ring length demonstrated a gradual ascending limb, a broad peak at L_o, and a steeper descending limb as L_o was exceeded *(Fig. 2)*. For tracheobronchial vessel rings, AT_{max} was 2379±289mg *(Table 1)*. For mesenteric rings, AT_{max} was 2501±320mg (P=N.S.).

Stress Generation

Tracheobronchial vessel ring mean wet weight was 3.88±0.50mg compared to that in mesenteric vessels of 2.98±0.40 (P=N.S.). Mean calculated cross-sectional area was 0.76±0.07 and 1.06±0.13mm^2 for tracheobronchial and mesenteric vessel rings, respectively ($p=0.06$). Stress developed at L_o was calculated to be 35.4±4.3mN mm^{-2} for tracheobronchial rings and 26.0±4.3mN mm^{-2} for mesenteric rings (*Table 1*; p=N.S.).

DISCUSSION

Rather than being passive conduits for lymph flow, lymph vessels are now known to be actively involved in lymph transport through a variety of mechanisms. Lymph vessels contain smooth muscle that is under myogenic control but also responds to neural and humoral factors (2-5,11). The majority of the data currently available regarding the function of lymphatic smooth muscle were derived from experiments using mesenteric lymphatics. Recent evidence indicates that regional variations exist in the anatomy and physiology of lymph vessels. For example, bovine thoracic duct contains relatively more fibrous tissue (6). In preliminary studies, we demonstrated that mediastinal and mesenteric lymph vessels have different sensitivities to contractile agonists (12). These findings suggest that lymph vessels may be heterogeneous in their structure and function.

Few data exist regarding the physiology of mediastinal lymphatic trunks other than the thoracic duct. Understanding the character of these vessels is becoming increasingly important as their potential role in regulating

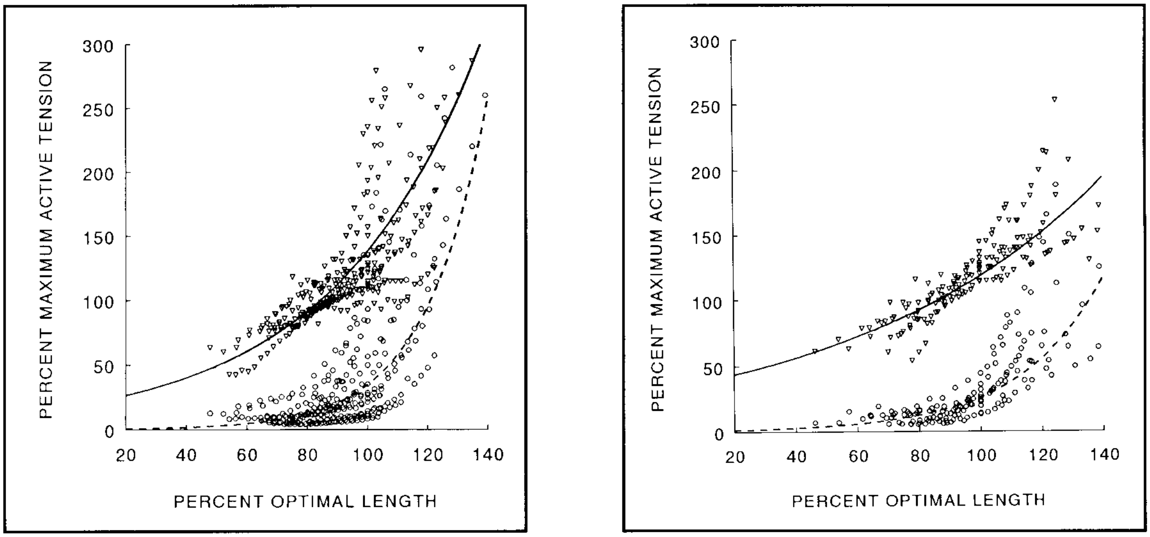

Fig. 1. Bovine tracheobronchial and mesenteric lymphatic vessel ring length-resting and length-total tension relationships. Data are expressed as percent maximum active tension (AT_{max}) vs percent optimal length (L_O). (Left): A total of 308 data points from 14 tracheobronchial vessel rings are included for each curve. The curves describe exponentials expressed as $AT_{max}=0.24\ e^{0.04}\ L_O$ (resting tension (o - - - - o); r=0.75) and $AT_{max}=17.7\ e^{0.02}\ L_o$ (total tension (Δ - - - - - Δ); r=0.84). (Right): A total of 180 data points from 11 mesenteric vessel rings are included for each curve. The curves describe exponentials expressed as $AT_{max}=0.65\ e^{0.03}\ L_o$ (resting tension (o- - - -o); r=0.86) and $AT_{max}=34.2\ e^{0.01}\ L_o$ (total tension (Δ - - - - - Δ); r=0.89).

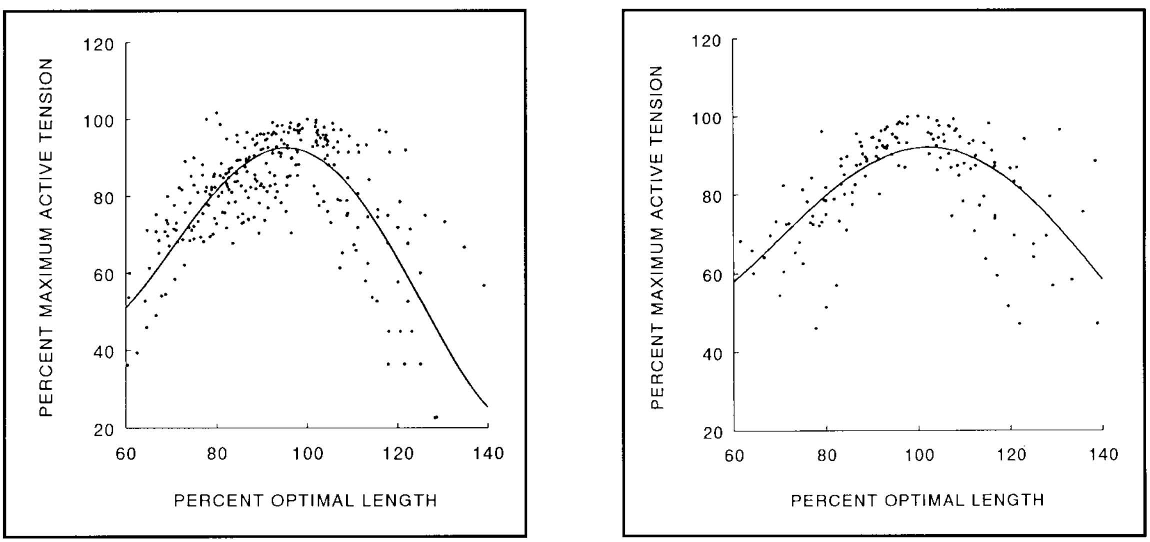

Fig. 2. Bovine tracheobronchial and mesenteric lymphatic vessel ring length-active tension relationships. Data are expressed as percent maximum active tension (AT_{max}) vs percent optimal length (L_o). (Left): A total of 308 data points from 14 tracheobronchial vessel rings are included. The curve was best described by a third order polynomial expressed as $AT_{max}=665.8 - 32.9\ L_o + 0.6\ L_o^2 - 0.004 L_o^3$ (r=0.80). (Right): A total of 180 data points from 11 mesenteric vessel rings are included. The curve was best described by a third order polynomial expressed as $AT_{max}=258.6 - 11.7\ L_o + 0.22\ L_o^2 - 0.001\ L_o^3$ (r=0.79).

interstitial fluid volumes of intrathoracic organs is recognized (8-10). We hypothesized that these lymphatics share many of the characteristics found in mesenteric lymph vessels. This study provides the first data regarding the length-tension characteristics of tracheobronchial lymphatic smooth muscle.

Bovine tracheobronchial and mesenteric lymph collectors were selected for comparison because 1) mesenteric lymphatics have been used previously in evaluating lymphatic smooth muscle activity, and 2) bovine mesenteric and tracheobronchial lymphatics are of sufficiently large diameter that dissection and mounting vessel rings is technically possible with a minimum of tissue damage. Only one of our prepared rings was excluded from analysis because of inadequate smooth muscle activity due to tissue damage, thus emphasizing the quality of tissue preservation and ease in preparation.

The data demonstrate that tracheobronchial lymphatics have a noteworthy capacity for smooth muscle activity. Gradual length increases of the vessel rings resulted in non-linear changes in both resting tension and total tension, indicating that tracheobronchial lymphatics are complex structures that possess both rigid and elastic components *(Fig. 1)*. The degree of tracheobronchial vessel ring elasticity is also similar to that found in mesenteric lymphatic tissue, as evidenced by resting tensions of 25-35% of AT_{max} at L_o. These data compare to values of 10% in airway (13), 30% in visceral (14), and 10% in arterial smooth muscle (media only) (15).

The curves generated for active tension developed in response to KCl activation during gradual lengthening in both tracheobronchial and mesenteric lymphatics are also similar *(Fig. 2)*. Both curves demonstrate a gradual ascending limb, a broad plateau near AT_{max} relative to that typically found in airway and other vascular smooth muscle, and a steep descending limb of the length-tension relationship.

The flatness of the plateau of the active length-tension curve demonstrated for mesenteric compared to thoracic lymphatic preparations is indicative of greater tissue compliance or reduced tissue elastic modulus in mesenteric lymph trunks. In general, tracheobronchial compared to mesenteric lymphatics measure less in cross-sectional area but generate similar AT_{max} (see Results). Force generation per cross-sectional area, however, is slightly greater for tracheobronchial than for mesenteric vessel rings, but not significantly.

Our values for stress generation, 25-35mN mm^{-2}, are less than those reported for other smooth muscles, including visceral (87mN mm^{-2}) (14), airway (108mN mm^{-2}) (13) and arterial (media only; 72-222mN mm^{-2}) (15,16). These findings probably reflect differences in 1) methods of specimen preparation, 2) lymphatic fragility, and 3) the ratio of smooth muscle to other non-contractile components of the preparations. However, accurate quantitative comparisons among these lymph vessels await complete morphometric analysis of lymphatic collectors.

The findings confirm our hypothesis that similarities exist in the length-tension characteristics of bovine tracheobronchial and mesenteric lymphatics. We have demonstrated that tracheobronchial lymph vessels are capable of producing significant active contractile force. The data suggest tracheobronchial lymphatics actively contribute to the flow of mediastinal lymph.

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REFERENCES

1. Zweifach, BW, JW Prather: Micromanipulation of pressure in terminal lymphatics in the mesentery. Am. J. Physiol. 228 (1975), 1326.
2. Allen, JM, NG McHale: Neuromuscular transmission in bovine mesenteric lymphatics.

Microvasc. Res. 31 (1986), 77.
3. Ferguson, MK, HB Shahinian, F Michelassi: Lymphatic smooth muscle responses to leukotrienes, histamine, and platelet activating factor. J. Surg. Res. 44 (1988), 172.
4. Ohhashi, T, S Kobayashi, S. Tsukahara, et al: Innervation of bovine mesenteric lymphatics: From the histochemical point of view. Microvasc. Res. 24 (1982), 377.
5. Ohhashi, T, T Azuma: Variegated effects of prostaglandins on spontaneous activity in bovine mesenteric lymphatics. Microvasc. Res. 27 (1984), 71.
6. Ohhashi, T: Comparison of viscoelastic properties of walls and functional characteristics of valves in lymphatic and venous vessels. Lymphology 20 (1987), 219.
7. Takahashi, N, Y Kawai, T Ohhashi: Effects of vasoconstrictive and vasodilative agents on lymphatic smooth muscles in isolated canine thoracic ducts. J. Pharmacol. Exper. Ther. 254 (1990), 165.
8. Nakahara, K, S Nanjo, M Maeda, et al: Dynamic insufficiency of lung lymph flow from the right lymph duct in dogs with acute filtration edema. Am. Rev. Resp. Dis. 127 (1983), 67.
9. Staub, NC: Pulmonary edema. Physiol. Rev. 54 (1974), 678.
10. Taylor, AE: The lymphatic edema safety factor: The role of edema dependent lymphatic factors (EDLF). Lymphology 23 (1990), 111.
11. Ohhashi, T, T Azuma, M Sakaguchi: Active and passive mechanical characteristics of bovine mesenteric lymphatics. Am. J. Physiol. 239 (1980), H88.
12. Ferguson, MK, U Williams, AR Leff, et al: Heterogeneity of tracheobronchial lymphatic smooth muscle responses to histamine and 5-hydroxytryptamine (abstract). Am. Rev. Resp. Dis. 143 (1991), A768.
13. Stephens, NL, E Kroeger, JA Mehta: Force-velocity characteristics of respiratory airway smooth muscle. J. Appl. Physiol. 26 (1969), 685.
14. Gordon, AR, MJ Siegman: Mechanical properties of smooth muscle. I. Length-tension and force-velocity relations. Am. J. Physiol. 221 (1971), 1243.
15. Herlihy, JT, RA Murphy: Length-tension relationship of smooth muscle of the hog carotid artery. Circ. Res. 33 (1973), 275.
16. Mulvany, MJ, W Halpern: Mechanical properties of vascular smooth muscle cells *in situ*. Nature 260 (1976), 617.

Mark K. Ferguson, M.D.
Associate Professor
Department of Surgery
The University of Chicago
5841 South Maryland, MC 5035
Chicago, IL 60637 USA