The human thoracic duct is functionally innervated by adrenergic nerves

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Telinius N, Baandrup U, Rumessen J, Pilegaard H, Hjortdal V, Aalkjaer, C, Boedtkjer DB. The human thoracic duct is functionally innervated by adrenergic nerves. Am J Physiol Heart Circ Physiol 306: H206–H213, 2014. First published November 8, 2013; doi:10.1152/ajpheart.00517.2013.—Lymphatic vessels from animals and humans have been shown to be innervated. While morphological studies have confirmed human lymphatic vessels are innervated, functional studies supporting this are lacking. The present study demonstrates a functional innervation of the human thoracic duct (TD) that is predominantly adrenergic. TDs harvested from 51 patients undergoing esophageal and cardia cancer surgery were either fixed for structural investigations or maintained in vitro for the functional assessment of innervation by isometric force measurements and electrical field stimulation (EFS). Electron microscopy and immunohistochemistry suggested scarce distribution of nerves in the entire vessel wall, but nerve-mediated contractions could be induced with EFS and were sensitive to the muscarinic receptor blocker atropine and the α-adrenoceptor blocker phentolamine. The combination of phentolamine and atropine resulted in a near-complete abolishment of EFS-induced contractions. The presence of sympathetic nerves was further confirmed by contractions induced by the sympathomimetic and catecholamine-releasing agent tyramine. Reactivity to the neurotransmitters norepinephrine, substance P, neuropeptide Y, acetylcholine, and methacholine was demonstrated by exogenous application to human TD ring segments. Norepinephrine provided the most consistent responses, whereas responses to the other agonists varied. We conclude that the human TD is functionally innervated with both cholinergic and adrenergic components, with the latter of the two dominating.

lymphatic innervation; human lymphatic vessels; isolated vessel; myograph; morphology

SPONTANEOUS CONTRACTIONS generated in the lymphatic vessel wall are essential for the transport of lymph back to the venous circulation, but the mechanisms involved in the initiation, coordination, and modulation of these contractions are not yet understood. Innervation of blood vessels is an important determinant of the vessels’ diameter and thus their resistance to flow. The extent to which vessels are innervated varies according to the type of vessel and localization; for example, small muscular resistance arteries are far more densely innervated compared with larger elastic arteries. While we have a comprehensive understanding of the influence nerves exert on arterial tone, our knowledge concerning lymphatic vessels is limited. Studies of lymphatic vessels in animals and humans using in vitro and in vivo techniques have suggested some functional contribution of nerves in these vessels, although the type and extent of innervation present and its role in pathophysiology of the lymphatic system are unknown.

Adrenergic, cholinergic, and nonadrenergic-noncholinergic (NANC) nerves have been described using fixed or living lymphatic vessels from different species (2, 13, 20). However, adrenergic nerve activity is thus far the only type that has been conclusively demonstrated by direct activation of nerves with electrical field stimulation (EFS) in living tissue. A functional role for adrenergic nerves has also been demonstrated in sheep in vivo (19, 34) and might also be supported by the finding that injection of norepinephrine (NE) increases lymph output in the thoracic duct in humans (18). The contribution of cholinergic and NANC nerves has been suggested by immunohistochemical reactions in fixed tissue or proposed based on reactivity to exogenous compounds administered to tissue in vitro (14, 15, 24, 35). Reports of human lymphatic vessel innervation are limited, with morphological studies describing either an absence of nerve fibers (28), restriction of fibers to the adventitia (4), or diffuse innervation of all layers (22). The human thoracic duct has already been suggested to possess nerves in the subendothelium, media, and adventitia (29). In support of this, recent immunohistochemical analysis of the human thoracic duct revealed markers of sympathetic and parasympathetic innervation in addition to substance P (SP)-, dopamine-, and vasoactive intestinal peptide-positive fibers (22). These findings suggest a complex innervation of adrenergic, cholinergic, and NANC nerves. However, no functional data exist supporting the extent to which these nerves contribute to the physiology of the human thoracic duct.

We (33) have previously demonstrated in vitro that the human thoracic duct contracts in response to NE, consistent with the presence of adrenergic innervation. The aim of the present study was to pursue this finding and corroborate the recent nerve supply pattern described for the human thoracic duct (22) by functionally characterizing the innervation of this vessel using EFS and reactivity to exogenously applied neurotransmitters.

MATERIAL AND METHODS

Tissue Preparation

Human thoracic duct tissue was obtained from 51 patients (35 men and 16 women) during esophageal and cardia cancer surgery at the Department of Cardiothoracic and Vascular Surgery of Aarhus University Hospital (Skejby, Denmark). Informed consent was obtained from each patient, and the protocol was reviewed and approved by the Ethical Committee for the Danish Regional Health Authority. This study was conducted in accordance with the principles of the Helsinki Declaration. The mean patient age was 63 yr (±10 SD).

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Tissue harvest was performed as previously described (6, 33). In brief, a separate disease-free tissue block at level T7–T9 was removed and immediately placed in cold (4°C) physiological saline solution (PSS; for composition, see Solution and Chemicals), and the thoracic duct was subsequently dissected free from surrounding connective tissue and fat under a stereomicroscope.

Isometric Tension Measurement

The portion of the thoracic duct dissected from the tissue block was typically 2–5 cm in length. Seventy-two thoracic duct ring segments (2 mm long, devoid of valves) were prepared and mounted on 40-μm wires in multichannel myographs (DMT 610M, Danish Myo Technology) for isometric force measurements. The preparation and mounting procedures as well as the experiments were performed in PSS. Vessels were maintained at 37°C in PSS equilibrated with a mixture of 21% O2 and 5% CO2 throughout the experiments (pH 7.4).

Vessels were permitted to equilibrate for 30 min at 37°C after being mounted. Each ring segment was normalized by setting them to an internal diameter where the wall tension was equivalent to a transmural pressure of 21 mmHg [the value at which vessels produce maximal active tension (33)] using the DMT Normalization module for Chart software. The average diameter of the vessels after normalization was 2.0 ± 0.2 mm.

Isometric force development (in mN) was recorded at 40 Hz with a Powerlab 4/25 (AD Instruments) using Chart (version 5.5.6) software. Data files were saved for offline analysis. Force data were converted to tension (in N/m) by dividing the force (in mN) by two times the segment length (in mm).

EFS

The standard stainless steel jaws of the myograph (Danish Myo Technology) were replaced with plastic jaws featuring integrated electrodes, which were connected to a current stimulator (DMT CS200). This configuration permits short-interval bursts of currents that induce electric fields concentrated around the vessel. After normalization, ring segments were allowed to equilibrate for a further 30 min. The startup procedure for all ring segments was stimulation with 10 μM NE for 3 min to determine vessel reactivity and, after washout, stimulation with 15 s of EFS with the following settings: 80 mA, 16 Hz, and 0.1-ms pulse duration. Thereafter, frequency-response curves (2, 4, 8, 12, 16, and 20 Hz) were obtained with 60-s stimulation duration and a 120-s interval between each step. The current was set to 80 mA with a pulse duration of 0.1 ms. Preliminary experiments with lower current intensity did not elicit any contractions.

Experimental Protocols

All vessels used in this study were assessed by an initial NE stimulation. Vessel segments demonstrating reactivity to NE were used for one of the following protocols.

Reactivity to neurotransmitters. PROTOCOL 1. Vessels were incubated first with 1 μM neurotensin Y (NPY; n = 7) and, after washout, 10 μM SP (n = 4).

PROTOCOL 2. Vessels were preincubated for 20 min with 100 μM N-nitro-l-arginine methyl ester hydrochloride (l-NAME; n = 6), which was also present for the remainder of the protocol. Vessels were incubated with 10 μM ACh (n = 6). Four of the vessels were also incubated with melanocline (MCH; n = 4). In the case of a response, the incubation was repeated in the presence of 1 μM atropine.

PROTOCOL 3. A possible role for fast voltage-gated Na+ channels (VGSC) in the generation of spontaneous contractions was evaluated by incubation with 0.1 and 1 μM tetrodotoxin (TTX; n = 6).

EFS protocols. EFS SERIES 1. After the startup procedure, three frequency-response curves were performed with 15-min intervals between each (n = 5).

EFS SERIES 2. In a separate set of vessels, an initial frequency-response curve was repeated in the presence of either 1 μM phentolamine (n = 10), 1 μM TTX (n = 4), or 1 μM atropine (n = 8). In vessels from four patients used for the atropine EFS series, a subsequent EFS series was performed in the presence of 1 μM atropine and 1 μM phentolamine (n = 4).

Non-EFS experiments. Neurotransmitter release was investigated indirectly with 125 mM K+–containing PSS (KPSS; n = 5, all NaCl substituted with KCl) and with 10 μM tyramine (n = 5). Ring segments were activated with KPSS, and the same procedure was repeated in the presence of a combination of 1 μM phentolamine, 10 μM propranolol, and 10 μM suramin. The same protocol was repeated but with tyramine stimulation instead of KPSS.

Solution and Chemicals

All salts and drugs were purchased from Sigma-Aldrich. All drugs were dissolved in distilled water, except for TTX (acetate buffer), and were stored in aliquots at −20°C until required. PSS of the following composition was used (in mM): 119 NaCl, 4.7 KCl, 1.17 MgSO4, 25 NaHCO3, 1.18 KH2PO4, 0.026 EDTA, 5.5 glucose, and 1.6 CaCl2. PBS was composed of 55 mM Na2HPO4, 13 mM NaH2PO4, and 57 mM NaCl adjusted to pH 7.50 with NaOH. Fixatives, paraformaldehyde, and glutaraldehyde (Alfa Aesar) were purchased from VWR Bi and Berntsen.

Immunohistochemistry

Eight thoracic ducts were fixed in 4% paraformaldehyde for 1 h and stored in PBS at 4°C until paraffin embedding and sectioning (4 μm) were performed. Sections were stained with 1:8,000 S100 antibody (z0311, Dako) for immunohistochemical detection of nerves. All sections were counterstained with Mayer’s hematoxylin. Immunopositive segments were counted in each coronal section using ImageJ (National Institutes of Health), and the density was determined by dividing the number of immunopositive segments with the volume of the section, i.e., total area × wall thickness (including all layers of the vessel wall).

Electron Microscopy

Electron microscopy was performed as previously reported (6). In brief, immediately after resection, pieces of the thoracic duct from two patients were removal immersed in a modified Karnovsky fixative (8 + 8% or 4 + 4% glutaraldehyde and paraformaldehyde). Thoracic duct tissue was fixed and stored at 4°C for several days before a thorough wash and immersion in PBS. Sections were cut, further processed, and examined in a Philips Morgagni 260 EM microscope at 80 kV.

Statistical Analysis

All responses presented represent average tension during the complete stimulation period. Relative tension is defined as the average tension during the period of interest divided by the average tension during the initial NE stimulation. Data were analyzed using Microsoft Excel and GraphPad Prism. Although a single duct specimen from one patient could provide many ring segments, statistical analysis was performed. Data were stored in Excel and GraphPad Prism. Although a single duct specimen from one patient except symbols with error bars in which the symbol values always refer to the number of patients in the group. All symbols in the figures represent one patient except symbols with error bars in which the symbol represents the mean for all patients. Data are provided as means ± SE. For EFS frequency-response curves, two-way ANOVA was used to test for a difference between groups. The remaining experiments were tested with either one-way ANOVA (with Bonferroni posttest) or a paired Student’s t-test, where P values of <0.05 were considered significant.
Contractile responses to ACh and MCh were blocked by atropine.

**EFS**

The EFS protocol induced phasic contractions with the average tension increasing with stimulation frequency (Fig. 2A). Average tensions at each step (2, 4, 8, 12, 16, and 20 Hz) were 5%, 10%, 27%, 48%, 47%, and 53% of the initial 10 μM NE contraction. The reproducibility of the EFS protocol (80 mA, 0.1-ms pulse duration, 2, 4, 8, 12, 16, and 20 Hz) was evaluated by repeating the series three times. The response was identical in this three consecutive stimulation series (Fig. 2B).

Blockade of VGSCs with TTX disables the normal means by which the nerves depolarize and release neurotransmitters. To elucidate whether the EFS-induced responses were dependent on TTX-sensitive VGSCs, the EFS series was repeated in the presence of 1 μM TTX, which completely abolished phasic contractions with only a minor increase in baseline tension (Fig. 3). In a separate series of experiments, TTX was added to spontaneously active vessel segments, which resulted in a complete inhibition of phasic contractions in four of six vessels at 0.1 μM (Fig. 4). After an increase in concentration to 1 μM, only one vessel remained spontaneously active but at half the initial frequency and unaltered amplitude. The inhibitory effect of TTX occurred within 1 min of application but was transient as the spontaneous activity slowly reinitiated after ~10 min in the continued presence of TTX. The inhibition of spontaneous contractions by TTX suggests that it affects not only the nerves but also smooth muscle cells directly. We then performed a series of experiments to block the postsynaptic effect of neurotransmitters released by noradrenergic and cholinergic nerves. The nonsubtype-selective α-adrenoceptor blocker

![Fig. 1](image-url) Raw data traces depicting the responses to 10 μM norepinephrine (NE), 1 μM neuropeptide Y (NPY), and 10 μM substance P (SP) (A) as well as 10 μM NE, 10 μM ACh, and 10 μM methacholine (MCh) (B), where the MCh response is inhibitable by atropine. For simplification, the periods between the stimulations have been cropped out and the time axis is therefore discontinuous in both traces. *Washouts.

**RESULTS**

**Reactivity to Neurotransmitters**

The neurotransmitters NE, SP, and NPY applied exogenously to ring segments of the human thoracic duct resulted in force development. NE induced high-frequency, small-amplitude oscillations upon an increased baseline tension (Fig. 1A), as previously described (33). NPY and SP produced more inconsistent responses. Phasic contractions were observed in six of seven vessels incubated with NPY (Fig. 1A). The number of contractions and time of onset varied: some vessels generated contractions after 10 s, others after 5 min, and contraction frequency varied between 0.1 and 5 contractions/min. In the case of SP, the time of onset did not show any variation, and contractions were induced immediately upon application. The contractions elicited by SP were, however, heterogeneous: some vessels reacted with a large-amplitude contraction with tension returning close to baseline within 3 min (Fig. 1A), whereas other vessels continued to maintain tension but with small-amplitude, high-frequency oscillations superimposed upon the tonic constriction, similar to that observed with NE. Average tensions during the incubation periods with SP and NPY were 105 ± 27% and 38 ± 8% of the initial 10 μM NE response, respectively. Analysis by one-way ANOVA found no significant difference between NE, NPY, and SP responses ($P = 0.0946$). Force production varied with ACh (in the presence of l-NAME to eliminate NO production from the endothelium): ACh induced oscillatory contractions equivalent to a high concentration of NE in one vessel (Fig. 1B) and one or two large contractions in two vessels while having no effect on tension in the remaining three vessels. When ACh induced a contractile response, MCh application also induced a response that had the same shape and magnitude (Fig. 1B).

![Fig. 2](image-url) A: representative trace of 10 μM NE stimulation and electrical field stimulation (EFS) frequency-response curves in the same vessel. B: cumulative data from three consecutive EFS frequency-response series demonstrating no decline in response after three series ($n = 5$). Data were normalized to the tension generated by the initial 10 μM NE stimulation from the startup procedure and are presented as relative tension. Error bars represent SEs.
phenolamine (1 μM) reduced the response after an EFS series
(P = 0.0395). The muscarinic receptor blocker atropine also
reduced the response (P < 0.0001), and the subsequent addi-
tion of phenolamine reduced the response even further (P <
0.0001); with the two blockers present, the response was
almost completely blocked (Fig. 5).

Non-EFS Experiments

Force development to KPSS, which depolarizes the mem-
branes of lymphatic smooth muscle cells and nerves, was
similar in the absence and presence of a combination of
nonspecific α-adrenoceptor, β-adrenoceptor, and ATP receptor
blockers (phenolamine, propranolol, and suramin, respectively;
Fig. 6A). Tyramine, a sympathomimetic and catecholamine-
releasing agent, stimulated contractions that were 40% of the
initial 10 μM NE-induced response. The tyramine response
could be significantly reduced by the combination of phentol-
amine, propranolol, and suramin (Fig. 6B).

Immunohistochemistry and Electron Microscopy

Staining with S100 antibody revealed few nerves diffusely
distributed in the entire vessel wall (Fig. 7A), i.e., no apparent
specific localization to the subendothelial area or the media-
adventitial border. Quantification of the nerve density revealed
3.3 ± 1.5 × 10⁻⁶ nerves/μm³. Corresponding to observations
with light microscopy, nerves were scarce in the electron
microscope analysis and generally not well preserved. Larger
nerve trunks containing glial cells, and nerve fascicles were
seen in the adventitia. These trunks were closely ensheathed by
long, flattened processes of interstitial Cajal-like cells (aster-
isk) with occasional caveolae (Fig. 7, B and C). Synaptic
configurations between nerve terminals and interstitial Cajal-
like cells were not seen.

DISCUSSION

The present in vitro study investigated the functional innerva-
tion of the human thoracic duct. We could elicit contractions
in a frequency-dependent manner with EFS and with the
catecholamine-releasing monoamine tyramine. EFS-induced
contractions were almost completely abolished with TTX and
with the combination of atropine and phenolamine.

Direct and Indirect Activation of Nerves

We found that tyramine-elicited contractions could be re-
duced with a broad spectrum of blockers and that EFS-induced
contractions could be reduced with phenolamine. Both find-
ings are consistent with the presence of adrenergic (sympa-
thetic) nerves. An almost complete inhibition of the EFS
response was achieved when phenolamine and atropine were
combined, which strongly argues that the EFS response is
nerve mediated. A sympathetic innervation would also be in
agreement with the findings of strong reactivity to NE (21, 27,
30), a finding that was confirmed in this study. Previous studies
by both Russell et al. (27) and Igarashi et al. (16) on animal
thoracic ducts have described EFS-induced contractions that
were blocked with both phenolamine and TTX. Of note, the
EFS protocols and stimulation parameters used in our study
were similar to those used in animal studies.

The finding that the EFS response was reduced with atropine
but that direct incubation with ACh or MCh (in the presence of
L-NAME) resulted in heterogeneous responses (ranging be-
tween strong contractions to no contractions) was surprising.
The ACh and MCh responses were, however, blocked by
atropine, thus confirming a muscarinic receptor-mediated ef-
fect. Our data suggest that functional cholinergic nerve fibers
are not consistently present in the thoracic duct. It is also
possible that part of the atropine effect on the EFS response

Fig. 3. A: representative trace of EFS series performed in the presence of 1 μM tetrodo-
toxin (TTX). The normal response for this vessel in the absence of TTX is shown in Fig.
2A. B: cumulative data from four vessels showing complete inhibition of the EFS-in-
duced response (P < 0.0002). Data were normalized to the tension produced after in-
cubation with 10 μM NE in the startup pro-
cedure and are presented as relative tension.
Error bars represent SEs.

Fig. 4. A: raw data trace depicting the effect of subsequent addition of 0.1 and 1 μM TTX on
spontaneous contractions. B: summarized data from six patients demonstrating a complete
abolishment of phasic contractions in five of
six patients.
could be due to an \( \alpha \)-adrenoceptor blocking effect (17), as atropine has been reported to reduce \( \alpha \)-adrenoceptor-mediated contractions by 20% in the concentration often used to block muscarinic receptors. Cholinergic nerve fibers have been previously demonstrated in the human thoracic duct from patients of the same age span as in this study using immunohistochemistry (22). These authors also demonstrated that the age-related decline of nerve fibers was more pronounced for cholinergic fibers than for adrenergic fibers. Cholinergic nerve fibers have been previously demonstrated in the human thoracic duct from patients of the same age span as in this study using immunohistochemistry (22). These authors also demonstrated that the age-related decline of nerve fibers was more pronounced for cholinergic fibers than for adrenergic fibers.

Fig. 5. Assessment of adrenergic and cholinergic components of the EFS-evoked response. A: incubation with the nonspecific \( \alpha \)-adrenergic blocker phentolamine significantly reduced the EFS response \( (P = 0.0395) \). B: the muscarinic receptor antagonist atropine also significantly reduced the EFS response \( (P < 0.0001) \). C: the subsequent addition of phentolamine upon atropine further reduced the response compared with atropine alone \( (P < 0.0001) \). Data were normalized to the tension generated by the initial 10 \( \mu \)M NE stimulation from the startup procedure and are presented as relative tension. Error bars represent SEs.

Reactivity to Neurotransmitters

To our knowledge, there have been no functional demonstrations of the presence of NANC nerves in lymphatic vessels. Several of the NANC neurotransmitters have been investigated by direct incubation (13, 14, 35). We also tested the reactivity of the human thoracic duct to SP and NPY and found that both agonists induced contraction. The SP response was approximately equivalent to that of NE, but NPY induced only about half of the tension produced by NE; the statistical analysis for this data did not reach significance. Reactivity to SP has been proposed to play a role in different pathophysiology affecting the collecting lymphatics (7). While reactivity to SP has been demonstrated in both rats (9) and guinea pigs (25), less is known about the reactivity of human lymphatics: our study demonstrates that SP is a vasoconstrictor in the human thoracic duct. It is possible that several of the less abundant neurotransmitters (i.e., NANCs) act as modifiers of the dominant neurotransmitters (e.g., NE). If this is the case, then the true reactivity of the duct to the combination release of these
neurotransmitters is not possible to assess by separate incubations with the various substances.

Morphology and Immunohistochemistry

Using immunohistochemistry, we found sparse positive staining for S100, a protein expressed in the nerve-supporting Schwann cells. An early anatomic study (29) of the human thoracic duct using silver nitrate staining described three extensive nerve plexi in the adventitia, the muscular layer, and the subendothelial layer. Our findings confirm the presence of nerves, but we did not find any plexi. It is likely that the previous description of the three plexi is due to labeling of interstitial Cajal-like cells, which have now also been described in the human thoracic duct (6), since it is known that silver nitrate may stain these cells as well as nerves (26). In contrast to arteries, where nerves are located to the media-adventitia border, nerves were primarily located in the muscular layer (32). In this aspect, the innervation resembles that of veins, which might be associated with the common embryonic origin of veins and lymphatic vessels (31). The estimated nerve density in our study was higher than that reported in a previous study (22) of human thoracic ducts. Possible reasons for this difference could be ascribed to our use of midthoracic thoracic duct segments compared with the lumbar and cervical regions of the previous report. Furthermore, we have analyzed a Schwann cell marker on thin coronal sections as opposed to a neuronal marker on thick sagittal sections. We therefore view our report as a valuable addition to the report by Mignini and colleagues (22).

TTX and Lymphatic Contractility

During EFS, an electrical field is created over the vessel that has the potential to depolarize all cells in the vessel wall. Nerves typically have a lower threshold than smooth muscle cells, which makes it possible to selectively activate nerves with EFS (23). The standard approach to confirm the nerve selectivity of the EFS protocol is to repeat the EFS series in the presence of TTX to block VGSCs, which are heavily expressed in nerves and rarely in smooth muscle cells. If TTX abolishes the EFS response, it is assumed that the response is nerve mediated, and any remaining response is presumed to reflect a direct activation of smooth muscle cells. This approach is often used for arterial preparations, where VGSCs are localized to nerves only. Although VGSCs have previously been described in animal lymphatic smooth muscle cells (3, 12), it is not known if human lymphatic smooth muscle cells express this channel. Patch-clamp experiments of sheep lymphatic cells have shown that 90% of the fast Na+ current is available at the resting membrane potential and that action potentials could not be elicited in the presence of TTX (12). Lymphatic vessels can contract synchronously over several centimeters, and VGSCs, given their fast activation kinetics, would be an ideal candidate to spread depolarization over such distances. The physiological role of lymphatic vessels is to pump fluid, and efficient activity is dependent on proper propagation of the contractile wave. The important role of VGSCs for lymphatic pumping has been demonstrated in a study by Convery and colleagues (8), in which TTX disrupted the contractile wave of a cannulated lymphatic vessel and reduced flow. Our preliminary findings from the human thoracic duct suggest that VGSCs play a major role in human lymphatic vessel contractility, and further investigations into the localization and role of this channel are warranted.

Physiological Significance

The exact role and importance of nerves in the lymphatic vessels remain unclear. In the arterial vasculature, nerves have been shown to be important for coordinating vasomotion (5), and a similar function in the lymphatic vasculature could be possible. Hayashi and colleagues (11) proposed that one of the functions of sympathetic nerves is to increase the return to the blood vasculature in extreme situations, such as hemorrhage. It may be that during blood loss, and similar conditions with reduced circulating blood volume, that the most important objective is to increase flow in as many small lymphatic vessels as possible. The resultant increase in fluid load to the thoracic duct may be adequate enough stimulus to increase activity in this vessel, making nervous control of the thoracic duct in this situation redundant. It could also be that the thoracic duct in a high-flow condition works as a conduit vessel instead of an active pump, and, in this instance, stimulating adrenoceptors (thus reducing diameter and increasing resistance) would be
deleterious to lymph flow (10). Despite the uncertainty regarding the exact physiological role of lymphatic innervation, several seminal articles (20–22) have already provided us with an extensive insights. It is still, however, critical to gain knowledge of the human innervation pattern and function since innervation can vary with species. Hollywood and McHale (13) showed that ATP is an important cotransmitter in sheep mesenteric lymphatic vessels, whereas NE is the sole transmitter in bovine lymphatic vessels (21). In contrast to previous investigations with animal lymphatics, in the present study, we provide data from EFS experiments suggestive of a functional cholinergic innervation in the human thoracic duct. Furthermore, we observed that the human thoracic duct differs with its canine counterpart by responding with phasic contractions to EFS: the thoracic duct in dogs does not produce any phasic contractions but only an increased baseline tension (16, 27). While studies on human tissues often, by virtue of the limitations to the population group available to study, provide less mechanistic or molecular insights than their animal-based counterparts, findings such as those we present here reiterate the importance in examining human tissues as fundamental differences in physiology exist between species.

Summary

Our study has addressed the functional impact of innervation for contractile function of the thoracic duct. We have shown that several neurotransmitters, when applied exogenously, elicit contractions in the human thoracic duct. Furthermore, we have shown that EFS induces nerve-mediated contractions. Contractions were reduced by α-adrenoceptor and muscarinic receptor blockers, demonstrating the presence of functional adrenergic and cholinergic nerves. However, the functional contribution of total innervation of the thoracic duct appears low. An interesting additional observation in this study was that Na⁺ channels are crucial for human lymphatic vessels to generate phasic contractions. This finding further highlights the disparity between blood and lymphatic vessels and provides us with insights into the excitation-contraction coupling of smooth muscle cells in the human lymphatic vascular wall.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


