The contribution of K⁺ channels to human thoracic duct contractility

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Telinius N, Kim S, Pilegaard H, Pahle E, Nielsen J, Hjortdal V, Aalkjaer C, Boedtkjer DB. The contribution of K⁺ channels to human thoracic duct contractility. Am J Physiol Heart Circ Physiol 307: H33–H43, 2014. First published April 28, 2014; doi:10.1152/ajpheart.00921.2013.—In smooth muscle cells, K⁺ permeability is high, and this highly influences the resting membrane potential. Lymph propulsion is dependent on phasic contractions generated by smooth muscle cells of lymphatic vessels, and it is likely that K⁺ channels play a critical role in regulating contractility in this tissue. The aim of this study was to investigate the contribution of distinct K⁺ channels to human lymphatic vessel contractility. Thoracic ducts were harvested from 43 patients and mounted in a wire myograph for isometric force measurements or membrane potential recordings with an intracellular microelectrode. Using K⁺ channel blockers and activators, we demonstrate a functional contribution to human lymphatic vessel contractility from all the major classes of K⁺ channels [ATP-sensitive K⁺ (KATP), Ca²⁺-activated K⁺, inward rectifier K⁺, and voltage-dependent K⁺ channels], and this was confirmed at the mRNA level. Contraction amplitude, frequency, and baseline tension were altered depending on which channel was blocked or activated. Microelectrode impalements of lymphatic vessels determined an average resting membrane potential of −43.1 ± 3.7 mV. We observed that membrane potential changes of <5 mV could have large functional effects with contraction frequencies increasing threefold. In general, KATP channels appeared to be constitutively open since incubation with glibenclamide increased contraction frequency in spontaneously active vessels and depolarized and initiated contractions in previously quiescent vessels. The largest change in membrane voltage was observed with the KATP opener pinacidil, which caused 24 ± 3 mV hyperpolarization. We conclude that K⁺ channels are important modulators of human lymphatic contractility. Lymphatic vessels; membrane potential; potassium channels; thoracic duct; human.

LYMPHATIC COLLECTING VESSELS have an intrinsic capacity to generate phasic contractions that enable lymph to be pumped away from tissue and eventually back to the venous circulation. The intrinsic contractile activity of lymphatic vessels is triggered by an underlying electrical activity in lymphatic smooth muscle cells (LSMCs) of the vessel wall. The electrical activity of LSMCs has been measured by various techniques in several animal species but has not yet been demonstrated in human lymphatic vessels. Animal LSMCs produce spontaneous depolarization and action potentials consisting of a rapid depolarization and repolarization of the LSMC membrane potential (Vm) without a plateau phase (30). Intracellular microelectrode impalements of bovine and guinea pig mesenteric collecting lymphatic vessels (70–500 μm in diameter) have shown that the resting membrane potential (RMP) of LSMCs averages around −60 mV (27–29, 32, 33), whereas the lymphatic endothelium in the guinea pig is more hyperpolarized at around −70 mV (32).

Four different classes of K⁺ channels have been identified in animal lymphatic smooth muscle: 1) Ca²⁺-activated K⁺ (Kca) channels (6), 2) voltage-dependent K⁺ (Kv) channels (2), 3) ATP-sensitive K⁺ (KATP) channels (16, 33), and 4) inward rectifier K⁺ (Kir) channels (1, 32). The presence of these distinct types of K⁺ channels has been demonstrated using pharmacological blockers and activators in different settings: either by measuring contractile activity in a wire or pressure myograph or by changes in electrical properties using intracellular microelectrodes, the double sucrose-gap technique, or patch-clamp electrophysiology on isolated LSMCs. Currently, we have no knowledge of the K⁺ channels expressed in human lymphatic vessels or what their functional importance may be. The present study investigated isometric force and Vm responses of human lymphatic vessels to blockade and activation of K⁺ channels.

MATERIALS AND METHODS

Tissue Preparation

Human thoracic duct tissue was obtained during esophageal and cardiac cancer surgery at the Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital (Skejby, Denmark). All patients were offered to participate in the study and no patient declined. Informed consent was obtained from each specific patient, and the protocol was reviewed and approved by the ethical committee for the Danish Regional Health Authority. The study was conducted in accordance with the principles of the Declaration of Helsinki. Thoracic ducts were harvested from 54 patients with an average age of 65 ± 1 yr (38 men and 16 women). It is important to note that the thoracic ducts came from aged patients with a malignancy; thus, local and systemic changes as potential influencers of our results cannot be excluded. Tissue harvest was performed as previously described (5, 25, 26). In brief, a separate tissue block at level T7–T9 was removed and immediately placed in cold (4°C) physiological saline solution (PSS), and the thoracic duct was subsequently dissected free from the surrounding connective tissue and fat under a stereomicroscope. In a few experiments (where indicated), mesenteric lymphatic vessels (internal diameter <500 μm) were harvested from patients undergoing bariatric surgery (3 women, age: 33, 36, and 45 yr) at the Department of Surgery, Viborg Hospital, and handled in the same way as the thoracic duct specimens.

Isometric Tension Measurements

The portion of the thoracic duct dissected from the tissue block was typically 2–5 cm in length. Ring segments of the thoracic duct (2 mm long, devoid of valves) were prepared and mounted on 40-μm wires in multichannel myographs (DMT 610M) for isometric force measurements. The preparation, mounting, and experiments were performed in PSS (for composition, see Solution and Chemicals). 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 30–60 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 were determined to produce peak active tension (26)] using the DMT Normalization module for Chart software.

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).

Contraction frequency (in contractions/min), contraction amplitude [defined as peak contraction tension minus baseline tension (in Nm⁻¹)], and baseline tension [defined as the resting tension between contractions (in Nm⁻¹)] were extracted and analyzed. All parameters were analyzed over a 5- to 10-min period when a steady-state condition was reached unless stated otherwise.

**V_m Measurements**

Thoracic duct segments (2 mm) were inverted to facilitate impalement, mounted in a single chamber wire myograph, and normalized to 21 mmHg, as described above. V_m values of LSMCs were measured using sharp glass microelectrodes (AS100F, WPI) pulled on a horizontal puller (P-97, Sutter). Voltage was recorded via an amplifier (Intra 767, WPI) connected to a PowerLab (model 4SP, AD Instruments) and recorded at a sampling speed of 1 kHz using Chart software. Microelectrodes were filled with 3 M KCl (giving an electrode resistance of 40–120 MΩ) and were flexible enough to maintain recording from a single LSMG even during depolarization and activation. The reference electrode was placed in the myograph chamber. Successful penetration and recording were defined as follows: 1) a rapid, negative change in potential upon penetration; 2) a rapid, positive return to the preimpalement level upon withdrawal of the electrode from the cell; and 3) a stable electrode resistance throughout the recording. Values more positive than −30 mV were excluded from analysis unless they displayed action potential or hyperpolarized to pinacidil. Only vessels displaying phasic contractions were analyzed.

**Pharmacological Protocols**

Vessels that developed phasic contractions with stable pattern for >15 min after the normalization were used for one of the following protocols.

**Nonselective K⁺ channel block.** Broad inhibition of K⁺ channels was attempted by either incubation with 1 mM tetrabutylammonium (TEA; n = 3) or 3 mM Ba²⁺ (n = 6). The effect of Ba²⁺ was compared with incubation with 10 μM NE.

**Kᵥ channels.** The large-conductance (BK) channel was blocked with 10 μM paxilline (n = 7) and opened with 10 μM NS-1619 (n = 7). Separate vessels were used for the paxilline and NS-1619 experiments. Small-conductance (SK) and intermediate-conductance (IK) channels were blocked with 0.1 μM apamin and 10 μM TRAM-34, respectively (n = 6), and both opened with NS-309. Vessels were first incubated with either apamin or TRAM-34 and then after 15 min the other drug was also added to the bath; the order the drugs were added was randomized. In a separate series of experiments, NS-309 was added in a cumulative concentration-response manner (0.1, 0.3, and 1 μM, n = 6). 

**Kₐtp channels.** 4-Aminopyridine (4-AP) was added to the myograph bath at a final concentration of either 40 μM (n = 7) or 1 mM (n = 4). Glibenclamide was added to some vessels after incubation with pinacidil to test for reversibility. V_m was measured after the addition of pinacidil and subsequent addition of glibenclamide in three vessels. In seven nonsynchronous vessels or vessels with limited activity that had stopped, 10 μM glibenclamide was added to the bath. V_m was measured in three nonsynchronous vessels before and after addition of 10 μM glibenclamide. Small lymphatic vessels from the human mesentery were tested with the Kₐtp opener cromakalim (1 μM, n = 3), and specificity was confirmed by the subsequent addition of 10 μM glibenclamide.

**Kᵥ channels.** Vessels were exposed to Ba²⁺ in a half-logarithmic cumulative concentration-response curve (1–30 μM, n = 8). Opening of Kᵥ channels was performed by increasing the K⁺ concentration in the bath to 10 mM (n = 7) either by adding a small volume of 125 mM KPSS (to give an isoosmolar elevation in the K⁺ concentration) or by slow superfusion of 10 mM KPSS through the chamber. RMP was measured after elevating the K⁺ concentration to 10 mM (n = 4) and after the addition of 50 μM Ba²⁺ (n = 3).

**Real-Time PCR**

After dissection, segments of the human thoracic duct (five patients) were placed in RNA later (Sigma) and thereafter stored at −20°C until RNA isolation. Total RNA was isolated using the RNeasy Mini Kit and QIAcube system (Qiagen), and first-strand synthesis performed using random decamers and SuperScript III reverse transcriptase (Invitrogen). cDNA was amplified with real-time PCR using the Mx3000P real-time thermal cycler (Agilient Technologies) according to the following protocol: initial activation of Maxima hot start Taq DNA polymerase (Thermo Scientific) and DNA denaturation at 95°C for 4 min and 50 amplification cycles of 10 s at 95°C, 20 s at 55°C, and 30 s at 72°C. The following Taqman gene expression primer/probe sets (Life Technologies) specific for human K⁺ channels were used: KCNA2 (K,1.2 channel; Hs00720656_s1), KCNA1 (K,1.1 channel; Hs01119498_m1), KCN2 (K,6 channel; Hs00265315_m1), KCNJ8 (K,1.6, KATP channel; Hs00958961_m1), and KCNJ11 (K,6.2, KATP channel; Hs00265026_s1). The housekeeping genes TATA box-binding protein (TBP; Hs99999990_m1) and β2-microglobulin (B2M; Hs009999907_m1) were used as internal controls. Duplicate PCR reactions (with reverse transcriptase) were performed for all patients for all genes in addition to no template (without reverse transcriptase) and H₂O controls. The same genes were assayed in the human carotid artery (Stratagene) in parallel. Data were analyzed using the comparative threshold cycle (Ct) method as follows: ΔΔCt = ΔCt (thoracic duct) − ΔCt (carotid artery), where ΔCt = Ct (of the gene of interest) − (average Ct for TBP and B2M). Data are expressed as fold changes using 2ΔΔCt.

**Solutions and Chemicals**

All salts and drugs were purchased from Sigma-Aldrich. With the following exceptions, all drugs were dissolved in distilled water: paxilline (DMSO), TRAM-34 (DMSO), NS-309 (DMSO), NS-1619 (DMSO), glibenclamide (DMSO), cromakalim (DMSO), and pinacidil (ethanol). Drugs were stored in aliquots at −20°C until required. Catalog numbers for the drugs purchased from Sigma-Aldrich were as follows: TEA (T2265), NE (A7257), pinacidil (P1545), paxilline (P2928), NS-1619 (N170), NS-309 (N8161), apamin (A9459), TRAM-34 (T6700), 4-AP (257857), glibenclamide (G6039), and cromakalim (C1055). 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 7.5 with NaOH. NaCl was substituted with KCl in 125 mM KPSS. PSS without sulphate was used for Ba²⁺ experiments and differed from PSS by the replacement of MgSO₄ with equimolar MgCl₂.

**Statistical Analysis**

Data were analyzed using Microsoft Excel and GraphPad Prism. Data are provided as means ± SE; n refers to the number of patients. Student’s t-test was used to compare groups with a P level of <0.05 defined as significant. If identical experiments were conducted on more than one
ring segment or several impalements were performed on the same ring segment, averages were calculated so that the value represents the average for that patient and \( n \) still represents the number of patients.

RESULTS

\( V_m \) Measurements

\( V_m \) values were measured in ring segments from 16 thoracic ducts. RMP ranged from \(-51\) to \(-32\) mV in a total of 70 impalements (Fig. 1B) with an average RMP of \(-43 \pm 3.7\) mV (\( n = 16 \)). Each action potential was followed by a contraction. Occasionally bursts of action potentials occurred, which were reflected in small oscillations on top of the initial contraction (Fig. 1D) in a manner reminiscent of an unfused tetanus-like contraction: in these instances, \( V_m \) returned to baseline before the vessel was completely relaxed and a new action potential was fired.

Baseline Contractile Activity

Vessels used in the pharmacology protocols to evaluate the functional effects of \( K^+ \) channel modifiers had a mean contraction frequency of \( 2.2 \pm 0.3 \) contractions/min and a contraction amplitude of \( 1.7 \pm 0.2 \) Nm\(^{-1}\) (Fig. 1A). Vehicle controls were run separately and showed no effect (data not shown).

Nonselective \( K^+ \) Channel Block

Both TEA and \( Ba^{2+} \) increased contractile activity. A high concentration of \( Ba^{2+} \) (3 mM) induced contractions with a mean tension almost comparable to NE incubation: the mean tension after \( Ba^{2+} \) was, on average, \( 80 \pm 10\% \) of the NE response. TEA induced an increase in contractile activity and occasionally a change in contraction pattern whereby the phasic contractions had small high-frequency oscillations superimposed on the phasic contractions (Fig. 2).

\( K_{Ca} \) Channels

We assessed the effect of isolated incubation with SK and IK blockers (apamin and TRAM-34, respectively) as well as coincubation with both blockers but found no effect on contraction frequency, contraction amplitude, or baseline tension (data not shown; \( n = 6 \)). NS-309, a SK/IK channel opener, also had no effect on any of these parameters (data not shown; \( n = 6 \)). Blockade of BK channels with paxilline increased the contraction frequency from \( 2.1 \pm 0.7 \) to \( 3.2 \pm 0.9 \) contractions/min (\( P = 0.0396 \) by paired Student’s \( t \)-test) without affecting contraction amplitude and baseline tension (Fig. 3). Conversely, opening of BK channels with NS-1619 did not change any of the parameters (data not shown; \( n = 7 \)).

\( K_v \) Channels

We blocked \( K_v \) channels with either a low concentration (40 \( \mu \)M) or a standard concentration (1 mM) of 4-AP. The low concentration increased contraction frequency from \( 1.6 \pm 0.4 \) to \( 3.3 \pm 1.4 \) contractions/min (\( P = 0.0313 \) by paired Student’s \( t \)-test) without altering the other parameters. Incubation with 1

Fig. 1. Cumulative data of contractile activity and membrane potential (\( V_m \)) measurements. A: distribution of the frequency and amplitude of spontaneous contractions in human thoracic ducts. Error bars represent means ± SE; B: distribution of 70 impalements in 16 patients showing a peak around \(-40\) mV. C: simultaneous \( V_m \) (top trace, left y-axis) and force recording (bottom trace, right y-axis) showing single action potentials and contractions. D: simultaneous \( V_m \) and force recording showing action potentials shooting off in bursts resulting in phasic contractions with small oscillations on top.

Fig. 2. Representative trace of incubation with the nonselective potassium channel blocker tetraethylammonium (TEA). The contraction pattern changed from singular phasic contractions into complexes consisting of longer-lasting contractions with burst-like activity superimposed. The appearance is similar to that in Fig. 1D.
mM 4-AP induced a transient increase in contraction frequency in all vessels from an average of $1.2 \pm 0.3$ to $3.5 \pm 0.6$ contractions/min ($P = 0.0703$), calculated during the first 3 min of incubation, which after a few minutes returned to preincubation levels and, in some cases, after $\sim 10$–15 min of exposure, fell below that (Fig. 4). After the initial transient increase in contraction frequency, the contraction amplitude increased from $2.9 \pm 0.3$ to $4.4 \pm 0.5$ Nm$^{-1}$ ($P = 0.0256$ by paired Student’s $t$-test).

**$K_{ATP}$ Channels**

Pinacidil and glibenclamide were used to open and close $K_{ATP}$ channels, respectively. Pinacidil application caused the

![Image](http://ajpheart.physiology.org/)
immediate cessation of spontaneous contractions and reduced baseline tension from 0.56 ± 0.16 to 0.31 ± 0.10 Nm⁻¹ (P = 0.0496 by paired Student’s t-test; Fig. 5). The inhibition of spontaneous contractile behavior was accompanied by a hyperpolarization of 24 ± 3 mV (P = 0.0158 by paired Student’s t-test). Spontaneous activity was reestablished within 5 min after the addition of glibenclamide, and V_m returned to a level similar to that seen before the addition of pinacidil (Fig. 5, A and D). Isolated incubation with glibenclamide increased contraction frequency from 2.6 ± 0.6 to 6.5 ± 1.5 contractions/min (P = 0.0156 by paired Student’s t-test) and reduced contraction amplitude from 2.8 ± 0.5 to 2.3 ± 0.5 Nm⁻¹ (P = 0.0006 by paired Student’s t-test) in spontaneous active vessels (Fig. 6) but did not affect baseline tension (P = 0.5029). In four of seven nonspontaneous vessels, glibenclamide initiated phasic contractions within 10 min. The contractions did not differ from normal spontaneous activity and had normal frequency and amplitude (data not shown). The addition of glibenclamide to vessels without spontaneous activity was accompanied by a 4.2 ± 0.8-mV depolarization. The importance of K_ATP channels for lymphatic function was confirmed in human mesenteric lymphatics in which 1 µM cromakalim abolished contractions completely (data not shown; n = 3). The effect was reversible and returned to normal upon additional incubation with glibenclamide.

**K_ir Channels**

We observed an increase in contraction frequency from 3.2 ± 0.8 to 6.1 ± 2 contractions/min when we blocked K_ir channels with 30 µM Ba²⁺ (P = 0.0429 by paired Student’s t-test), whereas contraction amplitude and baseline tension were unaffected (Fig. 7, A–C). Lower concentrations of Ba²⁺ did not seem to affect any of the parameters. We attempted to open K_ir channels by increasing the K⁺ concentration in the myograph chamber to 10 mM: six of seven vessels generated a transient wave of contractions immediately after the increase in extracellular K⁺ concentration ([K⁺]o), and five of those six vessels resumed normal phasic contractions after the initial transient contraction wave. Phasic contractions in the continued presence of 10 mM K⁺ now occurred with a higher frequency (3.8 ± 0.5 compared with 1.9 ± 0.3 contractions/minute).

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![Figure 5](http://ajpheart.physiology.org.org)

**Fig. 5.** Effect of the ATP-sensitive K⁺ (K_ATP) channel opener pinacidil on contractile activity and resting membrane potential (RMP) in human thoracic ducts. A: representative trace of incubation with pinacidil and subsequent incubation with the K_ATP blocker glibenclamide. B and C: cumulative data showing a cessation of contractions (B) and a significant reduction in baseline tension (C) in the presence of pinacidil (n = 6). D: V_m recording showing hyperpolarization by pinacidil that was reversed by the addition of glibenclamide. Error bars depict means ± SE. *Significant difference compared with control (P < 0.05) using a paired Student’s t-test.
min) and with a lower amplitude (1.4 ± 0.5 vs. 1.7 ± 0.5 Nm⁻¹, n = 5; Fig. 8, A–C). Two vessels changed their contraction pattern to either resemble a spasm (small high-frequency oscillations) or phasic contractions with small oscillations on top of each contraction. RMP depolarized by 3.8 ± 1 mV when we increased [K⁺]o to 10 mM (P = 0.0367 by paired Student’s t-test) and a further 3.7 ± 0.5 mV upon the subsequent addition of 50 µM a² (P = 0.0178 by paired Student’s t-test).

Expression Analysis for K⁺ Channels

Human thoracic duct RNA from five patients was investigated for the presence of the following K⁺ channel families: KCNA2 (Kv1.2 channel), KCNMA1 (BKCa channel), KCNJ2 (Kᵦ₆.1, KATP channel), and KCNJ11 (Kᵦ₆.2, KATP channel). Signals were amplified for all the channels in four of the five patients (Fig. 9), whereas one patient sample was positive for KCNA2, KCNMA1, and KCNJ8 but did not amplify KCNJ2 and KCNJ11. These data confirm that the thoracic duct expresses mRNA for the major classes of K⁺ channels for which we have observed functional pharmacological effects on contractility.

DISCUSSION

In this study, we present novel data from isometric force measurements and Vₘ measurements of human lymphatic vessels that describe the contribution and function of the major K⁺ channel families. Most studies of K⁺ channels in lymphatic vessels have focused on the electrical activity with less focus on the functional effect. In this study, we primarily focused upon the functional effects of disturbing K⁺ channel function, specifically regulation of baseline tension, contraction frequency, and contraction amplitude, and supplemented this with electrical measurements of Vₘ.

Vₘ Measurements

We found that the average RMP of LSMCs in the human thoracic duct (−43 mV) was more positive compared with previous measurements of bovine and guinea pig LSMCs (−60 mV) (27, 32, 33a). If we extend our comparison to other cardiovascular preparations, we find that human LSMCs are similarly or more depolarized than smooth muscle cells in resistance arteries (−45 to −60 mV) (3, 11, 19, 20) and cardiomyocytes (around −70 mV) (17) but comparable to smooth muscle cells in the portal vein (−37 mV) (10). Determining RMP enables us to speculate upon which voltage-gated ion channels could be important for function based on the activation and inactivation thresholds for these channels. We (25) have previously shown the importance of voltage-gated Na⁺ channels for generating spontaneous contractions. A RMP around −43 mV is close to the threshold for both voltage-gated Ca²⁺ channels and some voltage-gated Na⁺ channels, and small changes in RMP could therefore have a large impact on...
function: this was indeed seen as contraction frequencies increased two- to threefold when $V_m$ was depolarized $\geq 5$ mV.

While seminal electrophysiological studies have already provided us with much knowledge of the role of $V_m$ in LSMCs, we believe our work represents a valuable addition. Our electrical measurements were performed on vessels mounted in a wire myograph and stretched to the diameter allowing maximal force production. This provides a very standardized experimental situation with the recording conditions being complementary to those used for our pharmacological assessments on force alone. This is in contrast to previous electrical measurements from nonstretched or cut-open animal vessels (27, 32, 33a). The importance of combined measurements are exemplified by the tetanus-like contractions shown in Fig. 1D, which may not necessarily be predicted based on $V_m$ measurement alone. However, we did have to compromise somewhat in our $V_m$ recordings and mount inverted vessel segments for electrode impalement from the luminal side. This approach has also been successfully used in small resistance arteries, where it has been essential to approach the vessel from the endothelial side (3). Our primary tissue, the human thoracic duct, has a very thick layer of connective tissue in the adventitial layer (5) and electrode approach from the adventitial side consistently failed either due to breakage of the microelectrode or inability to impale a cell [the distribution of LSMCs is not ubiquitous in the thoracic duct wall (5)]. Notably, however, the inverted vessels did not differ from noninverted vessels with regard to their spontaneous contractility and agonist reactivity.

A limitation in our $V_m$ measurements is the lack of corroborative morphological evidence that we had impaled LSMCs. It seems unlikely, however, that we measured $V_m$ from endothelial cells as these are not coupled to LSMCs by myoendothelial projections or gap junctions (5). It would also be highly unexpected for endothelial cells to be electrically coupled to an action potential-generating cell as this would result in massive, rapid changes in endothelial intracellular Ca$^{2+}$ concentration and disturb the production and release of endothelium-derived factors. A similar conclusion was reached by von der Weid and Van Helden (32), where they recorded $10$ mV more hyperpolarized $V_m$ in the endothelium compared with LSMCs and found negligible electrical coupling between these cell types. The human thoracic duct is composed of a heterogeneous population of LSMCs and interstitial Cajal-like cells (5), and we cannot discount the possibility that some of the measurements described here are from the latter cell type. This possibility will be studied further in our laboratory by introducing fluorescent dye into the cytoplasm of the impaled cell via the microelectrode, thereby permitting a correlation between cell morphology and $V_m$. 

![Fig. 7. Effect of Ba$^{2+}$ on spontaneous contractions and RMP in the human thoracic duct. A: representative trace of a cumulative concentration-response curve to Ba$^{2+}$. B: cumulative data showing the increase in contraction frequency (black bars) with 30 $\mu$M Ba$^{2+}$ but unaltered contraction amplitude (gray bars) ($n = 8$). C: simultaneous $V_m$ (top trace) and force recording (bottom trace) showing a slight depolarization after the addition of Ba$^{2+}$. Error bars depict means $\pm$ SE. *Significant difference compared with control ($P < 0.05$) using a paired Student’s $t$-test.](http://ajpheart.physiology.org/)

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We found that all the major K\(^+\) channels (K\(_{ATP}\), K\(_v\), K\(_{Ca}\), and Kir channels) have a role in regulating spontaneous contractile activity. To identify and isolate the presence and contribution of distinct K\(^+\) channels, we used channel-specific drugs. The BK channel has been found in smooth muscle cells from most tissues, including lymphatic vessels, from animals (6). In this study, we confirmed that human lymphatic vessels possess these channels also. KCa channels are activated by an increase in intracellular Ca\(^{2+}\) and membrane depolarization and can potentially regulate baseline tension and contraction amplitude via negative feedback. In our preparation, the BK channel does not seem to have any of these roles but does act as a regulator of contraction frequency, with inhibition of BK channels leading to a 50% increase in contraction frequency. Since the BK channel is activated by depolarization and thus acts as a brake on depolarization, inhibition of this channel could allow the action potential threshold to be reached faster, permitting contraction frequency to increase. It is interesting, however, that the BK channel is not involved in the regulation of contraction amplitude or baseline tension. In both wire and pressure myographs, lymphatic vessels have, in a similar fashion to some arteries, an active component in addition to passive tension in the vessel wall due to stretch (7, 15). In arteries, myogenic tone is partly regulated by BK channels (4), but our wire myograph data do not support that this is the case for lymphatic vessels. It is possible that our use of an isometric wire myograph did not permit this effect to be seen: the pressure myograph is preferred for studying myogenic tone. Arteries have been shown to be more depolarized when maintained under isobaric conditions compared with isometric conditions.
ditions (8, 23), which would increase the number of active BK channels. The remaining K<sub>Ca</sub> channels (SK and IK channels) are rarely found in smooth muscle cells, and we did not find any evidence for a role in lymphatic contractility. However, K<sub>Ca</sub> channels have been described in interstitial Cajal-like cells (SK3 channels) (12, 21) and in lymphatic endothelial cells (32). The human thoracic duct possesses interstitial Cajal-like cells (5), but the functional data presented here suggest that these cells either do not have SK channels or that SK channels on these cells play no role in the spontaneous contractile activity of the tissue. The exact role of K<sup>+</sup> channels in lymphatic endothelial cells is not completely understood. Functionally, myoendothelial coupling appears sparse (1 in 12 endothelial/smooth muscle cells) (32) in animal lymphatics, and, morphologically, we have found no such connections in the human thoracic duct using electron microscopy (5), so it is possible that K<sub>Ca</sub> modifiers could affect endothelial cells without any measurable change in LSMC activity.

It has previously been shown by Beckett and colleagues (2) that 4-AP depolarizes sheep LSMCs. While the experiments of Beckett and colleagues confirm the presence of K<sub>Ca</sub> channels, it is relevant to investigate the effects of K<sub>Ca</sub> inhibition on contractile activity. We found that blockade of K<sub>Ca</sub> channels produced concentration-dependent effects, confirming an active role of these channels in regulating phasic contractions. The concentration-dependent effect could potentially be related to expanding the blocking spectrum of 4-AP to include additional K<sub>Ca</sub> channels, but nonspecific effects cannot be excluded. It is likely that K<sub>Ca</sub> channels are involved in both regulating the frequency and amplitude of spontaneous contractions in the human thoracic duct.

K<sup>ATP</sup> channels are the most thoroughly investigated K<sup>+</sup> channel in lymphatic vessels and have been shown to hyperpolarize and inhibit spontaneous contractions upon activation (16, 33). As expected, we confirmed that K<sup>ATP</sup> channels are present in both the human thoracic duct and mesenteric collecting lymphatics. Unexpectedly, we found that K<sub>ATP</sub> channels appear to be constitutively open since isolated incubation with glibenclamide increased contraction frequency in active preparations. This has not previously been demonstrated in animal lymphatics (16, 32) but has been previously described in the coronary arterial circulation (9, 22). Quiescent vessels (not having displayed any previous spontaneous activity) initiated phasic contractions in four of seven vessels after exposure to glibenclamide, indicating that open K<sub>ATP</sub> channels could be preventing the vessels from generating action potentials and phasic contractions. Glibenclamide depolarized vessels lacking spontaneous activity by just 4 mV, demonstrating how small alterations of RMP can have a significant influence on function. Besides their role in normal physiology, K<sub>ATP</sub> channels have been shown to be activated in a rodent model for inflammatory bowel disease causing the lymphatic dysfunction associated with this condition (14, 31). Given the powerful effect of opening K<sub>ATP</sub> channels (complete abolishment of spontaneous contractions and reduced myogenic tone), they could be candidates for mediating lymphatic dysfunction in humans as well.

As we found that LSMC RMP was around −40 mV, we did not expect a significant contribution of K<sub>ir</sub> channels. This was confirmed by a small depolarization of around −4 mV when [K<sup>+</sup>]<sub>i</sub> was increased to 10 mM, consistent with RMP not strongly influenced by K<sub>ir</sub> channels and governed by the permeability of several ions as determined by the Goldman-Hodgkin-Katz equation. The depolarization was accompanied by an increase in contraction frequency. While we found no significant contribution to RMP from K<sub>ir</sub> channels, a small, functionally relevant contribution did appear to be present: blockade of the channels with Ba<sup>2+</sup> almost doubled contraction frequency and depolarized the vessel ∼4 mV (in 10 mM K<sup>+</sup>).

**K<sup>+</sup> Channel Expression**

We confirmed expression of the major subtypes of K<sup>+</sup> channels in the human thoracic duct in the mRNA level by RT-PCR. The present analysis did not, however, permit us to draw any conclusions about the amount of these channels relative to each other. However, the molecular data do provide supportive evidence for the pharmacological interventions toward K<sup>+</sup> channels that we have made in the present study.

**Clinical Perspective**

Several human diseases caused by mutation in genes encoding K<sup>+</sup> channels exist, but none of these K<sup>+</sup> channelopathies have been linked to lymphedema (18, 24). This suggests that either other K<sup>+</sup> channels in lymphatic vessels compensate for the altered expression of the affected channel or that a lymphatic phenotype requires a more severe defect that is not compatible with life. There are several K<sup>+</sup> channel knockout mice available, but none have a lymphatic phenotype ascribed: this may be simply due to a lack of a lymphatic phenotype or that a phenotypic change has not been recognized. The latter is not unlikely since edema in a rodent is easily missed and the lymphatics are rarely a prioritized area when phenotyping animals, unless the animals are specifically designed for that purpose. It is complicated to isolate how modulation of K<sup>+</sup> channels eventually affect the tissue fluid balance since arteries, veins, and lymphatic vessels are all most likely affected. Both input and output, with an interaction between the two (13), affects the interstitial fluid level, and the exact contribution of the lymphatic vessels can be hard to pinpoint when the whole system is changed. We found that closing K<sub>ATP</sub> channels with glibenclamide converted nonspontaneous vessels into spontaneous vessels and increased contraction frequency in already spontaneous vessels. Glibenclamide is a sulfonylurea drug used for the treatment of diabetes. An interesting speculation is whether patients treated with these types of drugs have a lower risk of developing edema due to a positive influence on lymphatic pumping. In this study, we investigated the thoracic duct, the largest lymphatic vessel in the body. However, clinical edema is often located to the lower limbs and is likely not caused by thoracic duct dysfunction alone. The previous discussion is thus based on the assumption that our findings, which show the important regulatory roles for K<sup>+</sup> channels in human thoracic duct contractility, can be extrapolated to smaller collecting lymphatic vessels located in clinically affected areas. In this study, we also observed that contraction frequency increased significantly when [K<sup>+</sup>]<sub>i</sub> was elevated to 10 mM. A 10 mM plasma concentration of K<sup>+</sup> is a life-threatening condition due to the high risk of cardiac arrhythmias. However, the interstitial K<sup>+</sup> concentration can easily reach these levels during physical exercise, and the concentration in lymph will therefore be similarly increased. Elevated

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extracellular K⁺, in addition to the increased fluid extravasation that occurs with exercise, represents a physiologically relevant challenge to the collecting lymphatics, and it is interesting that we have observed a positive chronotropic effect upon lymphatic vessel activity with this perturbation.

Summary

Research on human lymphatic vessels is limited by access to tissue, and by virtue of the restricted source these studies are often intrinsically descriptive. Access to tissue from young healthy humans is almost impossible, and it is therefore necessary to accept that most research involving human tissue could potentially be influenced by factors including aging and comorbidities. In our study, we used vessels from aged patients with a malignancy; hence, systemic and local changes could potentially influence our findings. Animal models, on the other hand, provide the opportunity to isolate functional mechanisms in detail. However, the translational interest of animal-based data requires an understanding of the extent to which the animal reflects human function. A basic understanding of human lymphatic vessel physiology is therefore an absolute necessity to identify the similarities and differences between human and animal tissue and to provide a foundation for interpreting the clinical relevance of findings generated in animal models.

In this study, we described the expression of K⁺ channels in human lymphatic vessels and their functional role. We have, furthermore, as a first, measured Vm values of LSMCs from humans. Our comprehensive study represents an important addition to our knowledge of the physiological function of the human lymphatic vasculature. We conclude that human LSMCs are more depolarized than predicted by animal studies and that K⁺ channels are important regulators of lymphatic function by influencing all key parameters of lymphatic pumping.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: N.T., H.P., E.P., V.E.H., C.A., and D.M.B.B. conceived and design of research; N.T. and S.K. performed experiments; N.T. and D.M.B.B. analyzed data; N.T., V.E.H., C.A., and D.M.B.B. interpreted results of experiments; N.T. prepared figures; N.T. drafted manuscript; N.T., V.E.H., C.A., and D.M.B.B. interpreted data; N.T. and S.K. performed experiments; N.T. and D.M.B.B. approved final version of manuscript.

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