Myogenic constriction and dilation of isolated lymphatic vessels

Michael J. Davis,1 Ann M. Davis,1 Christine W. Ku,1 and Anatoliy A. Gashev2

1Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, Missouri; and 2Division of Lymphatic Biology, Department of Systems Biology and Translational Medicine, Cardiovascular Research Institute, Texas A & M Health Science Center, Temple, Texas

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LYMPH FORMATION AND PROPULSION are determined by the interplay of multiple factors, both passive and active, that control how lymphatic vessels behave as pumps and/or conduits (20, 23, 24, 44). Passive factors influencing lymph propulsion include hydrostatic pressure gradients across and along lymphatic vessels, tissue compression, respiratory movements, and gravitational forces (45, 46). Active lymph propulsion is achieved by the spontaneous, rhythmic contractions of collecting lymphatic vessels, which serve as an essential pump mechanism to propel lymph uphill against a hydrostatic pressure gradient from peripheral lymphatics through lymph nodes into the thoracic duct (29, 50).

The lymphatic pump exhibits cardiac-like behavior in several respects, such that pump performance can be analyzed using cardiac indexes (4, 26, 36, 42). For example, collecting lymphatics exhibit intrinsic compensation to changes in input (preload) or output (afterload) pressure (18, 21, 24, 39, 49). Other aspects of lymphatic vessel behavior resemble those of blood vessels. Similar to arterioles, lymphatics have a certain degree of basal tone (21, 22) and respond to imposed, intraluminal flow gradients (23, 33); in contrast to arterioles, lymphatics also respond to flow transients associated with the lymphatic contraction cycle (25).

Blood vessels, notably small arteries and arterioles, typically exhibit myogenic reactions to changes in intravascular pressure (3). Although the term “myogenic response” has been used to describe various responses of blood vessels to altered pressure or stretch, these are often distinct phenomena that more accurately fit under a broader heading of “myogenic behavior” (13). A narrower and more precise definition of the term myogenic response is constriction to elevated pressure or dilation to reduced pressure (12). In this sense of the term, myogenic responses are exhibited by arterioles, small arteries, and, to some extent, veins but are most pronounced in small- to intermediate-sized arterioles, where they are important for local control of blood flow and capillary pressure (13). Whether lymphatics exhibit myogenic responses in this stricter sense of the term is not known.

The term “lymphatic myogenic response” has been used previously in a broad sense to refer to a number of intrinsic adaptations of the lymphatic pump to hydrostatic forces (2, 42). Although the literature is replete with examples of lymphatic contraction patterns at various steady-state pressure levels (22, 23, 33, 39, 42), few continuous records of the lymphatic response to acute pressure changes have been published (40, 49). In the course of a recent study (10), we consistently observed what appeared to be myogenic constrictions to rapid pressure elevation. The constriction was evident as a time-dependent decrease in end-diastolic diameter (EDD) over a 1- to 2-min period following pressure elevation. The purpose of the present study was to determine the magnitude of the lymphatic myogenic response and the conditions under which it occurs.

METHODS

Vessel isolation. Male Sprague-Dawley rats (170–260 g body wt) were anesthetized with pentobarbital sodium (Nembutal; 100 mg/kg ip), and a loop of duodenum from each animal was exteriorized through a midline abdominal incision. All animal protocols were approved by the University of Missouri Animal Care and Use Committee and conformed to US Public Health Service policy for the humane care and use of laboratory animals (PHS Policy, 1996). Collecting lymphatic vessels (90–160 μm ID, 1–2 mm long) were dissected from their paired mesenteric small arteries/veins in MOPS-buffered, albumin-supplemented physiological saline solution (APSS) at room temperature. After removal of connective tissue and fat, each

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vessel was transferred to a 3-ml chamber for cannulation, pressurization, and subsequent isobaric studies. The animal was euthanized with pentobarbital sodium (200 mg/kg ic).

**Solutions.** APSS contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM Na₂HPO₄, 0.02 mM EDTA, 5.0 mM glucose, 2.0 mM sodium pyruvate, 3.0 mM MOPS, and 0.5 g/100 ml purified BSA (pH 7.4 at 37°C). Initially, pipette and bath solutions were APSS, but after vessel cannulation and equilibration, the external solution was changed to an identical solution without albumin. Ca²⁺-free APSS was identical to APSS, except for substitution of 3.0 mM EDTA for CaCl₂. All chemicals were obtained from Sigma (St. Louis, MO), except albumin (catalog no. 10856), which was obtained from US Biochemicals (Cleveland, OH).

**Pressure control method.** Vessel segments used for these protocols had at most one valve to ensure adequate pressure control in the entire set (1–2 mm total length). For study of isobaric contractions, the lymphatic segment was cannulated at each end with a glass micropipette on a Burg-style V-track system (17) and pressurized on the stage had at most one valve to ensure adequate pressure control in the entire set at 0.5 cmH₂O and then ramped to 3 cmH₂O at one of five different pressures (0.5, 1, 3, or 5 cmH₂O) for several minutes until a new contraction pattern stabilized. This pressure range overlaps the hydrostatic pressure range (2–5 cmH₂O) in vivo, under conditions approximating normal tissue hydration, based on the few published measurements of lymphatic pressure in rat mesentery (4, 26, 28). During this time, the diameter tracking system was adjusted to give accurate recordings of internal diameter. The bath solution was changed every ~30 min to minimize changes in osmolarity associated with evaporation. Criteria for viable vessels included the development of spontaneous tone, particularly at pressures <3 cmH₂O, the development of spontaneous contractions during the equilibration period, the amplitude of which was ≥30% of the maximal passive diameter at the equilibration pressure, and contractions that were reasonably uniform over the entire length of the vessel (i.e., not just in a single spot). Vessels that did not meet these criteria were discarded. In addition, data sets from vessels that developed irregular contraction patterns during an experiment were not used for subsequent analysis.

For step pressure protocols, pressure was set to one of four baseline levels (0.5, 1, 3, or 5 cmH₂O) for several minutes until a new contraction pattern stabilized. This pressure range overlaps the hydrostatic pressure range (2–5 cmH₂O) in vivo, under conditions approximating normal tissue hydration, based on the few published measurements of lymphatic pressure in rat mesentery (4, 26, 28). The vessel was then subjected to a series of rapid pressure steps (+2, +4, +6, +8, +10, and +12 cmH₂O), for 2–5 min at each step, with return to baseline pressure for 3–10 min before the next pressure step. After completion of the pressure step series, usually in sequential order, SP (3 × 10⁻⁸ M) was applied to the bath and the same series of pressure steps was repeated. For ramp pressure protocols, pressure was initially set at 0.5 cmH₂O and then ramped to 3 cmH₂O at one of five different rates, such that the ramp was complete in ~30, 15, 6, 3, or 1.5 min. A rapid pressure step from 0.5 to 3 cmH₂O was also tested on the same vessel. After 3–5 min at 3 cmH₂O, pressure was returned to 0.5 cmH₂O for 5–10 min until a stable contraction pattern redeveloped. Subsequently, another ramp was performed. This sequence was repeated until all five ramp rates were tested in pseudorandomized order.

At the end of each experiment, the vessel was equilibrated for 30 min in Ca²⁺-free APSS at 37°C, and then passive diameters were determined for each previous step or ramp pressure sequence.

**Data analysis.** After completion of an experiment, custom analysis routines written in LabView were used to detect the diameter maxima and minima associated with each contraction cycle and the contraction frequency (FREQ) before, during, and after the pressure steps or ramps. Frequency was computed on a contraction-by-contraction basis. Other contraction parameters were calculated as follows:

\[
\text{AMP} = \text{EDD} - \text{ESD}
\]

where EDD is end-diastolic diameter and ESD is end-systolic diameter at any given pressure.

\[
\text{normalized AMP} = \frac{\text{AMP}}{\text{MaxD}}
\]

where MaxD is the maximal passive diameter at the respective pressure.

\[
\text{normalized change in EDD} = 100 \times (\text{EDD} - \text{ESD}) / \text{MaxD}
\]

\[
\text{myogenic index} = \left[ 100 \times (R_i - R_j) / R_i \right] (P_f - P_i)
\]

where \(R_i\) is the initial end-diastolic radius, \(R_j\) is the final end-diastolic radius after a steady-state constriction was achieved, and \(P_i\) and \(P_f\) represent the baseline pressure and the final pressure after the pressure step (in mmHg).

Data sets were analyzed using Excel and JMP 5.1 (SAS, Cary, NC). In most cases, one-way ANOVAs were performed, with pressure designated as the independent variable. Tukey-Kramer or Dunnnett’s post hoc test was then used to test for significant within-group variation. For comparisons between control and SP data sets, matched-pairs tests were used. Significance was defined as \(P < 0.05\).

**RESULTS**

**Myogenic constriction.** Under in vitro conditions, the responses of mesenteric lymphatics to small positive pressure steps (≤3 cmH₂O) were associated with increases in EDD that were suggestive of passive diameter responses. However, in all cases, the change in EDD was less than the change in response to an identical pressure step in Ca²⁺-free APSS (Fig. 1A). In this regard, the lymphatic response to pressure elevation resembled that of some arteries (37) and veins (35) in what has been characterized as a “nonpassive” or “slight” myogenic response (13).

When larger pressure steps were imposed, the typical lymphatic response was an initial passive distension followed by a progressive decline in EDD over a time course of 1–3 min (Fig. 1A). Because overt constrictions to pressure elevation such as this more closely resembled the responses of small arteries and arterioles (8), we termed this behavior “lymphatic myogenic constriction.” In contrast to highly responsive arterioles (8, 38), however, we seldom observed lymphatic myogenic constriction to a diameter that was less than the initial diameter (i.e.,
Myogenic constrictions may not have been reported previously for lymphatic vessels, because their observation (based on our experience) required very clean and rapid pressure steps, which were not practical without the use of a servo system. Here, sustained myogenic constrictions were observed in 17 of 23 (74%) rat mesenteric lymphatic vessels (based on control vessels pooled from all protocols at 1 cmH$_2$O baseline pressure). However, subsequent analyses included all vessels whether or not they exhibited myogenic constriction, as long as the vessels met the other criteria for viability.

To quantify myogenic constriction, EDD, ESD, and AMP were measured for each spontaneous contraction following a positive pressure step. In preliminary studies, we found that myogenic constrictions were typically complete (i.e., with no further constriction) within 1–2 min (requiring 15–20 contrac-

A

![Diagram A](image)

B

![Diagram B](image)

Fig. 1. A: time course of lymphatic diameter response to an abrupt increase in pressure [+2 cmH$_2$O (left) and +6 cmH$_2$O (right)]. Passive diameter changes for the same pressure steps (recorded in Ca$^{2+}$-free bath at the end of the experiment) are overlaid and shown as maximal passive diameter (MaxD). EDD and ESD, end-diastolic and end-systolic diameter. Myogenic constriction is a time-dependent decline in EDD at constant pressure; a compensatory increase in AMP occurs when ESD decreases more than EDD over time. B: plot of normalized change in EDD for the first 25 contractions following positive pressure steps of +2, +4, +6, +8, +10, and +12 cmH$_2$O. Baseline pressure was 1 cmH$_2$O. Contraction frequency (FREQ) is higher with higher pressure (as in A), so time to complete 25 contractions is less with larger pressure steps. Error bars, SE.

less than EDD at the lower pressure). Myogenic constrictions were measured for each spontaneous contraction following a positive pressure step. In preliminary studies, we found that myogenic constrictions were typically complete (i.e., with no further constriction) within 1–2 min (requiring 15–20 contrac-

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trace) and corresponds to Time scales of A recording in identical in magnitude and length. All diameter scaling is the same as in Fig. 2. Protocol to test how timing of a pressure step might influence magnitude and/or time course of a change in EDD. Pressure step was initiated at 1 of 4 points within the lymphatic contraction cycle (C in each diameter trace) and corresponds to a–d. Pressure trace at top is associated with diameter recording in A (subsequent pressure steps are not shown but were essentially identical in magnitude and length). All diameter scaling is the same as in A. Time scales of B–D were adjusted slightly to align traces, and calibration bars indicate true scaling, 30 s. Actual sequence in which traces were collected was as follows: D, B, A, and C.

Compensatory AMP increase following pressure elevation. The first contraction after a positive pressure step was triggered by the increased distension/filling associated with the pressure change and, thus, was not a true isobaric contraction. Compared with the respective values at the baseline pressure, there was an initial increase in ESD, an increase in EDD, and a decrease in AMP after the pressure step. Subsequently, a progressive decline in EDD and ESD occurred, with the decline in ESD being greater, such that AMP progressively increased over time at constant pressure. EDD, ESD, and AMP stabilized after 25–30 contractions. A similar compensatory effect for AMP has been described previously in other lymphatic vessels (4, 10, 36), although the time course over which it occurs has not been documented. The phenomenon appears to represent an intrinsic compensation of the lymphatic pump to pressure elevation, perhaps analogous to heterometric regulation of cardiac muscle (47).

A compensatory increase in AMP was observed after positive pressure steps in nearly every vessel in response to most pressure steps between +2 and +12 cmH2O. It occurred even after pressure steps from 0.5 to 3 cmH2O or from 1 to 3 cmH2O, in which myogenic constrictions were seldom observed (e.g., Fig. 1A, left; Fig. 1B, open circles). The observation that this response was more consistent and robust than myogenic constriction prompted a comparison of its magnitude in vessels that did show myogenic constriction with that in vessels that did not show myogenic constriction. Figure 3 plots the time course of AMP (normalized to MaxD) as a function of time after a pressure step, with the data separated according to whether the vessel showed myogenic constriction to one or more pressure steps (Fig. 3A) or failed to show myogenic constrictions (Fig. 3B). Upon analysis, there were only slight differences between any of the respective pairs of curves for the two groups of vessels (e.g., comparison of the two curves at +4 cmH2O), although FREQ increased less in response to some of the higher pressure steps in the nonmyogenic vessels. These findings indicate that mesenteric lymphatics exhibited compensatory increases in AMP whether or not they showed myogenic constrictions and suggest that the two phenomena may be mechanistically distinct.

Pressure range for myogenic constriction. Next, we tested whether the magnitude of myogenic constriction was altered by the baseline pressure from which positive pressure steps were imposed. Vessels were equilibrated at one of the four baseline pressures (see METHODS) from which the standard set of positive pressure steps was imposed. The lowest practical baseline pressure was 0.5 cmH2O because of the very low FREQ of many vessels at this pressure (some contracted only once every 10 min in the absence of SP). Figure 4 summarizes the data for this protocol. In Fig. 4A, the average steady-state change in normalized EDD for each pressure step is plotted against the test pressure. The four sets of curves represent the data sets for the four different baseline pressures, with negative values indicating myogenic constrictions and suggest that the two phenomena are mechanistically distinct.

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Effect of SP. SP is a neuropeptide associated with lymphoid tissue and inflammatory responses. Not surprisingly, SP-containing nerves are closely associated with lymphatic vessels in the gastrointestinal tract (27, 30), and SP has been shown to modulate lymphatic contractility (1, 14). Agonists such as norepinephrine and angiotensin II enhance basal myogenic tone in arterioles and enhance myogenic constriction to pressure elevation (13, 19, 41). For these reasons, we hypothesized that SP would enhance the myogenic constriction observed in lymphatic vessels.

The effects of a moderately low concentration of SP (3 \times 10^{-8} \text{ M}) on the lymphatic response to rapid pressure elevation were studied in 11 vessels. This concentration of SP produced a consistent increase in spontaneous FREQ and a slight increase in tone at every baseline pressure, with very little change in AMP (14). Representative examples of the effect of SP on the lymphatic response to rapid pressure steps from a baseline pressure of 3 cmH\textsubscript{2}O are shown in Fig. 5, A and B. Ten of 11 lymphatic vessels showed enhanced myogenic constriction in the presence of SP. The results for all 11 vessels are summarized in Fig. 5, C and D. The chronotropic effect of elevated pressure is apparent from the shorter times required to achieve a fixed number contractions with higher pressure steps; for example, after a pressure step of +2 cmH\textsubscript{2}O, \sim 2.5 min were required to complete 25 contractions under control conditions, whereas only \sim 1 min was required in the presence of SP. More importantly, SP enhanced the rate and magnitude of constriction after pressure elevation for all pressure steps, as denoted by more negative values of normalized EDD at each time point. In most cases, the magnitude of the constriction increased by more than threefold in the presence of SP. Corre-

Fig. 3. Plots of normalized AMP as a function of time after rapid pressure elevation. Vessels were divided into 2 groups for separate analysis: vessels that showed myogenic constriction to at least 1 pressure step in the range +2 to +12 cmH\textsubscript{2}O (A) and vessels that showed no myogenic contractions to any pressure step over the same range (B). Scales are the same for A and B. Baseline pressure = 1 cmH\textsubscript{2}O for all vessels. Error bars, SE. Error bars on time axis are not shown for clarity.

Fig. 4. A: magnitude of myogenic constriction expressed as normalized change in EDD as a function of pressure. After equilibration at baseline pressures (0.5, 1, 3, and 5 cmH\textsubscript{2}O), pressure was stepped by +2, +4, +6, +8, +10, and +12 cmH\textsubscript{2}O. Values of normalized EDD were then averaged over contractions 15–25 after the pressure step to estimate the steady-state constriction achieved. B: change in diameter plotted as a function of the final pressure for the same vessels and pressure steps in A. Error bars, SE. *Significant difference (P < 0.05, by ANOVA followed by Dunnett’s post hoc tests) between the indicated mean and the mean at the lowest test pressure for a given baseline pressure; for clarity, significant difference is shown only for 0.5 cmH\textsubscript{2}O baseline pressure data set, but the pattern of significant differences was the same for all other data sets.
spondingly, the rate at which the constriction developed was also enhanced by SP. The slopes of the lines fitting the data sets in Fig. 5 ranged from $0.12$ to $0.48$ (change in normalized EDD/min) for the control data and from $2.89$ to $3.75$ for the SP data; i.e., constriction was 6- to 10-fold faster in the presence of SP.

The effects of SP on the change in normalized EDD in response to the same set of pressure steps are shown in Fig. 6 for four different baseline pressures. For this analysis, the steady-state change in normalized EDD was measured as described above. Under control conditions (in the absence of SP), the normalized EDD increased for the first pressure step ($+2$ cmH$_2$O) from a baseline of $0.5$ cmH$_2$O, but at all subsequent (higher) pressure steps, the vessels constricted (Fig. 6A, open circles). The same pattern was evident starting from a baseline pressure of $3$ or $5$ cmH$_2$O, regardless of the baseline pressure (not shown). Tone was $7$–$9\%$ at all baseline pressures ($0.5$–$5$ cmH$_2$O) in the absence of SP. In the presence of $3 \times 10^{-8}$ M SP, tone significantly increased to $12$–$13\%$ after all positive pressure steps, except after the lowest steps from baseline pressures of $0.5$ and $1$ cmH$_2$O, where even higher levels of tone ($17$–$18\%$) were recorded. This effect of SP at low pressures has been reported previously (14) and is not shown here, because the tone data sets at the four baseline pressure levels overlapped extensively and could not be easily distinguished on a single plot.

Myogenic dilation. Arterioles that show myogenic constriction to pressure elevation typically show myogenic dilation to pressure reduction (8). In the course of the present study, we observed that many lymphatic vessels showed what appeared
to be myogenic dilations in response to downward pressure steps. An example of this response is shown in Fig. 7. For comparison, Fig. 7A shows a vessel that did not exhibit myogenic dilation, because diameter slowly declined with time after the downward pressure step (arrow) over a time course similar to the diameter change for the same vessel in the Ca\(^{2+}\)-free bath (passive trace; superimposed on the active diameter trace). In contrast, the diameter of a myogenically

Fig. 7. Myogenic dilation after rapid pressure reduction. A: lymphatic vessel that exhibited a slight myogenic constriction to a pressure step from 1 to 6 cmH\(_2\)O but did not show myogenic dilation when pressure was returned to 1 cmH\(_2\)O. Passive response of the same vessel to the same-size pressure step, obtained at the end of the experiment, is overlaid on the active diameter trace. Change in EDD after pressure was reduced followed a time course similar to the passive trace. B: lymphatic vessel that showed myogenic dilations to the falling phase of each of 3 pressure steps. Passive traces for the same-size pressure steps are overlaid and labeled. After pressure was reduced, EDD rose with time during the 1st min, in contrast to the shape of the passive traces. Dilation was exaggerated with greater pressure steps.
in vivo hydrostatic pressure under conditions of normal tissue hydration, based on the few published measurements of lymphatic pressure in the intact rat mesenteric microcirculation. For example, Hargens and Zweifach (28) recorded lymphatic FREQ of 7 min\(^{-1}\) at 3 cmH\(_2\)O average end-diastolic pressure, and Granger et al. (26) recorded FREQ of 5 min\(^{-1}\) at 2 cmH\(_2\)O end-diastolic pressure. However, these in vivo measurements likely represent the high end of the lymphatic pressure range under conditions of normal tissue hydration, because they were, by necessity, made in superfused preparations in which the tissue was likely to be slightly hydrated, thus elevating lymphatic pressures and flow rates. Therefore, in vivo diastolic pressures in rat mesenteric lymphatics are probably often <2 cmH\(_2\)O, in a range where they would exhibit myogenic responses to transmural pressure increases.

How do lymphatic myogenic responses compare with those of arterioles and small arteries? Lymphatic vessels, similar to arterioles, show a lower and an upper limit to myogenic constriction. Lymphatic constrictions waned above pressures of \(-15\) cmH\(_2\)O and did not occur in response to the smallest pressure step (+2 cmH\(_2\)O), as consistently shown in Figs. 1B, 3, and 5A: similarly, even highly reactive arterioles do not show myogenic constrictions below a minimum pressure [e.g., <40 cmH\(_2\)O for hamster cheek pouch 2nd-order arterioles (8)]. To make more quantitative comparisons between lymphatic and arteriolar myogenic responses, we calculated the myogenic index according to the formula described in previous publications (8). The myogenic index represents the relative change in vessel diameter per unit change in pressure (traditionally in mmHg). The strongest myogenic constriction of lymphatic vessels occurred under control conditions with a pressure step from 0.5 to 10.5 cmH\(_2\)O, corresponding to a steady-state change in normalized EDD of approximately \(-3\) (Fig. 6A). We used the absolute lymphatic diameters (initial and final) for the 10 vessels in that protocol to calculate a myogenic index of \(-0.51\) (in mmHg). Of those 10 vessels, 6 constricted, 2 dilated, and 2 showed <1-\(\mu\)-m change in diameter upon pressure elevation (after initial distention). The myogenic index for the six vessels that constricted was \(-0.75\). Surprisingly, the values are quite comparable to those of first-, second-, and third-order arterioles from hamster cheek pouch (range \(-0.41\) to \(-0.85\)) and other tissues (8).

The mechanisms by which an increase in circumferential length evokes myogenic constriction in vascular smooth muscle are not fully understood, but it seems clear that muscle cell length per se cannot be the controlled variable (32). Burrows and Johnson (5, 6) demonstrated that wall tension correlates well with the responses of small arterioles to pressure elevation, where the vessels often constrict well below their initial diameters. The same principles presumably apply to lymphatic muscle, even though the compliance of lymphatic vessels is remarkably different from that of arterioles (48). In lymphatics, myogenic constrictions were strongest in response to pressure elevation from the lowest baseline pressure, from which pressure steps produced the largest amount of distension (Fig. 4). However, myogenic constrictions were not proportional to the degree of distension, nor were large distensions always required; for example, in the presence of SP, vessels showed larger myogenic constrictions starting from higher baseline pressures (Fig. 6, C and D), from which further pressure elevation evoked very little further distension (Fig. 4B).
Relationship of myogenic responses to lymphatic pump function. Lymphatic myogenic constrictions were not necessarily associated with, nor were they correlated to, time-dependent decreases in ESD after pressure elevation (Fig. 3). The examples shown here (Figs. 1A, 2, 5A, 5B, and 7) appear to be the first published descriptions of the time course of both phenomena in lymphatic vessels, even though steady-state AMP compensation has been documented previously (18, 21, 22, 40). The partial recovery (i.e., decline) of lymphatic vessel ESD (following its initial increase after a pressure step) presumably reflects underlying mechanisms that are analogous to heterometric regulation in the heart (34). In the isolated heart, an increase in preload produces an immediate increase in end-diastolic volume (i.e., EDD), with subsequent maintenance of end-systolic volume (ESD) if afterload is kept constant; thus cardiac stroke volume (AMP) increases in proportion to preload over a relatively wide preload range. If afterload is increased in the isolated heart (at constant preload), end-systolic volume increases and stroke volume is reduced (11, 47). A subsequent reduction in end-systolic volume does not occur unless there is an increase in contractility. However, if our protocols, preload and afterload were elevated simultaneously by increase of the pressure in both cannulating pipettes, so that further studies are required to distinguish their independent effects. Thus it is not clear whether an increase in lymphatic muscle contractility is associated with the subsequent decline in ESD after pressure elevation. However, given that myogenic constrictions in arterioles are associated with an increase in contractility of vascular smooth muscle (9, 32), the decline in lymphatic ESD probably does not reflect an increase in lymphatic muscle contractility, because we observed equivalent compensatory changes in ESD and AMP in vessels that showed myogenic constrictions and in vessels that did not show myogenic constrictions (Fig. 3), i.e., in vessels presumably exhibiting different levels of contractility.

Lymphatic myogenic responses are enhanced by SP. Multiple aspects of lymphatic myogenic constriction were enhanced by the inflammatory mediator SP: 1) the percentage of vessels in which it was observed, 2) its magnitude, 3) its rate, and 4) the pressure range over which it occurred. Recently, we demonstrated that SP induces positive inotropic and chronotropic responses in isolated rat mesenteric lymphatics. In addition, SP enhanced basal lymphatic tone, i.e., reduced EDD at constant pressure, in a dose-dependent manner (14). The latter effect is particularly interesting, because the development of basal tone is one characteristic shared by lymphatics and arteriolar blood vessels (21, 22), as opposed to lymphatic pump behavior, which is more cardiac-like (4, 26, 36). Since basal tone and myogenic responses have been shown to be closely related (but perhaps distinct) in blood vessels (13), it is not surprising that lymphatic vessels are able to demonstrate myogenic responses under the appropriate conditions and that those responses are enhanced by an agonist that also enhances basal tone. In addition, myogenic constriction has been shown to be associated with an increase in the activation state of smooth muscle (9, 31), so that agonists that enhance contractility might be expected to enhance myogenic responsiveness.

The effect of SP on the enhancement of lymphatic myogenic constriction was greatest at higher pressures (Fig. 6), extending the range over which myogenic constrictions occurred. One possible conclusion from this observation is that SP, and perhaps other inflammatory mediators that are known to modulate the lymphatic pump in vivo (1, 16), activate collecting lymphatics to enable them to resist distension at the higher intraluminal pressures that are encountered during inflammation/edema. Our studies have revealed this previously undescribed and possibly underappreciated mechanism that contributes to fluid homeostasis.

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