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MEASUREMENT OF FLOW CHARACTERISTICS DURING INDIVIDUAL CONTRACTIONS IN BOVINE MESENTERIC LYMPHATIC VESSELS*

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ABSTRACT

We developed a novel technique for measuring flow characteristics during individual contractions in lymph vessels. Bovine mesenteric lymph vessel segments (n=15) were mounted in organ baths and allowed to equilibrate for 1 hr. Transmural pressure was subsequently increased by 2 cm H_2O increments at 15 min intervals and vessel outputs were collected during the final 10 min of each period and measured. Flow also was continuously recorded with an in-line Doppler transducer connected to a flow analyzer, and flow characteristics were analyzed.

The two methods of flow measurement correlated well (r^2 = 0.92). Mean flow increased with increasing transmural pressure and reached a maximum of 0.5 ± 0.1 ml/min at a transmural pressure of 8 cm H_2O. The rate of spontaneous contractions, the peak flow during a contraction-induced wave, and the total volume of flow during a wave also increased with increasing transmural pressure and reached maximums of 12.4 ± 1.0 min-1, 8.2 ± 1.6 ml/min, and 0.21 ± 0.06 ml, respectively. Wave duration changed little in response to changes in transmural pressure.

Continuous in-line flow measurement is an accurate technique for assessing flow characteristics during individual contractions in lymph vessels in vitro. Transmural pressure regulates flow by influencing spontaneous contraction frequency and total and peak flows during contraction-induced waves.

Lymph vessels are capable of contracting and propelling lymph, which are vital elements in the transport of lymph from the interstitium to the bloodstream. The regulation of this spontaneous pumping activity is an important area of investigation into the physiology of lymph flow. Factors that affect spontaneous lymphatic smooth muscle activity are complex. The mechanisms that regulate flow typically have been investigated using relatively crude techniques such as drop counters. The identification of newer methods of assessing flow parameters through lymph vessels is necessary to permit more detailed analyses of the individual components of flow. In this report we describe the use of an in-line flow probe for assessing flow in isolated lymph vessels in response to changes in transmural pressure.

MATERIALS AND METHODS

Bovine mesenteric tissue was obtained fresh from an abattoir and immersed for up to 1 hr in 0.9% saline warmed to 38°C. Lymph nodes within the mesentery were injected with 0.5% Evans blue dye to delineate lymph vessels. Main efferent vessels and any side branch vessels measuring

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>10 cm length and >3 mm in diameter were separately dissected from surrounding tissue and all other side branches were ligated. The inflow and outflow ends of each vessel segment were cannulated with polyethylene tubing (PE-205; I.D. 1.57 mm, O.D. 2.08 mm) secured with silk ties. Each vessel segment was perfused gently with Krebs solution to check for leaks and was then mounted in a custom organ bath modeled after one previously described (1). The inflow tubing was connected to a circulating pump and reservoir containing Krebs solution (NaCl 118 mM, $NaHCO_3$ 24mM, dextrose 5.55 mM, KCl 4.7 mM, $MgCl_2$ 0.4 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 1.6 mM, pH 7.4) heated to 38°C and aerated with 95% O_2/5% CO_2. The vessel segments were briefly perfused at 6 cm hydrostatic pressure (inflow tubing and reservoir 6 cm above outflow level) and were then allowed to equilibrate at 0 cm H_2O transmural pressure (TMP) for 1 hr.

After equilibration flow was assessed using a Doppler flow meter (T206, Transonic Systems, Inc., Ithaca, NY) with a 2 mm bidirectional flow probe (2N, Transonic Systems, Inc., Ithaca, NY) mounted in-line with the outflow tubing, and data were acquired to a personal computer and stored for further analysis (WinDaq/200, Dataq Instruments, Akron, OH). The flow probes and analyzer were calibrated using Krebs solution propelled through the outflow tubing by a variable speed pump at flow rates of 0 to 1.5 ml/min for 5 min intervals. Total actual flow was measured by weighing the collected output for the interval. Mean flow was calculated for each interval at each flow rate and was compared to the analyzer flow rate. The mean correlation coefficients exceeded 0.95.

Transmural pressure (TMP) was increased by 2 cm H_2O increments every 15 min from 0 to 14 cm H_2O by simultaneously increasing the levels of the inflow and outflow tubing. Flow was measured during the final 10 min of each interval. The output from each vessel was also collected during each 10 min interval and was weighed. At the conclusion of the experiment the vessel segments were dismounted, measured for length and diameter, and opened longitudinally to permit counting their valves. Vessels were eliminated from analysis if they did not develop spontaneous contractions that persisted for the duration of the experiment or if they did not exhibit a mean flow during any TMP of at least 0.3 ml/min.

Computer-stored data were analyzed for mean flow during each 10 min interval using WinDaq/Ex (Dataq Instruments, Akron, OH) software. Spontaneous contractions produced antegrade flow as evidenced by discrete waves that were either monomorphic or polymorphic. The beginning of an individual wave was defined by a rise above a steady-state baseline value that persisted for more than 1 sec, and the end of the wave was defined as a return to baseline values for a period of at least 1 sec. There was sometimes minor retrograde flow between or during contractions. Peak flow and total flow during a contraction-induced wave as well as wave duration were measured for one contraction every 30 sec during each 10 min analysis interval. Each contraction that occurred during the 10 min intervals was counted to provide an assessment of contraction frequency.

Total collected output for each 10 min measurement interval was expressed as flow (ml/min) and compared to the computer-calculated mean flow by calculating a regression line and correlation coefficient (Minitab 12.0; Minitab, Inc., State College, PA). Data are expressed as mean ± SEM. Curves were fitted to the data sets using a Lorentzian 4 parameter peak function: $y = y_o + a/(1 + ((x-x_o)/b)^2)$ (SigmaPlot 5.0, SPSS Inc., Chicago, IL).

RESULTS

A total of 31 vessel segments were mounted and 15 were used for analysis. Ten vessel segments were omitted from analysis because they failed to demonstrate

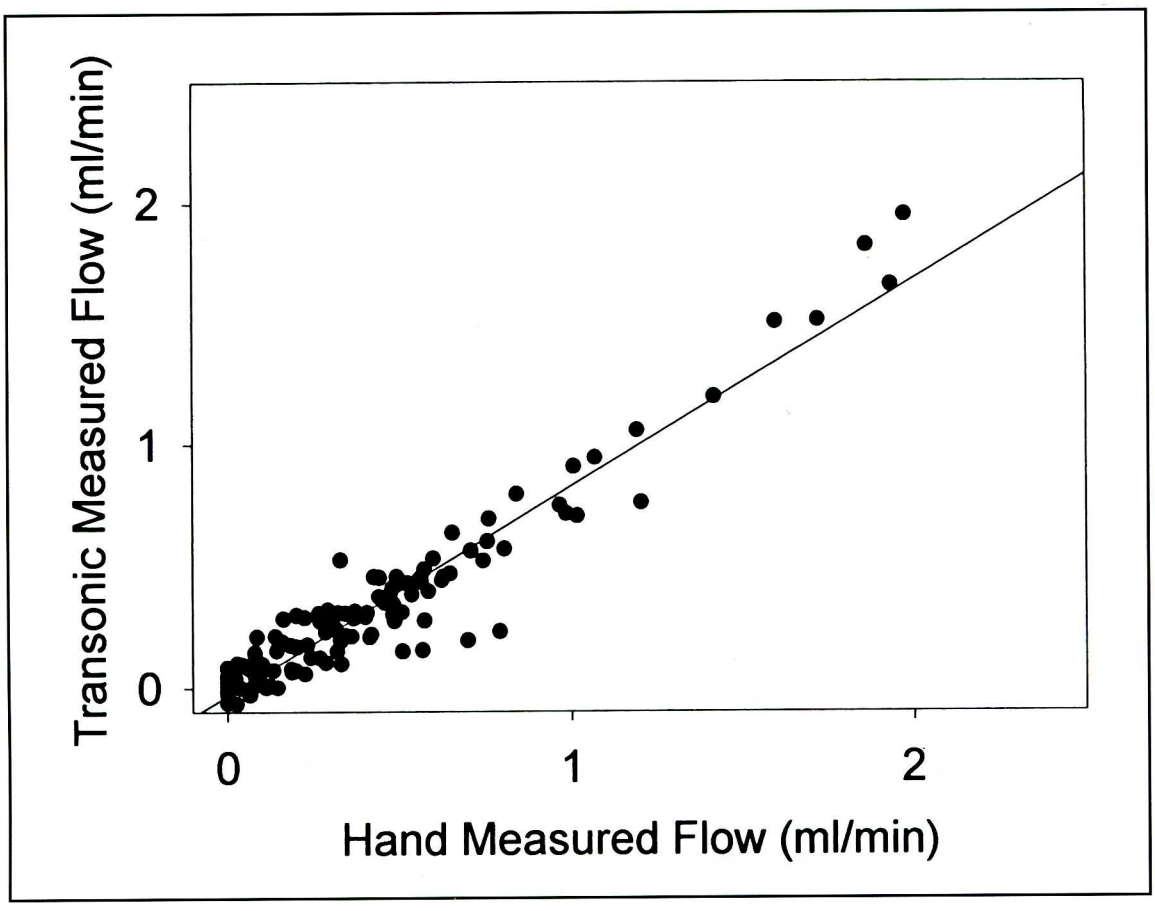

Fig. 1. Correlation of mean flow assessed by total vessel output during each measurement period (hand measured flow) and mean flow assessed by an in-line flow probe and analyzer (Transonic measured flow; r^2 = 0.92 for regression line) in bovine lymph vessel segments in vitro.

spontaneous contractions for the duration of the experiment and 6 did not exhibit sufficient flow to permit analysis. The mean vessel length was 16.5 ± 0.7 cm and the mean diameter was 3.9 ± 0.1 mm. The mean number of valves in each vessel segment was 7.9 ± 0.6. The length of an intervalvular segment (lymphangion) was 2.0 ± 0.2 cm and the volume of a lymphangion was calculated as 0.25 ± 0.03 ml. In all vessel segments spontaneous contractions began by the time TMP was raised to 6 cm H_2O and they began in 7 vessel segments at TMP = 0 cm H_2O. Waves were initially monomorphic in 9 vessel segments. In all but one vessel segment wave forms began as or converted to polymorphic configurations before the end of the experiment, usually within 15 min of the initiation of spontaneous contractions.

Mean flows calculated from the accumulated vessel output at each TMP correlated well with mean flows measured by the in-line flow probe and analyzer (*Fig. 1*; r^2 = 0.92). Flow-probe measured flows were used for the remaining analyses. The mean flow increased with increasing TMP to a maximum of 0.5 ± 0.1 ml/min at TMP = 8 cm H_2O and then slightly decreased as TMP was increased further. Similarly, the contraction frequency increased with increasing TMP to a

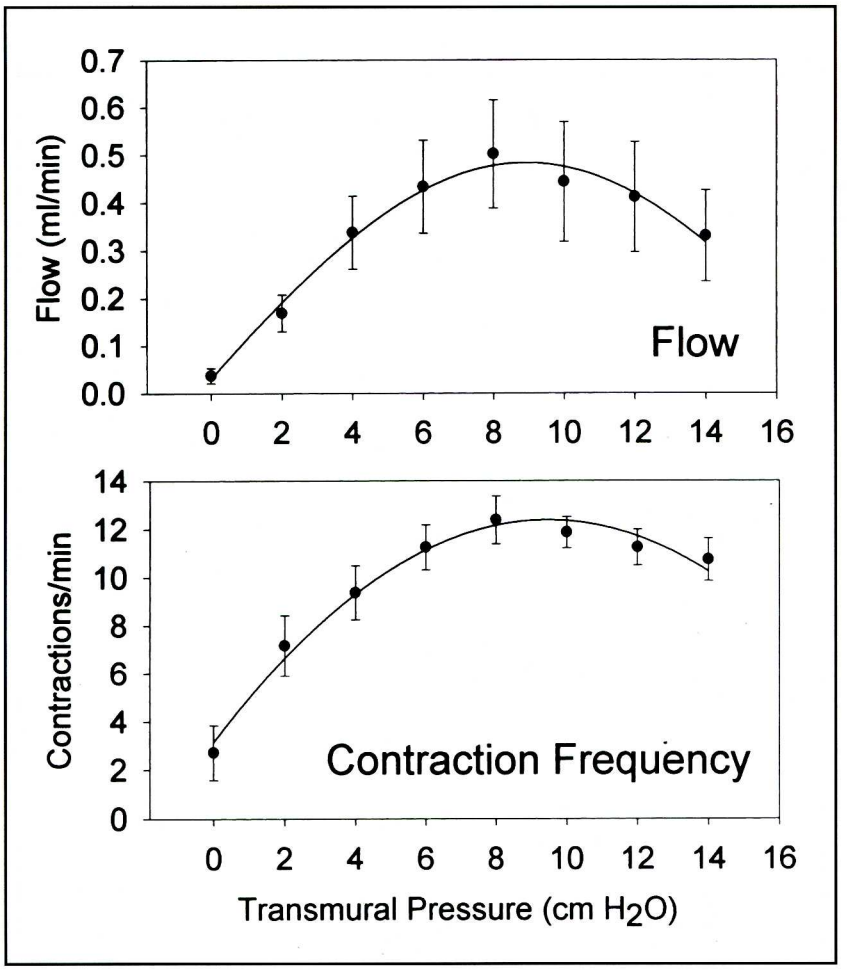

Fig. 2. Mean flow (measured by an in-line flow probe and analyzer) and contraction frequency calculated at progressively increasing transmural pressures in bovine mesenteric lymph vessel segments in vitro. The regression curve for mean flow is expressed as $y = -2.18 + 2.66/(1+ [(x - 8.92)/19.68]^2)$; $r^2=0.99$. *The regression curve for contraction frequency is expressed as* $y = -26{,}428 + 26{,}440/(1+ [(x - 9.46)/506]^2)$; $r^2=0.98$.

maximum of 12.4 ± 1.0 min^{-1} at TMP = 8 cm H_2O and then slightly decreased as TMP was increased further *(Fig. 2)*.

Analysis of individual waves revealed that the mean peak flow that occurred during a contraction-induced wave increased with increasing TMP to a maximum of 8.2 ± 1.6 ml/min at TMP = 10 cm H_2O and then slightly decreased as TMP was increased further. Total flow during a wave increased with increasing TMP to a maximum of 0.21 ± 0.06 ml at TMP = 6 cm H_2O and then decreased as TMP was increased further. The wave duration changed minimally in relation to TMP and averaged 4.6 ± 0.2 sec *(Fig. 3)*.

Mean flow, contraction frequency, total flow during a 10 min interval, peak flow during a wave, total flow during a wave, and wave duration were not statistically related to vessel length, vessel diameter, the number of valves within each vessel segment, or the lymphangion length and volume.

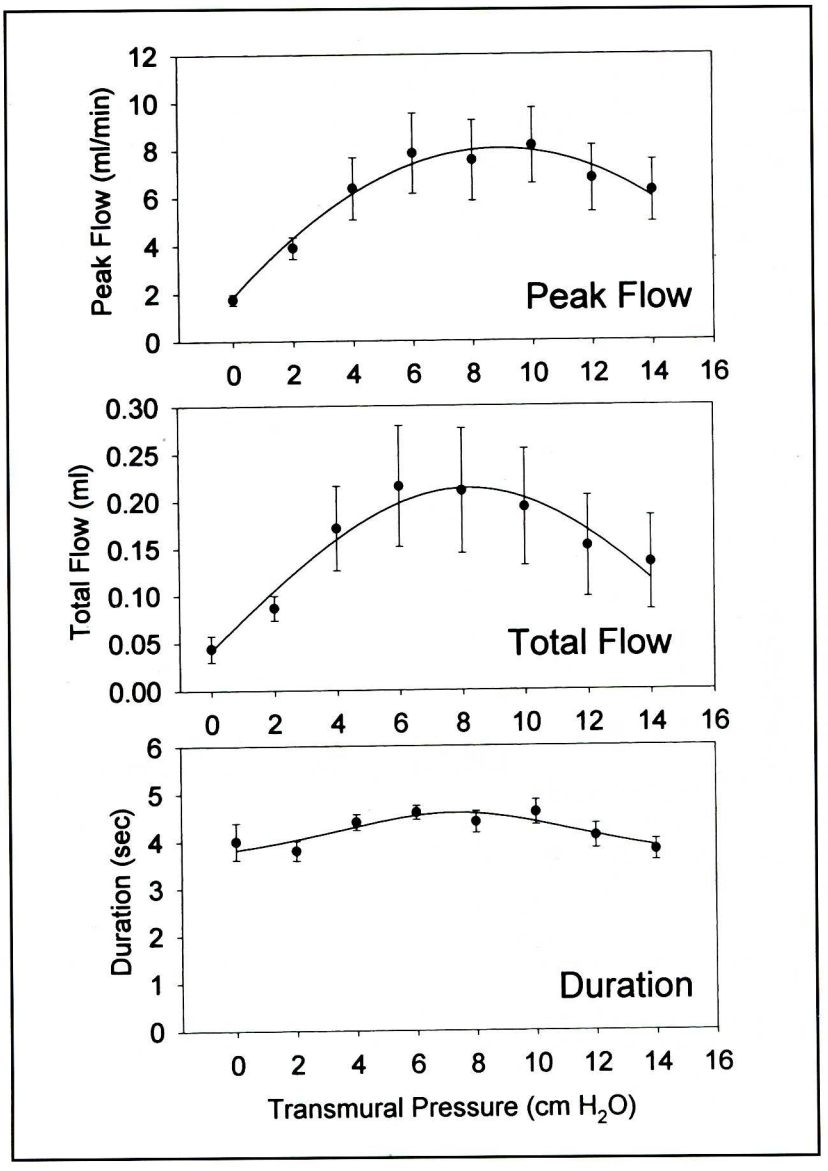

Fig. 3. Flow characteristics during contraction-induced waves calculated for peak flow, total flow, and wave duration at progressively increasing transmural pressures in bovine mesenteric lymph vessel segments in vitro. The regression curve for peak flow is expressed as $y = -376 + 384/(1+ [(x -9)/69]^2)$; $r^2=0.97$. *The regression curve for total flow is expressed as* $y = -0.55 + 0.76/(1+ [(x - 8.19)/15.19]^2)$; $r^2=0.97$. *The regression curve for wave duration is expressed as* $y = 3.2 + 1.4/(1+ [(x - 7.3)/6.5]^2)$; $r^2=0.87$.

DISCUSSION

Lymphatic vascular smooth muscle has mechanical properties similar to those of other vascular smooth muscle and is capable of generating forces sufficient to participate in the regulation of lymph flow (1,2). The spontaneous contraction of lymphatic vascular smooth muscle is a vital element in the transport of lymph, particularly in the mesenteric lymphatic circulation. The regulation of this spontaneous contractile activity has been

the focus of considerable investigation. Neural, pharmacologic, and mechanical factors have all been shown to participate in the control of mesenteric lymphatic vascular smooth muscle activity (1,3-8).

Unfortunately, the current techniques for assessing flow through lymph vessels are relatively crude, consisting of drop counters or collection of total flow over periods of time measured in minutes. More accurate methods provided by improved flow measurement techniques provide not only assessment of total flow over much shorter periods of time, measured in seconds, but also provide the ability to assess flow during individual contractions. The use of these techniques has the potential for providing information regarding subtle and possibly unique effects of contractile agonists and antagonists on flow through lymph vessels.

In the present experiments we describe the use of a commercial in-line Doppler probe and flow meter and commercial software for assessing lymph flow through mesenteric lymph vessels in vitro. Similar techniques have previously been reported for use in intact animal preparations for the measurement of lymph flow (9). We found that the measurements using the in-line system were similar to those obtained by measuring total output through the lymph vessels, confirming the accuracy of the technique.

The software permits analysis of mean flow, peak flow, and total flow during any selected time frame, including that equaling the duration of a single contraction-induced wave. Our findings that mean flow through bovine mesenteric lymph vessel segments averaged 0.5 ml/min at an optimal TMP were similar to those reported by other investigators of average flows of 0.6 to 0.8 ml/min (3,6). The optimal TMP for maximum flow was about 8 cm H_2O, confirming a value reported by others (3,6). We also found that contraction frequency increased with increasing TMP to a similar TMP limit of 8 cm H_2O, a finding that has been reported by others (1).

In the analysis of individual waves we identified several unique findings. The total volume of flow increased with increasing TMP to a maximum of 0.21 ml/min at a TMP of 6 to 8 cm H_2O. This increase was due primarily to changes in peak flow during a contraction rather than to changes in wave duration. The polymorphic configuration of waveforms in the majority of tracings suggests that the contractions of individual lymphangions within a vessel segment were somewhat coordinated but lacked true sequential activity that would produce a peristaltic wave.

These findings confirm previous reports that TMP has an important effect on the activity of mesenteric lymphatic smooth muscle. Our data suggest that the use of an in-line Doppler flow probe, flow analyzer, and software can provide unique information regarding flow in lymph vessel segments in vitro. This technology permits analysis of the effects of mechanical and pharmacologic influences on flow in a detailed and quantitative fashion. Such investigations may demonstrate unique effects of these different influences on the parameters that contribute to overall flow.

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