Co-ordination of contractile activity in guinea-pig mesenteric lymphatics

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1. Intraluminally perfused lymphatic vessels from the mesentery of the guinea-pig were examined *in vitro* to investigate their contractile activity and the co-ordination of this activity between adjacent lymphangions.

2. Lymphangions constricted at fairly regular intervals and exhibited 'refractory' periods of up to 3 s during which constrictions did not occur.

3. The contractile activity of adjacent lymphangions was highly co-ordinated.

4. The smooth muscle was found to be continuous between the adjacent lymphangions for the majority of valve regions examined morphologically (52 of 63 preparations).

5. Mechanical and electrical coupling between adjacent lymphangions was indicated, as some lymphangions underwent transient dilatations just prior to constriction, whereas direct electrophysiological measurements showed that the smooth muscle of most adjacent lymphangions was electrically coupled across the valve (15 out of 20 pairs of lymphangions).

6. It is concluded that perfused lymphangions of guinea-pig mesenteric lymphatic vessels rhythmically constrict, with the contractile activity of adjacent lymphangions highly co-ordinated. The findings also indicate that transmission of both mechanical and electrical signals between the adjacent lymphangions contribute to the co-ordination of their contractile activity.

Many lymphatic vessels have smooth muscle in their walls which contracts to pump lymph (Florey, 1927*a*, *b*; Smith, 1949; Horstmann, 1952, 1959; McGeown, McHale, Roddie & Thornbury, 1987; Eisenhoffer, Elias & Johnston, 1993). A net forward propulsion of lymph results, because unidirectional valves occur frequently and divide vessels into numerous small chambers or lymphangions (Mislin, 1972). The contractions of adjacent lymphangions are usually co-ordinated, constriction propagating either orthogradely (i.e. in the direction of the valves) or retrogradely along the length of the vessel (Benoit, Zawieja, Goodman & Granger, 1989; McHale & Meharg, 1992). Although it is known that contraction of lymphatic smooth muscle is triggered by action potentials (Kirkpatrick & McHale, 1977; Allen, McHale & Rooney, 1983; Van Helden, 1993), the mechanism underlying the propagation of contraction between lymphangions remains poorly understood. However, it has been suggested to involve mechanical stimuli and/or direct transmission of electrical activity between adjacent lymphangions (Horstmann, 1952, 1959; McHale & Meharg, 1992; Zawieja, Davis, Schuster, Hinds & Granger, 1993).

Horstmann (1952, 1959) suggested that each chamber of guinea-pig mesenteric lymphatic vessels forms a functional pumping unit and that initiation and propagation of contraction depends solely on mechanical stimuli. This conclusion was drawn because histological studies revealed that smooth muscle was scant or absent in the region of the valve. In addition, he observed *in vivo* that contractions of guinea-pig mesenteric lymphatic vessels propagated orthogradely and that constriction of an upstream chamber resulted in the passage of fluid into the adjacent downstream chamber causing it to distend. He proposed that stretching of the wall of the downstream chamber triggers it to constrict and propel fluid into the next chamber, and so on along the vessel.

Mechanical propagation of constriction along a lymphatic vessel assumes a reasonably large distending force to be present. However, lymphatic vessels have been observed to constrict in a co-ordinated manner in the absence of pressure stimuli (Mawhinney & Roddie, 1973; Hargens & Zweifach, 1977). This finding indicates that there must be some mechanism other than mechanical coupling which

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co-ordinates the activity of adjacent lymphangions along a lymphatic vessel. *In vitro* studies on bovine (McHale & Meharg, 1992) and rat (Zawieja *et al.* 1993) mesenteric lymphatic vessels have suggested that electrical coupling between adjacent lymphangions contributes to propagation of constriction. These studies demonstrated that constriction could propagate both orthogradely and retrogradely, and that application of the gap junction blocker heptanol disrupted propagation of contractile activity. In particular, the study of McHale & Meharg (1992) demonstrated that local application of heptanol to a small region in the centre of a vessel disrupted propagation of the contractile wave across the site of application.

In contrast with the findings reported by Horstmann (1952), we have found that the smooth muscle is continuous between most lymphangions of guinea-pig mesenteric lymphatic vessels. This finding raises the possibility that electrical activity is able to propagate directly between the lymphangions. Therefore, the present study has re-examined the contractile behaviour of guinea-pig mesenteric lymphatic vessels. In particular, the study has examined the contractile activity of individual lymphangions, as well as the co-ordination of constrictions between pairs of lymphangions and the ability to pass current between the smooth muscle of adjacent lymphangions. The two latter features have been correlated with the continuity of the smooth muscle in the region of the intervening valve. Preliminary communications of this work have been made in abstract form (Crowe & Van Helden, 1992; Crowe, Brock & Van Helden, 1993; von der Weid, Crowe, Brock & Van Helden, 1995).

METHODS

General

Guinea-pigs of either sex and age ranging from 2 to 10 days were killed by an overdose of the inhalation anaesthetic halothane and decapitation. The intestines were removed with the attached mesentery. A section of mesentery from the small intestine containing lymphatic vessels relatively free of covering adipocytes was removed from the gut and pinned to the Sylgard (Dow Corning)-coated base of a 2 ml recording chamber. The tissue was superfused with physiological buffered saline (PBS) of the following composition (mM): $CaCl_2$, 2·5; KCl, 5; $MgCl_2$, 2; NaCl, 120; $NaHCO_3$, 25; NaH_2PO_4, 1; glucose, 11. This solution was maintained at pH 7·2 by bubbling with 95% O_2–5% CO_2 and heated to 32–35 °C. The procedures were approved by the Animal Care and Ethics Committee of the University of Newcastle.

In experiments examining contractile activity, the lymphatic vessels were intraluminally perfused with PBS using an infusion pump connected to a fine glass cannula. The vessels were cut at the end closest to the intestine and the cannula loosely inserted into the lumen so that perfusion occurred in the direction of the valves. Using this system the flow into the vessels is not constant but depends on the intraluminal pressure. This results because the perfusate can back flow and this back flow increases as the intraluminal pressure rises. The perfusion rate through the cannula was increased to a level that induced continuous pumping activity (0·7–10·9 μl min^{-1}) and thereafter was not changed. Visual observations showed that the valves were still competent at these perfusion rates.

Constrictions of the vessels were monitored continuously using a video camera attached to an inverted microscope (Nikon Diaphot) and the output recorded on videotape. The data were subsequently analysed using a computer-based edge tracking system (sampling frequency, 25 Hz; see Beresford-Smith, Nesbitt & Van Helden, 1993). Depending on the experiment, constrictions were monitored at single or multiple locations along the vessel. In all records of contractile activity shown in the figures, downward-going deflections indicate constriction.

Contractile activity of single lymphangions

Single lymphangions were isolated from a vessel by cutting outside the valves at either end of the lymphangion. This procedure prevented damage to the valves at either end of the lymphangion but left segments of the adjacent lymphangions intact with the preparation. The perfusion cannula was loosely inserted into the distal end of the vessel with the tip located just before the upstream valve, and the lymphangion perfused at the minimum rate which induced constrictions. The contractile activity was recorded for periods of between 1 and 3 h. In parallel experiments the contractile activity of single lymphangions in intact lymphatic vessels (see above) was also studied.

Contractile activity of adjacent lymphangions

The contractile activity of short lengths of perfused lymphatic vessels was recorded for approximately 10 min and constrictions of adjacent pairs of lymphangions in these vessels analysed using the edge tracking system.

Electrophysiology

Determination of electrical coupling across valves was performed in non-perfused lymphatic vessels. Impalements were made from the adventitial side of the vessel with microelectrodes filled with 0·5 M KCl (resistance, 150–200 MΩ). Intracellular recordings were made from two smooth muscle cells simultaneously, one on either side of a valve. Negative current (1 s square pulse) of various amplitudes (−0·1 to −1·3 nA) was injected through one microelectrode and voltage responses recorded by the second. Only pairs of impalements where the resting membrane potential of both cells was more negative than −45 mV were included in the analysis.

Structural analysis of smooth muscle at the valves

To determine the amount of smooth muscle in the region of the valves between adjacent lymphangions, tissues were either stained with the fluorescent mitochondrial marker, diethyloxadicarbocyanine ($DiOC_2$; Molecular Probes) or fixed with glutaraldehyde. The $DiOC_2$ procedure involved immersing the tissue for 30–60 s in PBS containing 0·1 μM dye. Those tissues fixed with glutaraldehyde were left overnight in 5% glutaraldehyde in PBS and then kept in 0·1% PBS thereafter. This procedure produced aldehyde-induced fluorescence in the tissue (Reynolds, Little, Lin & Heath, 1994). For both procedures the fluorescence was observed using a laser scanning confocal imaging system (Laser-sharp MRC600, Bio-Rad, Hemel Hempsted, UK) attached to a Zeiss Axiophot 10 inverted microscope (BHS filter set with 488 nm excitation, 515 nm emission) with a ×40 oil-immersion objective lens (numerical aperture, 1·3). The presence or absence of smooth muscle fibres in the region of the valves was established by optical sectioning of the tissue.

Data analysis

Data are reported as means ± S.E.M. and, where appropriate, were compared using Student's t test.

RESULTS

General observations

The vessels studied were collecting lymphatic vessels which lay between the mesenteric lymph nodes and the wall of the small intestine. The vessels ranged in diameter from 75 to 350 μm (mean, 194·4 $\pm$ 5·7 μm; $n = 134$) and were divided into lymphangions by frequently occurring unidirectional valves at distances ranging from 200 to 2200 μm along the vessel (mean, 774·4 $\pm$ 34·3 μm; $n = 134$). When unperfused, most lymphangions were quiescent but a small number constricted at irregular intervals (see also Van Helden, 1993). Vessel perfusion induced contractile activity in the majority of lymphangions with the frequency of constrictions being dependent on the rate of perfusion (see also Mislin, 1983).

Contractile activity of single lymphangions

Small lymphangions (length, < 500 μm; diameter, < 250 μm) have been shown to be electrically short (Van Helden, 1993). Thus, the smooth muscle of these lymphangions constricts synchronously because the underlying electrical events cause an approximately uniform potential change throughout the smooth muscle. The contractile behaviour of these small lymphangions was studied in isolation by cutting away the adjacent lymphangions. However, to prevent damaging the isolated lymphangion it was necessary to leave a short length of the adjacent lymphangions intact (usually < 100 μm). In five of thirteen preparations, constriction of the remaining segment of an adjacent lymphangion often preceded and appeared to trigger constriction of the isolated lymphangion. These lymphangions were excluded from subsequent analysis. In five of the remaining lymphangions, constriction ceased before sufficient data were obtained for detailed analysis. However, three lymphangions exhibited continuous contractile activity for over 1 h. At the minimum perfusion rate that produced contractile activity, all three lymphangions constricted at relatively regular intervals. In one preparation the pumping rate remained fairly constant for a 2 h recording period. In the other two preparations, the constriction rate remained fairly constant for a period of over 1 h and then declined. The periods during which the frequency of constrictions remained relatively constant were analysed.

Figure 1 shows, for a single isolated lymphangion, a trace of the contractile activity (*A*), a plot of the cumulative number of constrictions during a 60 min period (*B*) and the frequency distribution of the intervals between constrictions for the same period (*C*). During this period the rate of occurrence of constrictions remained fairly constant (see Fig. 1*B*) and the frequency distribution of intervals was positively skewed with a mode interval of 4–4·5 s and a range of 3–14·8 s. The shapes of the interval frequency distributions for the other two lymphangions examined were also positively skewed, but the mode interval values and the ranges differed considerably between the three preparations examined (see Table 1). For the specific perfusion conditions utilized in this study (see Methods) all three isolated lymphangions exhibited a 'refractory' interval of 2–3 s during which they were not observed to undergo another

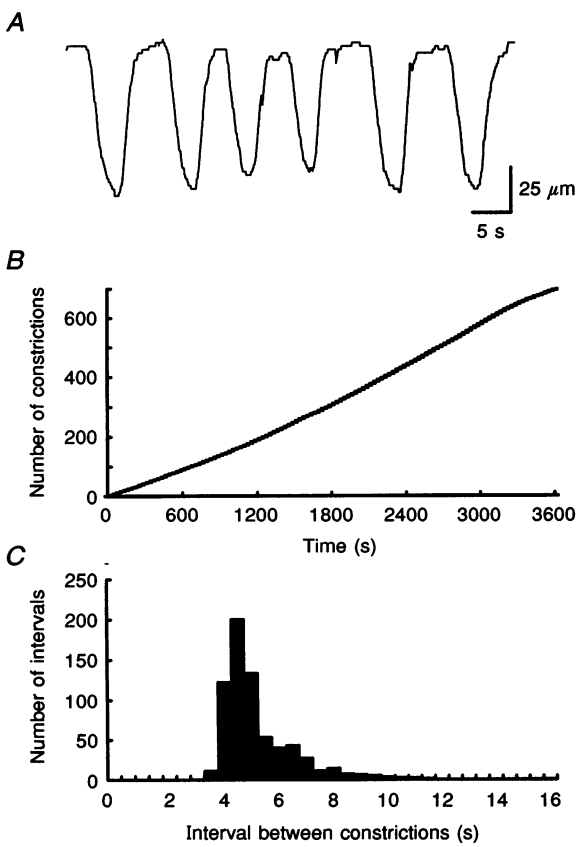

Figure 1. Contractile activity of a single isolated lymphangion

A, edge trace of the lymphangion showing the regularity of its constrictions (i.e. downward deflections). *B*, graph showing the cumulative number of constrictions of this lymphangion recorded over a 60 min period. *C*, histogram showing the frequency distribution of intervals between constrictions during the same 60 min period.

Table 1. Intervals between constrictions of single lymphangions

	Mode interval (s)	Range of intervals (s)
Lymphangion 1	3·0–3·5	2·5–106·5
Lymphangion 2	10·0–10·5	2·5–54·5
Lymphangion 3	4·0–4·5	3·0–14·8
Lymphangion 4	10·5–11·0	2·0–24·8
Lymphangion 5	5·5–6·0	1·8–146·3
Lymphangion 6	2·0–3·0	0·5–8·3
Lymphangion 7	4·5–5·0	1·5–16·0

Lymphangions 1–3 were isolated from the vessels and lymphangions 4–7 were in intact vessels.

constriction. However, this does not reflect an absolute refractory period, as increasing the perfusion rate can reduce the minimum refractory period (see review by Van Helden, von der Weid & Crowe, 1995).

The contractile activity of four small lymphangions in intact perfused vessels was also examined. The frequency of constrictions remained fairly constant for recording periods of between 1 and 3 h with two of the lymphangions showing a decline in the pumping frequency towards the end of the recording period. Like the single isolated lymphangions, the frequency distributions of intervals between constrictions for these lymphangions were positively skewed and the mode interval values and range of intervals varied considerably between preparations (see Table 1). These lymphangions also exhibited a refractory interval (0·5–2 s) during which the lymphangions were not observed to undergo another constriction.

Contractile activity of pairs of adjacent lymphangions

The contractile activity of twenty-two pairs of adjacent lymphangions from thirteen vessels was analysed. For these lymphangions the mean interval between constrictions of the individual chambers ranged between 3·0 and 20 s and the mean interval between all constrictions was 9·3 ± 0·4 s ($n = 2576$ intervals). Figure 2 shows histograms of the frequency distribution of intervals between constrictions of the upstream (L1) and the downstream (L2) lymphangions for two of these pairs of lymphangions (shown in Fig. 2*A* and *B*). In these histograms the intervals between constrictions of the upstream and the downstream lymphangions (i.e. L1 to L2) and between constrictions of the downstream and the upstream lymphangions (i.e. L2 to L1) are given positive and negative values, respectively. Figure 2 also shows traces of the contractile activity for the two pairs of lymphangions. In Fig. 2*A*, constrictions propagated orthogradely with contraction of the upstream lymphangion (L1) normally being followed within 0·5 s by that of the downstream lymphangion (L2). This is shown by the group of intervals located just positive to zero, the peak interval value being between 0 and 0·25 s. In addition, there is a second group of intervals with a peak value between −6·25 and −6·5 s, which reflects the intervals between constrictions of the downstream and upstream lymphangions (i.e. L2 to L1) and closely approximates intervals between constrictions of the individual lymphangions. In Fig. 2*B*, contractile activity

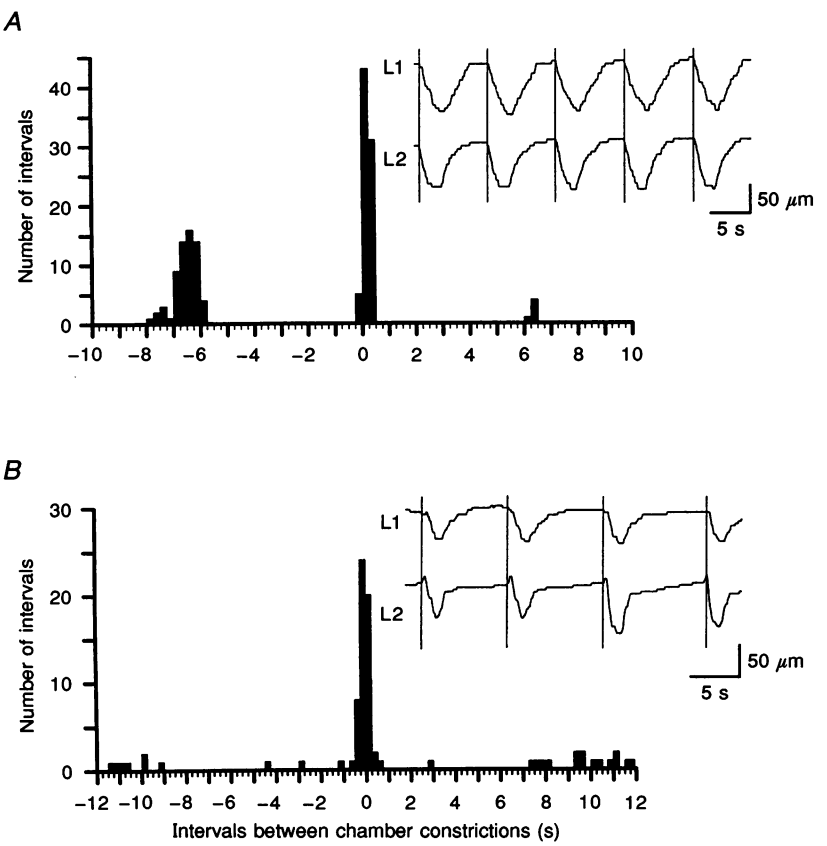

Figure 2. Contractile activity of adjacent lymphangions

A and *B*, edge trace recordings (insets) and interval frequency distributions for two pairs of lymphangions. The intervals between constrictions of the upstream and downstream lymphangions (L1 to L2) are presented in the positive-going direction and the intervals between the downstream and upstream lymphangions (L2 to L1) are presented in the negative-going direction. The vertical lines on the insets indicate the start of each pair of constrictions.

propagated in both the orthograde and the retrograde direction, there being a greater likelihood of the upstream lymphangion constricting before the downstream lymphangion as indicated by the peak interval being between 0 and −0·25 s.

In fourteen of the twenty-two pairs of lymphangions investigated, constriction of the upstream lymphangion was normally followed within a short interval by that of the downstream lymphangion, that is, constriction proceeded in the orthograde direction. However, in five pairs of lymphangions constriction normally proceeded in the retrograde direction. In the remaining three pairs of lymphangions the direction of propagation of contractile activity changed throughout the recording period.

To quantify the rate of propagation of contractile activity between the pairs of lymphangions, the percentage of constrictions that occurred within 1 and 2 s of each other was calculated. In seventeen pairs of lymphangions > 50% of constrictions occurred within 1 s of each other and in three pairs of lymphangions > 50% of constrictions occurred within 2 s of each other. The contractile behaviour of the remaining two pairs of lymphangions was not as tightly co-ordinated. For the seventeen pairs of lymphangions with > 50% of constrictions occurring within 1 s of each other, three and six pairs of lymphangions underwent > 50% of constrictions within 0·25 and 0·5 s of each other, respectively.

Mechanism of propagation of contractile activity across the valve

As perfusion of the lymphatic vessels was necessary to produce contractile activity in the majority of lymphangions studied, it is likely that mechanical stimulation had a major role in initiating constriction. In perfused vessels, constriction of many lymphangions is followed by a slow dilatation, with the initiation of the next constriction normally occurring at a relatively constant diameter (e.g. see insets in Fig. 2*A* and *B*). This finding suggests that the rate of lymphangion filling is important in determining the frequency of constrictions.

In thirteen of the twenty-two pairs of lymphangions investigated, > 70% of constrictions of one or both lymphangions were preceded by a transient dilatation or increase in the rate of dilatation (see Fig. 3). In the majority of cases, constriction of the upstream lymphangion caused a transient dilatation of the downstream lymphangion (e.g. Fig. 3*A*). However, in some cases where constriction propagated retrogradely, the upstream lymphangion was transiently dilated when the downstream lymphangion constricted. In this case, closure of the intervening valve during constriction of the downstream lymphangion caused an increased rate of filling of the upstream lymphangion. In addition, there were cases where both lymphangions underwent a near synchronous transient dilatation prior to constricting (e.g. Fig. 3*B*). In these cases constriction of a lymphangion further upstream or downstream resulted in an increased rate of filling.

In the remaining nine from twenty-two pairs of lymphangions, constriction was not normally preceded by a transient dilatation (e.g. see Fig. 2*A*). In particular this was true for the pairs of lymphangions in which constriction propagated rapidly across the valve. This suggests that mechanical stimulation may not be the only mechanism co-ordinating the contractile activity of pairs of lymphangions. One possibility is that electrical activity propagates directly between the lymphangions via smooth muscle cells in the region of the intervening valve. The following sections describe experiments designed to address this possibility.

Smooth muscle in the valve region

Morphological studies using the confocal microscope demonstrated that the smooth muscle fibres along the length of lymphangions were mostly circularly aligned in a

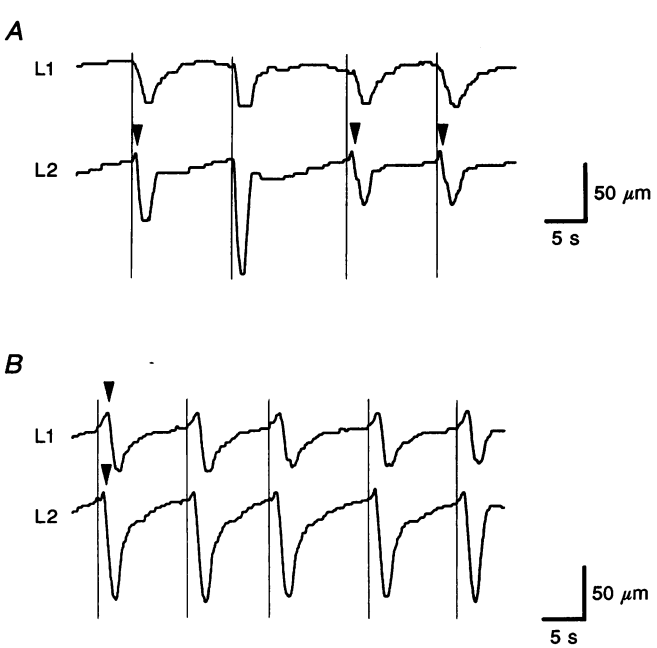

Figure 3. Edge traces of pairs of lymphangions showing a transient dilatation just prior to constriction

L1 indicates the upstream lymphangion and L2 indicates the downstream lymphangion. *A*, constriction of the upstream lymphangion caused a transient dilatation of the downstream lymphangion. *B*, both lymphangions simultaneously dilated just prior to constriction. The vertical lines indicate the start of each pair of constrictions. Arrowheads indicate transient dilatations.

continuous sheet, with occasional fibres lying longitudinally or obliquely along the vessel. The smooth muscle in the region of the intervening valve was examined for a total of sixty-three pairs of lymphangions. In eighteen of these pairs of lymphangions there was no interruption of the smooth muscle sheet in the region of the valve (continuous smooth muscle, Fig. 4*D*), while in thirty-four pairs of lymphangions the numbers of smooth muscle fibres decreased and their orientation became less regular such that they no longer formed a continuous sheet (partially continuous smooth muscle, Fig. 4*C*). The remaining eleven pairs of lymphangions had no detectable smooth muscle present in the region of the valve, with a gap of 17–140 μm between the smooth muscle of the adjacent lymphangions (totally discontinuous smooth muscle, Fig. 4*A*).

The pairs of lymphangions in which the smooth muscle in the region of the intervening valve was examined included the twenty-two pairs of lymphangions in which the co-ordination of the contractile activity was examined (see

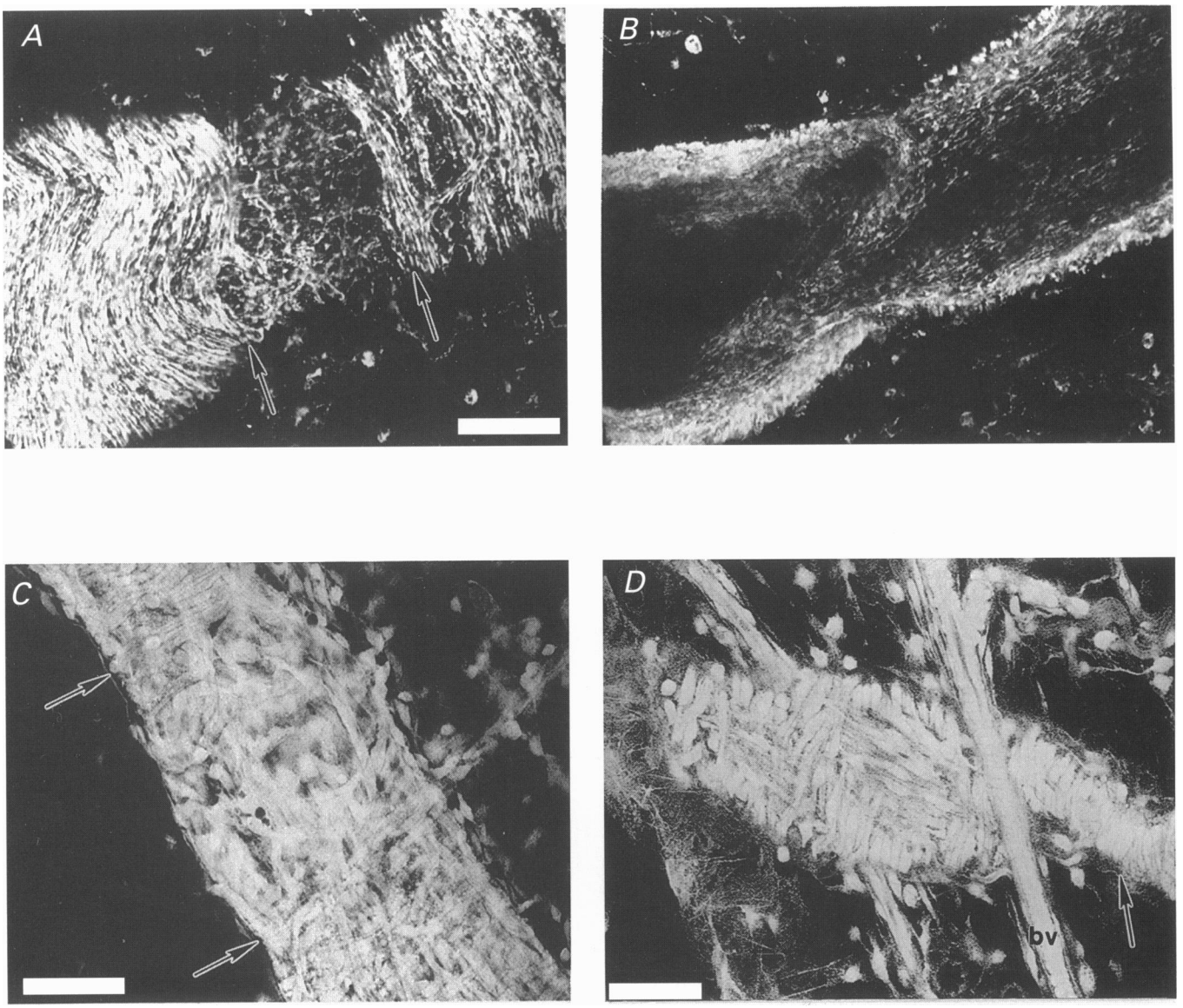

Figure 4. Confocal images of lymphatic vessels showing various arrangements of the smooth muscle in the region of the valve

Tissues were stained with $DiOC_2$ (*A* and *B*) or visualized by glutaraldehyde fixation (*C* and *D*). *A*, an optical section through the smooth muscle of a vessel showing a total discontinuity in the smooth muscle in the region of the valve. The arrows in *A* indicate the region where the smooth muscle is discontinuous. *B*, an optical section through the lumen of the vessel shown in *A*, revealing the structure of the valve. *C* and *D*, optical sections showing partial (*C*) and total (*D*) continuity of the smooth muscle in the region of the valve. In *C*, the arrows indicate the points where the smooth muscle becomes partially continuous and in *D* the arrow indicates the position of the valve region. The scale bars represent 50 μm. The scale bar in *A* also applies to *B*. bv, blood vessel.

Table 2. Comparison between the continuity of smooth muscle in the region of the valve and the intervals between constrictions of the adjacent lymphangions

Interval during which > 50% of constrictions occurred (s)	Continuity of smooth muscle at valve		
	Continuous	Partially continuous	Discontinuous
< 0·25	2	1	0
< 0·5	2	3	1
< 1	1	7	0
> 1	0	3	2

above). For these twenty-two pairs of lymphangions, five had continuous smooth muscle, fourteen had partially continuous smooth muscle and three had no smooth muscle connection between the lymphangions. Table 2 shows the intervals during which > 50% of constrictions occurred for pairs of lymphangions with continuous, partially continuous or totally discontinuous smooth muscle in the region of the valve. This table reveals that there was a tendency for the intervals between constrictions to be shorter for the pairs of lymphangions with continuous smooth muscle in the region of the valve.

Electrical coupling between adjacent lymphangions

Electrophysiological recordings were made from twenty-seven pairs of cells across twenty different valves. The extent of electrical coupling was investigated by injecting current pulses (−0·1 to −1·3 nA) into one microelectrode and recording voltage changes at the second microelectrode across the valve at distances of less than 1 mm (range, 170–800 μm). In fifteen of twenty pairs of lymphangions studied, electrical coupling was detected across the valve (see Fig. 5*A* and *B*). In eleven pairs of cells where current pulses were injected through both microelectrodes, similar voltage responses were recorded at the second microelectrode suggesting that there was no polarity to the coupling. In two of the fifteen pairs of lymphangions in which coupling across the valve was observed, the first set of impalements failed to reveal coupling across the valve but removal of one of the microelectrodes and impalement of a nearby smooth muscle cell subsequently revealed electrical coupling.

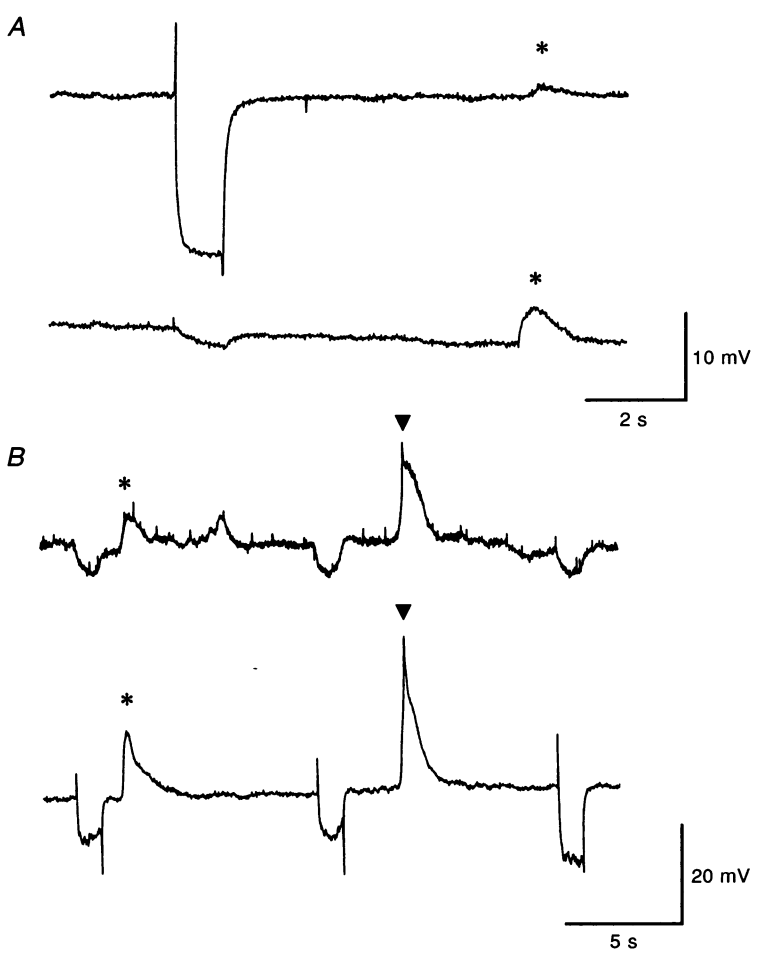

Figure 5. Examples of electrical coupling across lymphatic valves

A, simultaneous recordings made from two smooth muscle cells which were separated by a distance of 800 μm across a valve. A current pulse of −1 nA injected into cell 1 (top trace) caused a voltage change in cell 2 (bottom trace) and a spontaneous transient depolarization (STD; indicated by *) was simultaneously recorded from both cells. The resting membrane potentials were −54 and −60 mV for cell 1 and cell 2, respectively. *B*, recording from another pair of cells separated by a distance of 400 μm across a valve. Current injection of −0·9 nA into cell 2 (bottom trace) caused voltage changes in cell 1 and an action potential (indicated by an arrowhead) was recorded approximately simultaneously in both cells. This action potential was associated with constriction of both lymphangions (not shown). A subthreshold depolarization was also recorded in both cells (indicated by *), which may have resulted from the summation of STDs or an action potential conducted from further along the vessel. The resting membrane potentials were −59 and −55 mV for cell 1 and cell 2, respectively.

In at least one of these initial recordings that failed to show electrical coupling, the microelectrode may have impaled an endothelial cell, as the membrane potential recorded was relatively negative (−77 mV). Endothelial cells in guinea-pig mesenteric lymphatic vessels have a resting membrane potential of about −70 mV, which is significantly more negative than that for smooth muscle cells (see von der Weid & Van Helden, 1995). The mean resting membrane potential for all recordings in cells where coupling was detected was −59 ± 1 mV ($n = 40$). No significant difference in membrane potential was detected between the pairs of cells.

Recording from an endothelial cell may also have accounted for at least three of the five pairs of lymphangions where no electrical coupling was detected. This is concluded because the resting membrane potential recorded by one of the electrodes was markedly more negative than that recorded by the other electrode (−75 ± 2 mV compared with −55 ± 3 mV; difference, 20 ± 4 mV; $n = 3$).

Electrical coupling and smooth muscle in the valve region

For the pairs of lymphangions where double microelectrode recordings were made, the smooth muscle in the region of the valve was either continuous ($n = 10$) or partially continuous ($n = 10$). No recordings were made from valves where subsequent morphological analysis revealed the smooth muscle to be discontinuous. This presumably reflects the relatively low frequency of occurrence of this type of morphology (see above).

In pairs of lymphangions that were electrically coupled, there was no significant difference in the extent of electrical coupling for valves where the smooth muscle was either continuous or partially continuous. Where measured, the smallest current that produced a detectable voltage change across the valve was −0·3 ± 0·1 nA (range, −0·1 to −0·7 nA; $n = 9$) for valves with continuous smooth muscle and −0·4 ± 0·1 nA (range, −0·01 to 1·0 nA; $n = 11$) for valves with partially continuous smooth muscle. For these recordings the distances between electrodes did not differ significantly (continuous: 479 ± 65 μm, range 170–800 μm; partially continuous: 470 ± 38 μm, range 350–700 μm).

Transmission of cellularly generated electrical activity across the valve

Spontaneous transient depolarizations (STDs; see Van Helden, 1993) were recorded in seven of the electrically coupled pairs of lymphangions. In three of these pairs, STDs were recorded concurrently by both microelectrodes but with an obvious decrement in amplitude at one of the recording sites (see Fig. 5*A*). This is consistent with the premise that the currents underlying STDs are locally generated (Van Helden, 1991; Wang, Hogg & Large, 1992).

In three of the electrically coupled pairs of lymphangions, an action potential associated with constriction was recorded in one lymphangion whilst a potential change was simultaneously recorded in the adjacent lymphangion. However, the consequences of the action potential-associated depolarization in the adjacent lymphangions varied. In one pair of lymphangions in which action potentials were recorded at one microelectrode, the associated depolarization was always subthreshold and there was no corresponding action potential or constriction in the adjacent lymphangion. In the other two pairs of lymphangions, the associated depolarization sometimes triggered an action potential and constriction in the adjacent lymphangion (see Fig. 5*B*).

DISCUSSION

This study has investigated the contractile behaviour of guinea-pig mesenteric lymphatic vessels. When perfused, individual lymphangions constricted at fairly regular rates and the contractile activity of adjacent lymphangions was highly co-ordinated. The findings suggest that the propagation of contractile activity along these lymphatic vessels involves the transmission of both mechanical and electrical signals between the adjacent lymphangions. In particular, the study has provided a direct demonstration of electrical coupling between the smooth muscle of adjacent lymphangions.

Constriction of single lymphangions

Individual lymphangions constricted regularly and exhibited a refractory period following each constriction. This refractory period showed some variability between lymphangions. Presumably, this variability reflects the level of excitation of the pacemaker mechanism, as pumping rate can be readily modified by many factors including perfusion rate (see Florey, 1927*a*, *b*; Hall, Morris & Woolley, 1965; Hargens & Zweifach, 1977; Van Helden *et al.* 1995). However, using constrictions alone, it is not possible to determine the minimum refractory period of the pacemaker as the intervals between constrictions at higher levels of excitation could be limited by the refractory properties of the smooth muscle action potential and not necessarily the pacemaker mechanism *per se*.

At the minimum perfusion rate that induced continuous contractile activity, both the range of intervals between contractions and the mode interval varied considerably between the individual lymphangions studied. In the single isolated lymphangions these differences may, in part, reflect the rate of filling but may also be an intrinsic property of the pacemaker mechanism. However, in perfused vessels the constriction rate of most lymphangions appears to be determined by that of the neighbouring lymphangions, as their contractile activity is highly co-ordinated. This implies that a single lymphangion, located upstream or downstream, acts as a dominant pacemaker and that its constriction entrains the activity of a length of vessel (see also McHale & Meharg, 1992).

In bovine mesenteric vessels regular contractions occur in the absence of distending stimuli (Mawhinney & Roddie, 1973) and this activity is associated with pacemaker electrical

activity in the lymphatic smooth muscle (Allen *et al.* 1983; Ward, McHale & Sanders, 1989). In the smooth muscle of small unperfused guinea-pig mesenteric lymphangions or lymphangion segments, STDs are recorded at irregular intervals and are believed to result from activation of Ca^{2+}-activated excitatory channels by Ca^{2+} released from intracellular stores in a pulsatile manner (Van Helden, 1993). These events, if of sufficient amplitude, can trigger action potentials and constrictions. Therefore, STDs have been proposed to provide a basis for pacemaking in these vessels (Van Helden, 1993). This hypothesis assumes that STD activity synchronizes to give rhythmic depolarizations of sufficient magnitude to activate action potentials. As the present study demonstrated that vessel perfusion produces rhythmic constrictions, it may also be responsible for entraining STD activity. Consistent with this hypothesis is the finding that vessel distension increases STD activity in the smooth muscle of guinea-pig mesenteric veins (see Fig. 4 in review by Van Helden, von der Weid & Crowe, 1996).

Coupling between lymphangions

A particular emphasis of the present study was to examine the co-ordination of the contractile activity of adjacent lymphangions. Constriction of one lymphangion often resulted in a transient dilatation of an adjacent lymphangion, which then constricted, suggesting that, in these cases, distension triggered smooth muscle contraction. This finding is similar to that previously reported by Horstmann (1959). However, constrictions were not always preceded by a transient dilatation. In particular, this was the case for pairs of lymphangions in which contractile activity propagated rapidly across the valve.

In some 80% of pairs of lymphangions examined morphologically, the smooth muscle was connected across the valve. Furthermore, in the majority of pairs of lymphangions where electrophysiological measurements were made, hyperpolarizing current pulses were conducted across the valve. This finding indicates that most lymphangions are electrically coupled to their adjacent lymphangions. The study also demonstrated that STDs and action potential-associated depolarizations could propagate between the lymphangions. In some cases, the action potential-associated depolarization was of sufficient amplitude to trigger an action potential and contraction in the adjacent lymphangion.

While these findings indicate that electrically propagated signals can directly activate the vascular smooth muscle of a neighbouring lymphangion, it seems likely that these signals would also act together with those produced by mechanical stimulation. Vessel distension may produce depolarization of the lymphatic smooth muscle either directly by activation of stretch-activated channels and/or by inducing STD activity (reviewed in Van Helden *et al.* 1996). If this stretch-induced electrical activity sums with that conducted electrotonically between neighbouring lymphangions, this would more readily co-ordinate their pumping activity.

All pairs of lymphangions examined electrophysiologically had connecting smooth muscle. While no electrophysiological measurements were made across valves where the smooth muscle was totally discontinuous, it is unlikely that the co-ordinated contractile activity of such lymphangions would result from electrical coupling. This conclusion follows from the observation that the endothelium, which is continuous between all lymphangions, is not normally electrically coupled to the smooth muscle (von der Weid & Van Helden, 1995).

In conclusion, the present study has demonstrated that vessel perfusion induces rhythmic constrictions of individual lymphangions of guinea-pig mesenteric lymphatic vessels and that under these conditions the contractile activity of adjacent lymphangions is highly co-ordinated. In addition, the study has provided a direct demonstration of electrical coupling between the smooth muscle of adjacent lymphangions. The findings suggest that transmission of both mechanical and electrical signals between the adjacent lymphangions contributes to the co-ordination of their contractile activity.

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