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PACEMAKER POTENTIALS IN LYMPHATIC SMOOTH MUSCLE OF THE GUINEA-PIG MESENTERY

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SUMMARY

1. Intracellular microelectrode recordings have been made from lymphatic smooth muscle (SM) to investigate pacemaker mechanisms underlying lymphatic pumping.

2. The SM of small lymphangions or that of short segments, cut from lymphangions of any length, behaved similarly. Both preparations exhibited spontaneous transient depolarizations (STDs) and exhibited simplified electrical characteristics approximating those of a spherical cell.

3. STDs were found to underlie activation of action potentials and hence constrictions.

4. The level of STD activity correlated to the pumping activity of lymphangions, the SM from more active chambers exhibiting increased STD activity.

5. Lymphatic SM exhibited STDs with properties similar to the STDs of mesenteric veins. STDs appeared to be of myogenic origin as they were present despite denervation or substantial destruction of the endothelium.

6. Noradrenaline enhanced the size and frequency of STDs.

7. STD activity was abolished by chelation of cytosolic Ca^{2+}.

8. It is proposed that STDs provide a mechanism for pacemaking in the lymphatic SM studied here. Furthermore, it is postulated that STDs are the consequence of Ca^{2+}-dependent pulsatile release of an intracellular messenger, probably Ca^{2+} itself. This mechanism provides a novel means for pacemaking.

INTRODUCTION

It has been known for more than a century that many lymphatic vessels actively propel lymph. They do this through a division of lymphatic collecting vessels into literally millions of tiny 'hearts' termed lymphangions (Mislin, 1961, 1983). A lymphangion is comprised of a tubular chamber bounded by adjacent unidirectional valves and it has a central sleeve of smooth muscle (SM). In lymphatic tissues such as that of the guinea-pig mesentery the SM is either completely (Horstmann, 1959; Mislin, 1961, 1983) or partially (Crowe & Van Helden, 1992) discontinuous in the region of valves. As a result, many lymphangions have the capability of constricting independently of adjacent chambers. Constriction within a lymphangion is generally phasic, each caused by an action potential (Kirkpatrick & McHale, 1977).

A key determinant of lymph propulsion is the rate at which phasic constrictions

occur. Whilst it is known that intrinsic pumping occurs spontaneously, through perfusion or neural activity (Florey, 1927; Smith, 1949; Mislin, 1961; Hall, Morris & Woolley, 1965; Campbell & Heath, 1973; Mawhinney & Roddie, 1973; McHale & Roddie, 1976; McHale, Roddie & Thornbury, 1980; Casley-Smith & Casley-Smith, 1983; Schmid-Schonbein, 1990), there remains no understanding of the underlying pacemaker mechanisms. The present report addresses this long-standing question. Evidence is provided for myogenically generated potentials which are responsible for activation of action potentials and consequent constriction in the lymphatic SM studied in this report. Some of these results have been communicated elsewhere (Van Helden, 1989, 1990, 1991*b*).

METHODS

Experiments were performed *in vitro* on small (diameter < 200 μm) collecting vessels isolated from the jejunal lymphatics of young guinea-pigs (age < 1 month) killed by deep anaesthesia with halothane followed by cervical dislocation. The procedures used involved intracellular microelectrode recording and were similar to those used previously to study veins (Van Helden, 1991*a*). Experiments were performed either on small lymphangions (length < 500 μm) or on short segments (length < 300 μm) cut using a fragment of razor blade. Short segments were made in order to ensure simplified electrical properties of the SM. It has been shown in arterioles (Hirst & Neild, 1978) and in veins (Van Helden, 1988*a*, *b*) that vessel segments of similar dimensions provide SM with electrical properties approximating those of a spherical cell so that electrical activity, even though generated at localized foci within the SM, produces a similar voltage change in all the SM cells of the segment.

The physiological saline was applied at 34–36 °C and had the following composition (mM): $CaCl_2$, 2·5; KCl, 5; $MgCl_2$, 2; NaCl, 120; $NaHCO_3$, 25; NaH_2PO_4, 1; glucose, 11. The pH was maintained at 7·2 by bubbling with a 95% O_2–5% CO_2 gas mixture. Some experiments were made with vessels internally perfused with this same solution. A few experiments were made using low chloride solution in which all the NaCl of the physiological solution was replaced with sodium isethionate. Microelectrode recordings were made from the SM using electrodes filled with 1 M KCl and of resistance 90–140 MΩ. Vessel or segment constrictions were monitored by a video–computer edge detection system (Neild, 1989). The drugs used were: 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, acetyl methyl ester form (BAPTA AM; Molecular Probes, Eugene, OR, USA); L-noradrenaline bitartrate (NA, Sigma Chemical Co., St Louis, MO, USA); phentolamine mesylate (Sigma Chemical Co.); prazosin hydrochloride (Pfizer, Groton, CT, USA).

Denervation

Denervation was performed using the procedure of Hill, Hirst, Ngu & Van Helden (1985). Guinea pigs were anaesthetized with 60–100 mg/kg of ketamine hydrochloride. The small intestine was exposed with a midline incision and several jejunal mesenteric lymphatics were selected and marked with loosely tied ligatures. Denervation was effected by locally freezing each lymphatic for 15 s, proximally near to the mesenteric lymph node, using a liquid nitrogen-cooled steel rod tapered to a contact diameter of 1 mm. Experiments were performed *in vitro* on these animals 4–6 days after denervation.

Histochemical studies were made to determine the extent of the plexus of the sympathetic nerves remaining in the lymphatics after denervation. The method was based on the modified procedure of Furness, Costa & Wilson (1977) with a 3 h exposure to fixative consisting of 0·5% gluteraldehyde and 4% formaldehyde in phosphate buffer, pH 7·0. Subsequently, the preparations were dried over P_2O_5, mounted in paraffin oil and viewed under a Nikon Diaphot microscope fitted with a 100 W mercury lamp, a 390–420 nm bandpass excitation filter, a 455 nm dichroic mirror and a 490 nm barrier filter.

Disruption of the endothelium

The endothelium of lymphatics was disrupted *in vitro* by briefly (10–20 s) passing bubbles of gas (95% O_2–5% CO_2) into lymphatic vessels using a cannula placed distally (i.e. nearer the gut wall)

with other branches tied off. A glass cannula was then used to further the destruction of the endothelium. Histological examination confirmed that the combination of these two procedures removed the majority of endothelial cells (> 70%). More vigorous application of the procedures to completely remove the endothelium damaged the SM and hence was not used. The method provided short segments which had normal membrane potentials and input resistances and which exhibited increased pumping to application of 1 μM NA in the superfusate.

RESULTS

General observations

The lymphatic vessels studied were the collecting lymphatics (lacteals) of the jejunal region of the guinea-pig mesentery isolated from young animals. The small lymphatic vessels (diameter 50–150 μm) which emerged from the gut wall converged to form larger vessels (diameter 100–350 μm) which followed the vascular network up to the mesenteric lymph nodes. Most vessels had contractile capability, having a thin layer of SM in the vessel walls, and transiently constricted in localized regions to pump lymph. Lymphatic pumping was generally observed when vessels were perfused or during application of agonists such as NA, though some regions of vessel were observed to undergo constrictions in the absence of such stimulation.

There were marked discontinuities in the electrical coupling of SM in the region of the valves as constrictions only infrequently propagated across these regions (see below). The example in Fig. 1 demonstrates constrictions typical of medium (or smaller) sized lymphangions (length < 2 mm; diameter < 300 μm). The SM within each lymphangion constricted synchronously (as observed through the microscope) pumping lymph by transient reductions in chamber volume. Larger lymphangions (length > 2 mm) did not always constrict synchronously. Indeed a few very long lymphangions (length > 4 mm) exhibited waves of constriction which traversed the length of the chamber.

The records in Fig. 1 show three lymphangions of a perfused vessel demonstrating (top to bottom), the relaxed state, the central lymphangion constricted, the rightmost lymphangion constricted and all three lymphangions constricted. The independent constrictions of the chambers (Fig. 1*B* and *C*) indicate discontinuity in the electrical coupling at the valves between the chambers. However, the observation of near synchronous constriction (Fig. 1*D*), suggests that this discontinuity is only partial. Some feeling for the extent of electrical coupling across valves can be gleaned from the observation that all chambers (in more than 20 studied) were capable of constricting independently of adjacent chambers. Of these, less than 50% were observed to undergo near synchronous constrictions with adjacent chambers and they generally did this only infrequently. For example, the near synchronous constrictions, as shown in Fig. 1*D*, occurred 5 times in 100 constrictions of any of the three chambers. Thus, the valves which occur frequently along these vessels not only divide vessels into multiple chambers but are demarcation points for the continuity of the SM. Therefore, many chambers constrict independently of adjacent chambers. In so doing, they act as primitive hearts propelling lymph in a forward direction through the unidirectional valves.

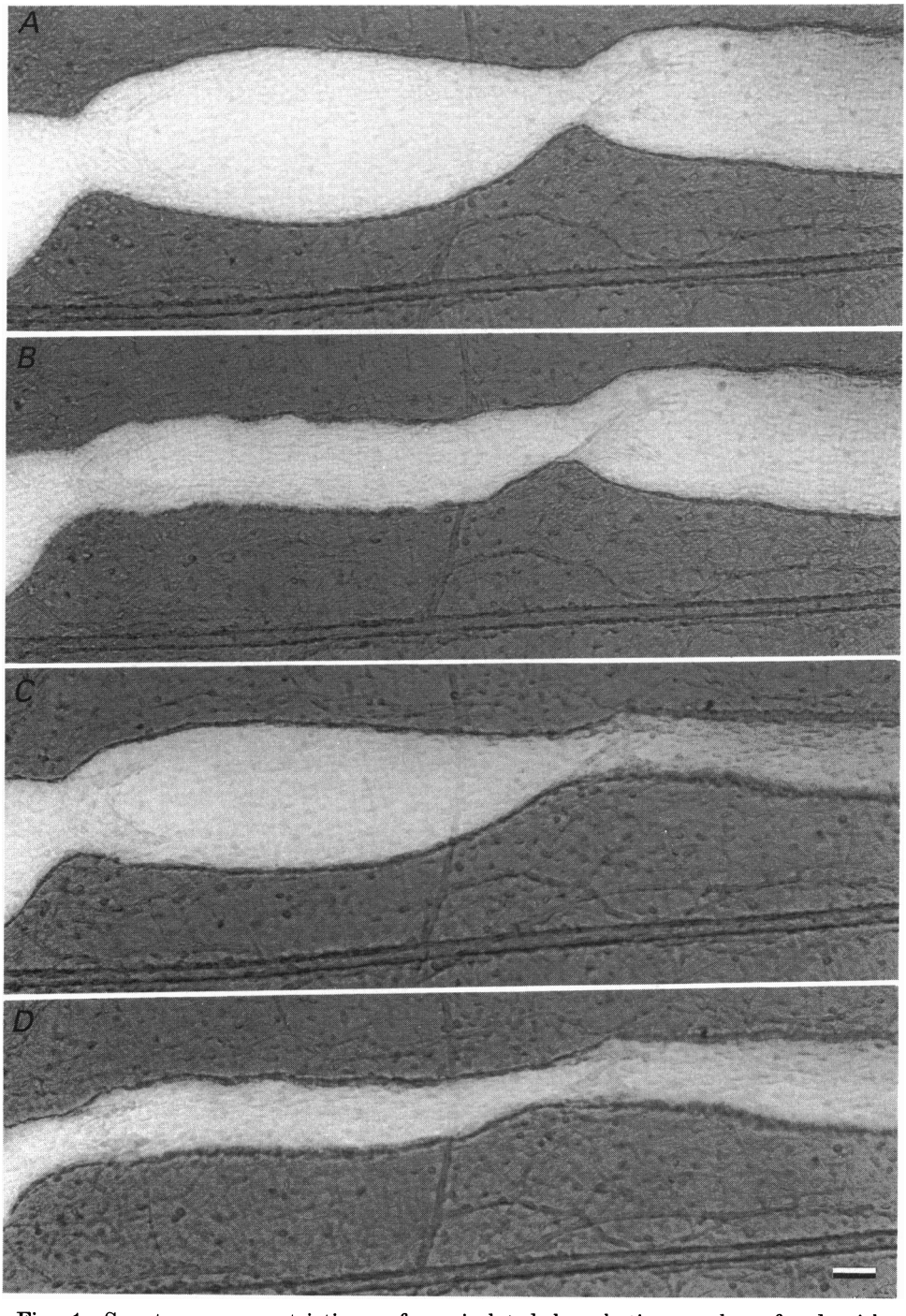

Fig. 1. Spontaneous constrictions of an isolated lymphatic vessel perfused with physiological saline containing 1 μM fluorescein and viewed through a fluorescence microscope (Nikon diaphot, fluorescein filter set). The vessel is shown in its relaxed state

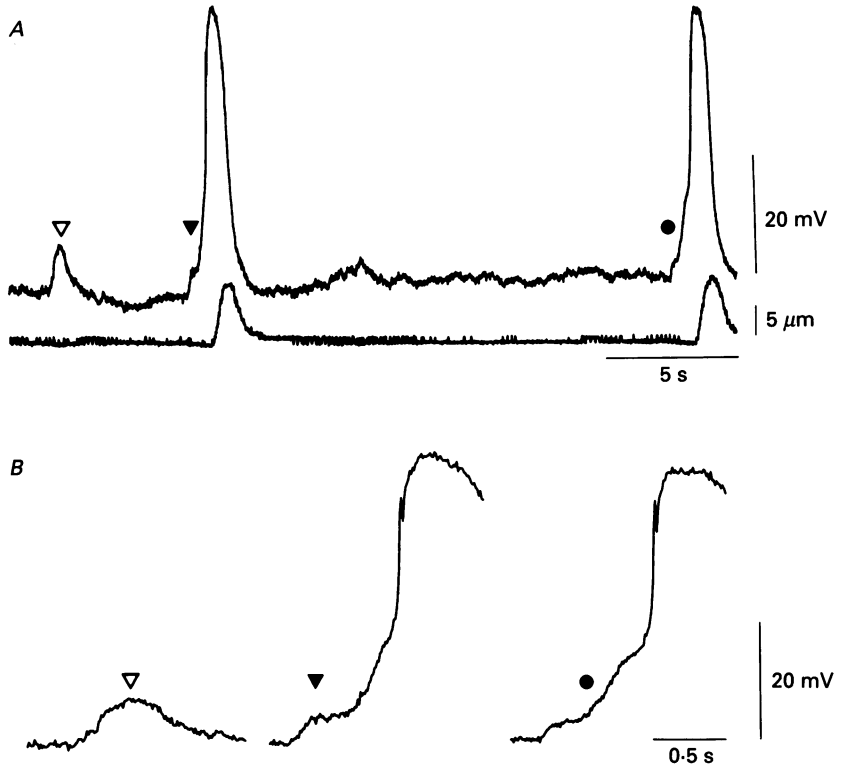

Fig. 2. Records of membrane potential and associated constriction of a short segment of lymphatic vessel (length 220 μm, diameter 130 μm) undergoing spontaneous constrictions. *A*, the SM exhibited spontaneous transient depolarizing potentials and action potentials. STDs (▽) were monophasic and did not cause constriction whereas action potentials (▼, ●) were biphasic and caused constriction. *B*, the STD and the action potentials marked in *A* are shown on an expanded time scale. The SM of the segment had a membrane potential of −61 mV and an input resistance of 44 MΩ.

Electrophysiology of short segments

The first approach taken to study the pacemaker mechanism underlying activation of constrictions was to cut lymphangions of small diameter (70–200 μm) into one or more short segments (length < 300 μm). The rationale for making these preparations was to create SM with simplified electrical characteristics so that electrical pacemaker activity, if present, would be recorded without decrement simply by placing a microelectrode into any of the SM cells present in a short segment of vessel. Short segments were found to be effectively isopotential since the response to injection of a current pulse was exponential (see Hirst & Neild, 1978; Van Helden, 1988*b*). Measurements made on twenty-one short segments provided an input resistance and a mean time constant of 36 ± 6 MΩ and 178 ± 14 ms respectively. The average

(*A*), with the middle chamber constricted (*B*), with the right chamber constricted (*C*) and with all three chambers constricted (*D*). The scale bar represents 70 μm. Maximum diameter of relaxed central chamber in *A* is 280 μm.

membrane potential of the SM of these short segments was $-65{\cdot}1 \pm 1{\cdot}5$ mV ($n = 21$), a value similar to that for intact vessels with a mean value of $-66{\cdot}4 \pm 1{\cdot}9$ mV ($n = 16$, all values presented as means ± 1 S.E.M.).

Pacemaking activity in short segments

As has been noted, lymphangions were not always quiescent under resting conditions (without internal perfusion or NA) with some chambers exhibiting spontaneous pumping. This activity was also observed in some of the short segments. Recordings from spontaneously active short segments provided insight into the mechanism responsible for pacemaking. An example of a recording of the membrane potential and associated constrictions of a segment is shown in Fig. 2. Two types of depolarizing event were evident. The first type, termed spontaneous transient depolarization (STD), was similar in form to the STDs first reported in veins (Van Helden, 1991*a*). STDs had a monophasic waveform and produced no constriction (e.g. the first depolarization marked in Fig. 2*A*). The second type of depolarization was an action potential. This was always of large amplitude and induced constriction. The action potentials were characteristic in shape, being biphasic and having rise phases much more rapid than those of STDs. As seen from the expanded records (Fig. 2*B*), each action potential was preceded by STD-like prepotentials suggesting that STDs are the basis for pacemaking in these cut segments.

Pacemaking activity in intact lymphangions

Evidence for pacemaker potentials has so far been presented in cut segments where electrical activity within the SM was recorded without decrement. Many small lymphangions, in constricting synchronously and independently of adjacent lymphangions, appeared to function as 'short' segments. Consistent with this premise, was the finding that the SM of the small lymphangions studied had measurable input resistance and responded with an exponential voltage transient to application of a current step (see Fig. 3*D*).

The results of intracellular recording from a small lymphangion before and during spontaneous constrictions are presented in Fig. 3. Both action potentials (Fig. 3*A*) and STDs (Fig. 3*B*) were observed. As for short segments, only action potentials induced constrictions. Insight into the mechanism responsible for generating the action potentials is provided by comparing the rising phase of the STDs and the foot of the action potential (Fig. 3*C*). The waveforms overlie each other indicating that the action potentials were triggered by STDs. The finding that STDs serve as pacemaker potentials was further confirmed in studies on three other small lymphangions. Taken together STDs in intact lymphangions were not obviously different to those recorded in short segments. Furthermore, the STD-like prepotentials generated action potentials suggesting that STDs provide a means for pacemaking in these small lymphangions.

Enhanced STD-activity in the SM of 'active' and 'inactive' lymphangions

To date, it has not been possible to maintain intracellular recordings of perfused lymphatic vessels. As vessel perfusion is an important means of activating lymphangions it was important to establish some correlation between STDs and

perfusion-associated activity. To do this, advantage was taken of the finding that lymphangions showed different responses to perfusion. For example, while the majority of lymphangions of a vessel commenced pumping upon perfusion, some remained quiescent. Therefore, a comparison was made between intracellular

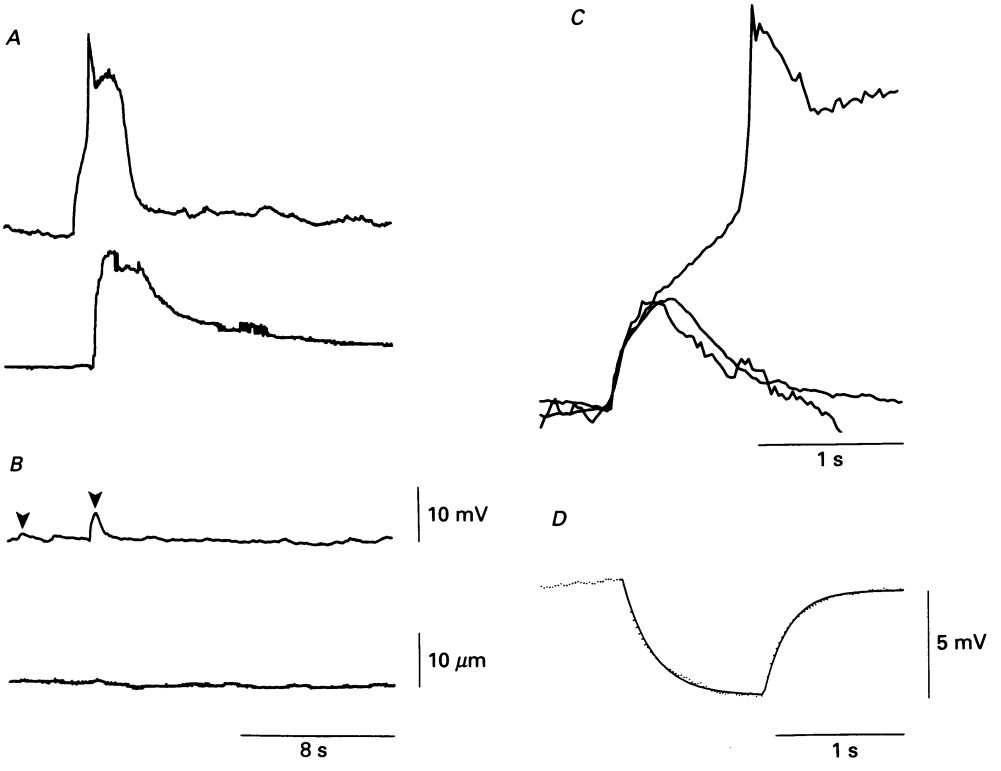

Fig. 3. Records of membrane potential and constriction in a non-perfused lymphangion (length 390 μm; diameter 150 μm). The resulting potential was −67 mV. *A*, the action potential was biphasic and caused constriction (scale bars of *B* apply). *B*, STDs were of variable size and were monophasic. They did not directly cause constriction. Two STDs have been marked (arrowheads). *C*, the action potential and STDs (of *A* and *B*). The STDs have been amplitude scaled to that of the foot of the action potential. *D*, the voltage response to injection of a current pulse (amplitude −0·2 nA). The solid curve represents the exponential of best fit to the 'on' and 'off' transients of the voltage response.

microelectrode recordings from the SM of 'active' segments (vessel segments cut from small lymphangions which had either commenced or increased pumping upon internal perfusion) and 'inactive' segments (vessel segments cut from small lymphangions which had remained inactive despite internal perfusion). Intact vessels of small diameter (< 200 μm) were internally perfused by locating a glass perfusion cannula into the distal end of a lymphatic vessel. This perfusion procedure activated most, although not all, of the lymphangions to undergo spontaneous pulsatile constrictions. Vessel segments were then cut from selected small lymphangions (length 300–500 μm) by cutting at the valves. This procedure isolated the lymphangion ensuring a stable preparation with no possibility of activation by

adjacent lymphangions either through distension or by any electrical coupling. The electrical characteristics of 'active' and 'inactive' segments were then compared.

Intracellular recordings from an 'active' and 'inactive' segment are presented in Fig. 4*A*. The 'active' segment, whilst no longer constricting in the absence of

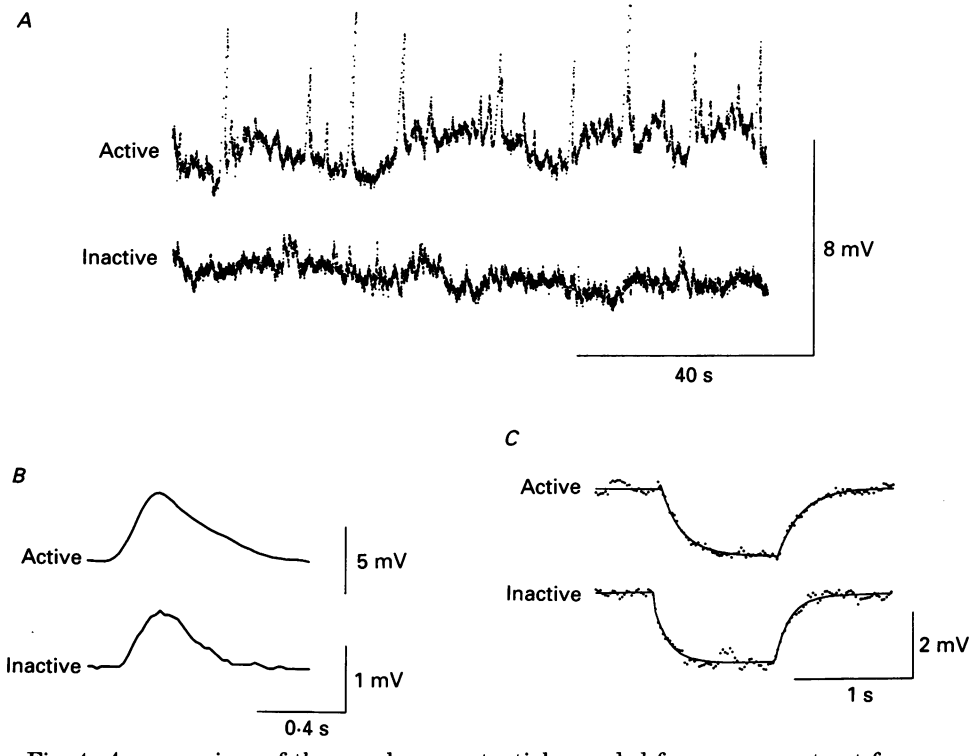

Fig. 4. *A*, comparison of the membrane potential recorded from a segment cut from an 'active' lymphangion (one which commenced pumping upon internal perfusion) to one cut from an 'inactive' lymphangion. The membrane potentials of the segments were held near −70 mV. *B*, the averages of 10 STDs from the 'active' and 'inactive' segments respectively (note the different voltage scales). *C*, the voltage responses to injection of a current pulse (amplitude 0·2 nA). The transient voltage changes were well fitted by an exponential waveform (continuous curves) for both 'active' and 'inactive' segments with time constants of 170 and 130 ms respectively.

perfusion, exhibited STDs. Such activity, was also present in the 'inactive' segment, although markedly smaller in amplitude. Figure 4*B* shows the comparison of the average of the ten largest STDs recorded over a 2 min period from the 'active' and 'inactive' segments respectively. The events, while about four times larger in the 'active' segment, exhibited a similar time course for both 'active' and 'inactive' segments. The difference in amplitude was not due to marked differences in input resistance of the two vessel segments as the response to a current pulse of the same amplitude produced similar steady-state voltage responses. Both segments were electrically short as the transient components of the voltage response were well fitted by an exponential function (Fig. 4*C*). Similar observations were made in two other experiments with STDs two to three fold larger in the 'active' segments.

Noradrenaline

The results so far are consistent with the hypothesis that STDs underlie pacemaking. A further test relates to the use of agonists such as NA which have been shown to enhance markedly lymphatic pumping (McHale & Roddie, 1983; McHale,

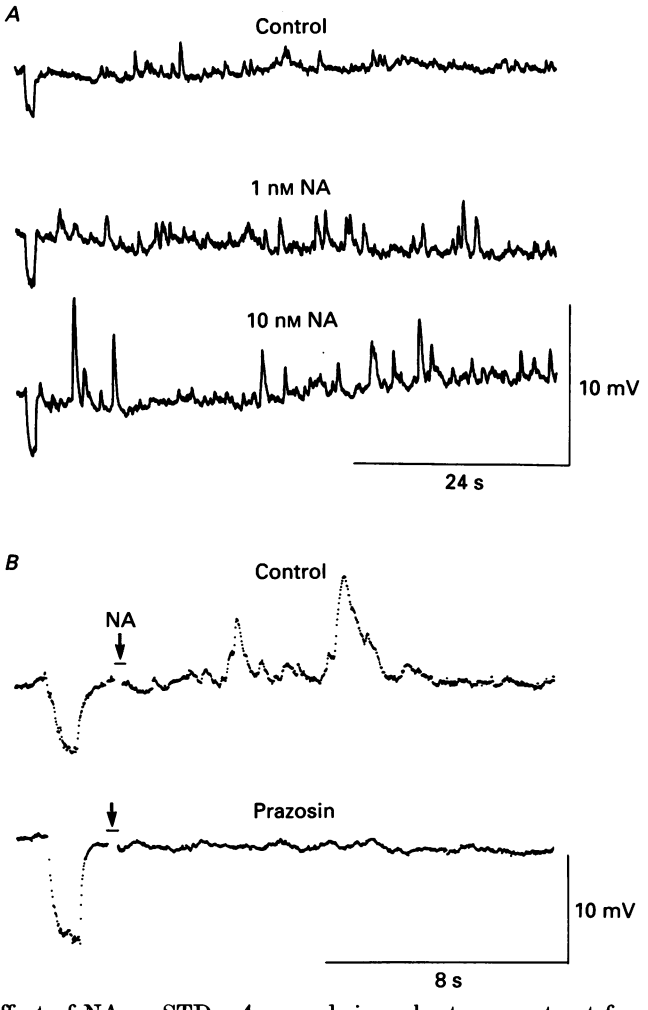

Fig. 5. The effect of NA on STDs. *A*, records in a short segment cut from a lymphatic vessel which had been denervated four days previously by surgical intervention. The membrane resistance was measured by injection of a current pulse (−0·1 nA) applied at the start of each record. The membrane potential of the segment was held near −65 mV throughout. *B*, STDs which resulted from application of NA (in this case by ionophoretic application to an innervated short segment; ionphoretic charge 0·5 nC) were blocked by prazosin (1 μM). The membrane resistance was monitored by application of a current pulse (−0·2 nA). The resting membrane potential of the segment was −63 mV.

Allen & Iggulden, 1987). The effects of NA on STDs and membrane resistance are shown in Fig. 5. Bath application of NA, in this case applied to a short segment from a denervated lymphangion, caused an increase in size and/or frequency of occurrence

of the STDs (Fig. 5*A*). This was not due to an increase in segment resistance as it increased by less than 40% compared with a more than 200% increase in the size of STDs. The records of Fig. 5*A* also show that STDs and their enhancement by NA were in no way related to innervation. The NA-induced STDs could also be induced

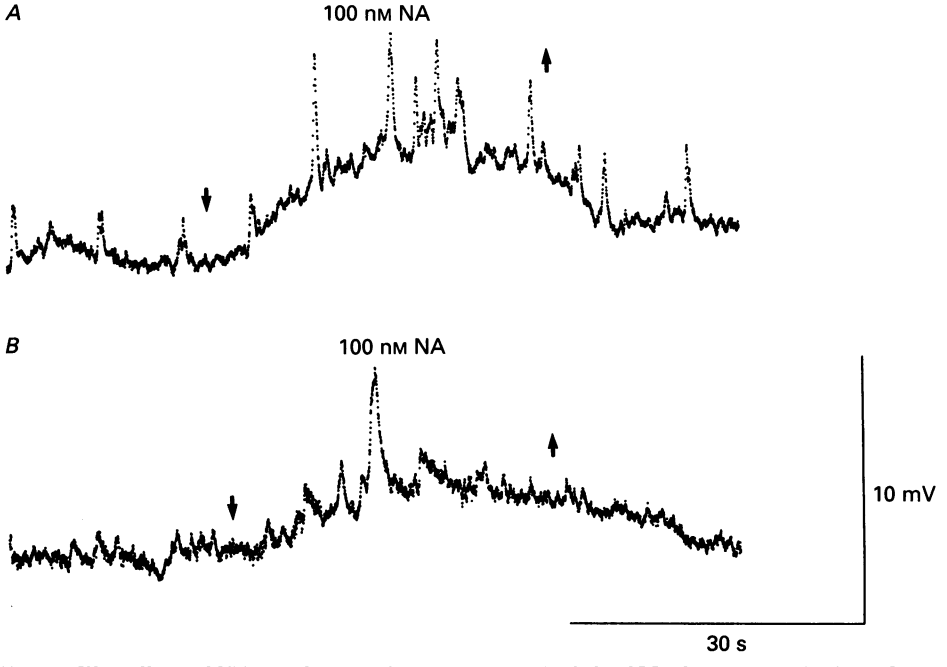

Fig. 6. The effect of NA on the membrane potential of the SM of an 'active' (*A*) and an 'inactive' (*B*) segment (see text). The membrane potentials of the segments were held near −70 mV before application of NA.

by brief ionophoretic application of NA with activity enhanced for 10–40 s afterwards ($n = 8$). They were a consequence of activation of α-adrenoceptors as NA-induced STDs were inhibited by application of the α-adrenoceptor antagonist prazosin (Fig. 5*B*).

A further test of this premise, namely that STDs are a general mechanism for pacemaking, relates to the observation that both 'active' and 'inactive' lymphangions (see above) could usually be induced to pump through exposure to agonists such as NA. While the 'effectiveness' of the NA to induce pumping as judged by the frequency of occurrence of constriction was lower for 'inactive' segments, the fact that NA could induce both perfused 'inactive' and 'active' segments to constrict or to constrict more frequently indicates that STDs are an inherent mechanism for pacemaking in most lymphangions. The likelihood that the pacemaker mechanism underlying this activity is STD related is supported by the records shown in Fig. 6. Shown are the NA-induced effects on the membrane potential in a segment cut from an 'active' lymphangion (Fig. 6*A*). There was a marked enhancement of the STDs in response to the NA. This record is to be

compared to that from an 'inactive' segment (Fig. 6*B*). Application of NA caused marked enhancement of STD activity in this latter segment although the response was less marked than that of the 'active' segment.

Some properties of STDs

Lymphatic STDs shared many of the properties of STDs in mesenteric veins (Van Helden, 1991*a*). STDs were recorded in all twenty-one short segments. They were

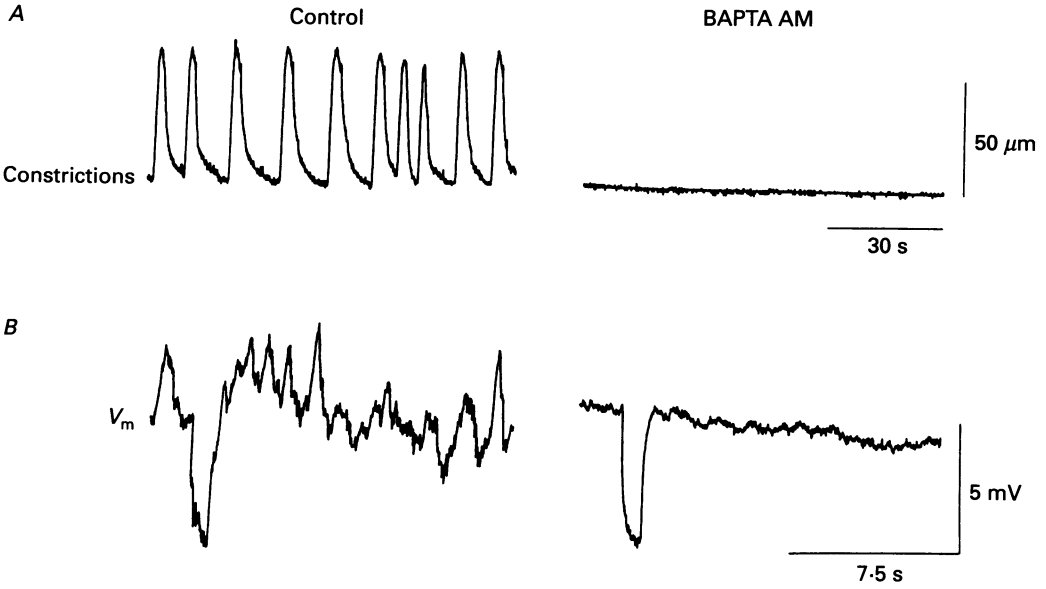

Fig. 7. The effect of chelation of intracellular Ca^{2+} on constriction and STDs. *A*, constrictions in a perfused lymphangion were inhibited by exposure to BAPTA AM (2 min exposure at 60 μM). The lymphangion was both superfused and perfused with physiological saline (Methods) but with $[Ca^{2+}]$ reduced to 1 mM. *B*, STDs which occurred at a high frequency in a short segment under control conditions (same superfusing solution as for *A*) were suppressed by BAPTA AM (3 min exposure at 60 μM). The membrane potential (V_m) of the segment was held near −65 mV.

always monophasic with slow onset (time-to-peak 100–300 ms) and duration between 0·3 and 1·5 s. The amplitude of STDs varied markedly both within and between segments. STDs persisted despite neural block with 1 μM tetrodotoxin ($n = 3$), denervation (Fig. 5*A*) or 1 μM phentolamine, a non-selective antagonist to α-adrenoceptors ($n = 4$). They also persisted even after severe damage to the endothelium ($n = 3$). Lymphatic STDs reversed at potentials near −35 mV (range −40 to −25 mV; $n = 4$) and were suppressed by exposure to low chloride solution. STDs were not carried by Ca^{2+} as removal of Ca^{2+} and substitution with a Ca^{2+} channel antagonists (Co^{2+} or Mn^{2+}) caused an initial (time < 1 min) enhancement of STDs while completely blocking the Ca^{2+} action potentials (4 segments, 95% bath changeover times < 6 s). The effect of longer term exposure (1–5 min) to either of the nominally Ca^{2+}-free solutions was to inhibit STDs. This may have been due to a

reduction of $[Ca^{2+}]_i$. Paralleling these studies were the observations that lymphatic pumping was not blocked by TTX, phentolamine or denervation but was blocked by exposure to low chloride solution. Lymphatic pumping was not inhibited by severe damage to the endothelium, a finding consistent with that of Hanley, Elias & Johnston (1992) who were able to destroy completely the endothelium of bovine lymphatics without inhibiting pumping.

A role for $[Ca^{2+}]_i$ was tested by chelating cytosolic Ca^{2+}. The procedure used was to superfuse the tissue with the Ca^{2+} chelator BAPTA in a membrane permeable form. BAPTA AM when applied at 60 μM for longer than 2 min abolished constrictions in a lymphatic vessel exposed to a physiological saline of reduced Ca^{2+} concentration (Fig. 7*A*). Most importantly the BAPTA AM abolished STDs (Fig. 7*B*), an action observed in the three tissues studied.

DISCUSSION

This report has presented evidence that spontaneous transient depolarizations, as first reported in the SM of mesenteric veins (Van Helden, 1991*a*), are also functional in lymphatic SM. The remarkable finding with respect to lymphatic vessels is that STDs are likely to be the pacemaker potentials responsible for initiating and controlling pumping in the small lymphangions studied. They do not subserve this role in guinea-pig mesenteric veins which do not normally undergo phasic constrictions (Suzuki, 1981; Van Helden, 1988*a*). The distinctive role in lymphatics compared to veins does not appear to be due to differences in the properties of STDs but rather to very different geometries and electrical characteristics of the SM. This difference is primarily a consequence of the division of lymphatic SM into segmented chambers (lymphangions) which act as electrically isolated units due to a marked discontinuity of the SM at the valves (see Mislin, 1983). The result is that the small lymphangions studied have higher input impedances such that the currents which underlie STDs cause relatively large potential changes and hence can activate action potentials.

The mechanism by which STDs are generated is indistinguishable from that in mesenteric veins. The data obtained from cellular recordings from lymphatic SM again supports the postulate that STDs are the consequence of pulsatile release of an 'intracellular messenger' which activates an excitatory conductance. In the studies on STDs in veins it was postulated that pulsatile release of Ca^{2+} from stores activated a conductance carried by excitatory Ca^{2+}-activated channels (Van Helden, 1991*a*). In this respect, except for being excitatory, STDs share many of the characteristics of spontaneously transient outward currents first reported by Benham & Bolton (1986). More recently, Wang, Hogg & Large (1992) have demonstrated the existence of spontaneous transient inward currents in SM cells isolated from the rabbit portal vein. Their data indicated that these currents were due to the opening of Ca^{2+}-activated chloride channels, each event resulting from pulsatile release of Ca^{2+} from intracellular stores. It may be that these channels also underlies lymphatic STDs as longer term exposure (> 5 min) to low chloride solutions caused marked suppression of STD activity.

The finding that chelation of Ca^{2+} abolished STDs further indicates a role of this ion as an 'intracellular messenger'. The postulate that calcium is the intracellular

activator would fit a wealth of data from other cells where direct demonstration of oscillatory and/or pulsatile release of Ca^{2+} have been made (e.g. see Berridge, 1990; Petersen, Gallacher, Wakui, Yule, Petersen & Toescu, 1991). A model that has received considerable support, involves Ca^{2+} release from inositol 1,4,5-trisphosphate (IP_3)-sensitive stores with the IP_3-induced Ca^{2+} release sensitized by $[Ca^{2+}]_i$ (see Missiaen, Taylor & Berridge, 1991; Parker & Ivorra, 1991; Miyazaki *et al.*, 1992). On the basis of this model, increases in either IP_3 or $[Ca^{2+}]_i$ cause an increase in pulsatile (or oscillatory) Ca^{2+} release. In lymphatic smooth muscle STD activity was increased by noradrenaline an agonist known to increase IP_3 and hence $[Ca^{2+}]_i$ in other SMs (Kowarski, Shuman, Somlyo & Somlyo, 1985; Hashimoto, Hirata, Itoh, Kanmura & Kuriyama, 1986).

The next issue to consider is the origin of STDs. These could be generated by the SM. Alternatively, as found in another SM (dog colon; see Ward & Sanders, 1980), there may be another class of cell which is electrically coupled to the SM and is responsible for generating the STDs and hence pacemaking. In the study of mesenteric veins, it was assumed that STDs were generated within the SM cells themselves (Van Helden, 1991*a*). The findings on isolated SM cells from the rabbit portal vein (Wang *et al.* 1992) support this premise. As for the mesenteric veins, recordings from the lymphatic SM also demonstrated a large variation in the size and/or frequency of STDs from different cut segments (or small lymphangions). This might be due to a heterogeneous population of SM cells which exhibit the generator mechanisms to varying extents. Alternatively, if a distinct population of pacemaker cells is responsible for generation of STDs then one or more of these cells must be present in each lymphangion and their activity must vary between lymphangions. To date, there have been no reports of a morphologically distinct cell type which might fulfil this role.

It is useful to consider whether the STD mechanism is a general pacemaker mechanism for all lymphangions. As has been noted, in small lymphangions the current underlying STDs is capable of producing depolarization of sufficient amplitude to generate action potentials. However, as chambers increase in size or in well coupled syncitia of lymphatics such as those of ruminants (McHale & Meharg, 1992), the currents underlying STDs will become increasingly ineffective in depolarizing the SM. A severe case is exemplified by mesenteric veins which have SM with the electrical properties of an infinite cable and as such have SM with very low input impedance (Van Helden, 1991*a*). Yet even in this circumstance relatively large (10–15 mV) STD-like events occur (Suzuki, 1981; Van Helden, 1991*a*) probably corresponding to conductance > 50 nS. The relative success of such depolarizations in producing action potentials will depend on the regenerative properties (rise phase) of the action potential, which are weaker (i.e. slower rise phase) in guinea-pig mesenteric veins than in the lymphatics (D. F. Van Helden, unpublished observations). It may be that the mechanisms underlying STD generation can act co-operatively either within or between cells to activate large conductances. The action of NA to cause a marked increase in the size of STDs may be a reflection of such co-operativity.

The data presented support the idea that STDs are responsible for pacemaking in the small lymphangions of the guinea-pig. They may represent a mechanism for pacemaking in lymphatics generally. The likelihood that pacemaking occurs

myogenically through a mechanism involving Ca^{2+}-dependent pulsatile Ca^{2+} release from stores provides an elegant means of controlling lymphatic pumping. Such a model may explain, at least in part, why many disparate stimuli (e.g. NA, stretch, etc.) which are likely to increase $[Ca^{2+}]_i$ have a similar outcome, namely to increase lymphatic pumping.

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