Use of sucrose-gap to measure the membrane potentials of the smooth muscle cells of the guinea pig thoracic duct

by

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INTRODUCTION

Although smooth muscles show great diversity in their properties, there are three physiologic characteristics of smooth muscle that occur in general. (1) Smooth muscles can maintain slow, sustained contractions with a minimum of expended energy. (2) They have exclusively autonomic motor innervation. (3) Smooth muscles all show a certain amount of basal resting tension on which contractions are superimposed (Marshall, 1974). Several years ago, it was proposed that smooth muscles be classified into two groups - unitary muscles and multi-unit muscles - according to certain physiologic properties (Bozler, 1941). A characteristic of unitary muscles is the presence of pacemaker areas which initiate spontaneous activity in the tissue which spreads throughout the whole muscle causing it to act like a single unit. Multi-unit muscles do not spontaneously contract and are normally activated by multiple motor nerves in more than one region (Marshall, 1974).

The lymphatic system plays a significant role in the fluid exchange between blood and interstitium (Nicoll and Taylor, 1977). Histological studies of lymphatic vessels show that the walls consist largely of smooth muscle (Pfleger, et al., 1967; Leak, 1972). It has been suggested that these smooth muscles are of the unitary type and serve to propel

lymph throughout the lymphatic system (Mislin, 1966). Despite evidence of such an intrinsic mechanism for lymphatic contractility (Florey, 1927; Smith, 1949; Mislin, 1966), there have been very few electrophysiological studies of lymphatic vessels. Knowledge of the electrical properties of the smooth muscle cells of lymphatics could lead to better understanding of the functioning of the lymphatic system.

REVIEW

Functions of the Lymphatic System

The lymphatic system is a group of vessels which function in conjunction with the blood circulatory system. It transports to the blood stream, the large molecules, including protein, which leak from the blood vessels, those which pass through the lining of the gut, and those released by various tissues. It also serves to remove excess fluid accumulated during intense tissue activity (Casley-Smith, 1967).

To perform its various functions, the lymphatic system is composed of three components: (1) an interconnected group of capillaries which collect lymph; (2) larger lymphatic vessels which return lymph to the bloodstream, eventually emptying into the great veins of the neck; and (3) lymph nodes which filter lymph (Cooper and Schiller, 1975).

The thoracic duct is the largest vessel of the lymphatic system. It returns the lymph from most of the body to the bloodstream. It serves to drain all of the body except the right side of the head, neck and right thoracic limb; these drain into the right lymphatic duct (cranial cervical node), in the guinea pig. In the guinea pig there are usually two thoracic ducts, one on each side of the dorsal aspect of the aorta (Cooper and Schiller, 1975).

Movement of Lymph

To accomplish its functions, the lymphatic system must have some mechanism by which it propels lymph through the lymphatic vessels. Several mechanisms have been suggested to explain lymph movement. Earlier, it was usually assumed that the regulating factor controlling the flow of lymph through the thoracic duct was the difference between the positive abdominal pressure and the negative intra-thoracic pressure (Acevedo, 1943). It has since been suggested that limb and skeletal muscle movement may affect lymph flow and composition. It was found that thoracic duct lymph flow increased during passive limb movement and anaesthesia caused suppression of lymphatic return of fluid and proteins in the dog; an effect attributed to immobilization (Schad and Brechtelsbauer, 1977). Lymph flow has also been associated with a change in transmural pressure (McHale and Roddie, 1976) or in hydrostatic pressure in the lymphatic segment (Hargens and Zweifach, 1977).

Some neurogenic regulation of lymph flow has also been suggested. In 1882, it was observed that electrical stimulation of mesenteric nerves caused constriction of lacteals; stimulation of splanchnic nerves caused dilatation of lacteals; and stimulation of the caudal end of the divided vagus nerve caused dilatation of the cisterna chyli (Bert and

Laffont, 1882). In 1894, stimulation of the distal end of the left splanchnic nerve was found to cause dilatation of the cisterna chyli (Camus and Gley, 1894). In 1927, Florey observed that stimulation of the vagus caused diminution in the amplitude of contractions in the mesenteric lacteals of the rat and guinea pig; while stimulation of the left splanchnic nerve caused an increase in the rate of beats (Florey, 1927).

In 1943, Acevedo found evidence of nerves in the walls of lymphatic vessels (Acevedo, 1943). But there was an even earlier detailed description of fine nerve endings in and around the walls of large lymphatic vessels, in particular the thoracic duct of the dog (Quenu and Darier, 1887). Acevedo observed that stimulation of the vagus nerve caused a constriction of the thoracic duct in cats (Acevedo, 1943). Later, spasms were observed in the lymphatics of a dog's leg during electrical stimulation of the lumbar sympathetic chain (Rusznyák, Földi and Szabó, 1950). Browse (1968) observed that stimulation of the lumbar sympathetic chain caused an increase in lymphatic pressure in the hind limb of a dog.

Although autonomic innervation has been shown to influence lymphatic vessels, there is evidence that the movement of lymph is not exclusively controlled by neurogenic mechanisms. Rhythmic contractility of lymph vessels was

seen in the mesenteric lymph vessels in the rat and guinea pig (Florey, 1927). Florey concluded that the rhythm resided in the lacteal wall itself since regular contractions continued for one-half to three-fourths hour after cessation of breathing. In 1935, Pullinger and Florey fed a guinea pig fat, killed it rapidly, opened the thorax and pulled the lungs aside; they observed spontaneous contractions of the thoracic duct. In 1949, Smith also noted the intrinsic nature of the lymphatic contractions when the vessels remained actively contractile for 30 to 45 minutes after the death of an animal during the experiment. His experiments suggested that the movement of lymphatic vessels, in those animals with spontaneous lymphatic contractility, is associated directly with the transport of lymph. He claimed that the rate of contraction and rate of formation of lymph are directly related, and the contractions are stimulated by an increase in intraluminal pressure.

In 1961, Mislin showed that the valved segment of a lymph vessel constitutes an autonomous functional unit, which was called a lymphangion. From his investigations of the structural-functional connections between the mesenteric lymph vessels, he suggested that the lymphagions are a contractile apparatus with an autonomous automaticity. He further concluded that the lymphagion is controlled by myogenic and neurogenic mechanisms (Mislin, 1966).

Spontaneous rhythmic contractility has been observed in many varieties of lymphatic vessels, for example, in the mesenteric lymph vessels of the rat and guinea pig (Florey, 1927; Mislin, 1966; Hargens and Zweifach, 1977); in bovine mesenteric lymph vessels (Mawhinney and Roddie, 1973; McHale and Roddie, 1976; Azuma, Ohhashi and Sakaguchi, 1977); in the main lymphatic vessels of rats and guinea pigs (Pullinger and Florey, 1935; Mandryko, 1975; Orlov, Borisova and Mundriko, 1976); the most peripheral lymphatic vessels of rats, mice and guinea pigs (Smith, 1949); in human lymphatics (Kinmonth and Taylor, 1956); and in sheep lymphatics (Hall, Morris and Wooley, 1965). This spontaneous contractility has been suggested as an intrinsic mechanism for the transport of lymph (Hall, et al., 1965).

There has also been data suggesting that lymphatic contractions are induced by increased tension in the layer of smooth muscle encircling the vessel, since it was found that a rise in intraluminal pressure and stretching of the lymphatic wall are associated with contractions (Smith, 1949; Hargens and Zweifach, 1977).

Characteristics of Vessels

Like blood vessels, the larger lymphatic vessel walls consist of three layers: intima, media and adventitia. The intimal layer is composed of endothelial cells (Leak,

1972). The media is composed entirely of muscle bundles enclosed in collagen tissue (Pfleger, et al., 1967). The vascular wall of blood vessels is composed mostly of smooth muscle cells arranged in a helical fashion. In the tunica media of the major lymphatic vessels, including the thoracic duct, the smooth muscle cells are fusiformed cylinders with tapering ends. The cells' fine structure is distinguished by numerous myofilaments ranging in widths from 60 to 80 Å (Leak, 1972). Between the intima and media a network of longitudinally arranged, loosely interlaced elastic fibers, is found; in the thoracic duct, the elastic membrane is not continuous, but occurs in bundles, as in large veins (Pfleger, et al., 1967; Leak, 1972).

The third and outermost layer, the adventitia, is composed of loosely arranged collagenous fibers which intersperse with each other and pass into the surrounding connective tissue (Pfleger, et al., 1967). This layer also contains nerves and nutritional blood vessels (Leak, 1972; Pfleger, et al., 1967).

The structural similarities between blood and lymph vessels suggests the possibility of similar properties. However, there are also features of lymphatic vessels which distinguish them from blood vessels. Some characteristics of lymphatic vessels not found in blood vessels are: (1) a

basket-like arrangement of muscle bundles in the media; (2) less pronounced development of elastic tissue; (3) wider and more irregular lumen; (4) loose structure of connective tissue in the wall of lymphatic vessels, and (5) a system of anchoring filaments terminating on the endothelial wall to bind the lymphatic endothelium to the adjoining interstitial areas (Pfleger, et al., 1967; Leak, 1970). Therefore, one might also expect some differences in the properties of blood vessels and lymphatic vessels. A study on bovine mesenteric arteries, veins and lymphatics confirms this. Serotonin caused constriction in all three vessels. But, overall, the lymphatics shortened, the arteries lengthened and the veins first lengthened, then shortened (Williamson, 1969). Williamson (1969) suggests that these responses and others may be related to the arrangement of smooth muscle fibers in the walls of the vessels. As stated by her, the lymphatic smooth muscle fibers are irregular and arranged loosely, the arteries contain only circular fibers, and the muscle wall of the vein consists of an inner circular coat and outer longitudinal bundles.

Because of the similarities between blood vessels and lymph vessels and because of the paucity of information on the electrophysiological properties of lymphatic smooth muscles, it is useful to look at the electrophysiology studies that have been done on the smooth muscles of arteries

and veins for indications of what might be expected from similar studies in lymphatics. Resting membrane potentials and action potentials have been measured under various conditions. Effects of norepinephrine and increased extracellular potassium on electrical and/or mechanical activity have also been studied. Electrophysiological properties are found to depend on drug concentration, ion concentration, type of tissue and the species of origin.

Membrane Potential

The most common electrophysiological parameter which is measured in vascular smooth muscle is the membrane potential. There are two techniques by which this measurement can be made, the microelectrode method and the sucrose-gap technique. Measurements of resting membrane potentials show great variability. For example, in the portal vein of the guinea pig a mean of -37 mV was measured with microelectrodes (Ito and Kuriyama, 1971). But measurements in the rabbit portal vein were between -40 and -50 mV with the sucrose-gap technique (Holman, 1969). A wide range, -30 to -65 mV, of measurements was found in the rat portal vein (Funaki and Bohr, 1964).

Different arteries in the same animal also do not always have similar resting membrane potentials. For arteries in the frog tongue, the range was -55 to -75 mV with a mean of

-64.7 mV, but the same researcher measured resting potentials in the arteries in the skin of the lateral abdomen of the frog and found a range of -35 to -50 mV with a mean of -43.6 mV (Steedman, 1966). These recordings were made with micro-electrodes. A sucrose-gap measurement of resting potential in the sheep carotid artery yielded recordings with a mean of -61 mV (Keatinge, 1964).

Drugs, such as norepinephrine, may have various effects on vascular smooth muscle, depending on the tissue and the dose. When norepinephrine was applied to the portal vein of the rabbit, at doses between 100 to 300 µg/liter (Holman, 1969) and to the portal vein of the rat, at a dose of 100 µg/liter (Johansson, et al., 1967), similar responses were obtained. In both, continuous spiking was observed. But at higher concentrations, different results were obtained. Doses of .5 to 2 mg/liter depressed spike activity in the rabbit portal vein, and in some, only a maintained depolarization, no spike activity, was observed (Holman, 1969). A similar response was seen with a dose of 10 mg/liter in the rat portal vein (Johansson, et al., 1967).

A comparison of different tissues in the same animal may show different responses to the same drug. Low doses, 2×10^{-8} (3.39 µg/l) to 10^{-7} M (16.9 µg/l) of norepinephrine, induce tension without depolarization in the rabbit pulmonary artery (Casteels, et al., 1977). This response is similar to

that of sheep carotid artery to a dose of 25 µg/l of norepinephrine (Keatinge, 1964). But in rabbit mesenteric veins, low doses of norepinephrine cause an increased frequency of action potentials concomitant with a rise in tension (Cuthbert and Sutter, 1965); a response which is similar to that of turtle veins at doses of 1 µg/ml to 100 µg/ml of norepinephrine (Roddie, 1962).

Responses of arteries to higher doses of norepinephrine resemble the responses of veins. A dose of 2.5 mg/100 ml of norepinephrine caused rapid depolarization followed by contractions in the sheep carotid artery (Keatinge, 1964). A similar response, increased tension development and depolarization of cells, occurred in rabbit pulmonary artery to doses between 2.5×10^{-7} M (4.2 µg/100 ml) and 5×10^{-6} M (85 µg/100 ml) (Casteels, et al., 1977).

The effect of increased external potassium (tenfold increase) on rabbit pulmonary artery (Casteels, et al., 1977) is similar to the effect of a 5.4-fold increase on rabbit mesenteric veins (Cuthbert and Sutter, 1965). In both, the effect is depolarization of the membrane and an increase in contractile activity. However there is a minimum depolarization which must occur in order to elicit the contractile activity. In the rabbit pulmonary artery, the minimum depolarization is 4 mV (Casteels, et al., 1977), and in the rabbit mesenteric veins, the minimum is 6 mV (Cuthbert and Sutter,

1965). Sometimes, the amount of depolarization can be used to quantify the amount of tension or force developed. For potentials between -45 mV and -18 mV after depolarization, it has been found that a potential change of 6 mV effects a detectable change in force development in the dog carotid artery (Siegel, et al., 1976). Keatinge (1964) found a great effect with addition of potassium-rich solution, an abrupt onset of depolarization which continued for up to 15 minutes until the potential was almost 0 mV. And in all samples tested, the potential at this stage was always near 0 mV, never more than +10 mV nor less than -10 mV. There were also persistent contractions which he found would continue for 24 hours if the arteries were left in the potassium-rich solution.

The degree of electromechanical coupling, that is, the extent to which mechanical changes are dependent on electrical potential changes, is of interest in vascular smooth muscle. There is some uncertainty about whether contractions caused by drugs in vascular tissue are always accompanied by an action potential discharge. Barr (1961) recorded no action potentials after treating the dog carotid artery with epinephrine. Similarly, no depolarization of cells nor action potentials were recorded in the main pulmonary artery after treatment with norepinephrine even though contractions were observed (Su, Bevan and Ursillo, 1964). However, in a later

study, Somlyo and Somlyo (1968) observed that norepinephrine depolarizes and causes oscillations of the membrane of these cells. But since the quantitative correlation between the tension development and depolarization was limited, and since drug-induced contractions may also occur in depolarized tissues, this type of excitation-contraction coupling was called pharmacomechanical coupling by these authors.

Cuthbert and Sutter (1965) found that low doses of norepinephrine caused an increased frequency of action potential discharge concomitant with a rise in tension caused by the drug. But this correlation held only for the initial stage of the drug effect, after which the action potential frequency fell to or below the control value even though the tension remained elevated. Higher doses (10 mg/l) of norepinephrine caused an even more marked dissociation between excitation and contraction. Steedman (1966) found when high doses (10^{-3} g/ml) of epinephrine or norepinephrine were dripped on arteriole vessels of the frog and rat, that the vessels constricted vigorously, but only rarely was spike activity recorded.

It is usually the case that electromechanical coupling occurs under normal conditions as spontaneous contractions or low doses of drugs. Roddie (1962) found that each action potential usually initiated an all-or-none contraction when norepinephrine, in low doses (1 µg/ml to 100 µg/ml), was

applied to turtle arteries and veins. Holman (1969) found that waves of depolarization usually occurred in a one-to-one relationship with spontaneous contractions. In the dog carotid artery, it was found that membrane potential and mechanical tension have a qualitatively equal dependence on external potassium concentration (Siegel, et al., 1976). This implies that in the dog carotid artery, tight electro-mechanical coupling exists.

Previous Lymphatic Studies

Some of the properties present in blood vessels may also be present in lymphatic vessels. And although few studies have been made on lymphatic vessels, some electrical-mechanical properties have been described for some of the vessels. The number of electrical studies that have been made on lymphatic vessels is very small. The first was done in 1966 by Mislin, who recorded, for the first time, extracellular action potentials of guinea pig mesenteric lymphatics with the use of suction electrodes. However, he did not state the level of the resting membrane potential nor did he describe the shape of the action potential. Ten years later, Orlov, et al. (1976) made simultaneous electrical-mechanical recordings of the main lymphatic vessels of rats. They recorded spontaneous rhythmic contractions at a rate of 12/minute, a much higher rate than recorded in bovine

mesenteric lymphatics, 2/minute, by Mawhinney and Roddie in 1973. Orlov, et al. (1976) recorded resting membrane potentials of -25 mV in the rat thoracic lymphatic duct, and -35 to -37 mV in the rat mesenteric vessels. Another electrical-mechanical study of lymphatics was made on bovine mesenteric lymphatic vessels where an average resting potential of -32.7 mV was recorded (Azuma, et al., 1977). Action potentials in a one-to-one correspondence to contraction waves were also recorded in bovine mesenteric vessels (Azuma, et al., 1977; Kirkpatrick and McHale, 1977).

Only one group has applied a high concentration of potassium to lymphatic vessels, in a study made of rat thoracic lymph vessels. It was found that a depolarizing solution of potassium chloride did not induce contractions but influenced spontaneous activity (Orlov and Borisova, 1974). They found the response to be dose-dependent. Low concentrations (10 mM) caused an increase in amplitude and frequency of the contractile waves, but high concentrations (20 mM and above) suppressed spontaneous contractions. These responses are similar to responses of vascular smooth muscle to potassium-rich solutions (Cuthbert and Sutter, 1965; Casteels, et al., 1977).

Effects of norepinephrine on lymphatic smooth muscle have been found to be dose-dependent. Mawhinney and Roddie

(1973) found that norepinephrine increased the frequency and decreased the amplitude of contractions of bovine mesenteric lymphatics. The smaller doses, 1 ng/ml (1×10^{-9} g/ml) to 5 ng/ml, only slightly increased the rate of contraction and slightly reduced the amplitude. But a higher dose, 25 ng/ml, caused the frequency of contraction to increase to 10/min from a baseline frequency of only 2/min. Also at this high frequency, the contractions had small amplitudes and could be seen only as a "ripple on the trace" (Mawhinney, et al., 1973). Similar results were obtained for the main lymphatic vessels of rats where a concentration of 1×10^{-6} M ($.169 \times 10^{-6}$ g/ml) of norepinephrine caused contracture and an increased frequency of spontaneous contractions (Orlov, et al., 1976). Mislin (1966) also found that norepinephrine, in concentrations of 10^{-8} M ($.169 \times 10^{-8}$ g/ml) to 10^{-9} M ($.169 \times 10^{-9}$ g/ml), had a positive inotropic effect on guinea pig mesenteric lymphatic vessels. A recent study on the effect of norepinephrine on lymphatic vessels corroborates the previous studies. A concentration of 5×10^{-7} g/ml of norepinephrine applied to bovine mesenteric lymphatics caused a slight but long-lasting depolarization with frequent action potentials, followed by a gradual increase in smooth muscle tone and the initiation of phasic contractions in one-to-one correspondence to the action potentials (Azuma, et al., 1977).

MATERIALS AND METHODS

Thoracic Duct

Prior to each experiment, a guinea pig was fed for two or more days with guinea pig pellets that had been soaked in olive or corn oil. This caused the thoracic duct to be distended with lymph, and consequently more readily visible for dissection. Without prior oil feeding, the duct was difficult to identify since it was quite small (less than .1 mm in diameter). With lymph distention, the duct was about .2-.5 mm in diameter and was white and bulbous in appearance. Male guinea pigs weighing at least 900 g. were used. It was found that identification and dissection of the thoracic duct was very difficult, even after oil feeding, in smaller animals.

Euthanasia was performed by placing the animal in a chamber which was then filled with CO_2. An incision approximately midway between the vertebral column and the sternum was made to gain entry into the thoracic cavity. This allowed exposure of the thoracic ducts, normally one on each side, dorso-lateral to the aorta. Two ligatures (as far apart as possible) were placed around one of the ducts, the aorta and the immediately adjacent tissue. Removal of this mass of tissue and dissection of the thoracic duct from this tissue was done using a dissecting microscope. During dissection, the tissue was placed in warm (37°C) saline which was

oxygenated with 95% O_2 and 5% CO_2. It was necessary to have a strip 3½ to 4 cm long for the recording.

After recording, the presence of the lymphatic vessel was verified by standard histological techniques. The tissue was fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Sucrose-gap

The sucrose-gap method for recording transmembrane potentials with external electrodes was introduced by Stämpfli (1954). The theoretical basis for this technique is explained in the Appendix (see Bureš, Petrán, and Zachar, 1967 for additional information). This method was used for bundles of myelinated nerve fibers in 1954 (Stämpfli and Straub), and for nonmedullated fibers (Ritchie and Straub, 1956, 1957). It was first applied to smooth muscle in 1958 (Burnstock and Straub).

The sucrose-gap apparatus is illustrated in Figure 1. This technique gives satisfactory results provided movement and flow artifacts are eliminated. Consequently, the tissue was fixed at the junctions between solutions and mixing of solutions was prevented by use of dental periphery wax or plasticine. One end of the thoracic duct was continuously superfused with saline at 37°C. The saline was heated to 37°C on a heater-stirrer and heat loss in the flow system

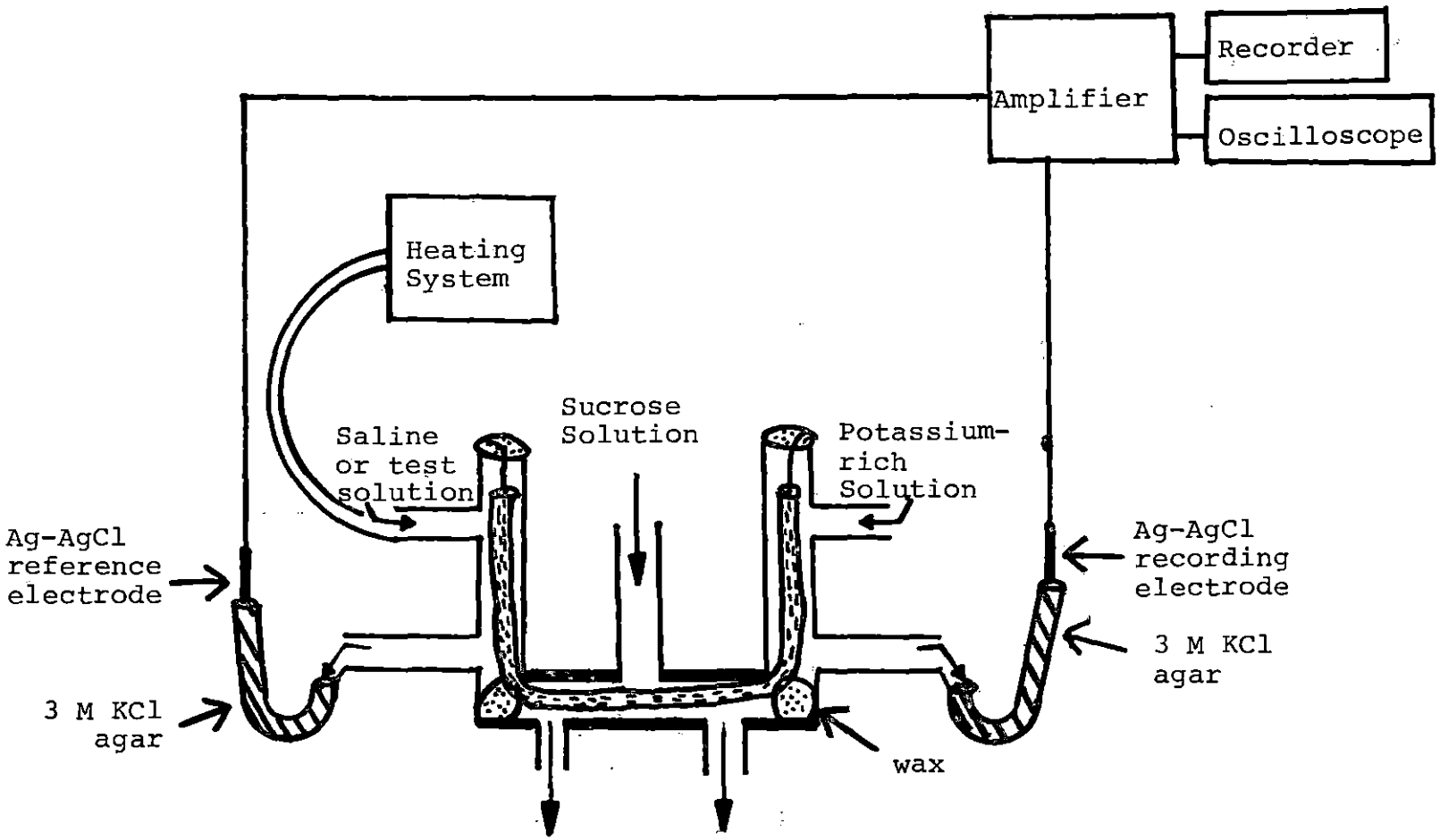

Figure 1. The sucrose-gap apparatus

was eliminated by circulating hot water around the saline line. A thermister was used to measure temperature at the duct. The temperature was maintained at 37±.5°C. A potassium-rich solution and a solution containing norepinephrine were introduced to the recording chamber by switching the saline in-flow line to a different container which already contained the test solutions. The other end of the duct was always superfused and depolarized by a potassium-rich solution. The sucrose was refrigerated and cooled to approximately 10°C before it entered the apparatus. This improves the stability and life of the preparations. It was passed through a deionizing column (Sybron/Barnstead) before use to increase its specific resistance. Silver-silver chloride electrodes were connected with the saline and potassium-rich outflow solutions through bridges of 3 M KCl agar. Micro-manipulators were used to hold the electrodes steady once they had been properly placed in the agar bridges. The outputs from the electrodes were amplified by a headstage preamplifier and signal conditioner (Brockman, 1971), displayed on an oscilloscope (Sony-Tekronix 323) and recorded on paper using a Hewlett Packard recorder (type 7402A). The solutions were pushed through the apparatus at a constant rate with a peristaltic pump.

Solutions

The standard saline solution contained (mM): NaCl 133; $NaHCO_3$ 16.3; NaH_2PO_4 1.38; KCl 4.7; $CaCl_2$ 2.5; $MgCl_2$ .105; dextrose 7.8. In making this solution, the last three compounds were added after the solution of the first four had been equilibrated with a mixture of 95% O_2 and 5% CO_2. The same gas mixture was bubbled through the solution continuously during experiments. The potassium-rich solution contained (mM): NaCl 3.3; NaH_2PO_4 1.38; K_2SO_4 89.6; $CaCl_2$ 2.5; dextrose 7.8; $MgCl_2$ .105: $KHCO_3$ 16.3. The sucrose solution contained 318.6 mM sucrose and 7.8 mM dextrose to help survival of the tissue in it (Keatinge, 1964). All solutions were made using glass-distilled water. The potassium and sucrose solutions were made the day before they were used. The saline solution was made the day it was used as was the solution (2.5 mg in 100 ml) of d,l norepinephrine in saline.

RESULTS

The thoracic ducts were placed in the sucrose-gap chamber and bathed by the three solutions for approximately 15-30 minutes before recording was begun. The resting membrane potentials ranged from -46 mV to -60 mV (mean -52.2 mV, 5 guinea pigs). In three of the five guinea pigs, the resting potential was not steady, but wavered by as much as 7 mV in either direction from its mean resting potential during the course of the experiment.

The effects of norepinephrine (2.5 mg/100 ml) were varied. In all four cases where it was applied some depolarization of the membrane occurred; in one experiment the resting potential depolarized 14 mV (see Figure 2). A summary of the resting potential effects of norepinephrine appears in Table 1. In all cases, superfusing with normal saline caused the resting potential to return to its value before the addition of norepinephrine. In some cases it appeared that there was some electrical activity present after the application of norepinephrine; there were some waves of depolarization or apparent spikes, as shown in Figure 3. But it is difficult to say whether this activity was of biological origin since movements of the system could also cause appearance of "spikes" on the recording. But these movement "spikes" had a different shape and larger amplitude (see Figure 4), than the activity shown in Figure 3. It

Table 1. Resting potentials before addition of norepinephrine and amount of depolarization after addition of norepinephrine (2.5 mg/100 ml)

Guinea pig	Resting potential before norepinephrine	Amount of depolarization
1	-50	-
2	-54	14 mV
3	-47 to -50	9 mV
4	-54 to -58	4 mV
5	-46 to -60	5 mV

would be necessary to do further studies to determine whether norepinephrine does indeed cause spike activity in the guinea pig thoracic duct.

Replacement of the saline solution with the potassium-rich solution (see Materials and Methods section for solution) caused depolarization of the membrane in all cases where applied. The membrane potential approached 0 mV with respect

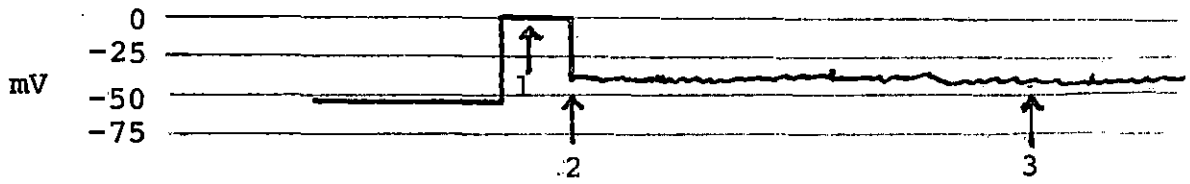

Figure 2. The cells show a resting membrane potential of -54 mV. At arrow 1, the recorder was turned off and the norepinephrine test solution (2.5 mg/100 ml) started. At arrow 2, the recorder was turned on. The application of the norepinephrine caused a depolarization of 14 mV, from -54 mV to -40 mV. The time between arrow 2 and arrow 3 was 60 seconds.

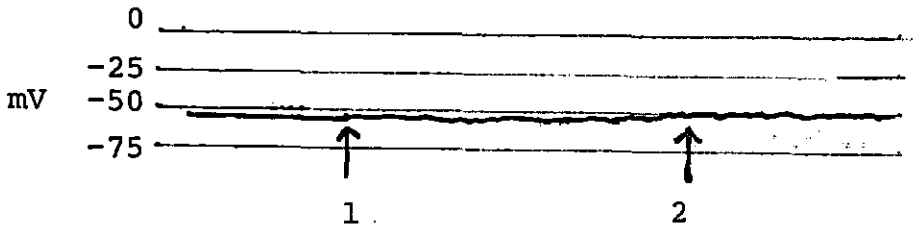

Figure 3. The cells show a resting membrane potential of -55 mV. At arrow 1 the norepinephrine test solution (2.5 mg/100 ml) was applied. At arrow 2, 45 seconds later, the cells had depolarized by 5 mV to -50 mV. Following application of the norepinephrine, the recording was less steady, showing possible spike activity.

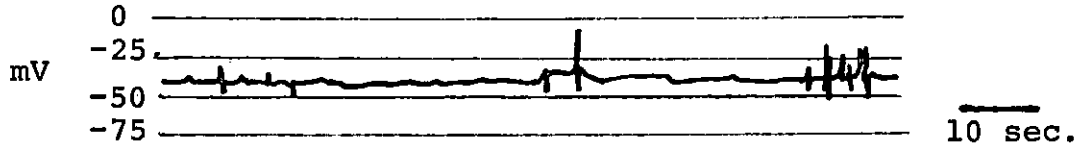

Figure 4. Movement "spikes" from mechanical interference to the system

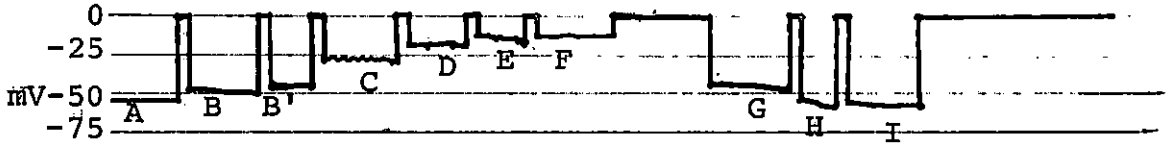

Figure 5. Depolarization caused by replacement of saline with potassium solution. Resting potentials were: (A) -55 mV before potassium solution; (B and B') -45 to -50 mV after 15 min; (C) -30 mV with spikes after 30 min; (D) -18 to -20 mV after 45 min; (E) -15 to -17 mV after 60 min; (F) -14 mV after 80 min. After replacement with saline, resting potentials were: (G) -46 mV after 3 min; (H) -55 to -60 mV after 20 min; (I) -57 to -60 mV after 25 min. Readings of 0 mV correspond to the recorder being turned off.

to time. There were also, at times, small waves of depolarization present with amplitudes of 2 to 5 mV (see Figure 5), which may have been due to biological electrical activity. Again, further study is required. In one case (guinea pig #5, in which the resting membrane potential varied from -46 to -60 mV), after depolarization to -14 mV with the potassium-rich solution, the potassium solution was replaced with the normal saline solution. Within 3 minutes, the membrane potential returned to its smallest previous resting potential, -46 mV. After 20 minutes, it returned to its largest previous resting potential, -60 mV as shown in Figure 5.

Although no spontaneous action potentials were observed during sucrose-gap recording, there was evidence of spontaneous contractions. These contractions were observed in the dead animal after the thoracic cavity had been opened and the thoracic duct exposed. After approximately one-half hour of observation, most of the lymph had passed through the thoracic duct.

To test the sucrose-gap apparatus for validity in recording resting membrane potentials, various small experiments were performed. Since the sucrose-gap technique had previously been applied to bundles of myelinated nerve fibers (Stämpfli and Straub, 1954), and to nonmedullated fibers (Ritchie and Straub, 1956, 1957), nerve bundles from separate experiments were placed in the sucrose-gap chamber.

Potentials of -60 to -80 mV were obtained, as was depolarization of the membrane when the potassium-rich solution replaced the saline. Replacement of the potassium-rich solution with saline caused the potential to return to its original resting potential.

Other experiments were performed to test whether the potentials measured were due to junction potentials or electrode differences. A tissue which was known to be dead, a nerve soaked in alcohol, was placed in the sucrose-gap apparatus. The result was a very small potential, in one case, it was -6 mV, and in another, +6 mV. It is likely that these values are recording artifacts caused by imperfect electrodes or slight mixing of solutions. To eliminate the effect of mixing of solutions, in the two cases mentioned here, measurements were also taken between the electrodes with no tissue and no sucrose in the chamber. Again, both readings were very small, -2 mV in the experiment which measured -6 mV with the dead tissue; and 0 mV for the second case.

In a third experiment, the potential measured between saline and potassium lines with no sucrose nor tissue in the middle chamber was -10 mV. In this case, another test for junction potentials was performed. An agar bridge was placed between a petri dish holding the saline and one holding the potassium-rich solution, the measurement was -4 mV. Similarly, both electrodes were placed in the potassium-rich

solution through agar bridges, and the measurement was again -4 mV. The sucrose-gap apparatus was also tested with an agar bridge replacing the tissue. In a case where the measurement between the saline and potassium lines with no tissue nor sucrose had been 0 mV, with an agar bridge, the measurement was very small, +12 mV. However, if plasticine was used in place of wax to block the leakage of solutions between chambers, this measurement dropped to +6 mV. Also, with the same electrodes, with dead tissue again, the measurement was +6 mV with wax, and 0 mV with plasticine. This suggests plasticine would serve as a better block to leakage than wax.

It is absolutely necessary to use agar bridges on the electrodes. They were eliminated at one point during the course of the experiments because of mechanical difficulty in keeping the electrodes positioned in the agar. But this gave readings of +30 to +50 mV whether there was tissue in the chamber or not. This problem was eliminated by re-designing the flow system to allow the use of micromanipulators to hold the electrode-agar bridge in contact with the solutions.

DISCUSSION

The results show that the resting potential for the guinea pig thoracic duct is in the range of -46 mV to -60 mV, using the sucrose-gap method. These values are larger than those of previous electrical recordings of lymphatic vessels. Orlov, et al. (1976), recorded a resting potential of the rat thoracic duct as -25 mV, and for mesenteric lymphatics as -35 to -37 mV. Azuma et al. (1977) measured a resting potential of -32.7 mV for bovine mesenteric lymphatic vessels. There are several possible reasons that these results gave a larger measurement than previous ones. First, it is possible that these measurements give a true indication of the resting potential of the guinea pig thoracic duct. As was pointed out earlier, resting potentials in vascular tissue may vary greatly between different blood vessels of the same animal, and between the same tissues of different animals. So a recording of -25 mV in the rat thoracic duct (Orlov et al., 1976) may not be an exact indication of what the resting potential of the guinea pig thoracic duct should be. It is also possible that a small part of the measurement was contributed by junction potentials, since it was found that the reading between the electrodes with no tissue or with dead tissue ranged from -10 mV to +6 mV. Subtracting these values, puts the measurements

more in the range of previous studies of lymphatics.

The results show that one of the effects of norepinephrine (2.5 mg/100 ml) was a depolarization of the membrane by 4 to 14 mV. In the only other study of the effect of norepinephrine on the electrical activity of lymphatic vessels (Azuma, et al., 1977) a long-lasting depolarization was also seen, but it was only by 2 mV. However, a lower concentration (.05 mg/100 ml) of norepinephrine was applied. In the other study of electrical activity of lymphatics, including the rat thoracic duct, no mention of depolarization of the membrane in response to norepinephrine was made although a similar dose (1.69 mg/100 ml) was applied (Orlov, et al., 1976). Depolarization of the membrane is also a response seen in vascular muscle. The same dose (2.5 mg/100 ml) caused depolarization by 10 to 15 mV in sheep carotid artery (Keatinge, 1964) and a similar dose (1.0 mg/100 ml) of norepinephrine also caused a depolarization in the membrane of rat portal vein (Johansson, et al., 1967).

The results also show that norepinephrine (2.5 mg/100 ml) may have caused some electrical activity in the membrane, although the evidence is not complete. In bovine mesenteric lymphatics, Azuma, et al. (1977) found that noradrenaline (.05 mg/100 ml) caused frequent action potentials in a one-to-one correspondence to phasic contractions. Even though action potentials were not substantiated by the results, it

is possible that contractions were present. Orlov, et al. (1976) found that norepinephrine (.02 mg/100 ml) caused contracture in the main lymphatic vessels of rats, including the thoracic duct. Cuthbert and Sutter (1965) found that high doses (1.0 mg/100 ml) of norepinephrine depressed action potential activity, in rabbit mesenteric veins, even though tension remained elevated. It is possible that a similar response occurred in these experiments. It is also possible that action potentials were occurring somewhere in the thoracic duct, but not in the segment from which these recordings were made.

The results show that the potassium-rich solution caused depolarization of the membrane to near 0 mV, possible electrical activity, and a return to normal with saline solution. This is similar to the effect of KCl on rat thoracic lymph vessels, in which depolarization occurred, although contractions were not induced (Orlov, et al., 1974). An eightfold increase in external potassium caused initially continued spike firing and contraction, and depolarization which continued after spike activity ceased; switching back to normal solution caused repolarization and return of spikes and phasic contractions in rat portal vein (Axelsson, et al., 1967). But the guinea pig responses measured here are most like those of sheep carotid artery in which depolarization resulted, most of it occurring in the first five

minutes, but slowly continuing for about 10 more minutes until the potential was close to 0 mV (Keatinge, 1964). Keatinge also found that contractions persisted if the arteries were left in the potassium-rich solution. The similarity between the guinea pig results and the results of the experiments described above ends in the time involved in the depolarization of the membrane. In the sheep carotid artery experiments, the depolarization occurred within 15 minutes, whereas the guinea pig thoracic duct depolarization took over an hour. One possible reason is a slower replacement of the saline with the potassium-rich solution because of differences in system design and because there was a certain amount of connective tissue still adhering to the surface of the thoracic duct.

To gain more complete knowledge of the electrophysiological properties of the guinea pig thoracic duct, further studies may be carried out. The effects of variations in external potassium concentration could be studied by use of graded increases in potassium concentration in the solution applied. Norepinephrine could also be added in graded amounts to determine its effect at various concentrations. A simultaneous recording of mechanical and electrical activity would also further enhance understanding of the resting properties of the guinea pig thoracic duct, as well as knowledge of its spontaneous activity, responses to in-

creased external potassium concentration, and responses to norepinephrine. Electrical recordings made in a different segment of the thoracic duct from which these recordings were made might show more electrical activity. Application of other drugs could also give more insight into the properties of the guinea pig thoracic duct.

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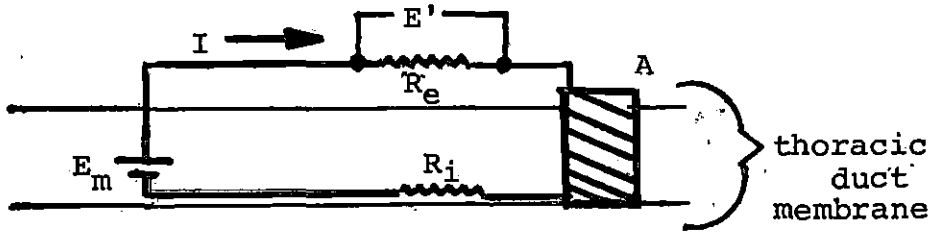
APPENDIX: THEORETICAL BASIS FOR SUCROSE-GAP



where:

E_m = transmembrane potential

E' = potential measured with extracellular electrodes

R_e = external resistance

R_i = internal resistance of the cells of the thoracic duct

A = area of tissue which has been depolarized

I = current

By Kirchhoff's laws, in this diagram:

$$E_m - IR_e - IR_i = 0$$

$$E_m = I(R_e+R_i)$$

but

$$E' = IR_e \quad \text{so} \quad I = E'/R_e$$

$$\rightarrow \quad E_m = \frac{E'}{R_e}(R_e+R_i)$$

$$E' = \left(\frac{R_e}{R_i + R_e}\right) E_m$$

The term $\frac{R_e}{R_i+R_e}$ is called the short circuiting factor. If R_e is made very high with respect to R_i, the short circuiting factor approaches 1. This allows E' and E_m to be approximately the same.