In situ observations of spontaneous contractions of the peripheral lymphatic vessels in the rat mesentery: Effects of temperature

A. Yasuda[1] and N. Ohshima[2]

Division of Biomedical Engineering, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki 305 (Japan),
30 January 1983

Summary. By means of an intracellular glass microelectrode, action potential changes were successfully recorded in situ from the endothelial cells of rat mesenteric lymphatics over the temperature range of 27–40 °C. The frequency of action potential and the lymphatic contraction rate correlated well with temperature.

The lymphatic circulation system provides an important route for mass transfer through which several substances such as fluids, proteins and large particulate matter are exchanged between interstitial spaces and the blood circulation. A well known controversy about aspects of the endothelial pathway[3] for the transport of substances through the endothelial wall has attracted considerable attention to the dynamics of the blood to lymph mass transfer. A number of experimental studies have been performed with respect to the dynamics of lymphatic contraction and lymph tansport[4–6]. Although many investigators have made efforts to measure lymph flow, pressure[7], and endothelial permeability[8] etc., few inferences have been made as to the mechanisms of lymphatic contraction, which undoubtedly affect lymph transportation and mass transfer. On the other hand, since the early work of Mislin[9], contractile phenomena in a segment of lymphatic vessel, (the 'lymphangion'), have been extensively studied from both the structural and functional viewpoints[10], using both in vitro and in situ preparations (for review see Mislin[11]). The present study attempts to elucidate the interrelationships between dynamic behavior and contractile mechanisms of microlymphatic vessels by using the intravital microscope technique. This paper reports an attempt to investigate the electrophysiological evidence for the contraction of peripheral lymphatic vessels observed in situ in the rat mesentery. In addition, the relevance of contraction to the dynamics of the microlymphatic vessels at different temperatures was investigated.

Materials and methods. Experiments were performed on white Wistar rats of either sex with body weights ranging from 150 to 250 g. Anesthesia was induced with sodium pentobarbital (50 mg/kg), and the animals were tracheotomized. An indwelling catheter was placed in the left common carotid artery to monitor arterial blood pressures. The mesentery was exteriorized by a common procedure detailed elsewhere[12]. A section of the mesentery having clearly identifiable, contracting lymphatic vessels of 30–200 μm in diameter was placed in a perfusion chamber on the stage of an Olympus intravital microscope[13]. The mesentery was continuously perfused with Tyrode's solutions of constant temperatures (27, 30, 33, 37 and 40 °C; pH 7.4). During each experiment, the dynamic courses of contraction and relaxation of the peripheral lymphatic vessels were continuously visualized by a television camera mounted on the top of an intravital microscope, and recorded by a VTR. The contraction rate, i.e. frequency of contraction, and changes in internal diameter at several temperatures of the perfusing solution were measured by replaying video-tapes.

In a separate series of experiments, the transmembrane potentials of endothelial cells of the peripheral lymphatic vessels at the above mentioned temperatures were also measured in situ, with intracellular microelectrodes. Glass microelectrodes (filled with 3 M potassium chloride; 60–100 MΩ) were inserted into endothelial cells of lymphatic vessels of 100–120 μm in diameter, through a 10-mm hole perforated in a plate laid over the mesentery. The electrode was flexibly mounted on the lymphatic vessel[14], and the insertion of the electrode was carried out under a binocular microscope.

Results and discussions. In mesenteric preparations, the lymphatic contraction mechanism seemed to be myogenic in ori-

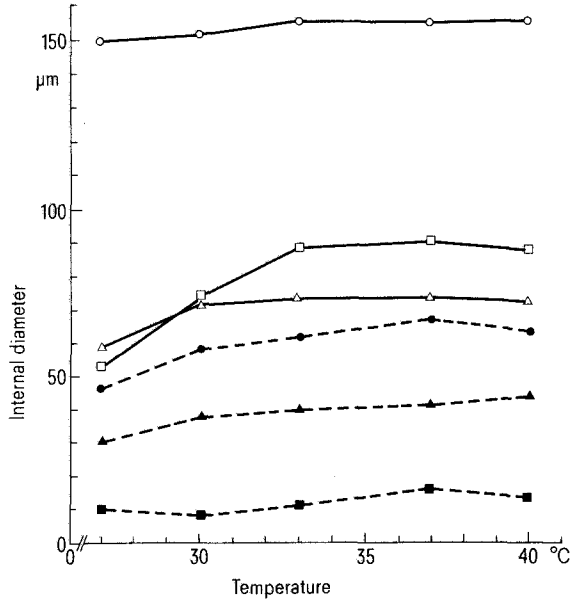

Figure 1. Effect of temperature on internal diameter of lymphatics. Measurements of internal diameter from 3 lymphatics were plotted. Open symbols represent measurements of internal diameter in the most dilated state and closed symbols in the most contracted state for any given lymphatic vessels.

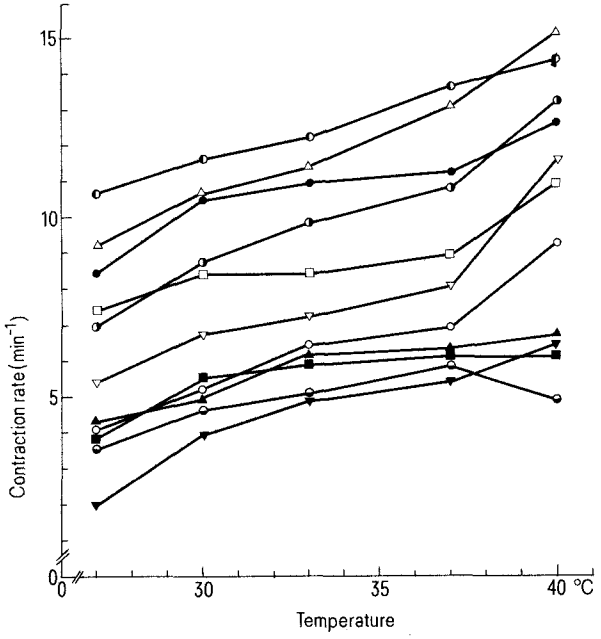

Figure 2. Effect of temperature on the contraction rate of peripheral lymphatics (pH 7.4; temperatures: 27, 30, 33, 37 and 40 °C).

gin[11]. In most experiments changes in the lymphatic diameter were not significantly altered in the temperature range studied (fig. 1). However, contraction rates were found to increase linearly with increase in temperature, and a significant correlation was found between the contraction rate and temperature (fig. 2). During these observations, arterial blood pressures remained almost unchanged.

In a previous paper[15], we proposed that lymphatic contraction indices are a quantitative measure of the dynamics of contraction;

Index I = $(b^2-c^2)/b^2$

Index II = $a \cdot (b^2-c^2)/b^2$,

where a is contraction rate, b is internal diameter at the most dilated state and c is internal diameter at the most contracted state. Thus, Index I is considered to represent a lymphatic fractional contraction in cross sectional area. While Index II, which is a product of Index I and the contraction rate, is a measure of overall lymphatic contractile activity. Figure 3 illustrates the effect of temperature on these lymphatic contraction indices estimated from the measured values of internal diameters of lymphatics. It is evident that no significant differences in the values of Index I were found with change in temperature between 33 and 40 °C. On the other hand, as can be seen in figure 3b, Index II showed a significant increase with increasing temperature within all diameter ranges studied. As is evident from the definitions of both indices, these differences must be attributed to the difference in contraction rate. These results seem to suggest that some 'autoregulatory' mechanism works to keep the fractional contraction in cross sectional area constant particularly in microlymphatics over 70 µm in diameter, and that the overall activity of lymphatic contraction is affected solely by the contraction rate.

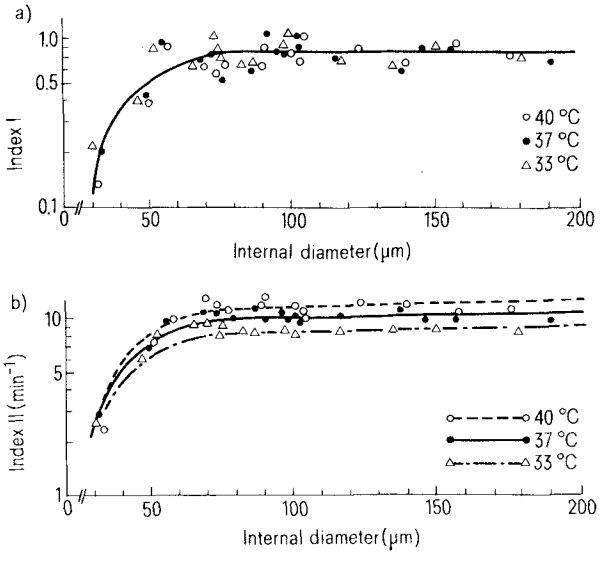

Figure 3. Lymphatic contraction indices plotted against the internal diameter of lymphatics measured at its standstill state. *a* Index I; *b* Index II.

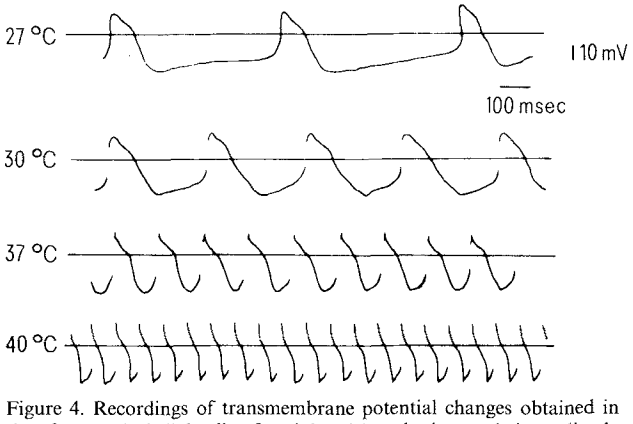

Figure 4. Recordings of transmembrane potential changes obtained in situ, from endothelial cells of peripheral lymphatic vessels immediately before contraction. Diameter of the lymphatics was 105 µm. Horizontal lines indicate the 0 mV level. In this case, resting potentials were −25 mV (27 °C), −25 mV (30 °C), −26 mV (37 °C), and −28 mV (40 °C). Action potentials were +42, +40, +40, and +42 mV, and durations were 550, 310, 150, and 80 msec, respectively. Measurements were made in Tyrode's solution containing (mM) Na^+, 147; K^+, 4; Cl, 133.4; HCO_3^-, 22; $H_2PO_4^-$, 0.7; Ca^{2+}, 2; glucose, 5 at pH 7.4, gassed with 95% O_2–5% CO_2.

Electrophysiological studies revealed that all the endothelial cells in the contracting regions of the peripheral lymphatic vessels showed a negative resting membrane potential against the external solution (mean: −26 ± 2.3 mV; n = 52). Most of these cells fired spontaneous action potentials which followed a slow depolarization, as shown in figure 4. These action potentials were recorded immediately before the lymphatic contraction. The amplitude of the action potentials showed almost the same value at the 4 temperatures studied, ranging from 20 to 42 mV (mean: 37 ± 2.9 mV; n = 50). However, the duration of action potentials was prolonged with decreasing temperature. The increase in temperature caused contraction rates to rise, and to increase the frequency of the action potentials. Frequencies of the potential were correlated well with temperature. The temperature coefficient, Q_{10}, calculated from the results presented in figure 4 was found to be 3.9–4.1, which is in good agreement with the reported values of 2.6–4.6 by Mislin[10, 11].

Prior to this study, there had been no in situ measurements of the transmembrane potential using glass intracellular microelectrodes[11], correlated with the effect of temperature on the frequency of spontaneous contraction. The present study shows that the changes in dynamic properties of the lymphatic endothelial cells with temperature are controlled by altering the rate of contraction rather than the diameter of the peripheral lymphatic vessels. In addition, the electrical activity of these cells directly controls the rate of contraction.

1 Present address: Department of Research and Development, St. Mary's Hospital, Fukuoka 830 (Japan).
2 To whom all correspondence should be addressed.
3 Renkin, E.M., Acta physiol. scand., suppl. *463* (1979) 81.
4 Mayerson, H.S., in: Handbook of physiology, Sect. 2, Circulation, vol. 2, p. 1035. Ed. W.F. Hamilton. American Physiological Society, Washington, D.C. 1972.
5 Casley-Smith, J.R., Experientia *32* (1976) 1.
6 Casley-Smith, J.R., in: Microcirculation, vol. 1, p. 423. Eds G. Kaley and B.M. Altura. University Park Press, Baltimore/London/Tokyo 1977.
7 Hargens, A.R., and Zweifach, B.W., Am. J. Physiol. *233* (1977) 1157.
8 Garlick, D.G., and Renkin, E.M., Am. J. Physiol. *219* (1970) 1595.
9 Mislin, H., Experientia *17* (1961) 29.
10 Mislin, H., in: Handbuch der Allgemeinen Pathologie, vol. 3/6, p. 219. Eds H.W. Altman et al. Springer, Berlin/Heidelberg/New York 1973.
11 Mislin, H., in: Lymphangiology, p. 165. Eds M. Földi and J.R. Casley-Smith. Schattauer Verlag, Stuttgart/New York 1983.
12 Frasher, W.G., Microvasc. Res. *5* (1973) 376.
13 Ohshima, N., and Sato, M., Angiology *32* (1981) 752.
14 Woodbury, J.W., and Brady, A.J., Science *123* (1956) 100.
15 Ohshima, N., and Yasuda, A., Proc. 1983 Biomechanics Symposium, AMD-vol. 56, FED-vol. 1, p. 75. Eds S.L.-Y. Woo and R.E. Mates. The American Society of Mechanical Engineers, United Engineering Center, New York 1983.

0014-4754/84/040342-02$1.50 + 0.20/0