Lymphology 14 (1981) 1–6

Cholinergic and Adrenergic Innervation of Mesenterial Lymph Vessels in Guinea Pig

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Summary

The innervation of lymph vessels in guinea pig mesentery was studied by light and electron microscope using histochemical techniques. The cholinergic fibers run prevalently longitudinal in the perivascular connective tissue, only brief segments show a loose network. The lymph vessels wall, compared with that of blood vessels, shows few adrenergic nerve fibers located in the adventitia outside the smooth muscle cells. The possible role of the nervous system in the motor control of the lymph vessels is discussed.

Introduction

To date there has been no complete and convincing description of the innervation of lymph vessel wall (*Kytmanof* 1901, *Lawrentjew* 1925, *Shdanow* and *Pawlitzkaja* 1949. *Kubik* and *Szabo* 1955).

Even the most recent contributions are not consistent and comparable with one another (*Schipp* 1965, *Vadja* 1966, *Shdanow* and *Wolodjko* 1967, *Furness* 1971, *Todd* and *Bernard* 1973, *Huth* and *Bernhardt* 1977). The aim of the present study is therefore to furnish new data using modern and selective techniques.

Material and methods

Studies were carried out on mesentery collectors of guinea pigs (150–200 g body weight) using light and electron microscopical methods.

Light microscopy

The animals were divided into five groups treated as follows:

Group 1: Karnovsky and Roots histochemical technique, for selectively evidencing the acetylcholinesterase positive nerve fibres, applied to stretched mesentery strips and sections of same.

Group 2: Falck and Owmann fluorescence histochemical technique applied as above to selectively demonstrate the adrenergic post-gangliar nerve fibres.

Group 3: Dopamine treatment, one 40 mg/kg i.p. injection 1 h before death to increase the adrenergic activity of the nerve endings, followed by fluorescence technique as for group two.

Group 4: 5-hydroxydopamine treatment, for 20 mg/kg i.p. injections at 12 h interval before death, followed by fluorescence technique as for group two.

Group 5: Treatment with two doses of 6-hydroxydopamine, an initial dose of 50 mg/kg i.p.; one week later, a second dose of 100 mg/kg i.p. 24 h before sacrifice of animals: 6-hydroxydopamine selectively destroy the adrenergic nerve fibres.

Electron microscopy

Six groups of animals were treated as follows:

Group 1: Routine electron microscopy techniques applied to small fragments of mesentery fixed by perfusion.

Group 2: Westrum and Black cytochemical method for demonstrating at the electron

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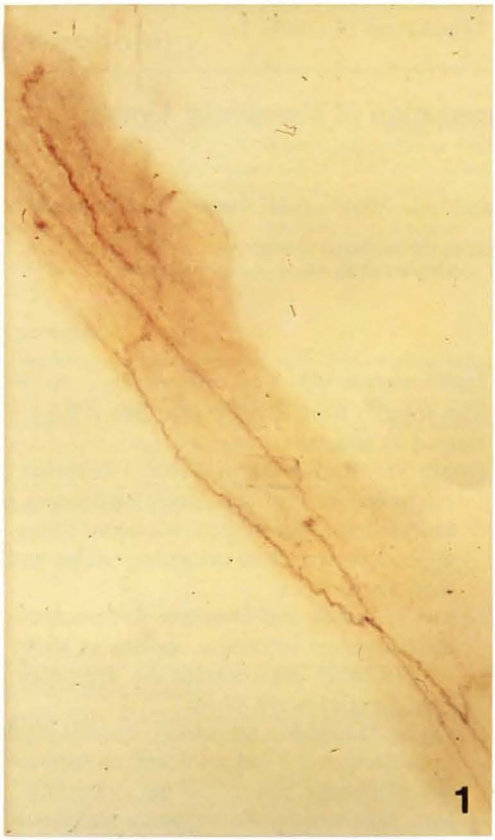

Fig. 1 Acetylcholinesterase. A stretch preparation of a mesenteric lymph collector. Small acetylcholinesterase positive fibres with prevalently longitudinal distribution form a loose network in the vascular wall 320x

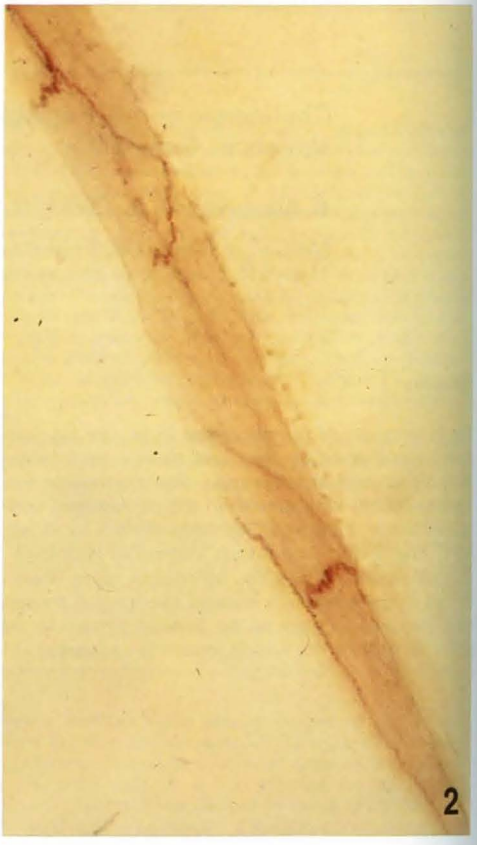

Fig. 2 Acetylcholinesterase. The acetylcholinesterase positive fibres running longitudinally are connected by anastomoses showing an intense enzyme activity 320x

microscopical level acetylcholinesterase activity in the nerve fibres.

Group 3: Tranzer and Richards cytochemical method for evidencing axonic microvesicles containing noradrenaline.

Group 4: Dopamine treatment, one 40 mg/kg i.p. injection 1 h before death, to reinforce adrenergic activity and Wood's cytochemical method for selectively evidencing the adrenergic microvesicles in the axonic varicosities.

Group 5: Reinforcing treatment with 5-hydroxydopamine, four 20 mg/kg i.p. injections at 12 h interval before death and Wood's technique as for group four.

Group 6: Treatment with 6-hydroxydopamine, an initial dose of 50 mg/kg; one week later a second dose of 100 mg/kg i.p. 24 h before death, to control the selective destruction of the adrenergic fibers at electron microscopical level.

Results

Light microscopy

In the lymph collectors, portions of wall free of innervation and portion showing acetylcholinesterase positive fibres alternate. These fibers often derive from large nerve trunks running parallel to the lymph collectors, being mostly disposed longitudinally (group 1), (Figs. 1, 2).

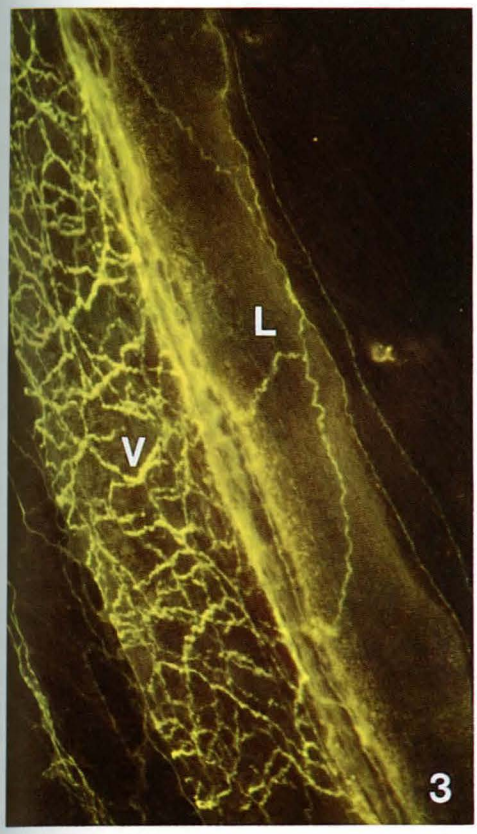

Fig. 3 Fluorescence. Venule and mesenteric lymph collector in a stretch preparation. Rare adrenergic fibres irregularly distributed are present in lymph vascular wall. Venous wall shows a close fluorescent network with uniform pattern. 320x. V = venule, L = lymph collector

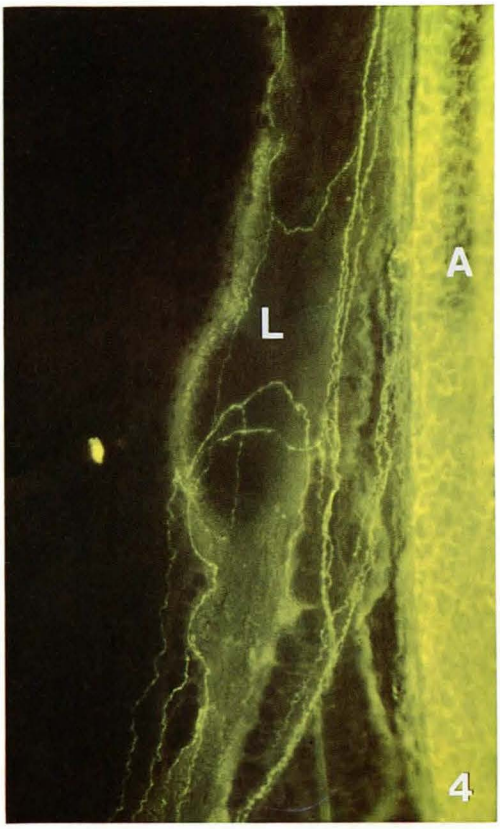

Fig. 4 Fluorescence. A stretch preparation of mesenteric small artery and lymph collector. Fine adrenergic fibres with varicosities form a loose network in the lymph vascular wall. The arterial wall shows a very close and intensely fluorescent network. 320x A = artery, L = lymph collector

The ligth fluorescence microscopy revealed an extreme scarcity of adrenergic fibres in the lymph collectors wall. Adrenergic fibres which contact the lymph vessels wall generally run lengthwise, and occasionally wind themselves more or less completely around the collector (Fig. 3). Quite frequently small closely spaced varicosities, intensely fluorescent, are manifest along the fibres (group 2) (Fig. 4). Sometimes lymph vessels wall for long extent is lacking of fluorescent nerve fibres. The observation of transverse sections of the lymph collectors confirm that acetylcholinesterase positive and fluorescent fibres are evident in the tunica adventitia only. The treatments with dopamine and 5-hydroxydopamine enhances the fluorescence in the adrenergic fibres and therefore make themselves readily evident (groups 3 and 4). After 6-hydroxydopamine treatment (group 5) the lymph collectors and mesentery blood vessels lose their fluorescent (adrenergic) nerve network but the acetylcholinesterase positive (cholinergic) remains.

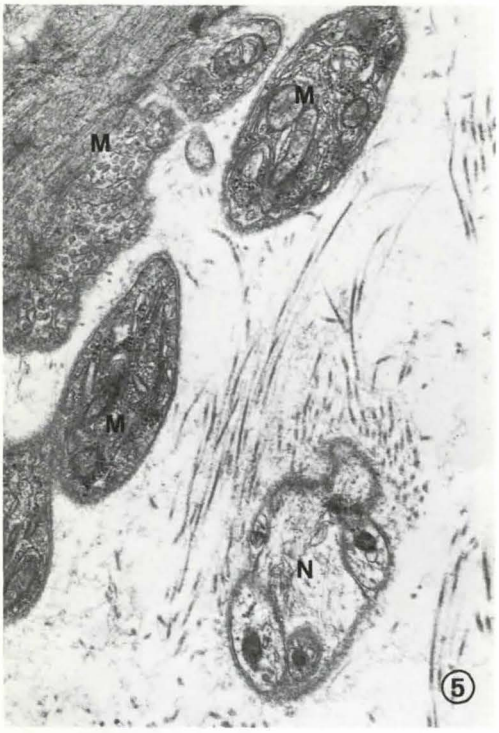

Fig. 5 Mesenteric lymph collector. Unmyelinated nerve fibre near the smooth muscle coat. 23250x M = muscle cell, N = nerve fibre

Electron microscopy

The few unmyelinated nerve fibres in the tunica adventitia of the lymph collectors, constituted by no more than 3 or 4 axons, lie at least 200–300 nm or even more from the basal membrane of the smooth muscle cells (Fig. 5). Not infrequently the mesentery collectors wall lack muscle cells and the nerve endings run close to the nude endothelial cells but not closer than 200 nm (group 1) (Fig. 6). These axons contain neurofilaments, neurotubules and form small varicosities containing mitochondria and few synaptic vesicles; it is difficult to understand the nature of mediator they contain. In the group 2 animals few and rare acetylcholinesterase positive fibres are visible near the lymph vessels wall (Fig. 7). The enzyme reaction product is an electron dense precipitate found in the space between axolemma and the Schwann cell membrane: this product has a characteristic "macula" distribution and morphology. The presence of adrenergic nerve fibres in the lymph collectors wall is confirmed by group 3 animals: in some axonic varicosities synaptic vesicles with strongly electron dense granules are evident. The adrenergic synaptic vesicles are particularly clear after dopamine (Fig. 8) and 5-hydroxydopamine treatment (group 4 and 5). The selective degeneration of the adrenergic axons is demonstrated after 6-hydroxydopamine treatment (group 6).

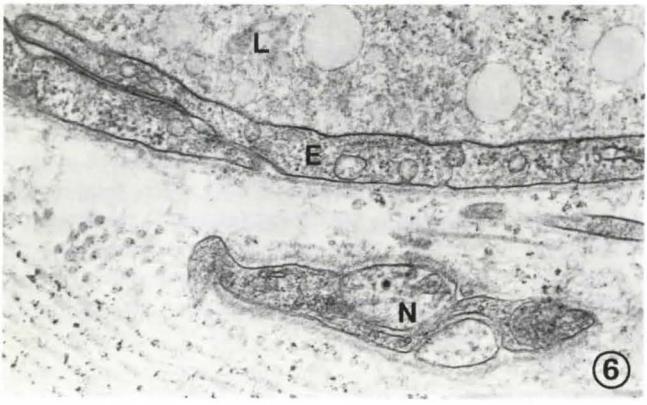

Fig. 6 Mesenteric lymph collector. Fine unmyelinated fibre near the endothelium. 36600x. N = nerve fibre, E = endothelium, L = lumen

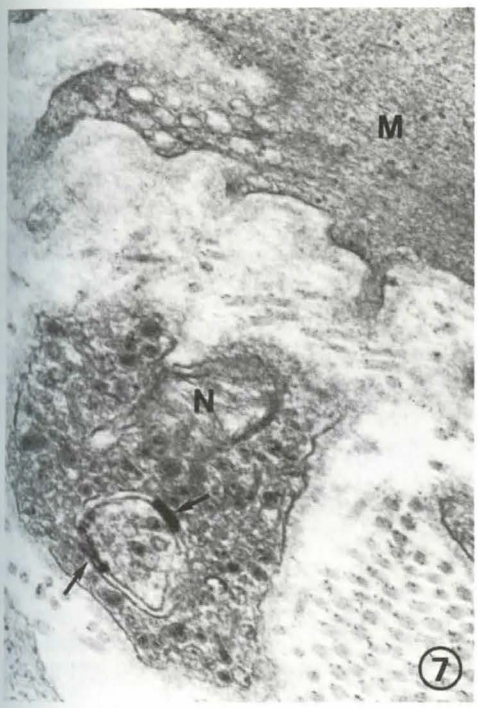

Fig. 7 Mesenteric lymph collector. Unmyelinated nerve fibre containing an acetylcholinesterase positive axon (arrows). 57500x. M = muscle cell, N = nerve fibre

Discussion and conclusion

The present study demonstrates that the few and rare nerve fibres in the lymph collectors wall lie at a distance of at least 200–300 nm from the muscle cells. The distribution of the nerve endings along the vessel wall is particularly irregular, considerable extents of lymph vascular wall being without innervation. Preferential innervation does not exist between the segment of vessel containing valves (where the wall is thinner) and segments lying between valves (where the wall has more muscle cells). The present results indicate that fibres of the parasympathetic and sympathetic nervous system are present in mesentery lymph collectors. The distance of the fibres from the basal membrane of the muscle cells is never less than 200 nm and membrane specialization typical of synapses is absent. It is known however, that at least chemical mediators of adrenergic type, even when liberated at a considerable distance (400–1000 nm) from the smooth muscle cells membrane, are still able of provoking contraction (*Burnstock* 1970, 1975). Isolated lymph vessels when stimulated in vitro with noradrenaline and acetylcholine, respond readily with accelarated pulsations (*Orlov* et al. 1976, *Ohhashi* et al. 1978). This could give a functional meaning to the present observations. The limited nervous network of lymph vessels wall could be correlated with the relative thinnes of the wall and thus with the scarcity of muscle cells,

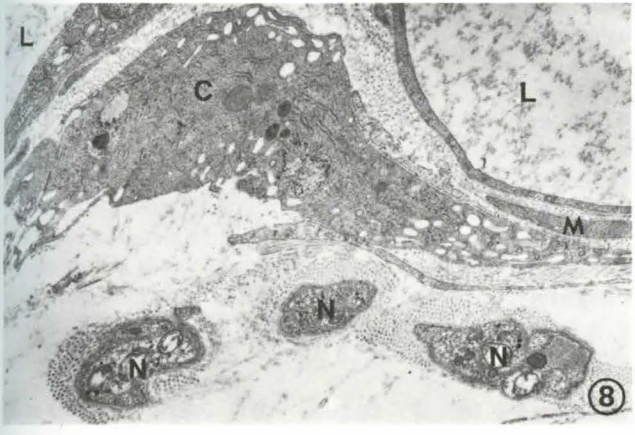

Fig. 8 Mesenteric lymph collector. Unmyelinated nerve fibre near the insertion of a valvular edge. 13500 x. M = muscle cell, N = nerve fibre, C = connective cell, L = lumen

already reported in numerous morphological studies (*Fruschelli* 1967, *Fruschelli* and *Simoni* 1970, 1971).

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