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Claudication Induces Systemic Capillary Endothelial Swelling

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An in vivo *model of intermittent claudication has been developed to investigate systemic reperfusion injury associated with transient muscle ischaemia. Rats were subjected to unilateral common iliac artery ligation and two weeks of intermittent hind limb muscle stimulation. Electron microscopy demonstrated a significantly increased percentage of swollen capillary endothelial cells both locally and systemically in these "claudicant" rats, compared with controls or those undergoing muscle stimulation or artery ligation alone. These results support human data suggesting that claudication induces an inflammatory response which results in systemic vascular injury.*

Key Words: Intermittent claudication; Cell swelling; Capillary permeability

Introduction

Recent evidence suggests that intermittent claudication is associated with a systemic inflammatory response, involving neutrophil activation and increased vascular permeability.[1–3] Investigation on humans is limited by ethical constraints, so in order to obtain more direct evidence of events in the microcirculation during claudication, an *in vivo* model was developed.

Reperfusion following ischaemia is complicated by capillary endothelial cell swelling, which results from increased cell membrane permeability.[4,5] The hypothesis that claudication induces systemic vascular injury was therefore tested initially by measuring the degree of muscle capillary swelling in the claudicant model.

Methods

The claudicant model

Male Sprague-Dawley rats (250 g) underwent laparotomy under halothane anaesthesia and the right common iliac artery was ligated 2 mm distal to the aortic bifurcation. A pair of multi-stranded steel wire electrodes (SS7T, Clark Electromedical Supplies, Reading) were then implanted adjacent to the lateral popliteal nerve in the right hind limb, via a lateral incision. The Teflon-coated wires were tunnelled subcutaneously to emerge through the nape of the neck. The muscles of the ischaemic right hind limb could then be stimulated by connecting the electrodes to a neuromuscular stimulator (Neuro Tech programmable system controller 16, Biochemical Research, Ireland). Stimulation was by square-wave impulses of 0.3 ms duration, 10 Hz frequency, at a voltage sufficient to induce palpable muscle twitching without apparent discomfort (1.5–2.3 V). Rats were subjected to this for 10 min, seven times daily for 7 days (with 90 min between each stimulation) and then for 15 min, seven times daily for a further 7 days; thus producing repeated contractions in a partially ischaemic muscle.

The circulatory and morphological changes observed in the hind-limb muscles of this model are similar to those seen in human claudicants. Resting muscle blood flow is reduced by 40% 2 weeks after common iliac artery ligation and during contraction falls to only 32% of that seen in controls.[6] The stimulation regimen outlined above increases resting limb blood flow 2 weeks after artery ligation, but during contractions blood flow is still only 40% that of control limbs.[7] Muscle biopsies of rats subjected to artery ligation and electrical muscle stimulation reveal increased oxidative enzyme activity, glycogen

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content and mitochondrial volume density, an increased percentage of oxidative muscle fibres and reduced ATP concentration; conforming to those changes seen in human claudicants.[8–10] The stimulation regimen described was chosen because more intensive, prolonged stimulation resulted in muscle necrosis and weight loss.[9,11] No such changes, however, occur as a result of this regimen, which produces a slight improvement in muscle performance.[7]

This rat model is obviously not identical to the situation in human claudicants. For example, the vessels are not atherosclerotic and the period of muscle contraction is not limited by pain. Animal investigation is justified, however, as more direct, invasive techniques can be used. Results obtained would be particularly valid if they supported human data obtained by indirect methods.

Capillary swelling

Four rats were subjected to the "claudication" regimen as described above and then deeply anaesthetised with intra-peritoneal pentobarbitone. Biopsies were taken from the extensor digitorum longus (EDL) muscles of both hind limbs and also the spinotrapezius muscle, the diaphragm and the left ventricular papillary muscle. The animals were allowed to exsanguinate following the final biopsy. All biopsies were immediately placed in gluteraldehyde fixative then osmic solution and dehydrated with ethanol and propylene oxide, before being embedded in resin blocks. The process was repeated in four rats that had undergone only artery ligation and sham stimulation, four subjected to stimulation alone, after sham laparotomy and four controls subjected to both sham laparotomy and sham stimulation.

The resin blocks were cut into ultrathin (98 nm) sections which were placed on filtered grids, stained with uranium and lead and examined by transmission electron microscopy (JEM 100CX II, Jeol, Japan). With the observer blinded as to the origin of the grids, 50 capillaries from each muscle of every rat were photographed in cross section and assigned to one of three groups:

(1) Normal: no endothelial swelling (Fig. 1(a)).
(2) Moderate swelling: less than one-third circumference swollen (Fig. 1(b)).
(3) Gross swelling: more than one-third circumference swollen (Fig. 1(c)).

Thus 200 capillaries were analysed from each muscle in all four groups. Total numbers of normal and swollen capillaries were recorded. Numbers of normal and swollen cells varied very little between the four rats in each group and so differences between groups were compared with the chi-squared test.

Results

Only 10% of capillaries had swollen endothelial cells in the right EDL muscle of control rats (Fig. 2). This rose to 34% on stimulation only and to 52% following artery ligation. Stimulation-induced swelling was mainly moderate, but ischaemia markedly increased the proportion of gross swelling. The combination of ligation and stimulation increased the number of swollen capillaries to 62%. This was significantly higher than the number seen in the control ($p < 0.001$), stimulated-only ($p < 0.001$) or ligated-only ($p < 0.05$) groups, although the percentage of grossly swollen cells was similar to that seen after ligation only.

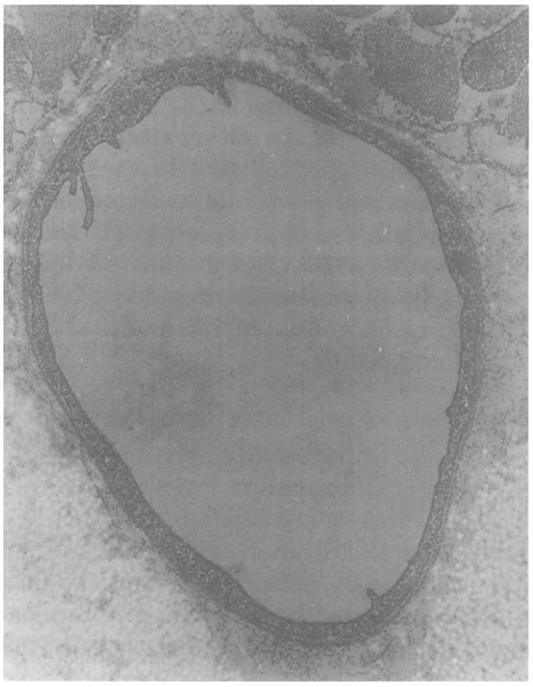
The results seen in the contralateral, left EDL muscle were similar (Fig. 3). Right-sided "claudication" increased cell swelling on the left to 57%, which was significantly higher than the other three groups ($p < 0.001$, all three).

Although the actual numbers were reduced when compared to the hind-limb muscles, the same trend was observed in the spinotrapezius muscle (Fig. 4), with increased swelling in the ligated-stimulated rats compared to the other three groups ($p < 0.001$, all three), including a marked increase in gross swelling. Fourteen per cent of endothelial cells seen were grossly swollen in these rats but there was no increase in gross swelling in the ligated-only or stimulated-only groups. Results obtained from the diaphragm and heart muscle were similar to those seen in the spinotrapezius.

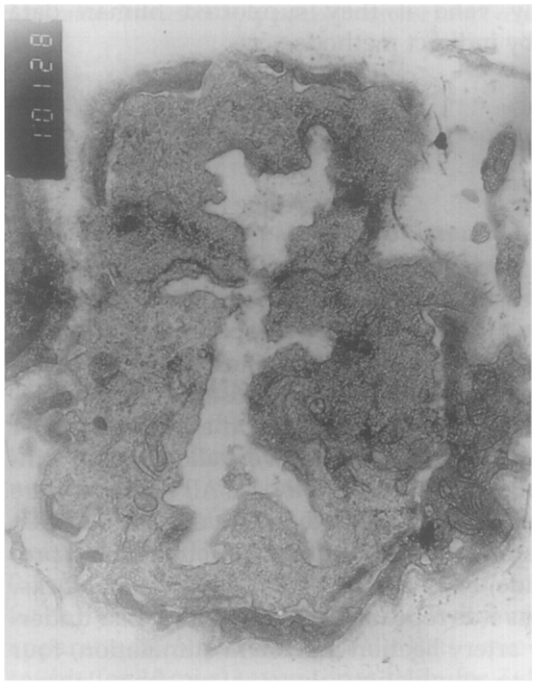
Discussion

These results show that ligation of the rat's common iliac artery, followed by intermittent stimulation of the hind-limb muscles, induces swelling of capillary endothelial cells. Swelling occurs both locally and systemically to a significantly higher degree than is seen after stimulation or artery ligation alone; indicating that the combination of ischaemia and exercise is important. Although caution must always be exer-

(b)



(a)



(c)

Fig. 1. (a) Normal, (b) moderately swollen and (c) grossly swollen capillary endothelial cell.

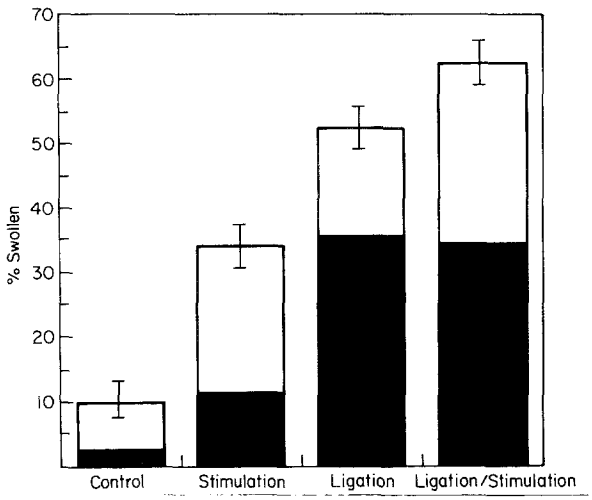


Fig. 2. Percentage of swollen capillary endothelial cells in the right-sided (ipsilateral) EDL muscle (mean ± S.E.). □: < one-third swollen; ■: > one-third swollen.

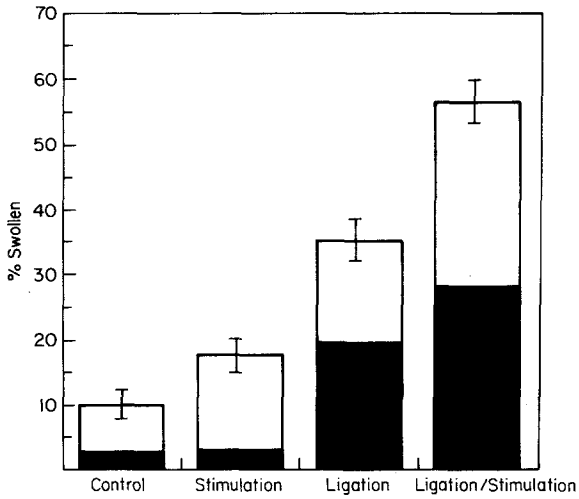


Fig. 3. Percentage of swollen capillary endothelial cells in the left-sided (contralateral) EDL muscle (mean ± S.E.). □: < one-third swollen; ■: > one-third swollen.

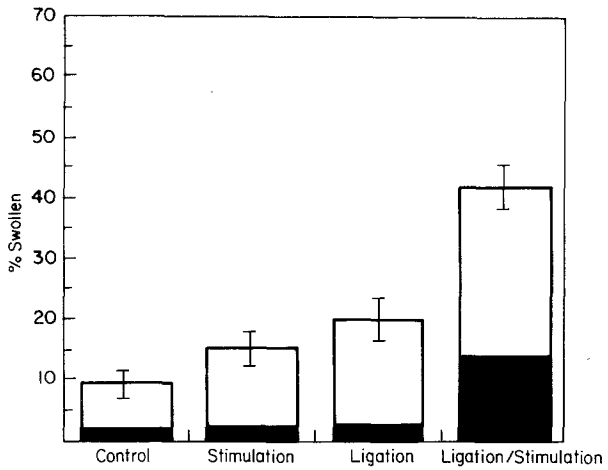


Fig. 4. Percentage of swollen capillary endothelial cells in the spinotrapezius muscle (mean ± S.E.). □: < one-third swollen; ■: > one-third swollen.

cised when interpreting the results of animal experiments, we feel that this supports our human data suggesting systemic vascular injury after claudication.[2]

The finding that around 10% of capillaries were swollen (mostly moderately swollen) in all the muscles of control animals is unexplained, but may represent increased vascular permeability in response to anaesthesia and surgery.[12] It is also unknown why the contralateral limb displayed greater swelling in response to unilateral claudication than the spinotrapezius and other muscles of the upper body. We are, however, investigating the possibility that stimulation of the sensory nerves in one hind limb may, by spinal reflex, produce motor stimulation in the contralateral limb.

Capillary endothelial cell swelling occurs during reperfusion following prolonged ischaemia; thus claudication may represent repeated ischaemia and reperfusion. Local swelling is likely to exacerbate ischaemia by luminal obstruction and secondary plugging with white cells.[13,14] The pathological significance of the systemic reperfusion injury is unknown but increased vascular permeability and leukocyte activation may accelerate atherogenesis.[15,16]

The phenomenon of claudication-induced systemic vascular injury is therefore worthy of further investigation. Work is continuing into the pathological mechanisms involved, including the role of white blood cells. It is likely that neutrophils, activated by inflammatory mediators released during reperfusion,

induce vascular injury by the release of oxygen-derived free radicals.[17] We feel that the rat claudicant model is suitable for this study and will also be used to assess whether the events observed are amenable to pharmacological manipulation.

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