Spinal Cord (1997) 35, 26–32

Regulation of vasomotion of arterioles and capillaries in the cat spinal cord: role of α actin and endothelin-1

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Ring-shaped vasoconstrictions of arterioles at their branching sites have often been reported in vascular corrosion casts of the brain and spinal cord in rats and cats. It is surmised that smooth muscle cells in arteriolar walls could regulate the blood flow by changing the diameter of the lumen (ie vasomotion). However, few reports have described vasomotion at the capillary (capillaries have no smooth muscle cells). Also, there have been no reports on endothelin-1 in the arterioles and capillaries of the spinal cord. This study was designed to determine (1) the electron microscopic architecture of vasomotion; (2) the immunohistochemical identification of α actin and endothelin-1 in the arterioles and capillaries of the spinal cord. Twenty-seven adult mongrel cats were used to study vascular corrosion casts at the lumbosacral spinal cord segments immunohistologically and through scanning electron microscopic observations. Sections of the spinal cord were stained with monoclonal anti-α actin and endothelin-1 antibodies.

Vascular corrosion casts demonstrated two types of vasomotion: a sausage-like peristalsis and a ring-shaped vasoconstriction at the arteriole and capillary levels. In the immunohistological study, α actin and endothelin-1 were identifiable in the vascular wall at the bifurcation, and pericytes were found to contain microfilaments of α actin. The ring-shaped vasoconstriction might be regulated by smooth muscle cells in arterioles and by pericytes in capillaries by releasing endothelin-1.

Keywords: spinal cord; vasomotion; vascular corrosion cast; endothelial cell; α actin; pericyte

Introduction

Certain regulation systems contribute to maintaining the spinal cord blood flow (SCBF) physiologically constant.[1,2] The amount of SCBF may depend on the size of vessels which changes, corresponding to the demands of the particular situations.[3–5] Although vasoconstrictions of arterioles at branching sites from their parent arteries has previously been reported to occur in rats and cats,[6–9] there are few precise anatomical details and clear explanation of the regulation mechanism in the capillaries of the spinal cord. Koyanagi *et al*[6,7] observed that the spinal cord arteries at the injury site frequently show constrictions. The study of the vascular regulation system is important to our understanding of the effects of pathological processes, such as the spinal cord injury in which vascular secondary effects have been implicated.[10,11]

The endothelium of vessels, which has hitherto been considered to exist between circulating blood and the smooth muscle, without playing any important role, has attracted attention due to its potential in regulating tissue blood flow.[12–13] The current study was designed to observe the ultrastructure of the microvascular corrosion casts of spinal cord vessels and to investigate, through immunohistochemical studies, the possible role of endothelial cells in the local regulation of blood flow.

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Materials and methods

Experiment 1: Observation of microvascular corrosion casts in spinal cord microvessels using a scanning electron microscope

Fifteen adult mongrel cats of both sexes, weighing from 2.6 to 3.8 kg (average: 3.2 kg), were used in this experiment. Each animal was anesthetized by the intraperitoneal injection of sodium pentobarbiturate (25 mg/kg body weight). A cannula 2.0 mm in diameter was then inserted in retrograde manner into the right femoral artery and a thoracotomy was conducted to expose the descending aorta, which was subsequently ligated at the level immediately above the diaphragm. The animal was perfused with 2000 ml of a 0.9% sodium chloride (NaCl) solution containing heparin (10 IU/ml) at a manually applied pressure of 100–110 mmHg. Using a method modified from that

of Murakami,[14] a vascular mould was made following the intra-arterial injection of resin (Mercox, Oken Co., Tokyo, Japan) mixed with methylmethacrylate monomer (Wako Chemical, Osaka, Japan) to reduce the viscosity. Care was taken to perfuse the resin to the extent that part of the agent overflowed from the cut end of the inferior vena cava. The spinal cord at the level of the lumbosacral segment was then removed and placed overnight in a hot-water bath (60°C) to polymerize the perfused resin. This spinal cord was immersed in a 30% potasium hydroxide (KOH) solution for 7 days to remove spinal cord tissue, and to leave resin. The vascular casts were cleaned in a gentle stream of running water. The vascular casts of the white matter was trimmed with a razor blade. The remaining vascular casts of the grey matter mounted on metallic board and coated with gold to be observed under a scanning electron microscope (Hitachi S-510, Hitachi Electrics, Tokyo, Japan).

Experiment 2: Immunohistochemical examination of regulation system in the spinal cord blood flow

I. Optical Microscopic Observation Twelve adult mongrel cats of both sexes weighing from 2.7 to 3.5 kg (average: 3.1 kg) were used in this experiment. After being anesthetized, animals were perfused with 2000 ml of a 0.9% sodium chloride (NaCl) solution containing heparin (10 IU/ml) and then with a fixative of 4% paraformaldehyde (Merck, Osaka, Japan) in 0.1 M phosphate buffer at pH 7.2–7.4. The spinal cord was removed in the same manner in experiment 1 and fixed with 4% paraformaldehyde for 2 h at 5°C. After dehydration with ethylalcohol, the spinal cords were embedded in paraffin wax and sectioned at a 10 μm thickness. The sections were placed on gelatin-coated slides for immunostaining. The indirect immunoperoxidase technique was employed as follows:

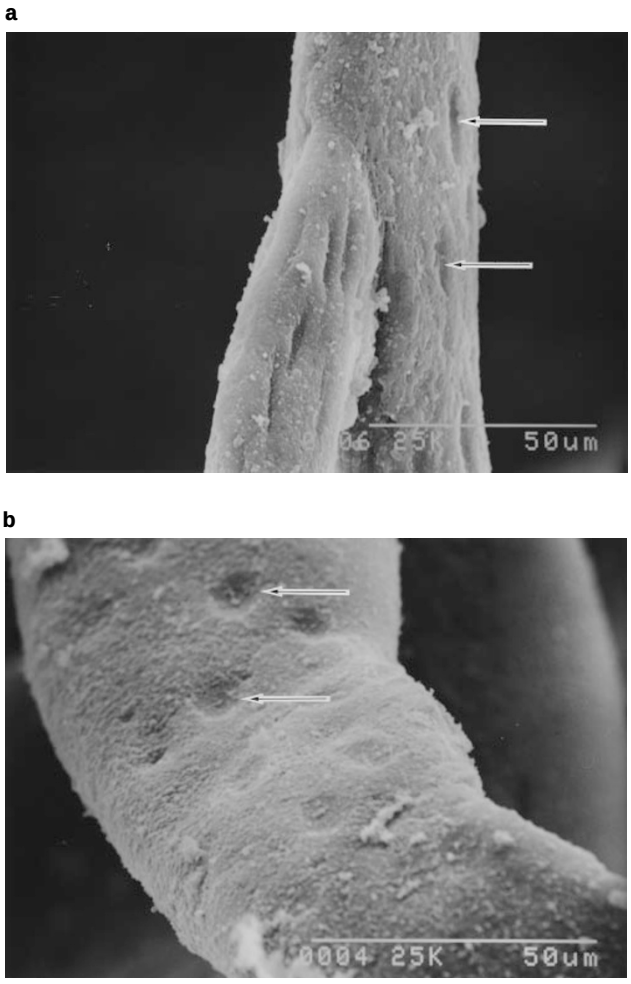

Figure 1 An arteriole and a venule in the grey matter of cat spinal cord, as seen with SEM of a vascular corrosion cast. (a) A number of ovoid depressions (arrow) is observed on the arteriolar surface. (b) The venular casts show more round depressions (arrow)

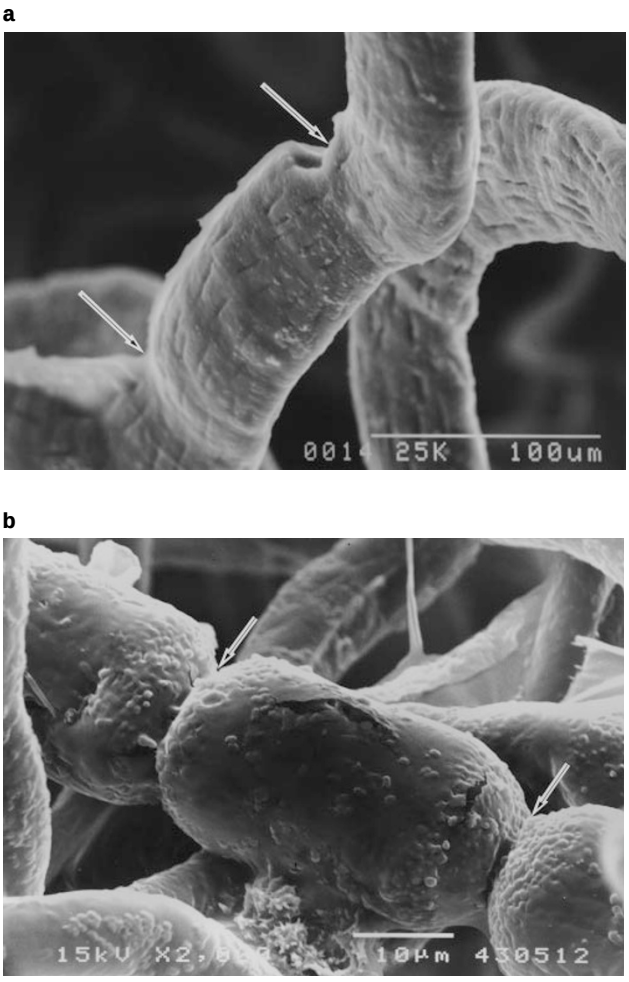

Figure 2 An arteriole and a capillary in the grey matter showing Type I vasomotion, which is observed at non-branching sites. (a) A sausage-like continuing vasoconstriction and vasodilation in the arteriole (arrow). (b) Type I vasomotion seen in the capillary projecting into the vascular lumen

Deparaffinized sections were incubated with anti-α actin monoclonal antibody (Boehringer Lab., Norristown, PA, USA) at a concentration of 1 : 400 and anti-endothelin-1 antibody (Yamasa, Tokyo, Japan) at a concentration of 1 : 100 in phosphate buffer saline, and incubated at 5°C for 8 h. Slides were rinsed three times (10 min each) in phosphate buffer saline and incubated for 1 h in 1 : 100 goat-antimouse IgG secondary antibody conjugated with horse radish peroxidase (Medical Biology Lab., Nagoya, Japan) at room temperature. After washing with phosphate buffer saline (10 min each), samples were subjected to a 3, 3′-diaminobenzidine tetrahydrochloride (DAB, Wako Chemical, Osaka, Japan) reaction for observation under an optical microscope (Model Labophoto-2, Nikon, Tokyo, Japan).

II. Electron Microscopic Observation Some of the samples embedded in paraffin wax were sectioned using a microslicer (Model DSK-1000, Dosaka Inc., Osaka, Japan) into 60 μm thicknesses. The appropriate sections were processed for immunohistological purposes to determine anti-α actin monoclonal antibody. They were then immersed in 1% osmium tetroxide for 1 h and dehydrated with graded ethanol to be embedded in epoxyresin. Ultrathin sections of these materials with 50 to 100 nm thicknesses were made with an ultratome (LKB 8800, Stockholm, Sweden) and observed using a transmission electron microscope (Hitachi TEM Model HS-8, Hitachi Electrics, Tokyo, Japan).

Results

Experiment 1
A number of ovoid depressions occurred on the surface of the arteriolar cast of the spinal cord (Figure 1a),

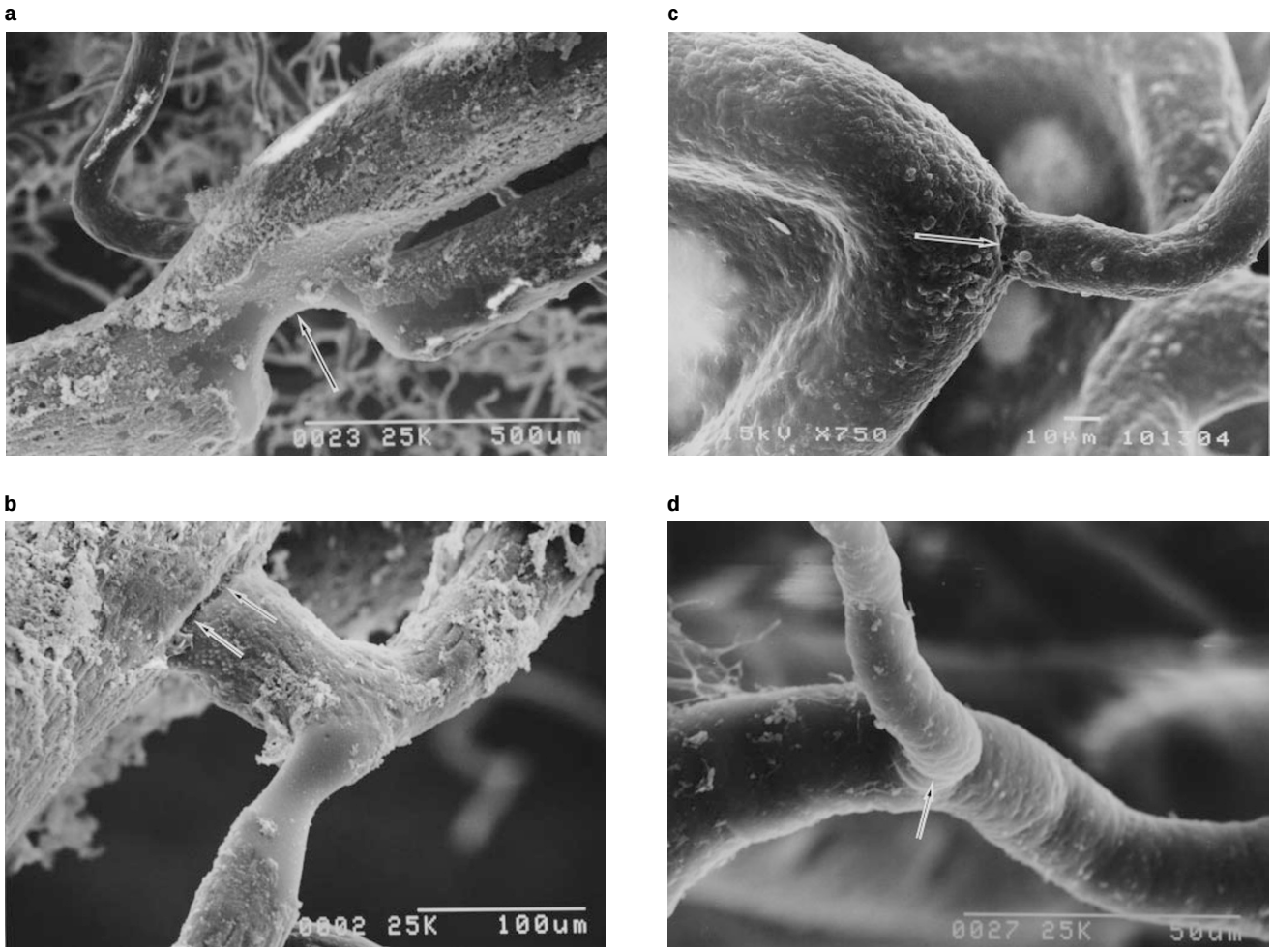

Figure 3 Arterioles and capillaries in the grey matter showing Type II vasomotion, which is observed at branching sites. (a) A valvular vasoconstriction (arrow) protruding often unilaterally into the vascular lumen, seen primarily in an arteriolar bifurcation yielding a branch having similar diameter. (b) A ring-shaped vasoconstriction (arrow) in an arteriolar bifurcation having a smaller branch. (c) Ring-shaped vasoconstriction (arrow) at the branching site of the capillary from the arteriole. (d) Type II vasomotion (arrow) is existent, but insignificant in the capillary bifurcation

while the venular cast showed round depressions (Figure 1b). The arteriolar casts indicate two types of vasomotion. Type I vasomotion was demonstrated as continuing intervals of vasoconstriction having a sausage-like appearance observed primarily in the arteriole (Figure 2a) and in the capillary (Figure 2b). This type of vasomotion is seen primarily in arterioles 20 to 100 μm in diameter and in capillaries 5 to 15 μm in diameter. Type II vasomotion demonstrated valvular or ring-shaped vasoconstriction mainly at branching

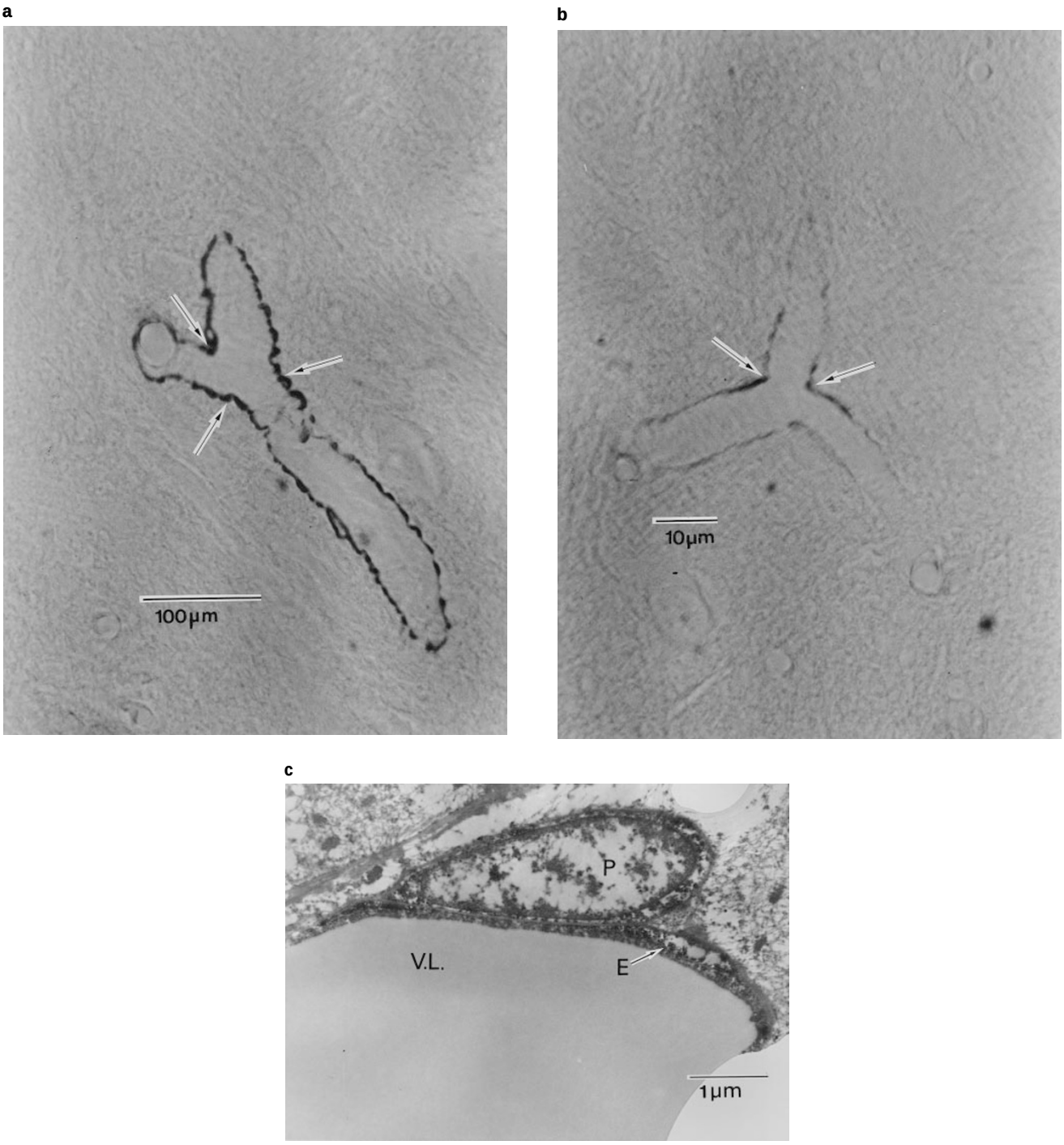

Figure 4 Immunohistologic evaluation of α actin in the grey matter of the spinal cord. (a) Microphotograph stained with anti-α actin antibody showing increased staining (arrow) at the site of arteriolar bifurcation. (b) Staining of α actin (arrow) at the capillary level is less significant than that seen in the arteriole. (c) A transmission electron microphotograph demonstrating microfilaments containing α actin deep in the endothelium and pericyte. V.L., vascular lumen; E, endothelium; P, pericyte

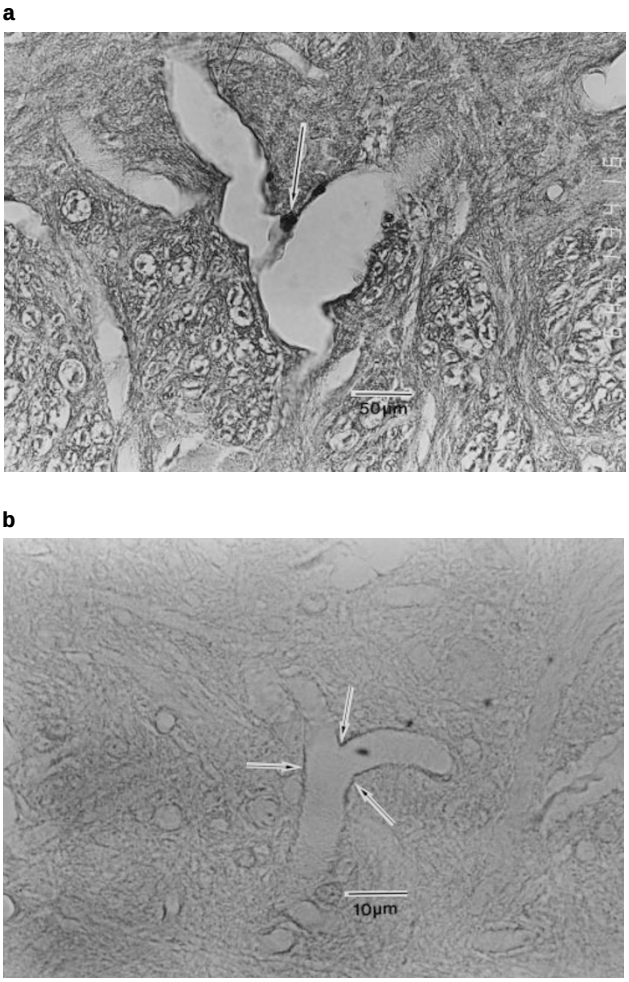

Figure 5 Immunohistologic evaluation stained with anti-endothelin-1 antibody. (a) Microphotograph showing increased staining (arrow) is observed at the site of the arteriolar bifurcation. (b) Decreased staining (arrow) is observed at the capillary level

sites with an increased frequency compared to other sites. At the site of arteriolar bifurcation yielding a branch of similar diameter, a valvular constriction often protruded into the vascular lumen that was sometimes observed unilaterally (Figure 3a). A circular indentation was often noted surrounding the branching site between the main trunk and a branch. Findings of this type were observed primarily at the arteriole (Figure 3b), at the origin of the capillary from the corresponding arteriole (Figure 3c), and at the capillary level (Figure 3d). The vasoconstriction of the capillary, however was not as significant as that seen at the arteriole level.

Experiment 2

Immunohistochemical observation with anti-α actin monoclonal antibody revealed a vessel wall deeply and clearly stained: (1) at the arteriolar level (Figure 4a), particularly at the site where the arteriole branches into capillaries; and (2) at the capillary level particularly at the site yielding a branch (Figure 4b). The transmission electron microscopic observations showed an increased intensity of staining with anti-α actin monoclonal antibody surrounding vascular pericytes and endothelial cells (Figure 4c). The immunohistology with anti-endothelin-1 antibody confirmed markedly increased staining in the inner wall of the arteriole and capillary. At the arteriolar level, the branching site was stained with antibody (Figure 5a) while, at the capillary level, the staining of the inner wall of the vessels was not as intense as that found in the arteriole. However, the staining at the branching site of the capillary was preserved (Figure 5b).

Discussion

The spinal cord has been physiologically shown to regulate and control its blood flow,[1,2] and the control of SCBF may depend on the change in vascular caliber (ie vasomotion).[3–5] The vasomotion has been observed directly only in the vessels of the mesentery[15,16] and the wing of the bat.[17] The vasomotion in the spinal cord, however, can be more accurately determined when observed stereoscopically using vascular corrosion casts and a scanning electron microscope,[6–9,14] and it is becoming better understood through immunohistochemical studies.[18]

The vasomotion at the arteriolar level was found to consist of two types: Type I vasomotion may represent the peristaltic movement by the arteriolar vessels that is hypothesized to have a pumping effect. Type II vasomotion is more frequently observed than Type I and is presumed to control the blood supply in accordance with local requirements. These phenomena may act as active control systems regulating the SCBF. Symon *et al*[19] noted possible myogenic factors, still not clearly identified, that changed the diameter of vessels. Furchgot *et al*[12] found that vascular endothelial cells secrete certain substances to relax the arterial smooth muscle by acethylcholine. Yanagisawa *et al*[13] identified the endothelium-derived-constricting factor, endothelin, which is believed to play an important role in local blood flow control. Gotoh *et al*[20] reported that the autoregulation system in the brain was destroyed by damaging the endothelium with exposure to ultraviolet beams, and thus again stressed the importance of the endothelial obligatory effect on the central nervous system. Our study demonstrates that vasoconstriction is most obviously recognized in the arteriolar bifurcations within the spinal cord. Vascular smooth muscles are abundant at the bifurcation site of the arteriole, designated as the vascular sphincter. Immunohistochemistry confirmed that α actin, a vasoconstricting protein, is more significantly identified at the branching site of the arteriole, showing the presence of increased sphincter muscles possibly causing a Type II vasomotion. Nicoll *et al*[17] suggested that vasoconstriction by sphincter muscles is con-

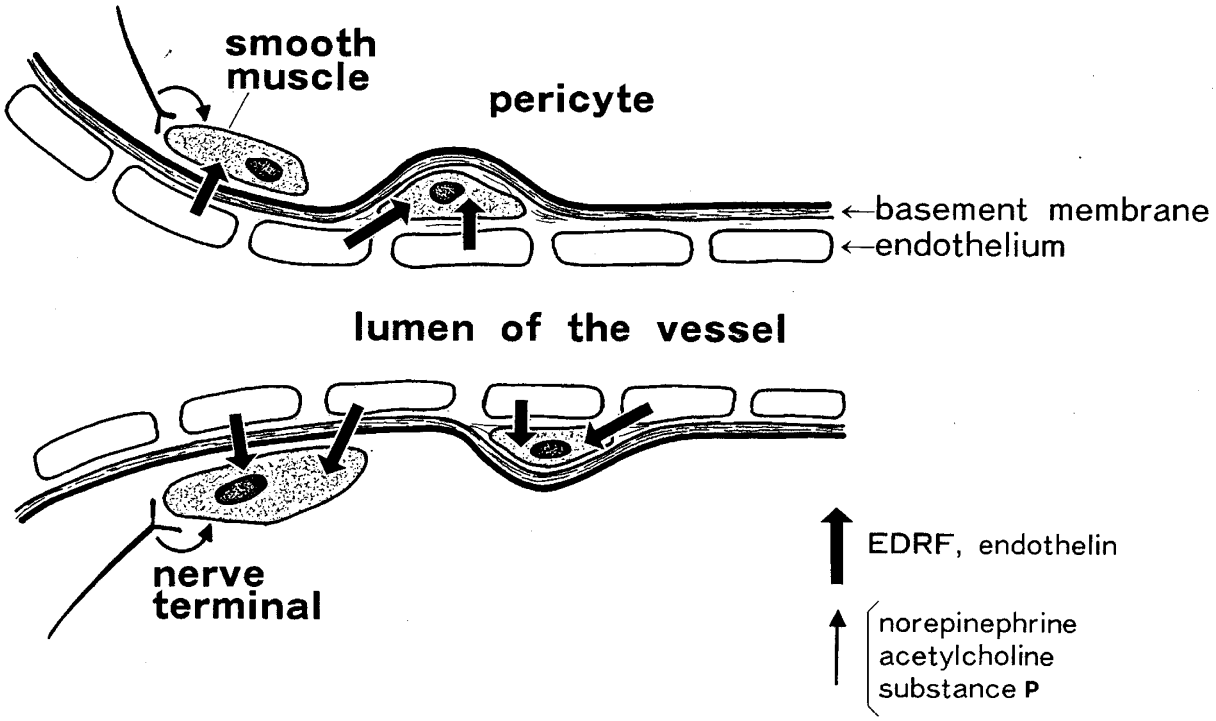

Figure 6 Scheme of the possible regulation system in the spinal cord. The endothelium of arterioles and capillaries in the spinal cord may perceive local changes in blood stream, and subsequently secrete vasoconstricting substances such as endothelin-1. They may act on smooth muscles and pericytes to induce vasomotion in arteriolar and capillary

trolled by local metabolic factors. Our present study with anti-endothelin-1 antibody showed significant incrementation in staining for endothelin-1 at the bifurcation site of the arteriole and capillary that strongly suggests that the activity of vasomotion is dependent on endothelial cells. Sakurai *et al*[21] proved that the type A receptor of the smooth muscle cell membrane, which has increased affinity for endothelin-1, contributes to vascular constriction. We suggest therefore that the endothelial cell of the spinal cord vessel produces such a constricting factor in response to changes in the blood flow and thus regulates vasoconstriction activity.

The diameters of capillaries between 5 and 15 μm were also found to change diameters in a sausage-like movement. It was determined that capillary vessels also make vasoconstrictive change at the level of bifurcation but to a less significant degree than that seen in the arteriole. Pericytes are assumed to regulate this activity at the capillary level, which has no vascular smooth muscle. Fujiwara *et al*[22] found from scanning electron microscopic observation that pericytes exist in the vascular wall and feature many cytoplasmic processes with octopus- or star-shaped extensions. Zimmermann[23] hypothesized that pericytes with cytoplasmic extensions vertical to the long axis of the capillary vessel may contribute to vasoconstriction. The validity of this assumption can be confirmed from findings as follows: First, a cell designated as 'transitional' with a morphology characteristic of both smooth muscle cell and pericyte has been observed at the distal site of the arteriole[24] and, second, certain proteins contributing to vasoconstriction in the smooth muscle are also found in pericytes as immunohistochemical evidence. Le Beux *et al*[25] and Joyce *et al*[26] advocate the role of vasoconstrictive activity of the pericyte based on evidence that microfilaments within the cell consist of many proteins such as actin, myosin, tropomyosin, and isomyosin. In our current study, we found α actin within the pericytes of the capillary vessel of the cat spinal cord. Gaudio *et al*[27] observed that the pericytes are primarily located at the branching site of the capillary, and thus deduced that they may act in vasoconstriction similar to smooth muscle cells at the arteriole level. Our observations support this assumption. Chakravarthy *et al*[28] observed that endothelin-1 contracted the pericytes in the retina of the cow. Yamagishi[29] found that the endothelial cell regulates the growth of capillary pericytes *in vitro* through the secretion of endothelin-1, and that pericytes produce mRNA coding to the type A receptor for endothelin-1. These observations suggest a regulation mechanism of the blood flow through an endothelium-pericyte interaction in capillary level.

Our results suggest that endothelial cells may be strongly associated with the regulation mechanism responsible for changes in capillary calibre. As yet,

however, this type of peripheral regulation system has not been recognized in the spinal segmental arteries or anterior spinal artery of the spinal cord with their wider inner diameters and thick smooth muscles. This suggests that these arteries are possibly under the control of the vasomotor center of the brain stem. In peripheral vessels within the spinal cord, this type of localized 'real-time' or 'on-site' regulation system may be more important in responding to rapidly changing requirements in blood flow. We conclude, as shown in Figure 6, that the endothelium may perceive local changes in blood flow and subsequently secrete vasoconstricting substances such as endothelin-1. These may act on smooth muscles and pericytes to induce changes in arteriolar and capillary calibre.

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