Contractile Proteins in Pericytes. II. Immunocytochemical Evidence for the Presence of Two Isomyosins in Graded Concentrations

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ABSTRACT This paper describes the localization of isomyosins in the pericytes of four rat microvascular beds: heart, diaphragm, pancreas, and the intestinal mucosa, by use of immunoperoxidase techniques and IgGs specific for either nonmuscle or smooth muscle isoforms. Based on the semiquantitative nature of the peroxidatic reaction, we concluded that the amount and distribution of these isoforms vary with the microvascular bed and also with vascular segments within the same bed. In the pericytes of small capillaries, nonmuscle isomyosin is the predominant form, whereas the smooth muscle isomyosin is present in very low concentration. A reversed relationship is found in the pericytes associated with larger capillaries and postcapillary venules. These results, taken together with previous findings on actin (Herman, I., and P. A. D'Amore, 1983, J. Cell Biol. 97:278a), tropomyosin (Joyce, N. C., M. F. Haire, and G. E. Palade, 1985, J. Cell Biol. 100:1379-1386), and cyclic GMP-dependent protein kinase (Joyce, N., P. DeCamilli, and J. Boyles, 1984, Microvasc. Res. 28:206-219), indicate that pericytes contain proteins essential for contraction in higher concentration than any other cells associated with the microvasculature, except smooth muscle cells. Pericytes appear to be, therefore, cells differentiated for a contractile function within the microvasculature.

It has been repeatedly assumed that the pericytes of blood capillaries and postcapillary venules function as contractile elements within the microvasculature because of their morphologic and topographic characteristics (3, 9, 12, 16, 18, 23, 33). To test this assumption, the presence of significant amounts of essential contraction-associated proteins in pericytes must be established, and eventually their contraction and its effects on microvascular blood flow must be demonstrated directly in vivo.

In our laboratory, two proteins associated with the regulation of contraction have been localized in microvascular pericytes by use of immunoperoxidase techniques. The companion paper (17) describes the localization of tropomyosin in the pericytes of four rat microvascular beds by use of an IgG specific for the smooth muscle isoform of this protein. In addition, we have localized cyclic GMP-dependent protein kinase, an enzyme postulated to function in the regulation of contraction in smooth muscle cells (14, 19, 27), in pericytes from a variety of tissues and organs (16). Actin, a major contractile and cytoskeletal protein, has been identified in pericytes by electron microscopic techniques (21, 32), and both smooth muscle and nonmuscle isoactins have been localized in pericytes in situ and in cell culture by immunofluorescence procedures (15).

To complete the identification of essential contractile proteins in pericytes, it is necessary to determine whether they contain myosin. Available information on this topic is either indirect or insufficient. In rat brain pericytes, LeBeux and Willemot (21) have observed thick, tapering fibrillar structures with morphological and solubility characteristics similar to those of myosin filaments, and brief preliminary reports (8, 25) have been published on the immunocytochemical localization of myosin in the pericytes of brain and lymph nodes. This paper describes results obtained in localizing isomyosins in pericytes by use of a light microscopic immunoperoxidase procedure and antibodies specific for either smooth muscle or nonmuscle myosin. We found the two isoforms to be present in different proportions in the pericytes of both capillaries and postcapillary venules of four rat microvascular beds.
MATERIALS AND METHODS

Antibodies: Two anti-myosin IgGs were used in these studies. The first, obtained from Biomedical Technologies, Inc. (Cambridge, MA), was raised in rabbits against the heavy chain of myosin isolated from human platelets. It was supplied as an IgG fraction precipitated from immune serum by ammonium sulfate and subsequently solubilized and dialyzed against phosphate-buffered saline (PBS). The second antibody was raised in rabbits in our laboratory against the heavy chain of myosin prepared from rat intestinal smooth muscle. The muscularis was dissected from the intestinal wall, minced, and extracted in SDS as described in reference 17; the resultant extract was electrophoresed on 5% Laemmli polyacrylamide preparative slab gels; the gel band containing myosin heavy chain was excised and macerated; and the protein was eluted by stirring in the presence of 10 mM sodium phosphate, pH 7.0, supplemented with 1% SDS, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM 2-mercaptoethanol, 0.05% sodium azide at room temperature for 24 h. The eluate containing myosin heavy chain was concentrated by pressure dialysis and dialyzed against 10 mM sodium phosphate, pH 7.0, containing 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.03 mM phenylmethyl sulfonylfluoride, and 0.02% sodium azide before injection as antigen by use of an immunization protocol similar to that described by Fujikawa and Pollard (11). IgG was precipitated from the immune serum, solubilized, and dialyzed as described above. For nonimmune IgG, serum collected from four normal rabbits was combined and the IgG fraction was precipitated, solubilized, and dialyzed as above.

To test the specificity of the two antimonyosins, extracts were prepared from the following cells and tissues known to be enriched in a specific isomyosin: human platelets (nonmuscle isomyosin); human uterus, rat intestinal smooth muscle, and rat aorta (smooth muscle isomyosins); rat heart (cardiac muscle isomyosin); and rat diaphragm (skeletal muscle isomyosin). Tissue specimens were extracted for 30 min at 100°C in a modified Ringer's-HEPES buffer supplemented with 2.5% SDS, 2.5% 2-mercaptoethanol, 1% bovine serum albumin, 8.0 μg/ml leupeptin, 20 μg/ml antipain, 1 μM peptstatin, 20 μM benzamidine, 0.1 mM phenylmethyl sulfonylfluoride, and 20 μg/ml soybean trypsin inhibitor. (All protease inhibitors were supplied by Sigma Chemical Co., St. Louis, MO.) After clarification of the extracts by centrifugation at 100,000 g for 30 min at 15°C, ~1.5 mg total protein from each extract was electrophoresed on individual 7% Laemmli polyacrylamide gels. Vertical strips were cut from the nitrocellulose transfers and incubated overnight at 45°C in PBS supplemented with 0.05% sodium azide and 1% hemoglobin to prevent nonspecific protein adsorption. After they were washed in PBS supplemented with 0.5% Triton X-100 and 0.05% sodium azide, strips were incubated for 2 h at room temperature in 0.5 μg/ml of either nonimmune IgG or anti-human platelet myosin heavy chain or anti-rat intestinal smooth muscle myosin heavy chain diluted in each case with wash buffer containing 1% hemoglobin. Bound IgG was detected by incubation of the strips in 125I-Protein A (New England Nuclear, Boston, MA) and subsequent autoradiography.

Nonimmune IgG was unreactive with all extracts (Fig. 1, lane 2; Fig. 2, lanes 2 and 6; Fig. 3, lanes 2 and 6). Anti-human platelet myosin heavy chain recognized only myosin heavy chain from the human platelet extract (Fig. 1, lane J). It did not react with myosin from rat intestinal smooth muscle (Fig. 2, lane 3), cardiac muscle (Fig. 3, lane 3) or skeletal muscle (Fig. 3, lane 7). This antibody was not tested against extracts of human uterus or rat aorta, nor was it tested against extracts containing rat nonmuscle myosin; however, it clearly reacted with rat “nonmuscle” cells in immunocytochemical experiments, which indicated its species cross-reactivity (see Results). Taken together, these results showed that anti-human platelet myosin heavy chain is specific for nonmuscle muscle.

FIGURE 1 Immunoblots illustrate the ability of anti–human platelet myosin heavy chain (a-PMHC) and anti–rat intestinal smooth muscle myosin heavy chain (a-ISMMHC) to recognize “smooth muscle” isomyosin. SDS extracts of rat intestinal smooth muscle and aorta were prepared and electrophoresed on 7% Laemmli polyacrylamide gels. Nitrocellulose transfers of the extracts and immunoblots were prepared as described in Materials and Methods. lanes 2–4, autoradiographs of nitrocellulose transfers of this extract incubated in nonimmune IgG (lane 2), a-PMHC (lane 3), and a-ISMMHC (lane 4). Lane 5, Coomassie Blue-stained profile of the intestinal smooth muscle extract. Lanes 6–8, autoradiographs of the corresponding nitrocellulose transfers incubated in nonimmune IgG (lane 6), a-PMHC (lane 7), and a-ISMMHC (lane 8). No staining was evident when either transfer was incubated in nonimmune IgG. A-ISMMHC recognized its homologous antigen, whereas a-PMHC could not recognize the “nonmuscle” myosin isoform. Arrowhead, position of myosin heavy chain.

FIGURE 2 Immunoblots illustrate the inability of anti–human platelet myosin heavy chain (a-PMHC) and anti–rat intestinal smooth muscle myosin heavy chain (a-ISMMHC) to recognize “cardiac” and “skeletal” muscle isomyosins. SDS extracts of rat cardiac muscle (as a source of cardiac isomyosin) and diaphragm (as a source of skeletal muscle isomyosin) were prepared and electrophoresed on 7% Laemmli polyacrylamide gels. Nitrocellulose transfers of the extracts and immunoblots were prepared as described in Materials and Methods. Lane 1, Coomassie Blue-stained profile of the cardiac muscle extract. Lanes 2–4, autoradiographs of the corresponding nitrocellulose transfers incubated in nonimmune IgG (lane 2), a-PMHC (lane 3), and a-ISMMHC (lane 4). Lane 5, Coomassie Blue-stained profile of the diaphragm extract. Lanes 6–8, autoradiographs of the corresponding nitrocellulose transfers incubated in nonimmune IgG (lane 6), a-PMHC (lane 7), and a-ISMMHC (lane 8). No staining was evident when either transfer was incubated in nonimmune IgG. Neither antibody could recognize cardiac or skeletal muscle isomyosin. Arrowhead, position of myosin heavy chain.
isomyosin; therefore, it will be referred to as anti-nonnucle muscle myosin throughout this paper.

The antibody raised against rat intestinal smooth muscle myosin heavy chain did not recognize myosin heavy chain in platelet (Fig. 1, lane 4), cardiac muscle (Fig. 3, lane 4), or skeletal muscle (Fig. 3, lane 8) extracts; however, it recognized specifically myosin heavy chain in extracts from rat intestinal smooth muscle (Fig. 2, lane 4), rat aorta (Fig. 2, lane 7), and human uterus (data not shown), indicating its specificity for smooth muscle isomyosin and its species cross-reactivity. Throughout the paper this antibody will be referred to as anti-smooth muscle myosin.

Immunoperoxidase Localization Procedure: Specimens from rat heart, diaphragm, intestine, and pancreas were fixed and processed for immunocytochemistry as described in detail in the companion paper (17). Progress of the peroxidatic reaction was monitored under a dissecting microscope (Wild Heerbrugg Instruments, Inc., Farmingdale, NY). Individual tissue sections were reacted for various times and then transferred to PBS. Optimal staining was obtained within 20 min for all specimens. Longer reaction times did not enhance reactivity but resulted in high background, due presumably to either nonspecific deposition or diffusion of the 3,3'diaminobenzidine (DAB) reaction product. The rest of the protocol (postfixation in osmium tetroxide and embedding in Poly/Bed 812 (Polysciences Inc., Warrington, PA) followed procedures previously described (17).

Enhancement Procedures: We tested several methods in an attempt to optimize the signal generated in the immunoperoxidase reaction. In preliminary experiments, specimens were fixed in (a) 4% formaldehyde for 30 min at room temperature; (b) 4% formaldehyde for 3 h at 0°C; (c) 4% formaldehyde, 0.1% glutaraldehyde for 30 min at room temperature; and (d) periodate-lysine-paraformaldehyde fixative (24), before incubation with primary antibody. Fixation in the presence of glutaraldehyde significantly reduced reactivity. We obtained the best results—in terms of preserved antigenicity and acceptable morphological preservation (for light microscopy)—by fixing the tissues in 4% formaldehyde for 30 min at room temperature. This protocol was used in all subsequent experiments. Our attempts to enhance the density of the DAB reaction product by using several published techniques (1, 13, 29) were unsuccessful in specimens prepared by our procedures.

Photography and Analysis of the Results: Thick (~1 #m) sections were cut from several blocks of tissue incubated with each of the primary antibodies, and bright field micrographs of the sections were taken on a Zeiss Photomicroscope II with a green filter and a 63x planar (nonphase) objective lens. Kodak Technical Pan film was used to provide optimal contrast and small grain size. Individual micrographs were printed to give a final magnification >1,000. Occasionally, a magnification greater than that optically useful was employed to facilitate the distinction of positive from negative cells (even at the price of image blurring).

Within each micrograph blood vessels were identified by criteria established for diaphragm microvessels (28). The outer diameter was measured on all vessels with readily identifiable associated pericytes and on all arterioles and muscular venules. (Measurement of vessel outer diameter was taken at the position on the vessel that most closely represented its transverse diameter, i.e., the short axis on obliquely and longitudinally sectioned vessels and any axis of vessels sectioned transversely.) All pericytes and vascular smooth muscle cells were rated according to their level of reactivity. Cells were considered negative when their staining (tan to light brown) was equal to background or not greater than the staining produced by nonspecific DAB adsorption. Cells were rated weakly positive when they appeared from light to medium brown and were darker than nonspecific DAB staining. Strongly positive cells were stained dark brown to black and were easily discernible even at low (16x) magnification.

The percentage of positive staining was calculated for all recognizable pericytes and vascular smooth muscle cells in each of the four microvascular beds and the data were expressed as a function of increasing vessel diameter. The number of strongly or weakly positive pericytes was totaled from all four microvascular beds and the percentages obtained were expressed as a function of increasing vessel diameter.

RESULTS

Control Experiments

Throughout the tissues examined, pericytes, vascular smooth muscle cells, endothelial cells, and fibroblasts were completely free of any form of background staining in specimens incubated with nonimmune IgG. Endogenous peroxidase activity was found in granulocytes (especially eosinophils) in the intestinal mucosa, in rare erythrocytes within capillary lumina, and in mast cells associated with blood vessels. DAB was apparently adsorbed nonspecifically in the fat droplets of adipocytes occasionally present in connective tissue, in lipid droplets within muscle fibers, and on the discontinuous internal elastic lamina of larger arterioles and muscular venules. In addition, staining due to nonspecific

1 Abbreviations used in this paper: DAB, 3,3'diaminobenzidine; OD, outer diameter.
adsorption of nonimmune IgG was observed on the luminal aspect of pancreatic acinar cells, in centroacinar cells, and in epithelial cells lining pancreatic ducts. Fig. 4 provides examples of heart, diaphragm, and pancreas incubated with nonimmune IgG. Additional micrographs of control specimens and a discussion of the limits of antibody penetration in these tissues can be found in the companion paper.

**Microvessel Identification**

Arterioles, mainly metarterioles, were identified by their outer diameter (OD) of 8 to 20 μm; their continuous muscle tunic, generally reduced to a single cell layer in the smaller arterioles; and the tendency of the endothelial nuclei to protrude into the lumen.

Capillaries were readily recognized by their small ODs which ranged from ~3 to 9 μm, most averaging ~3 to 4 μm in heart and diaphragm, and ~4 to 6 μm in pancreas and the intestinal mucosa.

Postcapillary venules were identified by their ODs which ranged from 8 to 25 μm; the relative thinness of their endothelium; and their increased coverage by pericytes with thicker processes than on capillaries.

Muscular venules varied in size from 18 to ~50 μm OD throughout the four microvascular beds. They had a continuous muscle tunic, consisting of one or more thin muscle layers and a discontinuous internal elastic lamina.

**Data Analysis**

12 arterioles and 12 muscular venules were identified in specimens reacted for nonmuscle myosin, and 41 arterioles and 27 muscular venules were identified in tissues reacted for smooth muscle myosin.

On capillaries, pericytes could be distinguished from other vessel-associated cells, such as adventitial fibroblasts, by their close apposition to the underlying endothelium. They were identified by their characteristic profiles, which included either the nuclear region and proximal portions of foot processes, or individual segments of more distal processes, depending on where along their lengths the cells were sectioned. When pericytes were unstained, only these morphologic cri-
Pericyte reactivity was scored only when these cells could be reliably identified and when reactivity in individual foot processes could be distinguished from areas of positivity within the endothelial cytoplasm. Larger segments of pericyte processes were relatively easy to identify; fine processes were more difficult to visualize and the more attenuated tips of these processes (which often interdigitate with the endothelial cells) went generally undetected; hence, they are not included in this analysis.

The pericyte sample size in each of the microvascular beds examined reflects the volume density of these cells and the criteria used for their reliable identification. An average of 341 capillaries was identified per microvascular bed for each antibody tested. Of these, an average of 108 capillaries (range 70–167), representing ~32% (range 21–58%) of the identifiable capillary population, had recognizable pericytes. This percentage compares favorably with the results obtained with the localization of smooth muscle tropomyosin (17) and cyclic GMP-dependent protein kinase (16). Nuclei were consistently unstained. The pericytes of small capillaries (3 to 6 μm diam) were strongly positive in all four microvascular beds (Figs. 5, a–d and 6b). Most pericytes on larger capillaries (6 to 9 μm diam) were also positively stained (Fig. 6a); however, as Fig. 6b indicates, an increased number of cells appeared weakly positive. (Compare the darkly stained pancreatic capillary pericyte in Fig. 5d with the less intensely stained pericyte on the intestinal capillary in Fig. 5e.)

The pericytes of postcapillary venules appeared to differ in their reactivity to anti-nonmuscle myosin, depending on the microvascular bed. In heart and diaphragm, pericytes on postcapillary venules of ≥10 μm OD appeared to be unreactive (Fig. 5, f–g and Fig. 6a), whereas those on vessels of the

Pericytes were more readily identifiable on postcapillary venules; however, since the frequency of these vessels was relatively low in the individual microvascular beds examined, and since the distinction between venular capillaries and postcapillary venules is often difficult by light microscopy, data from small postcapillary venules (8 and 9 μm OD) were included with those from capillaries in the same size range. Data for postcapillary venules with outer diameters of ≥10 μm were combined in a single pool.

**Immunoperoxidase Localization of Nonmuscle Myosin**

In all four microvascular beds, 80 to 90% of all capillary pericytes were positively stained for nonmuscle myosin as illustrated in Fig. 5, a–e and Fig. 6a. DAB reaction product was diffusely distributed within the perikarya and foot processes of these cells, thereby generating staining patterns similar to those previously described for the immunoperoxidase localization of tropomyosin (17) and cyclic GMP-dependent protein kinase (16). Nuclei were consistently unstained. The pericytes of small capillaries (3 to 6 μm diam) were strongly positive in all four microvascular beds (Figs. 5, a–d and 6b). Most pericytes on larger capillaries (6 to 9 μm diam) were also positively stained (Fig. 6a); however, as Fig. 6b indicates, an increased number of cells appeared weakly positive. (Compare the darkly stained pancreatic capillary pericyte in Fig. 5d with the less intensely stained pericyte on the intestinal capillary in Fig. 5e.)

The pericytes of postcapillary venules appeared to differ in their reactivity to anti-nonmuscle myosin, depending on the microvascular bed. In heart and diaphragm, pericytes on postcapillary venules of ≥10 μm OD appeared to be unreactive (Fig. 5, f–g and Fig. 6a), whereas those on vessels of the

![Figure 6](http://example.com/f6.png)

**Figure 6** (a) Percentage of pericytes or vascular smooth muscle cells positive for nonmuscle myosin in four rat microvascular beds. Both strongly and weakly positive cells are included in this calculation and results are illustrated as a function of increasing vessel diameter. ODs include a range of measured diameters rounded to the nearest micrometer. Indicated also are vessel size ranges in which capillary pericytes (C-P), postcapillary venule pericytes (PCV-P), and muscular venule smooth muscle cells (MV-VSM) are found. Arteriolar smooth muscle cells are not included to provide greater continuity with increasing vessel diameter. In all cases arteriolar and venular smooth muscle cells reacted identically. C-P in all four organs are positive for nonmuscle myosin. PCV-P in intestine and pancreas remain positively stained for this isoform, whereas no detectable staining is evident in heart and diaphragm. Vascular smooth muscle cells are consistently negative in all four microvascular beds. (b) Reactivity of pericytes for nonmuscle myosin expressed as a function of increasing vessel diameter. The number of strongly positive (solid bar) and weakly positive (striped bar) pericytes were totaled from all four microvascular beds. The percentage of total positive pericytes represented by each level of reactivity was calculated for each vessel diameter. Throughout the capillary bed (3–9 μm) pericytes are strongly positive for nonmuscle myosin; however, a slight decrease in reactivity occurs in pericytes associated with larger (6–9 μm) capillaries. The level of nonmuscle myosin reactivity is reduced in postcapillary venule pericytes of >10 μm OD. This last entry represents data from only intestine and pancreas, since no reactivity was observed in these cells in heart and diaphragm.
same size in pancreas and the intestinal mucosa remained positively stained (Figs. 5, h and i and 6a). A decrease in the staining intensity was observed, however, especially within the postcapillary venule pericytes of the intestinal mucosa (Fig. 5h and Fig. 6, a and b).

Vascular smooth muscle cells from both arterioles (Fig. 5, j and k) and muscular venules (Fig. 5, l and m) were consistently negative for nonmuscle myosin in all four microvascular beds (Fig. 6a).

Endothelial cells of both blood and lymphatic vessels stained positively for nonmuscle myosin, but the reaction was weak and definitely less intense than that of the corresponding pericytes. In most endothelial cells the positive reaction was just above background level throughout the cytoplasm (Fig. 5, a–m). Within some cells, however, discrete, small "spots" of more intense staining could be seen within both the perinuclear and more attenuated cytoplasm (Fig. 5, b–d, f, and g).

The endothelial cells of larger vessels such as arterioles, postcapillary venules, muscular venules, and lymphatics appeared diffusely and more intensely stained than those of capillaries (Fig. 5, j–m). Connective tissue fibroblasts gave a weak, diffuse reaction for nonmuscle myosin (as indicated at the arrows in Fig. 5, h and j).

**Immunoperoxidase Localization of Smooth Muscle Myosin**

The results of the immunoperoxidase localization procedure indicated that the concentration of smooth muscle myosin varied in a reproducible manner throughout the microvascular beds examined as illustrated by the gallery of microvessels in Fig. 7, a–l.

The number of positive pericytes (Fig. 8a) and the strength of their positive reaction (Fig. 8b) increased with increasing vessel size. Reactivity was generally undetectable or at very low levels in the pericytes of small (~3–4 μm diam) capillaries (Fig. 7a), whereas an increased frequency of weakly positive staining was evident in the pericytes associated with capillaries of moderate size (~5–7 μm diam); Fig. 7, b and c). Pericytes located on larger capillaries (~8–9 μm diam; Fig. 7, d and e) and on postcapillary venules (Fig. 7, f–i) were as strongly reactive for smooth muscle myosin as the vascular smooth muscle cells themselves (Fig. 7, j–l). The micrograph in Fig. 9 has been included to show that this distribution pattern could be clearly seen within a small area of a given specimen (in this case, a section through the rat myocardium): the pericyte on the smaller capillary (arrow) appears weakly re-

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**Figure 7** Gallery of representative microvessels from several rat organs illustrating the reactivity of vessel-associated cells for smooth muscle myosin. Capillary pericytes appear differentially reactive, the strength of their reaction depending on vessel size. Pericytes associated with small capillaries appear either unreactive as in a or weakly positive as in b and c. Note that the endothelium in c is unstained, whereas the pericycle process (arrow) is more darkly stained. This level of reactivity is considered weakly positive as compared with the dark, positive staining of the smooth muscle cell (arrowhead). Strong reaction is frequently seen in pericytes on larger capillaries (d and e). Postcapillary venule pericytes (f–i) are strongly reactive for smooth muscle myosin as are arteriolar (j) and (k) and venular (l) smooth muscle cells. In general, endothelial cells appear unreactive for this myosin isofrom. Pancreas: (a) x 1,890; (f) x 2,020; (l) x 1,010. Intestine: (b) x 1,990; (c) x 2,140; (g) x 1,490; (j) x 810; (k) x 1,540. Diaphragm: (d) x 2,120; (i) x 1,360. Heart: (e) x 2,640; (h) x 1,790.
active, whereas pericytes on the larger capillary (small arrowhead) and postcapillary venule (large arrowhead) are intensely stained for smooth muscle myosin. This appearance, which is representative for all specimens examined, rules out the possibility that the pattern described is an artifact generated by local variations in reagent concentrations.

In general, endothelial cells did not give a positive reaction for smooth muscle myosin (Fig. 7, a–l). In some cells, small areas of weak positivity could be observed, but, at the level of resolution provided by the light microscope, we could not determine whether the positive areas were located within endothelial cells or within closely apposed, thin pericytic processes. If we disregard these occasional and uncertain findings, the results showed that endothelial cells did not contain significant concentrations of smooth muscle myosin. Fibroblasts were also unreactive for this antigen.

**FIGURE 8** (a) Percentage of pericytes or vascular smooth muscle cells positive for smooth muscle myosin in four rat microvascular beds. Both strongly and weakly positive cells are included in this calculation and the results are expressed as a function of increasing vessel diameter. ODs were rounded to the nearest micrometer. The range of vessel diameters in which capillary pericytes (C–P), postcapillary venule pericytes (PCV–P), and muscular venule smooth muscle cells (MV–VSM) are found is also indicated. As in Fig. 6a, arteriolar smooth muscle cells are not included, since the response of all vascular smooth muscle cells was identical. In all four microvascular beds, the number of pericytes containing detectable levels of smooth muscle myosin increases with increasing vessel diameter. PCV–P ≥ 10 μm and all MV–VSM are positive for this myosin isoform. (b) Reactivity of pericytes for smooth muscle myosin expressed as a function of increasing vessel diameter. The number of strongly positive (solid bar) and weakly positive (striped bar) pericytes were totaled from all four microvascular beds and the percentage of total positive cells represented by each level of reactivity was calculated for each vessel diameter. The strength of the positive reaction for smooth muscle myosin increases in pericytes as the vessel diameter increases. PCV–P from all four microvascular beds are strongly positive for this myosin isoform.

**FIGURE 9** This micrograph of a thick (~1 μm) section of rat cardiac muscle incubated with anti-smooth muscle myosin illustrates the modulation in pericyte reactivity with increasing vessel size. The pericyte associated with the smaller capillary (arrow) appears weakly reactive, whereas dark DAB reaction product is visible within the pericyte on both the larger capillary (small arrowhead) and the postcapillary venule (large arrowhead), indicating a higher level of reactivity. M, mast cell. × 1,160.
distribution of isomyosins in pericytes and other vessel-associated cells.

The transition from capillaries to postcapillary venules occurred at the level of postcapillary venules. The change from capillaries to muscular venules.

A decrease in the concentration of the nonmuscle isoform occurred in the pericytes associated with large intestinal mucosa, it was shifted to the transition from postcapillary venules to another vascular segment within the same bed. Table I gives the relative distribution of isomyosins in pericytes and other vessel-associated cells.

Nonmuscle myosin was present in relatively high concentration in all capillary pericytes and absent in all smooth muscle cells. A rather abrupt increase in the level of nonmuscle myosin appeared to occur at the arteriolar end of the capillary bed in all four organs, since arteriolar smooth muscle cells were consistently unreactive for this antigen, whereas the pericytes on larger capillaries (8-9 μm OD), which may represent (in part) arteriolar capillaries, contained detectable amounts of nonmuscle isomyosin. Conversely, a pronounced decrease in the concentration of the nonmuscle isoform occurred at the level of postcapillary venules. The change from one isomyosin to the other was apparently coincidental with the transition from capillaries to postcapillary venules in the heart and diaphragm, whereas in the pancreas and the intestinal mucosa, it was shifted to the transition from postcapillary to muscular venules.

Smooth muscle myosin was present in high concentration in the smooth muscle cells of arterioles and muscular venules and in postcapillary venule pericytes. A somewhat lower concentration was found in the pericytes associated with large capillaries, whereas a very low concentration (if any) was detected in the pericytes of smaller capillaries, which indicates a more gradual shift in smooth muscle myosin concentration throughout the microvasculature. These findings indicate, therefore, that pericytes, or at least a fraction of their overall population, contain a mixture of nonmuscle and smooth muscle isomyosins.

The results obtained in this study, together with those reported in the companion paper (17) and those already published (15, 16), indicate that there are relatively high concentrations of contractile and regulatory proteins in microvascular pericytes. Taken together, the immunocytochemical data give us sufficient evidence to conclude that pericytes are potentially contractile cells. Moreover, by comparison with endothelial and other microvessel-associated cells, the pericytes appear to be differentiated cells specialized for a contractile function. The conclusion is in agreement with morphologic and other indirect evidence of pericyte contractility (9, 18, 23, 31, 33).

The presence of the smooth muscle isoforms of myosin, actin, and tropomyosin in pericytes and the fact that these cells also contain cyclic GMP-dependent protein kinase, an enzyme found in high concentration in both vascular and visceral smooth muscle cells (2, 5, 20), indicate that pericytes have a close biochemical relationship to smooth muscle cells. The presence of both isomyosins, together with their differential distribution in pericytes associated with different segments of the microvasculature, suggests, however, that the contractile properties of these cells may differ from those of smooth muscle cells and may be modulated from one segment to another throughout the microvasculature. Nonmuscle myosin and smooth muscle tropomyosin appear to co-exist in capillary and, to some extent, postcapillary venule pericytes. The finding deserves special attention: It may describe a novel combination of contractile and regulatory proteins, different from those already established in nonmuscle or smooth muscle cells. But it may also reflect differences in antibody-antigen affinity, as affected by our preparation procedures, in cells that may have isoforms of both antigens.

So far, the survey has been limited to proteins considered essential or of primary importance for contractile function. The presence, in pericytes, of secondary components of the contractile apparatus (α-actinin, vinculin, filamin, and other actin-binding proteins) remains to be investigated in the future.

There is morphologic evidence to suggest that pericyte contraction could have a significant effect on the underlying vessel wall. Electron microscopy has revealed that the tips of pericyte processes are closely apposed to the endothelium in regions from which the basement membrane appears to be excluded (26, 30). How this attachment is maintained has yet to be elucidated. Another form of pericyte-endothelial cell attachment has been found by Courtoy and Boyles (7). Their studies have localized fibronectin, a protein involved in the formation of adhesion plaques, to restricted areas of pericyte-endothelial cell apposition where fine cytoplasmic fibrils appear to insert into the pericyte plasma membrane. Both forms of adhesion would facilitate the transmission of the force generated by pericyte contraction to the underlying vessel wall. In addition, the basement membrane leaflets that enclose pericytes within the vessel wall would prevent their detachment from the endothelium during contraction.

There is, therefore, evidence that an elaborate contractile apparatus is present in pericytes and that structural mechanisms for force transmission from this contractile apparatus to endothelial cells are in place. Yet, notwithstanding these supportive findings, it should be remembered that the actual contraction of pericytes and its effect on blood flow remain to be demonstrated in vivo in the intact microvasculature.

Although regulation of blood flow by contractile responses to varied stimuli (10, 18, 31, 33) may be an important pericyte function, other roles are not excluded. Available evidence indicates that these cells are potentially phagocytic and become overtly so in inflammatory reactions (4, 6, 22).

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<th>Cell type</th>
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+++ strongly reactive; ++, moderately reactive; +, weakly reactive; -, unreactive.

DISCUSSION

With the immunoperoxidase procedures applied in this study we have localized both smooth muscle and nonmuscle isomyosins in pericytes, and, by estimating semiquantitatively the intensity of the positive reactions to each isomyosin, we have shown that the distribution of these antigens varies from one microvascular bed to another, as well as along vascular segments within the same bed. Table I gives the relative distribution of isomyosins in pericytes and other vessel-associated cells.

Nonmuscle myosin was present in relatively high concentration in all capillary pericytes and absent in all smooth muscle cells. A rather abrupt increase in the level of nonmuscle myosin appeared to occur at the arteriolar end of the capillary bed in all four organs, since arteriolar smooth muscle cells were consistently unreactive for this antigen, whereas the pericytes on larger capillaries (8-9 μm OD), which may represent (in part) arteriolar capillaries, contained detectable amounts of nonmuscle isomyosin. Conversely, a pronounced decrease in the concentration of the nonmuscle isoform occurred at the level of postcapillary venules. The change from one isomyosin to the other was apparently coincidental with the transition from capillaries to postcapillary venules in the heart and diaphragm, whereas in the pancreas and the intestinal mucosa, it was shifted to the transition from postcapillary to muscular venules.

Smooth muscle myosin was present in high concentration in the smooth muscle cells of arterioles and muscular venules and in postcapillary venule pericytes. A somewhat lower concentration was found in the pericytes associated with large capillaries, whereas a very low concentration (if any) was detected in the pericytes of smaller capillaries, which indicates a more gradual shift in smooth muscle myosin concentration throughout the microvasculature. These findings indicate, therefore, that pericytes, or at least a fraction of their overall population, contain a mixture of nonmuscle and smooth muscle isomyosins.

The results obtained in this study, together with those reported in the companion paper (17) and those already published (15, 16), indicate that there are relatively high concentrations of contractile and regulatory proteins in microvascular pericytes. Taken together, the immunocytochemical data give us sufficient evidence to conclude that pericytes are potentially contractile cells. Moreover, by comparison with endothelial and other microvessel-associated cells, the pericytes appear to be differentiated cells specialized for a contractile function. The conclusion is in agreement with morphologic and other indirect evidence of pericyte contractility (9, 18, 23, 31, 33).

The presence of the smooth muscle isoforms of myosin, actin, and tropomyosin in pericytes and the fact that these cells also contain cyclic GMP-dependent protein kinase, an enzyme found in high concentration in both vascular and visceral smooth muscle cells (2, 5, 20), indicate that pericytes have a close biochemical relationship to smooth muscle cells. The presence of both isomyosins, together with their differential distribution in pericytes associated with different segments of the microvasculature, suggests, however, that the contractile properties of these cells may differ from those of smooth muscle cells and may be modulated from one segment to another throughout the microvasculature. Nonmuscle myosin and smooth muscle tropomyosin appear to co-exist in capillary and, to some extent, postcapillary venule pericytes. The finding deserves special attention: It may describe a novel combination of contractile and regulatory proteins, different from those already established in nonmuscle or smooth muscle cells. But it may also reflect differences in antibody-antigen affinity, as affected by our preparation procedures, in cells that may have isoforms of both antigens.

So far, the survey has been limited to proteins considered essential or of primary importance for contractile function. The presence, in pericytes, of secondary components of the contractile apparatus (α-actinin, vinculin, filamin, and other actin-binding proteins) remains to be investigated in the future.

There is morphologic evidence to suggest that pericyte contraction could have a significant effect on the underlying vessel wall. Electron microscopy has revealed that the tips of pericyte processes are closely apposed to the endothelium in regions from which the basement membrane appears to be excluded (26, 30). How this attachment is maintained has yet to be elucidated. Another form of pericyte-endothelial cell attachment has been found by Courtoy and Boyles (7). Their studies have localized fibronectin, a protein involved in the formation of adhesion plaques, to restricted areas of pericyte-endothelial cell apposition where fine cytoplasmic fibrils appear to insert into the pericyte plasma membrane. Both forms of adhesion would facilitate the transmission of the force generated by pericyte contraction to the underlying vessel wall. In addition, the basement membrane leaflets that enclose pericytes within the vessel wall would prevent their detachment from the endothelium during contraction.

There is, therefore, evidence that an elaborate contractile apparatus is present in pericytes and that structural mechanisms for force transmission from this contractile apparatus to endothelial cells are in place. Yet, notwithstanding these supportive findings, it should be remembered that the actual contraction of pericytes and its effect on blood flow remain to be demonstrated in vivo in the intact microvasculature.

Although regulation of blood flow by contractile responses to varied stimuli (10, 18, 31, 33) may be an important pericyte function, other roles are not excluded. Available evidence indicates that these cells are potentially phagocytic and become overtly so in inflammatory reactions (4, 6, 22).
The results obtained for pericytes can be compared with those found for endothelial cells. The latter contain nonmuscle tropomyosin but lack smooth muscle tropomyosin (17), smooth muscle isomyosin, and cyclic GMP-dependent protein kinase (16). The presence of nonmuscle isomyosin, especially in the endothelial cells of the postcapillary venules, suggests that these cells can also contract but that the regulatory mechanisms and the properties of their contraction are different from those at work in pericytes and vascular smooth muscle cells.

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