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 sulfonyl fluoride, and 0.02% sodium azide before injection as antigen by use of an immunization protocol similar to that described by Fujisawa 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 antiamyosins, 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 μM antipain, 1 μM pepstatin, 20 μM benzamidine, 0.1 mM phenylmethyl sulfonyl fluoride, 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 preparative slab gels, and the resolved polypeptides were electrophotographically transferred to nitrocellulose. 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 2). 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...
0.1% glutaraldehyde for 30 min at room temperature; and (d) periodate-lysine-
periodate 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) t
reaction product. The rest of the protocol (postfixation in osmium tetroxide
formaldehyde fixative (24), before incubation with primary antibody.

Enhancement Procedures: We tested several methods in an attempt
to optimize the signal generated in the immunoperoxidase reaction. In prelimi-
nary 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.

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 peroxidase reaction was monitored under a dissecting micro-
scope (Wild Heerbrugg Instruments, Inc., Farmingdale, NY). Individual tissue
sections were reacted for various times and then transferred to PBS. Optimal
staining was obtained within 30 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)1
reaction product. The rest of the protocol (postfixation in osmium tetroxide
embedding in Poly/Bed 812 (Polysciences Inc., Warrington, PA) followed
procedures previously described (17).

Photography and Analysis of the Results: Thick (~1 μm)
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 63× 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 (16×) 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.

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 peroxi-
dase activity was found in granulocytes (especially eosino-
phils) 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, depending on where along their lengths the cells were sectioned. When pericytes were unstained, only these morphologic cri-
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. 6 a. DAB reaction product was diffusely distributed within the perikarya and foot processes of the 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 6 b).

Most pericytes on larger capillaries (6 to 9 μm diam) were also positively stained (Fig. 6 a); however, as Fig. 6 b indicates, an increased number of cells appeared weakly positive. (Compare the darkly stained pancreatic capillary pericyte in Fig. 5 d with the less intensely stained pericyte on the intestinal capillary in Fig. 5 e.)

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. 6 a), whereas those on vessels of the...
same size in pancreas and the intestinal mucosa remained positively stained (Figs. 5, h and i and 6 a). A decrease in the staining intensity was observed, however, especially within the postcapillary venule pericytes of the intestinal mucosa (Fig. 5 h 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. 6 a).

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 microvasculatures examined as illustrated by the gallery of microvessels in Fig. 7, a–l.

The number of positive pericytes (Fig. 8 a) and the strength of their positive reaction (Fig. 8 b) 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. 7 a), 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-
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-f). 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.
distribution of isomyosins in pericytes and other vessel-associated cells occurred at the level of postcapillary venules. The change from capillaries to muscular venules.

In the smooth muscle cells of arterioles and muscular venules, a rather abrupt increase in the level of nonmuscle myosin was found, whereas in capillary and (to some extent) postcapillary venule pericytes, a very low concentration (if any) was present. These findings indicate, therefore, that 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 Boles (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).

**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 isofrom 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).

**TABLE I**

| Cell type                  | Intensity of reaction |
|----------------------------|-----------------------|
|                            | To nonmuscle myosin   | To smooth muscle myosin |
| Pericytes                  |                       |                       |
| Small capillaries          | +++                   | - to +                |
| Large capillaries          | +++                   | +                     |
| Postcapillary venules      |                       |                       |
| Pancreas and intestine     | ++                    | +++                   |
| Diaphragm and heart        | -                     | +++                   |
| Vascular smooth muscle cells | -                   | +++                   |
| Arterioles                 | -                     | +++                   |
| Muscular venules           | -                     | +++                   |
| Endothelial cells          | ++                    | -                     |
| Fibroblasts                | +                     | -                     |

+++ strongly reactive; ++, moderately reactive; +, weakly reactive; -, unreactive.

**REFERENCES**

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REFERENCES

1. Adams, J. C. 1977. Technical considerations on the use of horseradish peroxidase as a neuronal marker. Neuroscience 2:141-145.
2. Boyles, J., N. Joyce, P. DeCamilli, U. Walter, and S. Mentone. 1983. Immunocytochemical localization of high levels of cyclic GMP-dependent protein kinase in vascular and somatic smooth muscle cells, myofibroblasts and myoepithelial cells. Adv. Cyclic Nucleotide Res. 17A:65. (Abstr.)
3. Boyles, J. R., and G. E. Palade. 1968. Studies on blood capillaries. I. General organization of blood capillaries in muscle. J. Cell Biol. 37:244-276.
4. Cancilla, P. A., R. N. Baker, P. DeCamilli, and S. P. Frommes. 1972. Reaction of sarcoplasmic reticulum with aflinity-fractionated antititin. J. Cell Biol. 70:278a. (Abstr.)
5. Cancilla, P. A., R. N. Baker, P. DeCamilli, and G. E. Palade. 1985. Contractile proteins in pericytes. I. Immunoperoxidase localization of tropomyosin. J. Cell Biol. 100:1379-1386.
6. Chang, S. 1982. The cytoarchitecture of the wall and the innervation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and methemoglobin. J. Pharmacol. Exp. Ther. 219:181-186.
7. Cho, Y. 1978. Actin and myosin-like filaments in rat brain pericytes, like vascular smooth muscle cells, are immunocytochemically positive for cyclic GMP-dependent protein kinase. Microvasc. Res. 28:206-219.
8. Cho, Y. 1983. Cyclic GMP-dependent protein kinases in mammalian tissues. Proc. Natl. Acad. Sci. USA. 71:4037-4041.
9. LeBeux, Y. J., and J. Willenot. 1978. Actin and myosin-like filaments in rat brain pericytes. Anat. Rec. 190:811-826.
10. LeBeux, Y. J., and J. Willemot. 1978. Actin and myosin-like filaments in rat brain pericytes. Anat. Rec. 190:811-826.
11. Maggi, G., and G. E. Palade. 1961. Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. J. Biophys. Biochem. Cytol. 8:571-605.
12. Mazanet, R., and C. Franzini-Armstrong. 1982. Scanning electron microscopy of postcapillary venules in mouse heart. J. Ultrastruct. Res. 76:206-219.
13. Palade, G. E. 1968. Studies on blood capillaries. I. General organization of blood capillaries in muscle. J. Cell Biol. 37:244-276.
14. Palade, G. E. 1968. Studies on blood capillaries. I. General organization of blood capillaries in muscle. J. Cell Biol. 37:244-276.
15. Simoncencu, N., S. P. Frommes, and M. E. Balas. 1979. Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens. Nature (Land). 265:730-731.
16. Simoncencu, N., M. Simoncencu, and G. E. Palade. 1978. Structural basis of permeability in sequential segments of the microvasculature of the diaphragm. Microvasc. Res. 15:1-16.
17. Sternberger, L. A., P. H. Hardy, Jr., J. C. Cuculis, and H. G. Meyer. 1970. The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complexes (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18:315-333.
18. Tilton, R. G., C. Kilo, and J. R. Williamson. 1979. Pericyte-endothelial relationships in cardiac and skeletal muscle capillaries. Microvasc. Res. 18:325-335.
19. Tilton, R. G., C. Kilo, J. R. Williamson, and D. W. Mueh. 1979. Differences in pericyte contractile function in rat cardiac and skeletal muscle microvasculatures. Microvasc. Res. 18:336-352.
20. Wallow, J. H., and B. Burnside. 1980. Actin filaments in retinal pericytes and endothelial cells. Invest. Ophthalmol. Vis. Sci. 19:1433-1441.
21. Zimmerman, K. W. 1923. Der feinere Bau der Blutcapillaren. Z. Anat.-Entwicklungs- gesch. 68:3-109.