Myosin Isozymes in Rabbit and Human Smooth Muscles

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Although multiple forms of myosin in cardiac and skeletal muscles have been identified, it has not been firmly established that myosin isoforms are present in adult smooth muscle. Myosin, extracted from human thoracic aorta and lower saphenous vein and rabbit aorta and uterus, was analyzed by pyrophosphate gel electrophoresis to determine if myosin isoforms are present in these tissues. In all smooth muscle tissues studied, two myosin isoforms were detected and labeled as smooth muscle 1 and smooth muscle 2, smooth muscle 2 being the faster migrating isozyme. Bovine cultured smooth muscle cells from the media of thoracic aorta also contained two forms of myosin. However, cultured fibroblasts contained only one form of myosin. Extracting myosin from either relaxed or contracting rabbit aortic smooth muscle did not influence the mobilities of smooth muscle 1 and smooth muscle 2 on pyrophosphate gels, suggesting that the degree of light chain phosphorylation did not significantly alter the electrophoretic mobility under our conditions. Smooth muscle 1 and smooth muscle 2 myosins each contain heavy chains (200,000 daltons) and light chains (20,000 and 17,000 daltons) in addition to filamin (235,000 daltons), which is closely associated with the native protein. Myosin peptide maps of rabbit aorta and uterus revealed areas of substantially different banding patterns between smooth muscle 1 and smooth muscle 2 from the same tissue. Similar peptide maps of smooth muscle 1 bands were produced from the different tissues, but the smooth muscle 2 maps were dissimilar. Since the speed of shortening of striated muscle appears to be influenced by the myosin isozyme patterns, the possibility exists that the contractile properties of various smooth muscle may also be influenced by the relative amounts of myosin isoforms present. (Circulation Research 1986;59:115-123)

Myosin isoforms have been identified in skeletal1 and cardiac2 muscles using pyrophosphate gel electrophoresis. Cardiac myosins appear to differ in their heavy chain composition,3 whereas skeletal myosins appear to differ in their light chain composition.4 In contrast to striated and cardiac muscle myosin isoforms, it has not been firmly established if smooth muscle and nonmuscle cells contain more than one type of myosin. Tanaka-Ohmuro et al5 reported that embryonic chicken gizzard, but not adult chicken gizzard, contained three myosin isozymes that differed in their light chain composition when analyzed by pyrophosphate gel electrophoresis. Beckers-Bleukx and Marechal6 have recently reported the presence of two myosin isoforms in various mammalian and avian smooth muscles that appear to differ in their heavy chain composition.7 Using immunohistochemical techniques, Larson et al7,8 demonstrated that smooth muscle media from aorta and mesenteric artery contain two antigenically distinct myosins; however, only one myosin was found using pyrophosphate gel analysis. Studies of non-muscle cells suggest that acanthamoeba9 and human platelets10 seem to contain more than one type of myosin; however, fibroblast cells appear to contain only one type of myosin.5

Our study examines extracts prepared from human aorta, human vein, rabbit aorta, and rabbit uterus to determine the presence of myosin isoforms. Samples were analyzed by pyrophosphate gel electrophoresis. Myosin bands were then further analyzed by SDS gel electrophoresis and one-dimensional peptide mapping. Also, myosin extracts from both relaxed and epinephrine-contracted smooth muscle segments were compared to determine if any differences in levels of light chain phosphorylation altered the electrophoretic pattern of myosin. Some of the findings communicated here have already been reported.11

Materials and Methods

Smooth Muscle Tissue

A total of five male and five female New Zealand White rabbits, 4 months or 18 months old, were quickly sacrificed by either transection of the cervical spinal cord or by lethal injection of T-61 Euthanasia solution (Hoechst Corporation). A segment of the thoracic aorta and uterus were removed within 5 minutes and washed in a cold physiologic salt solution containing 144 mM NaCl, 1.5 mM CaCl₂, 0.1 mM EGTA, 4.7 mM KCl, 5.0 mM 3-N-morpholinopropanesulfonic acid (MOPS), 1.2 mM MgCl₂, and 5.6 mM dextrose, pH 7.40 at 4°C. Segments of the aorta, used to compare myosins from relaxed and contracting tissue, were placed in cold, oxygenated physiologic solution (Po₂ = 650 Torr). Segments of human thoracic aorta and lower saphenous vein, obtained from a total of 7 male and 3 female patients undergoing coronary artery by-pass surgery,
were stored in iced normal saline and received within 4 hours following removal.

Cultured Cells
Smooth muscle cell cultures were kindly donated by Drs. Robert Rosenberg and James Marcum (Massachusetts Institute of Technology; Cambridge, Mass). Normal human fibroblast cells (Skin, GM 3349) were purchased from the Institute of Medical Research, Camden, N.J. Smooth muscle cells from bovine thoracic aorta were taken from internal layers of the tunica media to avoid fibroblast contamination from the adventitia. Cells were grown in 8-cm-diameter culture dishes using Dulbecco’s modified Eagles medium containing 10% calf serum, 100 units/ml penicillin, 0.1 mg/dl streptomycin sulfate, and 20 mM HEPES, pH 7.40, and incubated in a moist 5% CO2 atmosphere at 37° C. The culture medium was changed 3 times weekly. Cultured smooth muscle and fibroblast cells were examined by light microscopy to verify characteristic growth patterns and cell morphologies as described by Ross and Kariya12 and Chamley-Cambell et al.13

Preparation and Treatment of Smooth Muscle Tissue
Using a microscope, rabbit and human smooth muscle tissue were cleaned of fat and connective tissue. A 6- to 8-cm-long segment of rabbit aorta or human saphenous vein was placed in a dissection dish that contained iced physiologic salt solution. The vessel was opened longitudinally, then pinned down so that the luminal surface was directed upward. Under 40 X magnification, the margin between the media and adventitia was easily identified and separated using fine forceps as described by Wolinsky and Daly.14 This method, also referred to as the “internal method”, has been shown to be very effective in removing the fibroblast-rich adventitia from the intima media. Approximately 20% of the media adjacent to the adventitia was also removed and discarded. The luminal surface was vigorously scraped with a Teflon spatula to avoid fibroblast contamination from the adventitia. Cells were grown in 8-cm-diameter culture dishes to avoid fibroblast contamination from the adventitia. Cells were grown in 8-cm-diameter culture dishes using Dulbecco’s modified Eagles medium containing 10% calf serum, 100 units/ml penicillin, 0.1 mg/dl streptomycin sulfate, and 20 mM HEPES, pH 7.40, and incubated in a moist 5% CO2 atmosphere at 37° C. The culture medium was changed 3 times weekly. Cultured smooth muscle and fibroblast cells were examined by light microscopy to verify characteristic growth patterns and cell morphologies as described by Ross and Kariya12 and Chamley-Cambell et al.13

Extraction of Myosin
Smooth muscle tissue, cultured smooth muscle cells, or cultured fibroblasts were homogenized in 5 volumes (w/v) of myosin extraction solution consisting of 100 mM Na2P2O7, 5 mM EGTA, 50 mM NaF, 15 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonylfluoride (PMSF), 0.1 mM leupeptin, and 10% glycerol, pH 8.80 at 4° C. The frozen tissue suspension was placed in a glass homogenizer and thawed by the heat generated from rotating a Teflon pestle for about 5 minutes. The homogenate was refrigerated at 4° C for 10 to 15 hours, centrifuged at 14,000g for 15 minutes, and the supernatant fraction was mixed with an equal volume of glycerol. Extracts from each tissue were stored individually at −20° C and analyzed within 36 hours.

Separation of Myosin Isozymes
Myosin isozymes were separated by pyrophosphate polyacrylamide gel electrophoresis as originally described by Hoh et al15 with minor modifications.19 The pyrophosphate gel was prepared using 20 mM Na2P2O7, 10% glycerol, 4.1% acrylamide, and 0.13% bis-acrylamide. The electrophoresis buffer contained 20 mM Na2P2O7, 7 mL-cysteine, 10% glycerol. Electrophoresis was performed using a Pharmacia GE-2/4 LS electrophoresis chamber enclosed in a plexiglas/styrofoam insulation box. The temperature of the electrophoresis buffer was maintained at 0° C ± 0.5° C by circulating 20% ethylene glycol in water through the coils of the chamber using an LKB Multitemp II thermostatic circulator set at −4.3° C. A pump recirculated the buffer between the lower and upper electrophoresis chambers in order to maintain a constant pH. Either 2.7 mm or 4.0 mm i.d. × 7.0-cm-long glass rod tubes were used to cast the polyacrylamide gels to a length of 6.0 cm. The narrow tubes were used in order to cut myosin bands from the pyrophosphate gels and layer them on sodium dodecyl sulfate (SDS) slab gels.

Extracts (1–20 μl) were loaded on pyrophosphate gels and electrophoresed for 4 hours at a constant current setting. The current was set at 1 mA/tube when using 2.7-mm-diameter gels and 2 mA/tube when using 4.0-mm-diameter gels. Samples were electrophoresed for an additional 16 hours at a constant voltage setting of 84 V (14 V/cm gel length).

Gels were scanned on a Gifild Scanning Densitometer at a wavelength of 680 nm using a 0.05 X 2.36 mm aperture. The relative amount of each isozyme was determined as previously described by Martin et al.18

Separation of Myosin Heavy and Light Chains by SDS Gel Electrophoresis
Isozyme bands in pyrophosphate gels were either stained for about 10 minutes with a 0.05% Coomassie Blue G-250 solution containing 5% perchloric acid or for 2 hours with 0.1% Coomassie Blue R-250 as described previously.19 Some gels were stained using a modified version of the silver-stain method of Morrissey20 described below. Protein bands that appeared on the gels following staining with Coomassie Blue G-250 were sliced with a razor, layered on 14% polyacrylamide slab gels, and electrophoresed in the presence of SDS as described by Laemmli.21 When required, the

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silver-stained pyrophosphate gels were used as a template to aid in the identification of myosin bands barely detected following Coomassie Blue G-250 staining. Heavy chain analysis was performed in the same fashion except that a 5% polyacrylamide gel concentration was used.

**Peptide Mapping of Myosin Isozymes**

Two myosin isozymes were detected (see "Results") and labelled smooth muscle 1 (SM1) isozyme and smooth muscle 2 (SM2) isozyme. One-dimensional peptide mapping of SM1 and SM2 isozymes from rabbit aorta and uterus was performed using the method of Cleveland et al with slight modification. SM1 and SM2 isozymes were isolated by slicing the bands out of Coomassie Blue R-250 stained pyrophosphate gels. Each slice was placed in a separate well of a microculture plate (NONE, Denmark) and incubated at 4°C for 16 hours in a 250 μl solution containing 50 mM tetrapotassium phosphate, 50% glycerol, 1% 2-mercaptoethanol, 5 mM dithiothreitol (DTT), 10 ng papain, 10 ng trypsin, and 10 ng chymotrypsin, pH 8.8. The slices were then washed in the same buffer solution without enzymes, and 4–6 slices of each isozyme band were layered atop the stacking gel. The samples were electrophoresed at 20 mA/plate for approximately 3 hours, then 30 mA/plate until the dye marker migrated off the gel slab. The temperature of the buffer solution was maintained at 15°C. Polypeptide maps were silver stained as subsequently described.

**Silver-Stain Method for Pyrophosphate Rod Gels**

A modified silver-staining method was used in the analysis of myosin isozymes and their protein components when small amounts of sample were loaded. Immediately following electrophoresis, gels were soaked in 1 hour in two changes of deionized distilled water. The gels were drained, immersed in 10% glutaraldehyde, shaken for 1 hour, and washed for 4 hours in deionized distilled water with changes every 45 minutes. The gels were soaked for 30 minutes in a solution containing 5 μg/ml DTT. Without washing the gels, the DTT solution was replaced with 10 ml of 0.1% AgNO₃, and the gels were soaked for 30 minutes. After removing the AgNO₃ solution and rinsing the gels with a Na₂CO₃-formalin solution (8.8 g Na₂CO₃, + 0.125 ml formalin/250 ml H₂O) for about 10 seconds, the color reaction was started by adding 10 ml of the Na₂CO₃-formalin solution. The stained myosin bands usually appeared within 1–6 minutes. The color reaction was stopped by the addition of 0.5 ml of 2.3 M citric acid. After 15 minutes, the gels were stored in distilled water and the background cleared with full-strength photographic fixer.

**Relaxed and Contracted Segments of Media from Aorta**

In the experiments using epinephrine, samples from isolated rabbit aortic media were divided into two segments. Both segments were made to relax by incubation at 37°C in oxygenated physiologic salt solution, pH 7.4. This treatment has also been shown to reduce the level of light chain phosphorylation. After 45 minutes, one segment of relaxed media was quickly frozen in liquid nitrogen. The other segment was placed in an experimental chamber and firmly secured to a force transducer as previously described for cardiac muscle. The segment was made to contract by exposure to 10 μM epinephrine solution, and the force generated by the muscle was recorded using a Nicolet Digital oscilloscope. Then, the contracted segment was quickly cut from the transducer and placed in liquid nitrogen.

**Chemicals and Regents**

Leupeptin, pepstatin PMSF, MOPS, papain, trypsin, chymotrypsin, fibronectin, and silver nitrate were purchased from the Sigma Chemical Company. Chicken gizzard filamin was purchased from Calbiochem, Inc. Reagents used for preparing polyacrylamide gels were purchased from Bio-Rad Laboratories. All other chemicals were reagent grade and purchased from Fisher Laboratories.

**Results**

The adventitial layer in arteries and veins contains predominantly fibroblasts whereas the intima media layer contains exclusively smooth muscle cells. To obtain an estimate of the percent of fibroblast cells relative to smooth muscle cells present in our intima media preparations following dissection, sections were examined by scanning electron microscopy and light microscopy. Examination of the surface of the adventitial side of the intima media preparation indicated that a very small number of fibroblast cells were present, as seen in Figure 1. A reasonable estimate for the number of fibroblast cells present is between 2 and 30 cells per 1.2 × 10⁶ μm² sections of aortic media as counted from the electron microscope prints. Assuming that the volume of a smooth muscle cell is about 200 μm³, the maximum number of intima media smooth muscle cells in a volume of 1.2 × 10⁶ μm³ would be approximately 6,000. Using an estimate of 50% for the volume of intima media occupied by smooth muscle cells, approximately 3,000 smooth muscle cells would be present in these segments. Therefore, the percent of fibroblast cells relative to smooth muscle cells in the intima media preparations would range from 0.06 to 1.0%.

Segments from these intima media preparations as well as samples from the uterus were homogenized and the myosin extracted for analysis by pyrophosphate gel electrophoresis. In Figure 2, Lanes a through e show myosin isozymes from segments of rabbit aorta, human thoracic aorta, human saphenous vein, and rabbit uterus. These results show the presence of two electrophoretically distinct myosin isozymes. SM1 is the slower migrating myosin, and SM2 is the faster migrating myosin. The relative amounts of SM1 and SM2 differed in the rabbit aortas studied. For example, in Figure 2, Lane a shows myosin isozymes from the aortic media of a 4-month-old rabbit, while Lane b...
shows myosin from the aortic media of an 18-month-old rabbit. No consistent relation was found between the amount of SM1 and SM2 myosin present and the age of a rabbit, although this was not extensively studied.

Filamin is a dimer of 240,000 dalton subunits associated with smooth muscle. According to Huszar and Bailey, it remains in extracts prepared from smooth muscle. Therefore, filamin was co-electrophoresed with rabbit uterus extract to determine if one of the myosin bands could be filamin as suggested in recent preliminary reports. Under our conditions, filamin clearly migrated as a single band well above both the SM1 and SM2 myosin isozyme bands as shown in Figure 2, Lane f (arrow).

Myosin extracts from rabbit ventricular and psoas muscles were electrophoresed on pyrophosphate gels and compared with the results from smooth muscle myosin. The comigration shown in Figure 2, Lane g, demonstrates the marked difference in electrophoretic mobility between ventricular and uterus myosins obtained from the same rabbit. In all samples tested, smooth muscle myosin isozymes showed a faster electrophoretic mobility than either ventricular myosin (Figure 2, Lane h) or psoas muscle myosin (Figure 2, Lane i), inferring that smooth muscle myosins are either more highly charged, smaller in molecular weight, or possess a different quaternary structure resulting in faster mobility.

Fibronectin, a dimer of 200,000–220,000 dalton subunits, is synthesized primarily by fibroblasts in quantities of 1–3% of the total cellular protein. Filtered fibronectin was electrophoresed on pyrophosphate gels in order to exclude the possibility that small amounts of this protein coming from the slight fibroblast contamination present in our extracts might alter the smooth muscle myosin isozyme patterns either qualitatively or quantitatively. As shown in Figure 2, Lane j, fibronectin migrates relatively rapidly to form a diffuse band well below the myosin bands. In addition, no fibronectin bands were detected in the gels from the smooth muscle samples, further suggesting that fibroblast contamination does not influence our results.

Cultured smooth muscle and fibroblast cells were examined by light microscopy prior to fixation. Smooth muscle cells grew in a characteristic pattern of hills and valleys, whereas fibroblast cells grew in a characteristic swirling pattern as described by Ross and Kariya. Cultured smooth muscle cells were fixed in glutaraldehyde, embedded in Epon 812, and sliced into 0.5-μm-thick sections. Sections were then stained with Toluidine Blue and examined by light microscopy. Representative sections were essentially free of fibroblast cells.

Myosin extracts from rabbit aortic media, cultured smooth muscle cells, and cultured fibroblast cells were electrophoresed on pyrophosphate gels as shown in Figure 3. Two myosin isozyme bands are apparent for both rabbit aortic media and cultured smooth muscle cells; however, only one myosin band is present for fibroblast cells. Co-electrophoresis of cultured smooth muscle myosin and fibroblast myosin showed that fibroblast myosin migrates with the slower migrating myosin (SM1) from cultured smooth muscle cells. The densitometric scans shown beneath the corresponding gels in Figure 3 demonstrate that there is a similarity in the relative concentrations of SM1 and SM2 when comparing rabbit aortic myosin with myosin from bovine aortic smooth muscle cultures. By calculating the ratio of the area under each peak with the total area under both peaks, the relative proportion of each isozyme can be estimated. SM1 and SM2 myosins from rabbit aorta comprised 51 and 49% respectively of the isolated myosin fraction, while SM1 and SM2 myosins from bovine smooth muscle culture comprised 46 and 54% respectively of the isolated myosin fraction.
The charge on the light chains of cardiac, skeletal, and smooth muscle myosins becomes more negative when they are phosphorylated as shown by altered electrophoretic mobility on isoelectric focusing gels. Myosin isozymes also separate on pyrophosphate gels due to charge differences. Recent evidence by Trybus and Lowey suggests that in vitro light chain phosphorylation can alter the electrophoretic mobility of nondenatured turkey gizzard myosin on pyrophosphate gels. Therefore, myosin extracted from relaxed and contracting segments of media from rabbit aortic smooth muscle was examined by pyrophosphate gel electrophoresis to determine if light chain phosphorylation could alter the electrophoretic mobility. As shown in Figure 4, an aortic media segment in response to epinephrine developed force rapidly to reach a stable level. It has been reported by Askoy et al. that the level of light chain phosphorylation increases with the development of force. It is reasonable to assume, therefore, that if smooth muscle segments are quickly frozen in liquid nitrogen soon after the initial stimulation by epinephrine, the level of light chain phosphorylation should be substantially higher than in resting segments. Myosin extracts obtained from these relaxed and contracted segments were both co-electrophoresed and run separately on pyrophosphate gels.

Figure 3. Myosin isozymes were separated by electrophoresis on pyrophosphate gels; the isozymes are indicated by arrows (top arrow, SM1; bottom arrow, SM2). The corresponding densitometric scan is shown beneath each gel. The following myosin extracts were analyzed: a) rabbit aorta, b) cultured smooth muscle cells from bovine aorta, c) cultured human fibroblast cells, and d) cultured smooth muscle plus fibroblast cells. Gels were silver-stained as described in "Materials and Methods". In Lane d, small microliter amounts of both smooth muscle cell myosin and fibroblast myosin were loaded on the same gel in order to perform comigration studies. Consequently, the SM1 band stains heavily demonstrating similar electrophoretic mobility. The SM2 band is faintly stained because of the relatively small quantity of this isozyme in comparison to the amounts of SM1 and fibroblast myosin present.

Figure 2. Myosin extracts from various smooth muscles were electrophoresed on 4% pyrophosphate gels as described in "Materials and Methods". The lanes contained myosin from a) 4-month-old rabbit aorta, b) adult rabbit aorta, c) human aorta, d) human saphenous vein, e) rabbit nonpregnant uterus, f) rabbit uterus plus filamin (chicken gizzard), g) rabbit ventricle plus rabbit uterus, h) rabbit ventricle, i) rabbit psoas muscle. Fibronectin, lane j, was also electrophoresed under similar conditions (see text for interpretation of results). Gels a through c were silver-stained while gels d through j were stained with Coomassie Blue R-250.
FIGURE 4. A record of force development as a function of time for a segment of rabbit aortic media that was made to contract by exposure to a 10 μm epinephrine solution. The muscle was incubated in oxygenated physiologic salt solution, pH 7.40 at 37°C (see "Materials and Methods"), slightly stretched, then allowed to equilibrate for 45 minutes. Following exposure to epinephrine, a 50–60 mg segment was detached from the force transducer and rapidly frozen in liquid nitrogen.

There was no alteration in either the migration pattern or the number of myosin isozyme bands detected when compared with the bands obtained when each sample was electrophoresed separately. Thus, under our conditions, differences in myosin light chain phosphorylation do not appear to alter the relative mobilities of the SM1 and SM2 myosin isoforms.

Human smooth muscle myosin isozyme bands obtained by pyrophosphate gel electrophoresis were isolated as described in "Materials and Methods" and placed atop a 14% SDS gel slabs. This was done to verify that each band contained myosin and to detect any differences in heavy chain or light chain patterns between these isoforms. Results shown in Figure 5 indicate that both human aortic and saphenous vein SM1 and SM2 myosin isozyme bands contain heavy chains of approximately 200,000 daltons, and a pair of light chains of approximately 20,000 and 17,000 daltons. Co-electrophoresis of human aortic SM1 and SM2 isozymes revealed no change in electrophoretic mobilities of either the heavy chains or light chains when compared with the migration patterns of each isozyme run separately. A higher molecular weight protein of approximately 235,000 was also detected in the SDS gels from both human aorta and vein myosin isoforms. This high-molecular-weight protein was evaluated by 5% SDS gel electrophoresis to determine whether it was a second myosin heavy chain or filamin. Aliquots of smooth muscle myosin extracts along with rabbit psoas and ventricular myosin extracts were electrophoresed with and without the addition of 10 μg of chicken gizzard filamin. Figure 6 shows that the 235,000 dalton protein was present in all smooth muscle myosin extracts but not in the rabbit psoas or ventricular myosin extracts. Added filamin both comigrated with the high molecular weight protein present in the smooth muscle extracts as well as migrating to the same distance as the high molecular weight band when run separately (Figure 6, Lanes f and k).

These data strongly suggest that filamin is present in the smooth muscle extracts we studied. Moreover, both myosin isoforms appear to have filamin associated with the native protein, since each myosin band obtained from pyrophosphate gel electrophoresis showed a filamin band in addition to the myosin heavy and light chain bands when analyzed by SDS gel electrophoresis. The amount of filamin in each tissue appears to vary since human aorta myosin displays a faintly stained filamin band, while human vein and rabbit aorta myosins display darkly stained filamin bands. We do not know whether this variation is due to differences in the amount of filamin present in each tissue, the binding relationship with the myosins in each tissue, or the effect of the myosin extraction method in each tissue.
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Figure 6. The results from 5% SDS gel electrophoresis of the following myosin extracts: a) myosin standard from rabbit skeletal muscle (200,000 daltons); b) rabbit psoas, c) rabbit ventricle, d) rabbit uterus, e) rabbit uterus plus filamin, f) filamin, g) rabbit aorta plus filamin, h) rabbit aorta, i) human aorta, j) human aorta plus filamin, k) filamin, l) human saphenous vein plus filamin; and m) human saphenous vein. The gels were stained with Coomassie Blue R-250. The filamin content in those samples in which filamin was not added (Lanes d, h, i, m) varies greatly since rabbit myosin from aorta (Lane h) has a darkly staining filamin band, while myosin from human aorta (Lane i) contains only a faintly staining band.

One-dimensional peptide mapping was performed on rabbit aorta and uterus smooth muscle myosin isozymes to determine whether the SM1 and SM2 bands are structurally different. Figure 7 shows the peptide maps produced by treatment with papain, trypsin, and chymotrypsin. There are four major areas, as indicated in Figure 7, where significant differences exist between the peptide maps of SM1 and SM2 from the same tissue. Peptide maps from rabbit ventricle myosin isozymes V1 and V2 are also included for comparison. SM1 bands from different smooth muscle tissues appear to have similar banding patterns, while SM2 bands appear to be different. All of the smooth muscle myosin isozyme peptide maps differ considerably when compared to those from rabbit ventricle.

Discussion

Hoh et al reported that rat ventricle contains three myosin isozyms. Further studies examining rabbit ventricle myosin isozymes have shown that two of the three isozyms separated on pyrophosphate gels are heavy chain homodimers consisting of either two α heavy chains (V1), or two β heavy chains (V2); while the third isozyne is a heterodimer consisting of an α and β heavy chain (V3). Recently, Samuel et al reported that single cardiac muscle cells contain both α and β myosin heavy chains as shown by immunohistochemical staining. In the case of our smooth muscle isozyms, further work needs to be done in order to resolve their heavy chain composition. At this point, we could only speculate concerning the absence of a third isozyne in smooth muscle equivalent to that occurring in cardiac muscle. Also, we do not know whether the two isozyms come from two different cell populations or are present in each smooth muscle cell.

In cases where smooth muscle tissues also contain fibroblast cells, tissue extracts may have both fibroblast and smooth muscle myosin isozyms present. Our results indicate that fibroblast myosin comigrates on pyrophosphate gels with SM1 myosin from cultured smooth muscle cells. Tanako-Ohmuro et al also reported that fibroblast myosin comigrates with myosin from adult chicken gizzard smooth muscle. Therefore, the SM1 band could contain significant amounts of fibroblast myosin. However, our results indicate that the smooth muscle preparation from aorta used for myosin isozyme analysis contains a fraction of fibroblast cells amounting to about 0.06–1.0%. Thus, it seems unlikely that this small fraction of non-muscle cells could significantly influence the myosin isozyme profiles in the smooth muscle preparations from aorta.

Moreover, our results shown in Figure 3 of the myosin isozyms from rabbit aortic smooth muscle and cultured cells from bovine aortic smooth muscle emphasize another point. As indicated by the densitometric scans, rabbit aortic smooth muscle cells produce both...
SM1 and SM2 in virtually the same ratio as the cultured smooth muscle cells. This finding suggests that the isozymes in the rabbit aorta are derived primarily from smooth muscle cells with little, if any, significant contribution from the small fibroblast contamination.

Recent evidence by Trybus and Lowey has demonstrated that light chain phosphorylation can alter the mobility of avian smooth muscle myosin heavy chains on pyrophosphate gels. Therefore, to determine if heavy chain mobility was being altered by possible differences in the state of light chain phosphorylation, we attempted to produce different degrees of light chain phosphorylation for control and treated segments. Myosin extracts were prepared using two techniques, one that reportedly enhances myosin light chain phosphorylation and another that causes significant light chain dephosphorylation. After comigration studies were performed on pyrophosphate gels, no difference in electrophoretic mobility of the two myosin isozymes from either of the above preparations was detected. Although we did not make direct measurements of degree of light chain phosphorylation, studies by Silver and Stull have established that the techniques we used should have produced significantly different levels of light chain phosphorylation. Therefore, our data indicate that differences in the levels of light chain phosphorylation do not alter the mobility of myosin in mammalian smooth muscle using our pyrophosphate gel technique.

Each of the isolated SM1 and SM2 myosin isozyme bands contains heavy chains of approximately 200,000 daltons and pairs of light chains of 20,000 and 17,000 daltons in addition to filamin. Schildmeyer and Seidel have also reported that filamin isozymes from either of the above preparations were detected. Although we did not make direct measurements of degree of light chain phosphorylation, studies by Silver and Stull have established that the techniques we used should have produced significantly different levels of light chain phosphorylation. Therefore, our data indicate that differences in the levels of light chain phosphorylation do not alter the mobility of myosin in mammalian smooth muscle using our pyrophosphate gel technique.

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Several observations can be made on the basis of our results obtained from peptide map analysis of myosin isozymes from rabbit aortic media and rabbit uterus. These are that (1) SM1 and SM2 isozymes from the same tissue show structural heterogeneity, (2) the SM1 maps from the different tissues studied indicate similar patterns, (3) the SM2 isozyme maps have different patterns, and (4) the maps from SM1 and SM2 iso-zymes are distinctly different from maps of myosins from rabbit ventricle. We found that papaia and trypsin treatment of smooth muscle myosin isozymes did not produce satisfactory peptide maps. However, the addition of chymotrypsin resulted in a more complete proteolysis producing a detailed map.

In summary: This study provides evidence indicating that the speed of shortening is influenced by the type or relative amounts of myosin isozymes present. Therefore, it is possible that the presence of more than one myosin isozyme in smooth muscle could play a role in physiological function. Current models for regulation of force development and speed of shortening in smooth muscle do not specifically include the presence of different myosin isozymes. For example, Dillon et al have proposed that two populations of myosin cross-bridges, i.e., phosphorylated and nonphosphorylated, are involved in shortening and force maintenance in activated smooth muscle. Siegman et al and Paul et al have also proposed a direct effect of calcium on smooth muscle myosin cross-bridge cycling rate. Further work is needed to resolve these issues.

In summary: This study provides evidence indicating that two myosin isozymes are present in human and rabbit smooth muscle. Both SM1 and SM2 isozymes contain 200,000 dalton heavy chains and two pairs of light chains of 20,000 and 17,000 daltons. In addition, filamin, a protein of molecular weight 235,000 daltons, is present either bound to or closely associated with the native myosin protein. Structural differences between the two isozymes can be shown using the method of peptide map analysis. Further work is needed to clarify the functional roles played by each of the isozymes in the intact cell.

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References

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