

The Heavy-chain Stoichiometry of Smooth Muscle Myosin is a Characteristic of Smooth Muscle Tissues

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Abstract

The stoichiometry of the two heavy chains of myosin in smooth muscle was determined by electrophoresing extracts of native myosin and of dissociated myosin on sodium dodecyl sulfate (SDS) 4%–polyacrylamide gels. The slower migrating heavy chain was 3.6 times more abundant in toad stomach, 2.3 in rabbit myometrium, 2.0 in rat femoral artery, 1.3 in guinea pig ileum, 0.93 in pig trachea and 0.69 in human bronchus, than the more rapidly migrating chain. Both heavy chains were identified as smooth muscle myosin by immunoblotting using antibodies to smooth muscle and non-muscle myosin. The unequal proportion of heavy chains suggested the possibility of native isoforms of myosin comprised of heavy-chain homodimers. To test this, native myosin extracts were electrophoresed on non-dissociating (pyrophosphate) gels. When each band was individually analysed on SDS–polyacrylamide gel the slowest was found to be filamin and the other bands were myosin in which the relative proportion of the heavy chains was unchanged from that found in the original tissue extracts. Since this is incompatible with either a heterodimeric or a homodimeric arrangement it suggests that pyrophosphate gel electrophoresis is incapable of separating putative isoforms of native myosin.

Introduction

Myosin is a polymorphic contractile protein found in all muscular tissues and in non-muscular tissues (Pollard and Weihing 1974). It exists as isozymes or isoforms in skeletal (Hoh and Yeoh 1979) and cardiac muscle (Hoh *et al.* 1978), which differ in their ATPase activity and in their mobility on non-dissociating (pyrophosphate) gel electrophoresis (Pope *et al.* 1980). The distribution of these isoforms is influenced by the innervation (Pette and Vrbova 1985), functional demand (Gorza *et al.* 1981), hormones (Hoh *et al.* 1978) and development (Hoh and Yeoh 1979). The type of myosin determines some of the fundamental characteristics of contractility. The ATPase activity of the skeletal myosin is proportional to the speed of contraction of the muscle (Barany 1967). Since smooth muscles show a wide functional diversity, e.g. maximum velocity of shortening (V_{\max}) varies over a forty-fold range (Hellstrand and Paul 1982), it has been hypothesized that myosin may exist in smooth muscle as isoforms but the evidence to date is controversial.

Using pyrophosphate–polyacrylamide electrophoresis, which permits separation of native isoforms of skeletal and cardiac myosin, Hoh and Yeoh (1977) reported as many as four myosin isoforms in rabbit smooth muscle tissues. Takano-Ohmuro *et al.* (1983) found one isoform in adult chicken gizzard compared with three in chick embryo, and Beckers-Bleux and Marechal (1985) reported two isoforms in smooth muscle from dogs and guinea pigs. However, these studies did not distinguish between filamin (Persechini *et al.* 1986; Sparrow *et al.* 1987) and unphosphorylated and phosphorylated species of myosin (Persechini *et al.* 1986; Takano-Ohmuro and Kohama 1986) in the electrophoretic analysis.

Smooth muscle myosin is composed of two different heavy chains (Burridge and Bray 1985; Malik *et al.* 1982; Rovner *et al.* 1986*b*) with a molecular weight of 204 and 200 kDa (Kawamoto and Adelstein 1987). They are present in unequal proportions in many tissues (Burridge and Bray 1975; Cavaille *et al.* 1986; Kawamoto and Adelstein 1987; Malik *et al.* 1982) but in pig tissues Rovner *et al.* (1986*b*) found that they were present in equal proportions and proposed a single native myosin molecule that was organized as a heavy-chain heterodimer. Notwithstanding, there are many reports that indicate heterogeneity in the myosin expressed in smooth muscle: in the isoelectric isoforms of the 17-kDa light chain (Cavaille *et al.* 1986; Hasegawa *et al.* 1988) and the 20-kDa light chain (Erdodi *et al.* 1987); in its structure or enzymatic properties in pregnant and non-pregnant uterus (Huszar *et al.* 1980), rabbit stomach (Nohmi and Kuriyama 1979) and scallop adductor (Kondo and Marita 1981); and in the unequal proportion of heavy chains in airways, blood vessels and myometrium (Cavaille *et al.* 1986; De Marzo *et al.* 1987; Kawamoto and Adelstein 1987; Sparrow *et al.* 1987). The lability of these heavy chains is shown by a marked change in the relative abundance of these heavy chains in airways smooth muscle during development from the neonate to the adult pig (Mohammad and Sparrow 1988). Also by the change from approximately equal amounts in rat aorta *in vivo* compared with the predominance of the slower migrating heavy chain in cultures of passaged aortic cells (Rovner *et al.* 1986*a*) and cultured tracheal cells (Taylor and Stull 1986). The cultured aortic cells also express a third heavy chain, which is a non-muscle isoform, electrophoretically and antigenically the same as in platelets (Kawamoto and Adelstein 1987; Rovner *et al.* 1986*a*). Heterogeneity of myosin antigenic expression has been reported in elastic arteries (Larson *et al.* 1984) and isoforms of non-muscle myosin in platelets (Pegel *et al.* 1983).

We report here that the two heavy chains of myosin occur in quite different proportions in smooth muscle tissues. This suggested the possibility of isoforms of native myosin rather than a single myosin comprised of a heavy-chain heterodimer (Rovner *et al.* 1986*b*) although it did not exclude both arrangements occurring together. To investigate whether these heavy chains were arranged as homodimers, native myosin extracts were electrophoresed on non-dissociating (pyrophosphate) gels. When each band of native myosin was individually analysed on SDS-polyacrylamide gels the unequal proportion of the heavy chains was unchanged from that found in the original tissue extracts, implying that the pyrophosphate gel technique was not capable of resolving isoforms of native myosin from smooth muscle.

Methods

Myosin Preparation

Guinea pigs of either sex and weighing between 300 and 400 g were anaesthetized with ether. Approximately 15 cm of distal ileum adjacent to the ileocaecal junction was quickly removed, a cold glass rod inserted into the lumen so that the smooth muscle comprising both longitudinal and circular muscle approximately 0.15 mm in thickness could be dissected free of mucosa and submucosa. Female New Zealand White rabbits, weighing approximately 3 kg, were anaesthetized, the uterine horns removed and sections slipped over a cold glass rod. With the aid of a dissecting microscope bundles of smooth muscle fibres were teased away from the underlying tissue. Toads (*Bufo marinus*) were pithed, the stomach excised, washed out with normal saline and mounted on a glass rod. The serosa was removed then the circular muscle teased away from the submucosa to give narrow strips 0.6 mm thick. Pig tracheae were obtained from the local abattoir, transported in a Krebs solution at 0°C on ice and within 2 h of death the tracheal smooth muscle was dissected out into small strips about 0.5 mm thick from the tunica fibrosa at ventral aspect and connective tissue at dorsal aspect. Rats of either sex and weighing approximately 350 g were killed by cervical dislocation, and the femoral arteries dissected out from their origin at the abdominal aorta down to the lower thigh. They were dissected free of adventitia under the microscope. Human lung tissue was obtained from adults undergoing thoracotomy due to carcinoma, and transferred to the laboratory in Krebs solution at 0°C. The walls of bronchi of 3–5 mm in diameter were dissected free of lung parenchyma. All the tissues were kept moist with Krebs solution during dissection, and these procedures and subsequent extraction of proteins were carried out in the cold room with the tissue kept on ice.

For extraction of native myosin the smooth muscle, which weighed from 50–100 mg, was finely chopped with a razor blade on a cold plate, homogenized for 15 s in an ice-cold ground-glass homogenizer with 10 volumes of 50 mM NaH_2PO_4 , 10 mM phenylmethylsulfonyl fluoride (PMSF) at pH 7.0 and centrifuged at 12 000 g for 10 min at 4°C. The pellet was resuspended in a Guba-Straub solution containing 150 mM NaH_2PO_4 , 300 mM NaCl, 10 mM Na_2ATP , 0.125 mM PMSF and 1 mM 2-mercaptoethanol adjusted to pH 6.8, stirred occasionally for 1 h at 4°C, then centrifuged again. The supernatant was mixed with an equal volume of glycerol and stored at –20°C. When a second extraction of the pellet was carried out with Guba-Straub solution for a further hour it yielded a little more native myosin, albeit in greater dilution. This fraction was identical with the first fraction with respect to its heavy-chain composition on SDS 4%–polyacrylamide gel. When the native myosin was required to be maintained in the unphosphorylated state, 1 mM $\text{K}_2\text{H}_2\text{EGTA}$ (pH 7.0) was present in the above solution and Ca^{2+} was deleted from the Krebs solution. In some experiments the freshly dissected smooth muscle was instantly frozen at –170°C. It was subsequently crushed in a stainless steel mortar at –170°C and the frozen powder extracted in the above solutions. This modification had no effect on the profile of the protein bands seen on the pyrophosphate–polyacrylamide gels or on the proportion of the myosin heavy-chain bands seen on SDS 4%–polyacrylamide gels.

For extraction of dissociated myosin the smooth-muscle tissue was finely chopped in 10 volumes of sodium dodecyl sulfate (SDS) sample buffer containing 1% (w/v) SDS, 2.5% (v/v) 2-mercaptoethanol, 50 mM Tris buffer (pH 6.8) and 10% (v/v) glycerol, incubated in a boiling water bath for 2 min and then cooled, centrifuged and the supernatant stored at –20°C for SDS–polyacrylamide gel electrophoresis.

Electrophoresis of Native Myosin

Pyrophosphate–polyacrylamide electrophoresis was carried out by the method of Hoh *et al.* (1978), using a Pharmacia gel electrophoresis apparatus GE-2/4, with the following modifications: the length of the gel was reduced to 2.75 cm to optimize the separation of the bands, a constant current of 3 mA/tube for 22 h was used, $T = 4\%$ with cross linking 2.6%, the temperature of the running buffer, which was continuously recirculated from the lower to the upper tank was 0.5°C. Its composition was 30 mM $\text{Na}_4\text{P}_2\text{O}_7$, 1 mM ethylenediaminetetraacetic acid (EDTA), 10% glycerol and 2 mM cysteine adjusted to pH 8.8 with phosphoric acid. The gels were stained with 0.1% Coomassie brilliant blue R250 (Bio-Rad) for 3 h and then destained with 40% ethanol and 10% acetic acid. When the protein composition of the bands was to be analysed they were visualized by staining briefly (approx. 5 min) with 0.1% Coomassie brilliant blue G250 in 3% perchloric acid, then each band was excised separately with a razor blade, rinsed in buffer pH 7.5 to neutralize the perchloric acid, frozen in liquid N_2 at –170°C, then five frozen gel slices were pooled, crushed and taken up in SDS sample buffer, heated on a boiling water bath for 2 min, cooled and the polyacrylamide centrifuged down. The supernatant was stored at –20°C.

SDS–Polyacrylamide Electrophoresis of Heavy Chains of Myosin

The native myosin in Guba-Straub extract was dissociated into its sub-units by incubation in a boiling water bath with SDS sample buffer for 2 min. Samples were loaded on high-porosity SDS–polyacrylamide gel (stacking gel: $T = 3\%$, $C = 2.6\%$; resolving gel: $T = 4\%$, $C = 2.6\%$; slab thickness 1.5 mm), and electrophoresed at 22.5 mA/slab for 5–7 h as described by Laemmli (1970), and stained as above.

Immunoblotting

The protein bands in the SDS–polyacrylamide gel and in the pyrophosphate–polyacrylamide gel were transblotted onto nitrocellulose paper (Towbin *et al.* 1979) using a Bio-Rad apparatus for 4 h at 4°C. Transblotting was checked by staining a strip of nitrocellulose paper with amido black stain. The polyclonal antibodies were raised against purified myosin from chicken gizzard (smooth muscle myosin antibodies) and against human-platelet myosin (non-muscle myosin antibodies) in rabbits by Groschel-Stewart *et al.* (1985). The antibodies gave the best reaction at a protein concentration of 1–2 $\mu\text{g ml}^{-1}$ and were detected using a peroxidase-conjugated second antibody (goat anti-rabbit antibodies, Institut Pasteur, Paris) by incubation with horse-radish peroxidase developer (Bio-Rad).

Densitometric Quantitation

Densitometry of the Coomassie-blue-stained bands on the SDS–polyacrylamide gel was performed

on an LKB 2202 Ultrascan laser densitometer and absorbance of the peaks recorded on an LKB 2220 recorder with integrator. For quantitation purposes the areas under the peaks of the myosin heavy chains were measured. Each lane was scanned at three different places and the areas averaged. The areas were computed by the LKB 2220 by dropping a perpendicular to the horizontal baseline from the trough between the two peaks. The reliability of these ratios was confirmed independently using a graphics tablet with computer-programmed analysis.

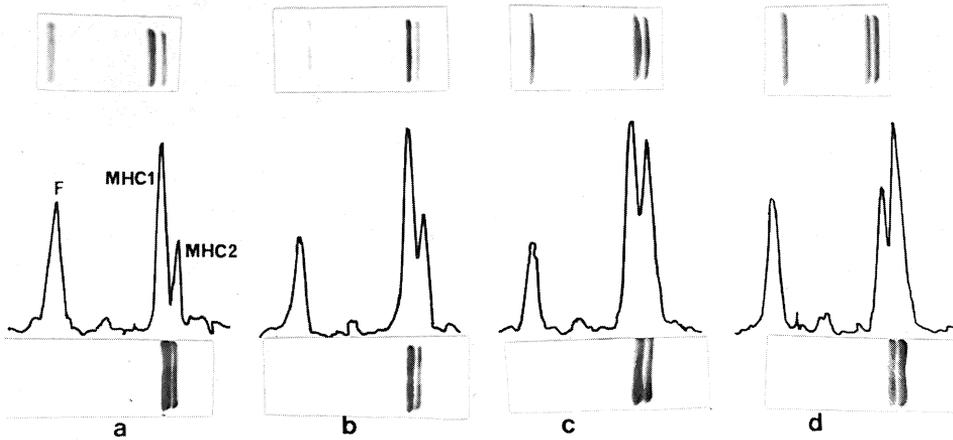


Fig. 1. Upper panel: SDS 4%-polyacrylamide gel electrophoresis of Guba-Straub extracts of smooth muscle from (a) toad stomach, (b) rabbit myometrium, (c) guinea pig ileum, (d) pig trachea. Middle panel: densitometric scans of filamin (F); and myosin heavy chains (MHC1 and MHC2). Lower panel: immunoblots with antibody to smooth muscle myosin.

Results

SDS-Polyacrylamide Electrophoresis of Guba-Straub Extracts of Smooth Muscle Tissues

SDS 4%-polyacrylamide gel electrophoresis of Guba-Straub extracts (native myosin) treated with SDS loading buffer prior to running, or of SDS extracts of homogenates of the whole tissue, revealed the two heavy chains of myosin previously described (Rovner *et al.* 1986b). These were referred to as MCH1 and MCH2 in order of increasing mobility. Fig. 1 shows sections of the gels (upper panel) and densitometric scans (middle panel) of the bands arising from the two heavy chains of myosin and from the filamin present in the Guba-Straub extracts from the smooth muscle of toad stomach, rabbit myometrium, guinea pig ileum and pig trachea. Location of the position of the bands was achieved using standards of purified myosin from rabbit skeletal muscle (heavy chain 200 kDa) and chicken gizzard (heavy chains 204 and 200 kDa) and purified filamin (230–240 kDa) also from chicken gizzard, which were run concurrently. Confirmation that both the 200-kDa bands were myosin was accomplished by indirect immunological labelling after electroblotting the proteins onto nitrocellulose paper (Fig. 1, lower panel). Both MHC1 and MHC2 reacted strongly with polyclonal antibodies to smooth muscle myosin at 1:2000 dilution but did not react with non-muscle myosin antibodies at this dilution, which gave a strong positive reaction with platelet myosin electroblotted concurrently.

The relative proportions of the heavy chains differed in each of the smooth muscle tissues examined. This was visually obvious by inspection of the gels (Fig. 1). In the circular smooth muscle of toad stomach, MHC1 was always in much greater abundance than MHC2, whereas in tracheal smooth muscle from pigs the proportion of MHC1 was less than MHC2. Quantitative assessment of the relative proportions was obtained by measuring the area

under each peak from densitometric scans (Table 1). The ratio of areas under the peaks of MHC1 and MHC2 was greatest in toad stomach, 3.6:1, and least in human bronchial smooth muscle, 0.69:1. The stoichiometry of the heavy chains was unchanged when determined over a three-fold range of protein loadings. The proportions of the heavy chains was the same irrespective of whether the myosin was extracted in the native form (Guba-Straub extract) or in the dissociated form (SDS extract of tissue). These differences in the

Table 1. Stoichiometry of the heavy chains of myosin (MHC) in the smooth muscle of various tissues

Values expressed as means \pm s.e.

Smooth muscle	<i>n</i> ^A	Area of MHC1 ^B	Ratio MHC1 : MHC2
Toad stomach	7	78.2 \pm 1.97	3.6 : 1
Rabbit myometrium	6	70.0 \pm 1.56	2.3 : 1
Rat femoral artery	4	67.0 \pm 1.53	2.0 : 1
Guinea pig ileum	9	55.8 \pm 1.08	1.3 : 1
Pig trachea	9	48.3 \pm 1.42	0.93 : 1
Human bronchus	14	40.7 \pm 1.32	0.69 : 1

^A Number of animals.

^B Expressed as % of total area of MHC1 plus MHC2.

relative proportions of the heavy chains in the tissues suggested that two native myosin molecules, in which the heavy chains were arranged as homodimers, could exist. This possibility was tested by examining their stoichiometry in the bands of native myosin obtained using non-denaturing (pyrophosphate) gel electrophoresis.

Pyrophosphate-Polyacrylamide Gel Electrophoresis of Guba-Straub Extracts

Fig. 2 shows examples of the band patterns obtained on pyrophosphate-polyacrylamide gels using Guba-Straub extracts containing native myosin. Three bands were most commonly resolved (Figs 2a, 2b, 2c) when the tissue was not in the relaxed state and was extracted without 1 mM K₂H₂EGTA in the solutions used (see 'Methods'). The slowest migrating band migrated with the same mobility as a filamin standard (Fig. 2c) and was shown to comprise filamin (see below). Rabbit myometrium showed two bands when extracted under Ca²⁺-free conditions (Fig. 2d). The relative intensities of the bands varied considerably in the tissues from the six species. To obtain the separation of the bands shown, short gels of 2.75 cm in length were used. Fig. 2e shows the effect of varying the length of the gel on the separation obtained. When gels of standard length, 6 cm, were used (Hoh *et al.* 1978), the bands were less well separated and the filamin band tended to fuse with the adjacent band. In view of the recent reports (Persechini *et al.* 1986; Takano-Ohmuro and Kohama 1986) it seemed most likely that the faster migrating bands comprised myosin in different states of phosphorylation. Support for this was obtained by extracting native myosin from tissues dissected in a Ca²⁺-free Krebs and maintained in the presence of 1 mM K₂H₂EGTA thereafter. Two bands were obtained from these relaxed tissues (Fig. 3) whereas tissues that were dissected in a Krebs solution containing 2 mM Ca²⁺ most often gave three bands but on some occasions four bands were obtained (Fig. 3). When this occurred the second and third bands were very close together and would fit the description given by Persechini *et al.* (1986) of the unphosphorylated and monophosphorylated myosins, with the fourth and fastest migrating band being attributable to the diphosphorylated myosin. This last band migrated 1.48 times faster than slow myosin (SM₁) from the anterior latissimus dorsi of the chicken wing, which was used as a marker (Fig. 3). However, identification of the state of phosphorylation of the bands was not pursued because the object was to estimate the stoichiometry of the heavy chains in the native myosin bands regardless of their state of phosphorylation.

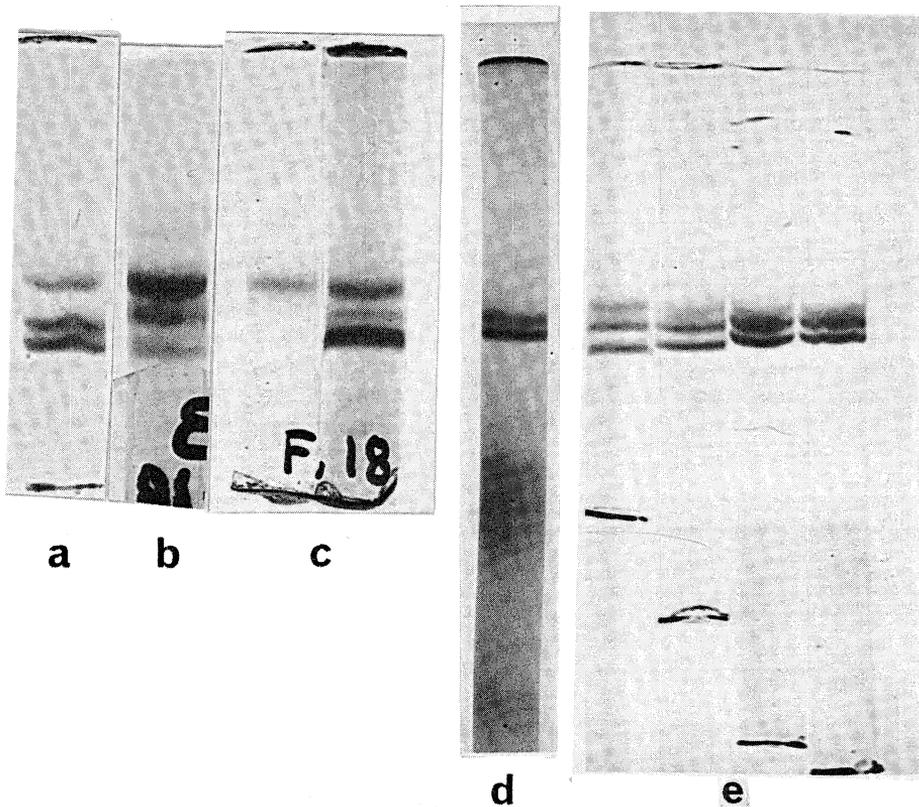


Fig. 2. Left: Pyrophosphate-polyacrylamide rod gels of 40 μ l of Guba-Straub extract of smooth muscle from (a) toad stomach, (b) pig trachea, (c) a filamin standard (F) 10 μ g run concurrently with guinea pig ileum (#18). Gel length 2.75 cm in (a) to (c). (d) Rabbit myometrium, 6 cm gel. (e) The effect of reducing the length of the gel on separation of bands using 50 μ l of Guba-Straub extract from another guinea pig. Left to right: 2.75 cm, 3.25 cm, 3.75 cm and 4 cm.

The protein composition of the native bands was investigated by electrophoretic analysis on SDS-polyacrylamide gels and by immunoblotting. Each band in the native gel was excised, the protein extracted and applied to SDS 4%-polyacrylamide gel. Cutting out the bands accurately was greatly facilitated by using Guba-Straub extract, which gave three bands, rather than Guba-Straub extract from relaxed tissue, which frequently gave poorly separated bands due to the preponderance of the putative unphosphorylated myosin fusing with the filamin as the densitometric traces of gels shown in Fig. 3 (left and middle) illustrate. Fig. 4 shows that in guinea pig ileum and toad stomach the uppermost band comprised almost entirely filamin and the second and third bands comprised principally myosin with a trace of filamin sometimes associated with the second band. The relative proportions of the heavy chains was the same in the middle band as in the fastest band and was the same as that in the original Guba-Straub extract applied directly to the SDS-polyacrylamide gel (Fig. 1). Similar findings were made for the stoichiometry of the heavy chains of pig trachea and rabbit myometrium. The protein composition of the bands on the native gels was also confirmed by electroblotting onto nitrocellulose, where the second and third bands reacted positively with the smooth muscle myosin antibodies but the filamin band showed no reaction, nor was there a reaction with the non-muscle antibody.

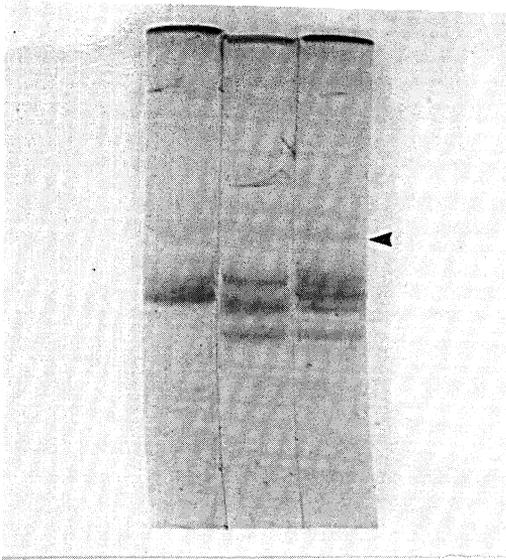
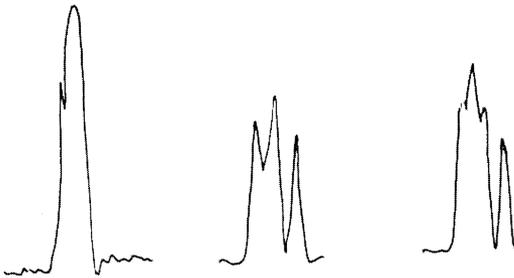


Fig. 3. Pyrophosphate-polyacrylamide rod gels and densitometric scans of Guba-Straub extract of pig trachea. Left: prepared under Ca^{2+} -free conditions from relaxed tissue; middle and right, prepared from contracted tissue exposed to Ca^{2+} . Arrow shows the position of the marker protein used, avian slow myosin (SM_1).



Discussion

The proportions of the two heavy chains of myosin, MHC1 and MHC2, have been estimated in smooth muscle in six species and found to differ widely. In toad stomach muscle MHC1 exceeded MHC2 by almost four times, whereas in the human bronchus the ratio was much less than one and was the same as in human tracheal smooth muscle obtained at post-mortem from infants dying of sudden infant death syndrome. We have attempted to provide quantitative data for the stoichiometry of the heavy chains to substantiate earlier, more qualitative reports. In the past, photographic records of the heavy chains on SDS-polyacrylamide electrophoresis gel showed that $\text{MHC1} < \text{MHC2}$ in chicken gizzard (Burrige and Bray 1975; Malik *et al.* 1982), and more recently densitometric scans showed that $\text{MHC1} \gg \text{MHC2}$ in rat and rabbit myometrium, $\text{MHC1} > \text{MHC2}$ in guinea pig myometrium (Sparrow *et al.* 1987). Kawamoto and Adelstein (1987) have quantified the relative amount of these heavy chains in rat aorta, where $\text{MHC1} \gg \text{MHC2}$, and in turkey gizzard $\text{MHC1} \ll \text{MHC2}$. Also we have confirmed that $\text{MHC1} = \text{MHC2}$ in pig carotid artery (Rovner *et al.* 1986b). Thus the ratio between MHC1 and MHC2 appears to be characteristic for a smooth muscle tissue, i.e. it is reasonably constant for a particular tissue.

These differing proportions of heavy chains are unlikely to arise through methodological artifacts. The pattern of heavy chains was identical irrespective of whether the myosin was

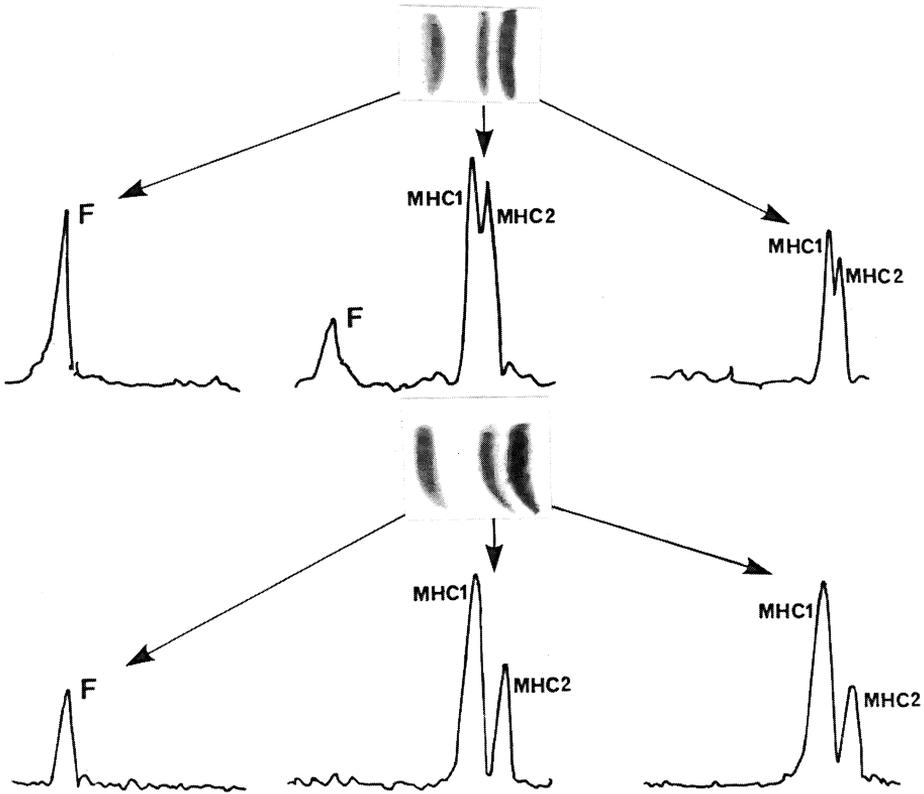


Fig. 4. Analysis on SDS 4%-polyacrylamide gel (densitometric scans) of each band cut from the pyrophosphate-polyacrylamide gel after electrophoresis of Guba-Straub extract of guinea pig ileum (upper) and toad stomach (lower). The slowest migrating band comprises filamin, the middle band myosin with a trace of filamin, and the fastest migrating band myosin. The ratio of MHC1 : MHC2 in the middle band is the same as in the lower band.

extracted from the tissue as native myosin using a Guba-Straub solution or as dissociated myosin using an SDS buffer. Using either a freshly chilled preparation or an instantaneously frozen tissue with or without a proteolytic inhibitor cocktail comprising leupeptin, PMSF, and pepstatin in the extracting solution made no difference. Furthermore, the same heavy-chain pattern was retained after the myosin-containing band from pyrophosphate-polyacrylamide was excised and re-electrophoresed on SDS-polyacrylamide gel. Both heavy chains reacted equally strongly with a smooth muscle myosin antibody after immunoblotting but not with a platelet myosin antibody, and we have also shown this in the other tissues referred to above, so that their identification as myosin heavy chains rests on substantial evidence. The MHCs may arise from two different genes, or perhaps from the same gene by alternative splicing (Breitbart *et al.* 1987). The similarity between the peptide maps of the two MHCs has not resolved this issue as yet (Kawamoto and Adelstein 1987).

The finding of a marked inequality in the relative proportions of the heavy chains typified by the toad stomach muscle suggested the possibility of isoforms of native myosin composed of heavy-chain homodimers as an alternative to the proposal of a single native myosin isoform that is a heavy-chain heterodimer (Rovner *et al.* 1986*b*). The existence of both heterodimer and homodimer molecules is not excluded. Nevertheless neither a heavy-chain homodimer nor a heterodimer arrangement could be demonstrated in the native

myosin bands on pyrophosphate–polyacrylamide gel. Each native band would have had to contain either one or the other chain only when it was re-electrophoresed on the SDS–polyacrylamide gel for a homodimeric arrangement to be the case: for a heterodimer the equal amounts of both MHCs would have been seen. Instead, the ratio of the heavy chains was the same as in the original tissue extracts whether extracted as native myosin or dissociated myosin from the tissue. Furthermore the ratio was the same in each band of the pyrophosphate gel irrespective of the state of the phosphorylation of the myosin, which is undoubtedly the reason for the separation of the myosin-containing bands (Persechini *et al.* 1986; Takano-Ohmuro and Kohama 1986). Any charge difference arising from the difference in amino acid composition of the isoforms of the 17-kDa light chain of myosin (Hasegawa *et al.* 1988) does not appear to affect migration of native myosin on pyrophosphate gels (Cavaille *et al.* 1986). The stoichiometry of the heavy chains in the late pregnant rat myometrium where MHC1 \gg MHC2 was also unchanged after pyrophosphate–polyacrylamide electrophoresis (Sparrow *et al.* 1987). Therefore, it would appear that the pyrophosphate technique does not resolve isoforms of native myosin in smooth muscle despite the high resolution of separation obtained in the current experiments. In skeletal muscle, embryonic myosins cannot be resolved from adult myosins by this technique despite differences in the structure of their heavy chains (Pette and Vrbova 1985). The inability to separate native myosins could arise firstly because the charge difference between the putative isoforms arising from different amino acid substitutions in the heavy chains was insufficient to significantly affect their mobility, or secondly because of the conformation of the myosin molecule: if it were in the folded 10S (Onishi and Wakabayashi 1984) form this could reduce the number of potentially available charged sites. High ionic strength and phosphorylation of the myosin favour the extended form (Trybus and Lowey 1984), whereas the high pH and the pyrophosphate concentration favour the folded form (Craig *et al.* 1983). Given the uncertain effects of the low temperature ($<2^{\circ}\text{C}$), which is a critical requirement for band separation, and also the presence of filamin, the actual form in which the myosin migrates is difficult to predict. To what extent the much faster migration rate of the smooth muscle myosin compared with skeletal myosin reflects their different amino acid compositions, although this difference is minor (Barany *et al.* 1966), or a 10S versus 6S conformation needs to be determined.

The presence of filamin in the Guba-Straub extracts of smooth muscle is undesirable when electrophoresing native myosin on pyrophosphate–polyacrylamide gels. Filamin has a molecular weight of 500 kDa, only slightly greater than smooth muscle myosin (470 kDa) and their mobilities are very similar. Unless the electrophoresis conditions are optimal, filamin and myosin do not separate sharply (see Fig. 2) or may not separate at all on the longer 6-cm gels used by Hoh *et al.* (1978) so that a band is obtained that is not homogeneous. The failure to recognize the presence of filamin in the myosin band has led to the claim that a 230-kDa protein component is a heavy-chain myosin isoform (Beckers-Bleux and Marechal 1985) instead of the monomeric subunit of filamin (Persechini *et al.* 1986).

In conclusion, differing proportions of heavy chains are a feature of myosin in smooth muscle tissues. The arrangement of the heavy chains in molecules of native myosin awaits the development of a biochemical technique that will separate putative native isoforms. As soon as antibodies specific for each heavy chain are produced then visualization of the heavy chains as homodimers or heterodimers by monoclonal antibody epitope mapping should be possible (Dechesne *et al.* 1987) and possibly their cellular location. A relationship between the ratio of the heavy chains in a tissue with its function is not as yet evident but may become so as more tissues are studied.

Acknowledgments

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