The distribution of heavy-chain isoforms of myosin in airways smooth muscle from adult and neonate humans

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Changes in the expression of heavy chains of myosin during development determine the functional characteristics of striated muscles. The distribution of heavy-chain isoforms of smooth-muscle myosin was determined in the airways of adult and infant humans to see whether it might underlie the hyperreactivity of human airways. The protein bands corresponding to myosin were separated using SDS/polyacrylamide-gel electrophoresis (4% gels) and identified by immunoblotting using both monoclonal and polyclonal antibodies against smooth-muscle myosin and non-muscle myosin. The relative proportion of each heavy chain stained by Coomassie Blue was measured by densitometric scanning. Three major bands corresponding to myosin heavy-chain isoforms were found; the two slower migrating bands (MHC1 and MHC2) were smooth-muscle myosin, and the third band was non-muscle myosin. The MHC1/MHC2 ratio was 0.69:1 in adult bronchus, and in infant bronchus and trachea. This contrasted with the airway smooth muscle in pigs, which was run concurrently, where the smooth-muscle heavy-chain ratio changed with development [Mohammad & Sparrow (1988) FEBS Lett. **228**, 109–112]. The non-muscle myosin heavy chain comprised 63% of the smooth-muscle myosin. In both adult and infant lungs an additional putative myosin heavy chain which migrated slightly more rapidly than non-muscle myosin heavy chain was identified using the monoclonal smooth-muscle myosin antibody BF 48. This was unique to the human species.

INTRODUCTION

Isoforms of myosin have been extensively characterized in skeletal and cardiac muscle, and their distribution correlates closely with function, such as speed of shortening (Pette & Vrbova, 1985; Sweeney *et al.*, 1986). During development three heavy chains appear sequentially in skeletal muscle (Whalen *et al.*, 1981) and this transition is associated with corresponding changes in the maximum velocity of shortening (Reiser *et al.*, 1988). Smooth muscles show a considerable functional diversity—the maximum velocity of shortening varies by more than 40-fold in different tissues (Hellstrand & Paul, 1982) – but the relationship between isoforms of myosin and function, if any, is undefined. This is partly due to the limited information available concerning myosin expression in smooth muscle. Evidence for the existence of isoforms of native myosin is still controversial (Takano-Ohmuro *et al.*, 1983; Beckers-Bleukx & Marechal, 1985; Lema *et al.*, 1986; Rovner *et al.*, 1986a) but the heavy-chain isoforms are now well documented (Rovner *et al.*, 1986a). These comprise two heavy chains (designated here as MHC1 and MHC2) of molecular mass 204 kDa and 200 kDa respectively (Kawamoto & Adelstein, 1987) in all vertebrate smooth muscles examined so far. The ratio of MHC1/MHC2 varies among species and is 2.6:1 in rat gravid uterus (Sparrow *et al.*, 1988), 1:1 in the media of pig carotid artery (Rovner *et al.*, 1986a) and 0.6:1 in turkey gizzard (Kawamoto & Adelstein, 1987). The proportions of the myosin heavy chains change with development in pig airways (Mohammad & Sparrow, 1988) and during tissue culture (Rovner *et al.*, 1986b; Taylor & Stull, 1986; Kawamoto & Adelstein, 1987) but not during hypertrophy (Sparrow *et al.*, 1988; Hewett

et al., 1988). The smooth-muscle myosin heavy chains are structurally different from the non-muscle myosin heavy chain (designated here as NM MHC) of molecular mass 196 kDa (Kawamoto & Adelstein, 1987) which is frequently found in tissues containing smooth muscle. It can be expressed by vascular smooth muscle cells in culture (Kawamoto & Adelstein, 1987; Rovner *et al.*, 1986b) and may be present in the intact media of arteries (Larson *et al.*, 1984).

A third smooth-muscle MHC has recently been reported in human airways tissue (De Marzo *et al.*, 1987) and in human vascular, uterine and intestinal tissues (Sartore *et al.*, 1987), based on immunological evidence, which has an electrophoretic mobility slightly greater than that of MHC2. This prompted us to examine the distribution of heavy chains in the airways of humans during development, particularly because the expression of this contractile protein might contribute to some of the functional characteristics of human airways, for example, hyperreactivity which is seen in adults, and more frequently in children (Hopp *et al.*, 1985) and in infants (Prendiville *et al.*, 1987).

Accordingly, we have measured the proportions of the heavy chains of myosin in the airways smooth muscle of adult and neonate humans, firstly to compare the distribution of MHC1 and MHC2 with other species and also to observe the expression of the putative third heavy chain, and secondly to quantify the amount of NM MHC relative to MHC1 and MHC2. Airways from adult and neonate pigs were concurrently examined to provide a comparison with a mammalian smooth muscle in which a marked change in the proportions of heavy chains occurs during development (Mohammad & Sparrow, 1988). We found that the ratio of MHC1/MHC2

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was 0.69:1 in adult human bronchus and was almost the same in infant bronchus and trachea. The putative third smooth-muscle myosin heavy chain migrated faster than non-muscle myosin and was detected in most preparations using the monoclonal antibody BF 48 raised against bovine foetal myosin, but was absent in pig and rat smooth muscle tissues.

MATERIALS AND METHODS

Human lung segments consisting of bronchus and lung parenchyma were obtained from 14 adult patients of either sex, average age 63 years (range 56–70 years) during surgical resection of part of the lung due to carcinoma. The specimens were immediately conveyed to the laboratory in ice-cold Krebs' solution where pieces of bronchus 3–6 mm in diameter were dissected out free of lung parenchyma, frozen in liquid N₂ and stored at –70 °C until use. Lung parenchymal tissue cut from the periphery of the lung was freed of airways greater than 1 mm in diameter and similarly stored. A whole lung and trachea was obtained within 6 h of death from eight infants of either sex, average age 4 months (range 1–8 months) who had died from Sudden Infant Death Syndrome. The specimens were placed in Krebs' solution at 0 °C immediately after autopsy and collected soon after. Pieces of bronchi were dissected out free of lung parenchymal tissue and tracheal smooth muscle (trachealis) was also dissected from the trachea. They were stored in liquid N₂ until used.

Extraction of myosin

For extraction of native myosin from the airways, the tissue was finely chopped with a razor blade on a cold plate, homogenized for 15 s in an ice-cold ground glass homogenizer with 10 vol. of phosphate buffer comprising 10 mM-sodium phosphate buffer (pH 7.0), 1 mM-EGTA and 0.125 mM-phenylmethanesulphonyl fluoride (PMSF) and centrifuged at 12000 g for 10 min at 4 °C. The pellet was resuspended in a Guba–Straub extraction solution containing 150 mM-sodium phosphate buffer, 300 mM-NaCl, 10 mM-Na₂ATP, 1 mM-EGTA, 0.125 mM-PMSF and 1 mM-2-mercaptoethanol adjusted to pH 6.8, or in pyrophosphate extraction solutions containing 40 mM-Na₂P₂O₇, 1 mM-MgCl₂, 1 mM-EGTA and 0.125 mM-PMSF with the pH adjusted to 8.5 or 9.5. The tissue was extracted at 0 °C for 1 h with continuous stirring and centrifuged again. The supernatant was mixed with an equal volume of SDS sample buffer containing 1% (w/v) SDS, 2.5% (v/v) 2-mercaptoethanol, 50 mM-Tris/HCl 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. In other experiments the freshly dissected bronchial and tracheal segments were finely chopped in 10 vol. of SDS sample buffer as described before, left on ice for 0.5 h with occasional stirring, 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. These different methods of extraction did not affect the profile of the myosin heavy-chain bands seen on SDS/4%-polyacrylamide gels (see Fig. 3), nor did omitting the washing step in 10 mM-sodium phosphate buffer (pH 7.0). The advantage of the latter

was that it removed a great many soluble proteins, particularly in the case of parenchyma, which otherwise are carried through to the electrophoresis. Samples containing dissociated myosin were loaded on a high porosity SDS/4% polyacrylamide gel (stacking gel: T = 3%, C = 2.6%, resolving gel: T = 4%, C = 2.6%, slab thickness 1 mm), and electrophoresed at 22.5 mA/slab for 3 h and stained as described by Mohammad & Sparrow (1988).

Immunoblotting

The protein bands in the SDS/polyacrylamide gel were transblotted on to nitrocellulose paper (Towbin *et al.*, 1979) using a Bio-Rad apparatus at 150 mA for 15 h at room temperature. A strip of nitrocellulose paper was stained with Amido Black to check the transblotting. The monoclonal antibody BF 48 (IgG₁ type) was generously supplied by Dr. S. Sartore, Institute of General Pathology, University of Padova, Italy, and was raised by intraperitoneal injection of 2-month-old Balb/c mice with bovine foetal (3-months gestation) myosin in complete Freund's adjuvant (Sartore *et al.*, 1989).

The polyclonal antibodies were produced in rabbits against purified myosin from chicken gizzard (smooth-muscle myosin antibodies) and against human platelet myosin (non-muscle myosin antibodies) by Dr. U. Groschel-Stewart, Institut für Zoologie, Technische Hochschule, Darmstadt, Germany (Groschel-Stewart *et al.*, 1985). A second smooth-muscle myosin polyclonal antibody raised in rabbits against myosin from calf aorta was used (designated AP2) by courtesy of Dr. N. De Marzo, Institute of Occupational Medicine, University of Padova, Italy (De Marzo *et al.*, 1987). It showed the same binding and specificity as the antibody raised to the myosin from chicken gizzard. The specificity of the antibodies was determined by immunoblotting using myosin preparations from smooth and striated muscle and platelets. Indirect immunofluorescence was performed on fresh-frozen cryostat sections (about 10 µm) of the same airway preparations used in the electrophoresis and immunoblot experiments as described by Everett & Sparrow (1987). The antibodies gave the best reaction for immunoblotting at a protein concentration of 1–2 µg/ml and were detected using a peroxidase-conjugated second antibody [goat anti-rabbit antibodies (Institut Pasteur, Paris) in the case of polyclonal antibodies and goat anti-mouse antibodies for monoclonal antibodies (Bio-Rad)]. The colour development was revealed by incubation with 0.05% 3,3-diaminobenzidine (Sigma) in 50 mM-Tris/HCl buffer at pH 7.4 and 0.035% of 30% hydrogen peroxide.

Densitometric quantification

The Coomassie Blue-stained bands on the SDS/polyacrylamide gel were scanned to estimate the relative amounts of proteins using an LKB 2202 Ultrascan laser densitometer, and the absorbance of the peaks was recorded on a LKB 2220 recorder with integrator. For quantification purposes, the areas under the peaks of the myosin heavy chains were measured. The values for each band scanned at three different places in each lane were averaged. The area was computed by the LKB 2220 by dropping a perpendicular from the trough between the two peaks to the horizontal baseline. The reliability of these ratios was confirmed independently using a graphics tablet with computer-programmed analysis.

Fig. 1. Separation of MHCs in Guba–Straub extracts of human and pig airways by electrophoresis on SDS/4%-polyacrylamide gels and subsequent immunoblotting

(a) Lane 1, heavy chains of smooth muscle myosin (MHC1 and MHC2) and non-muscle myosin (NM MHC) in the bronchus from an adult human; F is filamin monomer, approx. 240 kDa; lane 2, MHCs in another adult human bronchus; lane 3, MHC1 and MHC2 in adult pig trachealis smooth muscle; lane 4, NH MHC in pig platelets; lane 5, MHCs in infant trachealis; lane 6, MHCs in infant bronchus; lane 7, MHCs in neonate pig trachealis. (b) Immunoblots of Guba–Straub extracts from adult human bronchus. Lanes 1 and 2, reaction with MHC1 and MHC2 using polyclonal antibodies to smooth muscle myosin; lanes 3 and 4, reaction to MHC1, MHC2 and NM MHC using both smooth muscle myosin and non-muscle (platelet) myosin antibodies (the latter showed no reaction with MHC1 and MHC2 – see Fig. 2c). Note: lanes 2 and 3 were a single lane on the original polyacrylamide gel.

Fig. 2. Airway smooth-muscle MHCs in humans and pigs

(a) SDS/4%-polyacrylamide-gel electrophoresis of MHCs in Guba–Straub extracts. Lane 1, adult human bronchus in which an additional putative MHC band is just resolved; lane 2, trachealis of adult pig (MHC1 = MHC2); lane 3, trachealis of neonate pig (MHC1 > MHC2). (b) Section of a gel lane (adult human bronchus) from filamin to myosin enlarged to show four bands corresponding to MHCs, and the corresponding densitometric scan. (c) Immunoblot of MHCs of adult human bronchus. Lanes 3 and 4, reaction using monoclonal antibody BF 48 showing MHC1, MHC2 and a BF 48-sensitive third band; lanes 1 and 2, the non-muscle heavy chain NM MHC is revealed using the platelet myosin antibody. It is shown lying between the MHC2 and BF48-sensitive third band, and could just be resolved as a doublet. This antibody did not stain the smooth muscle heavy chains. (d) Immunoblot showing BF 48-sensitive bands in bronchus and parenchyma from another adult human lung. Lane 1, bronchus (30 μ l) loaded; lane 2, parenchyma (20 μ l) loaded; lane 3, parenchyma (25 μ l) loaded.

RESULTS

Myosin from adult and infant human bronchus extracted in the native form, or in the dissociated form

with SDS, showed three major protein bands with molecular mass of approx. 200 kDa on SDS/4%-polyacrylamide gels. The electrophoretic mobility of the

Table 1. Stoichiometry of the heavy chains of myosin in adult bronchus, infant bronchus and trachealis

The area of MHC1 is expressed as a percentage of total area of MHC1 plus MHC2. Values are expressed as mean $\pm$ S.E.M.; n = number of humans.

Group	n	Area of MHC1	MHC1/MHC2
Adult bronchus	14	40.7 ± 1.3 %	0.69:1
Infant bronchus	8	40.9 ± 1.1 %	0.69:1
Infant trachea	8	41.3 ± 2.1 %	0.70:1

slower-migrating first and second bands was the same as that of the heavy chains of smooth-muscle myosin from other mammalian species, while that of the third band was the same as that of the heavy chain of myosin from human platelets, which has a molecular mass of 196 kDa (Kawamoto & Adelstein, 1987). Fig. 1 shows the three bands, designated MHC1, MHC2 and NM MHC, together with myosin standards from pig trachealis and human platelets. Confirmation that the first two bands were smooth-muscle myosin was achieved by immunoblotting with polyclonal antibodies specific to smooth-muscle myosin from two sources, chicken gizzard and calf aorta (AP2). Both MHC1 and MHC2 reacted strongly with the antibodies at 2 μ g/ml (Fig. 1b, lanes 1 and 2). The third band, NM MHC, gave a strong reaction with myosin antibodies against platelets at the same dilution (Fig. 1b, lanes 3 and 4) but did not react with smooth-muscle bands (Fig. 2c, lanes 1 and 2). Table 1 shows the proportions of myosin heavy chains in adult bronchus and in bronchus and trachea from infants. The percentage of MHC1 of the smooth muscle myosin was 40.7 ± 1.3 % ($n = 14$) giving a MHC1/MHC2 ratio of 0.69:1. The non-muscle myosin chain comprised 63 ± 5.4 % ($n = 8$) of the total smooth-muscle myosin (MHC1 + MHC2). In the trachea and bronchus from eight infants, the distribution of MHC1 and MHC2 was the same as in the adult, namely 41.3 ± 2.1 in trachea and 40.9 ± 1.1 of MHC1 in bronchus. The proportion of the heavy-chain isoforms was very similar in the more distal parts of the airways. In one adult lung the proportion of MHC1 was 33 % in the bronchus, 26 % in the small airways and 28 % in the parenchyma. The non-muscle myosin appeared to be a little more abundant in the parenchyma (72 %) than in the bronchus (63 %).

The proportion of the myosin bands MHC1, MHC2 and NM MHC found in the airways tissue was unaffected by the mode of extraction of the myosin (see the Materials and methods section). For example, when bronchial tissue was subdivided, homogenized and divided into three portions, the proportion of MHC1 was 40 % after extraction with Guba–Straub (pH 6.7), 37.7 % with the pyrophosphate buffer (pH 9.5) and 39.4 % when the homogenate was extracted directly into SDS loading buffer. The proportion of NM MHC was similarly unchanged. Likewise, there was no difference in the band pattern when bronchus or lung parenchyma was extracted in pyrophosphate buffer at pH 8.5 or 9.5.

In some lung preparations a fourth band could just be resolved which ran slightly ahead of the non-muscle band (Fig. 2a). It was difficult to discern from the NM MHC band in most gels, but occasionally separation was

Fig. 3. A comparison of MHCs in airways from adult pig extracted using three different procedures

Each tissue was extracted with Guba–Straub (G–S) solution, pH 6.7 (lane 1), with pyrophosphate (PP_i) buffer, pH 9.5 (lane 2), or with SDS sample buffer (lane 3). (a) Trachealis; (b) bronchus; (c) parenchyma.

sufficient for it to be detected by the densitometer (Fig. 2b). The amount present was quite variable from one lung to another and it did not react with the non-muscle myosin antibody, but along with MHC1 and MHC2 it reacted with a monoclonal antibody to smooth-muscle myosin referred to as BF 48 (De Marzo *et al.*, 1987). Fig. 2(c) shows a nitrocellulose immunoblot from a 4 % polyacrylamide gel in which the bands are particularly well separated. The third band, NM MHC, is stained by the non-muscle-myosin antibody while MHC1, MHC2 and the fourth band are stained by the BF 48. The intensity of the reaction displayed by this fourth band varied from lung to lung, showing that the amount present varied relative to the amounts of MHC1 and MHC2. In three out of six adult bronchi and in two out of six infant bronchi tested with BF 48 it stained very strongly, and in two other infant bronchi it stained weakly. In lungs in which the BF 48-sensitive band was detected in the bronchus, this band was also present in the small airways and the parenchyma; in the latter its intensity was generally similar to that of MHC1 and MHC2 (Fig. 2d, lanes 2 and 3). In other lungs, BF 48 stained only MHC1 and MHC2, as did the smooth-muscle-myosin polyclonal antibody, and the third band stained only with the platelet-myosin antibody.

Fig. 4. Cryostat section of a human bronchiole stained for indirect immunofluorescence using BF 48 antibody

Only the smooth muscle of the bronchiole and nearby blood vessels are stained.

The BF 48-sensitive band was unique to the human species; it was not present in rat uterus (pregnant or non-pregnant), nor in pig smooth muscle tissue extracted under identical conditions. Fig. 3 illustrates the heavy chain pattern of pig trachea, bronchus and parenchyma on SDS/4%-polyacrylamide gels using the three extraction procedures referred to above. Only MHC1 and MHC2 were present in trachea, whereas three bands, of molecular mass about 200 kDa, were seen in bronchus and parenchyma. These were identified as MHC1, MHC2 and NM MHC using the smooth-muscle-myosin and platelet-myosin polyclonal antibodies. The monoclonal antibody BF 48 reacted with only two bands, MHC1 and MHC2.

Immunofluorescence studies were performed using cryostat sections of airways to examine the specificity of the smooth-muscle antibodies in human lung tissue. The monoclonal antibody BF 48 and the polyclonal antibody showed the same strong reaction with the smooth muscle cells of the airways and blood vessels of human lung (Fig. 4). They did not stain the epithelial or the connective tissue. Both antibodies showed a strong reaction with smooth muscle cells only in tracheal sections from pig and myometrium from rat.

DISCUSSION

In this study, three myosin heavy chains were found in human trachea, bronchi and lung parenchyma. The two slower-migrating heavy chains were smooth-muscle myosin, and the third heavy chain was non-muscle myosin. These appear to be very similar to those described in rat aorta and pig carotid based on their electrophoretic mobility (Kawamoto & Adelstein, 1987; Rovner *et al.*, 1986a, b). The source of this non-muscle myosin, which was abundant in bronchi and parenchyma, may be either the epithelium of airways, or fibroblasts present in the connective tissue of these structures. The possibility that it comes from the muscular layer of the airways itself has to be considered in view of immunocytochemical evidence for its presence in the media of elastic arteries (Larson *et al.*, 1984) and more recently by electrophoresis in adult swine carotid smooth muscle (Eddinger *et al.*, 1988). Immunocytochemical evidence for its location could not be obtained using our platelet-myosin antibody which,

despite its specificity for immunoblotting, gave a diffuse non-selective reaction with cryostat sections of airways.

The ratio of the heavy chains MHC1 and MHC2 in adult human bronchus was 0.69:1 which differs from the majority of mammalian smooth muscle tissues where MHC1 predominates (Sparrow *et al.*, 1987, 1988) or is equal to MHC2 (Rovner *et al.*, 1986a). This ratio was the same in infant bronchus and trachea and is very similar to that reported by De Marzo *et al.* (1988) in the trachea and bronchus of adult asthmatics, namely 0.72:1. This observation contrasts with pig trachea where the distribution changes from 2.1:1 in neonates to 0.95:1 in adult pigs (Mohammad & Sparrow, 1988). A similar shift occurs in the smooth muscle from pig carotid, stomach and uterus (Eddinger & Murphy, 1988). Concurrently, the sensitivity of the contractile proteins to Ca^{2+} is increased several times in the trachea of the neonate pig compared with the adult as we have shown using chemically-skinned fibres (M. A. Mohammad & M. P. Sparrow, unpublished work). The absence of any change in humans may indicate that development of the contractile machinery is essentially complete in these infants (mean age 4 months), but further studies are needed at much earlier stages in the development of the human. Histological sections show that the smooth muscle is well developed around the airways in these infants. The pattern of the heavy chains was unlikely to be influenced by tissue degradation between death and autopsy in the infants, which in most cases was quite short (3–6 h). Airways from all tissues were tested in an organ bath for viability by eliciting responses to electrical-field stimulation of the smooth muscle, which gave rapid and strong contractions. There is little likelihood of degradation occurring during the rapid extraction procedure carried out in the cold under Ca-free conditions and with PMSF present. The procedure used here was checked exhaustively using four different extraction regimes with the same results.

The ratio of these heavy chains is remarkably constant in any one smooth muscle tissue yet varies widely within and across species (Kawamoto & Adelstein, 1987; Sparrow *et al.*, 1987), but the significance of this tissue-specific heavy-chain ratio is unclear at present. Although a homodimer arrangement of the heavy chains has been suggested in adult pig tissues where the two heavy chains are present in equal proportions (Rovner *et al.*, 1986a) this seems unlikely to be the case in the majority of tissues where the proportions vary from as high as 4:1 in toad stomach muscle to 0.7:1 reported here for human airways. Progress in understanding this phenomenon is unlikely until identification of the myosin heavy chains is undertaken using monoclonal-antibody epitope mapping as has been done in cardiac myosin (Dechesne *et al.*, 1987). Possibly the heavy-chain ratio reflects the assembly of the myosin molecules into a thick filament as in the body wall muscle of *C. elegans*, where the two myosin heavy-chain isoforms determine the zone length of the filament such that the zone length increases markedly as the heavy chain ratio increases from 1:4 in the N2 strain to 1:0 (i.e. no MHC 2) in a mutant strain (Epstein, 1986).

The putative third heavy chain of smooth muscle myosin (i.e. BF 48-sensitive) was found in bronchi and parenchyma of the lungs of a number of adults and also in young infants, and was unique to the human species. It has been principally characterized in human pulmonary artery (Sartore *et al.*, 1989), and has been observed in

human uterine, intestinal and airways tissue (Sartore *et al.*, 1987; De Marzo *et al.*, 1987). It was difficult to quantify because of its close proximity to the abundant non-muscle heavy chain. Using immunological staining it stained as strongly as MHC1 or MHC2 in some lungs but in others stained very weakly. In some lungs it was not detectable as De Marzo *et al.* (1988) have recently reported. We could not detect it in the airways of pig lung nor in the gravid and non-gravid rat myometrium. The origin of this band is difficult to pinpoint. Why it did not stain with the polyclonal antibodies to smooth muscle myosin used in this study, one raised against chicken gizzard myosin and the other against calf aorta myosin (AP2), is difficult to understand. This evidence argues against it being a proteolytic product of MHC2. On the other hand, the possibility that this BF 48-sensitive band is a different protein (not a myosin heavy chain) which has an epitope in common with MHC1 and MHC2 also seems remote. It is essential to compare its peptide map with that of MHC1 and MHC2 in order to resolve this. As it is, the peptide maps of MHC1 and MHC2 are very similar in rat aorta and in turkey gizzard. Small differences are seen between the corresponding chains in visceral and vascular smooth muscle within a species, and major differences occur between avian and mammalian species (Kawamoto & Adelstein, 1987). Evidence for the existence of two MHC mRNA species has been obtained by S1 nuclease mapping techniques in rabbit uterus myosin (Nagai *et al.*, 1988) which may correspond to the two MHC protein isoforms. However, Yanagisawa *et al.* (1987) have sequenced the heavy-chain genome of chicken gizzard myosin and it appears to be a single copy gene unlike the multigene family of striated muscle myosin heavy chains, leading those authors to suggest that MHC2 may arise as a result of post-translational modification of MHC1, although the possibility of alternate splicing giving rise to these chains could not be excluded. It is important to know whether there is one or more genes which expresses myosin heavy chains in the human species.

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